Arterial Hemodynamics in Aging Populations
From genes to clinical practice

Germaine Claudette Verwoert

The work described in this thesis was conducted at the department of Epidemiology in collaboration with the department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands.

The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), the Netherlands Genomics Initiative (NGI), and the Municipality of Rotterdam.

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

Cover design and layout by Ferry Drop

Print: Optima Grafische Communicatie, Rotterdam

ISBN: 978-94-6169-513-0

Arterial Hemodynamics in Aging populations

From Genes to Clinical Practice

Arteriële hemodynamiek in vergrijzende populaties

Van genen naar kliniek

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
Op gezag van de Rector magnificus

Prof.dr. H.A.P. Pols

En volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op
woensdag 11 juni 2014 om 13.30 uur

Door

Germaine Claudette Verwoert
Geboren te Vlaardingen, Nederland

ERASMUS UNIVERSITEIT ROTTERDAM

Promotiecommissie

Promotores:	Prof.dr. E.J.G. Sijbrands
	Prof.dr. C.M. van Duijn
Overige Leden:	Prof.dr. O.H. Franco
	Prof.dr. F. Zijlstra
	Prof.dr. G.A. Rongen
Copromotor:	Dr. F.U.S. Mattace-Raso

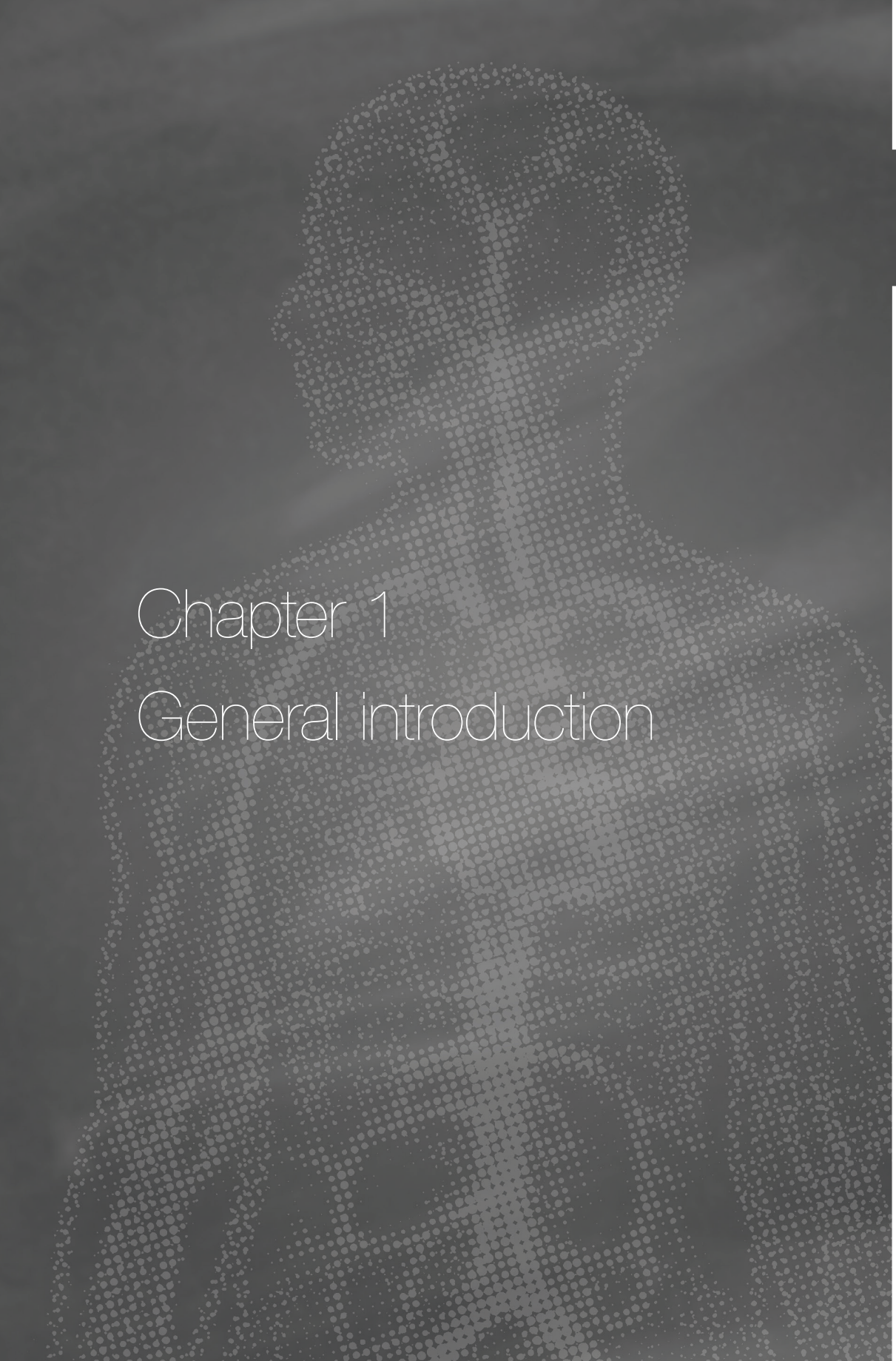
Financial support for the publication of this thesis was kindly provided by the Erasmus Medical Center.

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

‘Waar je aan begint, moet je ook afmaken’

Ter nagedachtenis aan mijn vader

Contents



Chapter 1
General introduction

Cardiovascular disease is the number one leading cause of death globally and estimations showed that the number of people, who will die of cardiovascular disease, will increase in the coming years.[1,2] Large part of cardiovascular diseases can be prevented by reducing the absolute risk by addressing multiple cardiovascular risk factors such as smoking, unhealthy diet, obesity, diabetes mellitus, raised lipids and hypertension.[3]

In this thesis, we will focus on the age related changes of the vascular tree in relation to cardiovascular disease. First, we will discuss a number of general principles of the hemodynamic system and the influence of advancing age. Second, we will explain general principals of genetic analyses. Third, we will describe the clinical consequences from the age related vascular changes and genetic analyses. Lastly, we describe the scope of this thesis.

Blood pressure

Blood pressure is the pressure exerted by circulating blood upon the walls of blood vessels. It is one of the vital signs and a certain value of arterial pressure is needed for the perfusion of organs. During each heartbeat, blood pressure varies between a maximum (systolic) and a minimum (diastolic) pressure.
Blood pressure is determined by:

1) Cardiac output, determined by frequency and stroke volume of the heart
2) Arterial resistance, which depend on the diameter of the artery
3) Central venous pressure
4) Circulating volume

Blood pressure varies widely. This variation is regulated by two feedback systems:

1) The central nervous system allows very precise, short-term blood pressure regulation through sympathetic and parasympathetic divisions of the autonomic nervous system. There is an intricate and interactive set of feedback from baro-, chemo- and osmoreceptors to continuously monitor blood flow and pressure. The autonomic nervous system adjusts the mean arterial pressure by altering, frequency and stroke volume of the hearts contraction as well as the total peripheral resistance.
2) The long-term adjustment of arterial pressure is performed by the renin-angiotensin system (RAS). This system allows the kidney to compensate for loss in blood volume or drops in arterial pressure by activating an endogenous vasoconstrictor, angiotensin II.[4]

The fluctuation of the arterial pressure results from the pulsatile nature of the cardiac output. The difference of the measured systolic and diastolic pressure is called pulse pressure and the average pressure over a cardiac cycle is the mean arterial pressure.

Hypertension

Elevated blood pressure, called hypertension, is a common and strong cardiovascular risk factor and responsible for many cardiovascular events.[5] Hypertension is defined as systolic blood pressure (SBP)≥ 140 and/or diastolic blood pressure (DBP)≥ 90mmHg.[6] The pathophysiology of hypertension is not yet fully explained however research indicates multi-etiological origin, including genetic susceptibility and lifestyle factors.

With advancing age the prevalence of hypertension increases and the nature of hypertension changes. In the middle-aged population, hypertension is predominantly a diastolic or combined systolic and diastolic hypertension. Isolated systolic hypertension (ISH) is the most frequent type of hypertension in the elderly[7], due to the continuous increase in systolic blood pressure with advancing age whereas diastolic blood pressure tends to remain constant or declines with advancing age.[8] The most likely explanation for the rise in systolic blood pressure and fall in diastolic blood pressure is large artery stiffening.[8-10]

Arterial stiffness

The cardiovascular system can be described as a closed conduit with a central pump with the principal function of supplying blood to all parts of the body. The cushion function of the arteries, transforming pulsatile flow at the ascending aorta into steady flow through the arterioles, provides that mean pressure is maintained throughout the whole arterial tree and pulsation around the mean in the ascending aorta is minimized. This cushioning function of the function of the arteries is dependent on the viscoelastic properties of the arterial walls.[11,12]

Disturbance of the optimal function of heart and arterial tree are seen with hypertension and aging. Wave reflection is apparent as a secondary boost to pressure at some part of the cycle. With aging, there is an increase in pulse wave velocity and therefore earlier return of the reflected wave in late systole instead of in diastole. This will boost the systolic blood pressure while reducing pressure during diastole and increasing aortic pulse pressure.[13,14] (Figure 1)

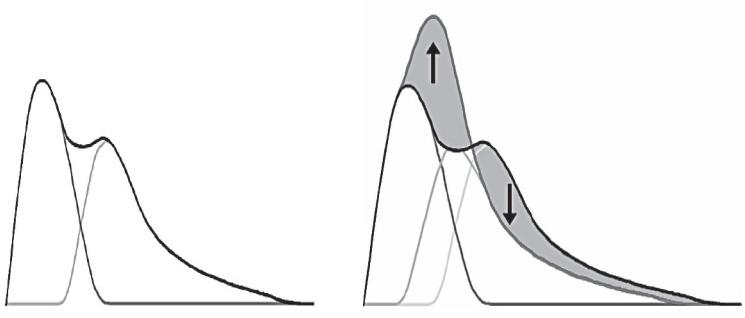


Figure 1a. Pressure wave in elastic arteries, first wave is the forward wave and second wave the reflected wave, blue line represents the arterial pressure during cardiac cycle.

Figure 1b. Pressure wave in stiffer artery, reflecting a boost in pressure during systole and decrease in pressure during diastole.

Arterial stiffness measurements

The elastic properties of conduit arteries vary along the arterial tree: the proximal arteries are more elastic, and the distal arteries stiffer. This heterogeneity is caused by the molecular, cellular and histological structure of the arterial wall, which differs between the various parts of the arterial tree.[15-18] There are several ways to measure arterial stiffness. Systemic arterial stiffness can only be estimated from models of the circulation[19], whereas regional and local arterial stiffness can be measured directly and non-invasively at various site along the arterial tree.[20,21] The aorta is a major vessel of interest when determining regional arterial stiffness, because of the large contribution of the thoracic and abdominal aorta in buffering pressure wave.[18,22] Measurement of local stiffness at the carotid artery may also provide important information, due to the atheroma formation at this site.[23]

Two of the most frequently used methods to assess arterial stiffness are the measurement of pulse wave velocity over a certain part of the arterial tree (regional arterial stiffness) and the measurement of changes in arterial diameter due to changes in arterial pressure over the cardiac cycle at one specific point in the arterial tree (measurement of local stiffness).

1) Carotid femoral pulse wave velocity
   This regional measurement of aortic stiffness is generally accepted as the most simple, non-invasive, robust and reproducible method to determine arterial stiffness.[23]
   Within our framework, the carotid femoral pulse wave velocity was assessed with an automatic device (Complior® Artech Medical, Pantin – France)[24] that measures the time delay between the rapid early upstroke of the pulse pressure waves recorded simultaneously in the carotid artery and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape over the surface of the body. The pulse wave velocity was calculated as the ratio between distance and the foot-foot time delay and was expressed in meter per second. (Figure 2)

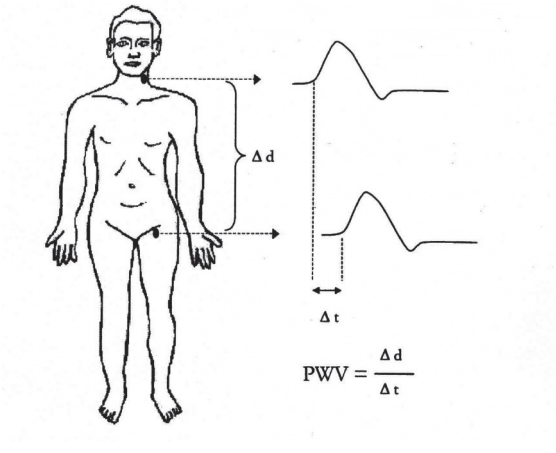
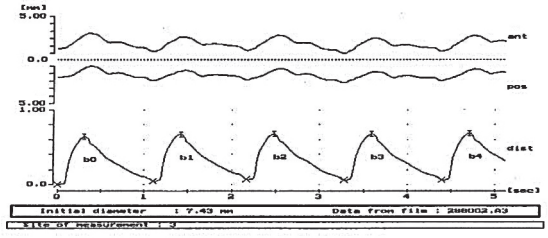


Figure 2: schematic representation of measurement of carotid-femoral pulse wave velocity

2) Distensibility of the common carotid artery

Local arterial stiffness of the carotid artery can be determined by using ultrasound devices. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. A region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified with the use of B-mode ultrasound, where after the system was switched to M-mode. The displacement of the arterial walls was obtained by processing the radiofrequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of 4 cardiac cycles of 3 successive recordings. The cross-sectional arterial wall distensibility coefficient, expressed in MPa-1, was calculated according to the following equation: distensibility coefficient=2ΔD/(D×pulse pressure).[25-27](Figure 3)



beat	dist (µm)	diam (mm)	dist (%)	dA/A (%)	RR-int (ms)	rise-time (ms)	ECG-10% (ms)
0	637	7.43	8.58	17.88	1100	143	97
1	634	7.47	8.49	17.71	1065	145	91
2	612	7.49	8.16	17.00	1110	142	96
3	624	7.48	8.34	17.38	1115	143	91
4	630	7.48	8.42	17.54	---	146	93
mean	627	7.47	8.40	17.50	1098	144	94
stdev	10	0.02	0.16	0.34	23	2	3

Figure 3 Example of common carotid arterial distensibility measurement

The two upper lines show the movement of the anterior and posterior arterial wall during the cardiac cycle. The bottom line shows the change in lumen diameter resulting from the movement of the arterial walls. The program automatically calculated several parameters for every heartbeat among which the absolute change in lumen diameter (dist), the end diastolic lumen diameter (diam) and the change in lumen diameter relative to its end-diastolic diameter, which indicates the distension of the artery (dist in %).

Clinical consequences

The increase in arterial stiffness has deleterious effects on the left ventricular cardiac function. An increase in pressure wave amplitude and early wave reflections, seen with arterial stiffening, increased peak- and end-systolic blood pressure in the ascending aorta, contributing to an increased myocardial oxygen consumption.[28] Increased systolic blood pressure induces myocardial hypertrophy[29], impairs diastolic myocardial function and ventricular ejection.[30] In addition, increased systolic blood pressure and pulse pressure accelerates arterial damage, increasing the fatigue of biomaterials, causing degenerative changes and further arterial stiffening.[31] Finally, the stiffness-induced reduction of diastolic blood pressure alters the driving pressure of the coronary circulation, favoring myocardial ischemia.[32] These alterations taken together explain why aortic stiffness is an independent risk factor of cardiovascular disease risk.

A number of studies have demonstrated that increased arterial stiffness is associated with elevated risk of cardiovascular disease in high-risk samples, including patients with hypertension[33], with end-stage renal disease[34], with diabetes mellitus[34] as well in community-based samples.[35-38]

However, several questions remain unanswered. Large population studies have focused on cardiovascular risk and in particular on myocardial infarction. No study focused on heart failure as outcome, which is a prevalent cardiovascular disorder and represents a major problem in aging populations. Moreover, new developed methods of clinical utility have not been studied with arterial stiffness.

Genetic background

The underlying mechanism of hypertension and arterial stiffening is multi-factorial and complex. Exploring genetic loci associated with hypertension and arterial stiffening, provides new insights in biological process and may suggest novel strategies for prevention and treatment of increased arterial stiffness and hypertension.

Genetic studies traditionally used candidate gene or family-based linkage studies to search for novel genes. Despite the substantial heritability and considerable knowledge about pathways that are critical to blood pressure homeostasis, studies of candidate genes produced few reproducible results.[39] And although arterial stiffness has shown to be, at least in part, heritable, the molecular mechanisms underlying aortic stiffness remained largely undefined.[40]

The variation of blood pressure and arterial stiffness is probably due to multiple variants with small effects that are hard to detect with the traditional genetic methods. With the introduction of Genome-Wide Association (GWA) studies, there has been considerable progress in identification of common genetic variants underlying common complex disorders.[39] Application of this approach to arterial hemodynamic measures reveal novel genes and contribute to the understanding of the complex nature underlying hypertension and arterial stiffening.

Scope and outline of this thesis

This thesis is divided in two parts. In the first part, we aimed to search for new genetic risk factors for age related changes in the vascular system including blood pressure and arterial stiffness. In the second part of this thesis, the cardiovascular consequences of arterial stiffness will be studied. The studies presented in this thesis used data from the Rotterdam Study, from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, from the International Consortium of Blood Pressure GWAs (ICBP-GWAs) and from AortaGen consortium.

The Rotterdam study is a large prospective population-based cohort study among initially 7,983 inhabitants of Ommoord, a suburb of Rotterdam, who were 55 years or older. Since the start of the study in 1989, participants have visited the research center up to 5 times. In 2000, the first extended cohort was enrolled, which included 3,011 inhabitants aged 55 years and older at that time, who visited the research center up to 3 times. In 2006, a second extended cohort was enrolled, which included 3,932 inhabitants aged 45 years or older, who are visiting the research center for the second time.[41] Figure 4 shows the structure of the Rotterdam Study.

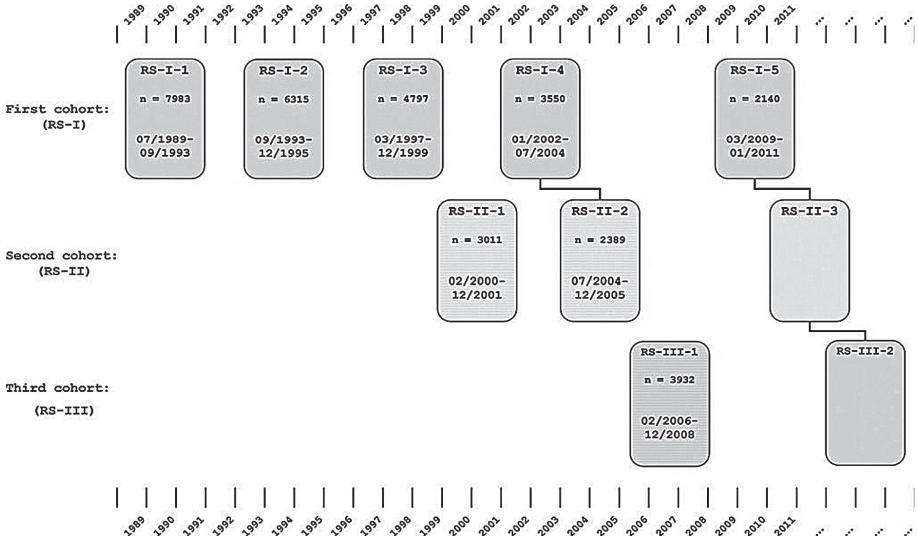


Figure 4. Diagram of the examination cycles of the Rotterdam Study (RS).

The CHARGE consortium comprises of 5 large international population-based cohort studies, which originally included the Atherosclerosis Risk in Elderly study (ARIC), Age, Gene/Environment Susceptibility-Reykjavik study(AGES), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS) and the Rotterdam Study (RS), and was designed to investigate genetic risk factors for cardiovascular diseases.[42]

The ICBP-GWA's is a multi-staged consortium with a combining sample > 200.000 subjects which aims to further the understanding of the genetic architecture underlying blood pressure.
The AortaGen consortium includes 9 cohort studies that completed genome-wide genotyping and had measured carotid femoral pulse wave velocity and two additional cohort studies for replication.

Part 1 of this thesis is focused on the genetic risk factors for age related changes of the vascular system. **Chapter 2** presents new discovered genes involved in blood pressure regulation and hypertension. In **chapter 2.1** we describe the principal findings of common genetic variation with blood pressure in CHARGE and in **chapter 2.2** we describe the extension of this work in a larger cohort, including the cardiovascular disease risk of these genetic variants. In **chapter 2.3** we described the relation between a candidate gene HSD3B1 and blood pressure. **Chapter 3** presents recently discovered genes involved in arterial stiffness. In **chapter 3.1** we describe new genes related to pulse pressure (PP) and mean arterial pressure (MAP) and in **chapter 3.2** we describe new genes related to pulse wave velocity.

Part 2 of this thesis is focused on the cardiovascular consequences of age related changes of the vascular system. **Chapter 4** is focused on the relation between arterial stiffness and hypertension. In **chapter 4.1** we describe the relation between hypertension and arterial stiffness. In **chapter 4.2** we describe the cardiovascular consequences of orthostatic hypotension. In **chapter 5** is focused on the clinical consequences of arterial stiffness. In **chapter 5.1** we describe the elevated risk of heart failure in relation to arterial stiffness. In **chapter 5.2** we describe the additive value of arterial stiffness in predicting cardiovascular disease.

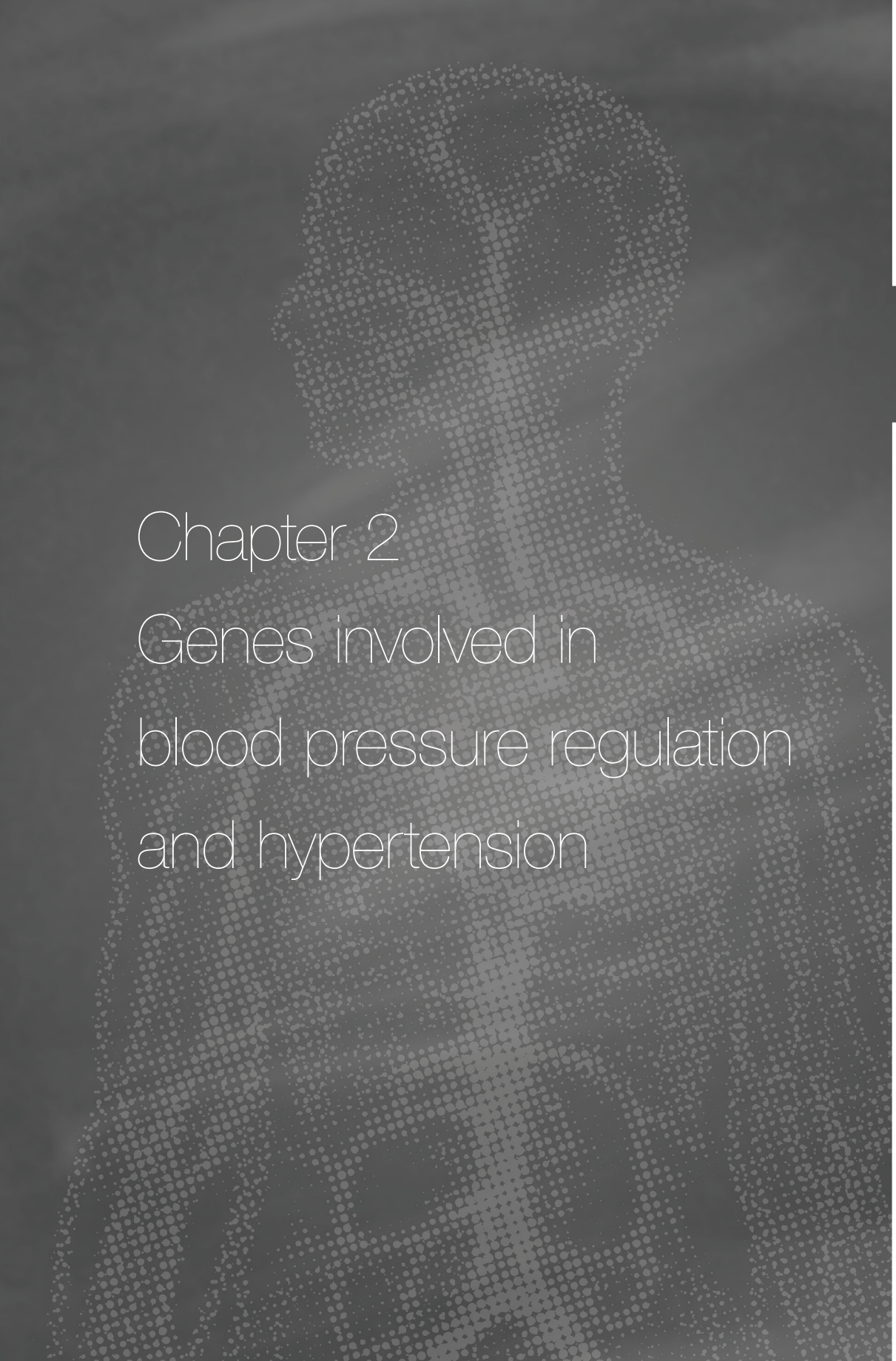
In **Chapter 6**, we discuss the main findings of this thesis and we provide suggestions for future research.

References

1. Alwan A, Maclean DR, Riley LM, et al. Monitoring and surveillance of chronic non-communicable diseases: progress and capacity in high-burden countries. Lancet. Nov 27 2010;376(9755):1861-1868.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS medicine. Nov 2006;3(11):e442.
3. Beaglehole R, Bonita R, Horton R, et al. Priority actions for the non-communicable disease crisis. Lancet. Apr 23 2011;377(9775):1438-1447.
4. Guyton AC. The surprising kidney-fluid mechanism for pressure control--its infinite gain! Hypertension. Dec 1990;16(6):725-730.
5. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. Lancet. May 3 2008;371(9623):1513-1518.
6. Mancia G, Fagard R, Narkiewicz K, et al. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. Journal of hypertension. Oct 2013;31(10):1925-1938.
7. Franklin SS, Jacobs MJ, Wong ND, L'Italien GJ, Lapuerta P. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. Hypertension. Mar 2001;37(3):869-874.
8. Franklin SS, Gustin Wt, Wong ND, et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation. Jul 1 1997;96(1):308-315.
9. Wallace SM, Yasmin, McEniery CM, et al. Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. Hypertension. Jul 2007;50(1):228-233.
10. Yasmin, McEniery CM, O'Shaughnessy KM, et al. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. Arterioscler Thromb Vasc Biol. Aug 2006;26(8):1799-1805.
11. Buntin CM, Silver FH. Noninvasive assessment of mechanical properties of peripheral arteries. Annals of biomedical engineering. 1990;18(5):549-566.
12. O'Rourke MF, Blazek JV, Morreels CL, Jr., Krovetz LJ. Pressure wave transmission along the human aorta. Changes with age and in arterial degenerative disease. Circulation research. Oct 1968;23(4):567-579.
13. Avolio AP, Deng FQ, Li WQ, et al. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. Circulation. Feb 1985;71(2):202-210.
14. Kelly R, Hayward C, Avolio A, O'Rourke M. Noninvasive determination of age-related changes in the human arterial pulse. Circulation. Dec 1989;80(6):1652-1659.
15. Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. Hypertension. Jun 2005;45(6):1050-1055.

16. Bezie Y, Lamaziere JM, Laurent S, et al. Fibronectin expression and aortic wall elastic modulus in spontaneously hypertensive rats. Arterioscler Thromb Vasc Biol. Jul 1998;18(7):1027-1034.
17. Fischer GM, Llaurado JG. Collagen and elastin content in canine arteries selected from functionally different vascular beds. Circulation research. Aug 1966;19(2):394-399.
18. Latham RD, Westerhof N, Sipkema P, Rubal BJ, Reuderink P, Murgo JP. Regional wave travel and reflections along the human aorta: a study with six simultaneous micromanometric pressures. Circulation. Dec 1985;72(6):1257-1269.
19. Nichols WW, McDonald DA. Wave-velocity in the proximal aorta. Medical & biological engineering. May 1972;10(3):327-335.
20. O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values. Am J Hypertens. May 2002;15(5):426-444.
21. Pannier BM, Avolio AP, Hoeks A, Mancia G, Takazawa K. Methods and devices for measuring arterial compliance in humans. Am J Hypertens. Aug 2002;15(8):743-753.
22. Isnard RN, Pannier BM, Laurent S, London GM, Diebold B, Safar ME. Pulsatile diameter and elastic modulus of the aortic arch in essential hypertension: a noninvasive study. Journal of the American College of Cardiology. Feb 1989;13(2):399-405.
23. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. Nov 2006;27(21):2588-2605.
24. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. Sep 1995;26(3):485-490.
25. Hoeks AP, Brands PJ, Smeets FA, Reneman RS. Assessment of the distensibility of superficial arteries. Ultrasound Med Biol. 1990;16(2):121-128.
26. Kool MJ, van Merode T, Reneman RS, Hoeks AP, Struyker Boudier HA, Van Bortel LM. Evaluation of reproducibility of a vessel wall movement detector system for assessment of large artery properties. Cardiovascular research. May 1994;28(5):610-614.
27. Reneman RS, van Merode T, Hick P, Muytjens AM, Hoeks AP. Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol. Jun 1986;12(6):465-471.
28. Lakatta EG. Changes in cardiovascular function with aging. Eur Heart J. May 1990;11 Suppl C:22-29.
29. Girerd X, Laurent S, Pannier B, Asmar R, Safar M. Arterial distensibility and left ventricular hypertrophy in patients with sustained essential hypertension. American heart journal. Oct 1991;122(4 Pt 2):1210-1214.
30. Mottram PM, Haluska BA, Leano R, Carlier S, Case C, Marwick TH. Relation of arterial stiffness to diastolic dysfunction in hypertensive heart disease. Heart. Dec 2005;91(12):1551-1556.
31. Dao HH, Essalihi R, Bouvet C, Moreau P. Evolution and modulation of age-related medial elastocalcinosis: impact on large artery stiffness and isolated systolic hypertension. Cardiovascular research. May 1 2005;66(2):307-317.
32. Saeki A, Recchia F, Kass DA. systolic flow augmentation in hearts ejecting into a model of stiff aging vasculature. Influence on myocardial perfusion-demand balance. Circulation research. Jan 1995;76(1):132-141.

33. Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. Jan 2002;39(1):10-15.
34. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. Kidney Int. May 2003;63(5):1852-1860.
35. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. Feb 7 2006;113(5):657-663.
36. Sutton-Tyrrell K, Najjar SS, Boudreau RM, et al. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation. Jun 28 2005;111(25):3384-3390.
37. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol. Dec 2001;21(12):2046-2050.
38. van Popele NM, Mattace-Raso FU, Vliegenthart R, et al. Aortic stiffness is associated with atherosclerosis of the coronary arteries in older adults: the Rotterdam Study. Journal of hypertension. Dec 2006;24(12):2371-2376.
39. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genetics in medicine : official journal of the American College of Medical Genetics. Mar-Apr 2002;4(2):45-61.
40. Mitchell GF, DeStefano AL, Larson MG, et al. Heritability and a genome-wide linkage scan for arterial stiffness, wave reflection, and mean arterial pressure: the Framingham Heart Study. Circulation. Jul 12 2005;112(2):194-199.
41. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. Aug 2011;26(8):657-686.
42. Psaty BM, O'Donnell CJ, Gudnason V, 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. Feb 2009;2(1):73-80.



Chapter 2
Genes involved in blood pressure regulation and hypertension

Chapter 2.1

Genome-wide association of blood pressure: CHARGE consortium

Based on

Genome-wide association study of blood pressure and hypertension.

Nat Gen 2009 May; 41:677-687.

Abstract

Background

Blood pressure is a major cardiovascular disease risk factor. To date, few variants associated with inter-individual blood pressure variation have been identified and replicated.

Methods

We conducted a meta-analysis of genome-wide association study of systolic (SBP) and diastolic (DBP) blood pressure and hypertension from six cohort studies in the setting of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (n = 29,136). We sought additional evidence to support our findings with the Global BPgen Consortium (n = 34,433)

Results

We identified 13 single nucleotide polymorphisms (SNPs) for SBP, 20 SNPs for DBP and 10 SNPs for hypertension at P <4x10-7 threshold. The top ten loci for SBP and DBP were incorporated into a risk score; mean blood pressure and prevalence of hypertension increased in relation to the number of risk alleles carried. When ten CHARGE SNPs for each trait were included in a joint meta-analysis with the Global BPgen Consortium, four CHARGE loci attained genome-wide significance (P < 5 x 10-8) for SBP (ATP2B1, CYP17A1, PLEKHA7, SH2B3), six for DBP (ATP2B1, CACNB2, CSK-ULK3, SH2B3, TBX3-TBX5, ULK4) and one for hypertension (ATP2B1).

Conclusions

Identifying genes associated with blood pressure advances our understanding of blood pressure regulation and highlights potential drug targets for the prevention or treatment of hypertension.

Introduction

High blood pressure affects about one-third of adults and contributes to 13.5 million deaths worldwide each year and about half the global risk for stroke and ischemic heart disease.[1,2] Clinical trials, dating back more than 40 years, have proven that drug treatment to lower blood pressure markedly reduces the risk of cardiovascular events in people with hypertension.[3,4]

The substantial (30–60%)[5] heritability of blood pressure has prompted extensive efforts to identify its genetic underpinnings. Several complementary approaches have been used to search for genes associated with inter-individual variation in blood pressure in the general population, but these have yielded relatively few clues. Despite considerable knowledge about pathways that are critical to blood pressure homeostasis, linkage and candidate gene studies have provided limited consistent evidence of blood pressure quantitative trait loci.[6-8] The study of families with rare mendelian high or low blood pressure syndromes has identified mutations with gain or loss of function in about a dozen renal sodium regulatory genes[9], and common variants in two renal sodium regulatory genes have been found to be associated with blood pressure in the general population.[10] The vast majority of the genetic contribution to variation in blood pressure, however, remains unexplained.

Large-scale genome-wide association studies (GWAS), in which hundreds of thousands of common genetic variants are genotyped and analyzed for disease association, have shown great success in identifying genes associated with common diseases and traits.[11,12] Six GWAS published to date, however, have not identified loci associated with blood pressure or hypertension at $P < 5 \times 10\text{-}8$, raising concerns about the utility of this approach for these traits.[13-18]

If blood pressure variation in the general population is due to multiple variants with small effects, very large study samples are needed to identify them. We established the Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE)[19] Consortium to identify common genetic variation associated with complex traits. The CHARGE Consortium consists of 29,136 participants of European descent who had undergone standardized blood pressure measurements in six population-based cohort studies: the Age, Gene/Environment Susceptibility Reykjavik Study (AGES), Atherosclerosis Risk in Communities (ARIC) Study, Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Rotterdam Study (RS) and the Rotterdam Extension Study (RES). We report the top findings of our GWAS of systolic blood pressure, diastolic blood pressure and hypertension, provide replication results for our most promising loci in the Global BPgen Consortium[20], another GWAS consortium of similar size, and report combined meta-analysis findings of the two consortia for the most promising loci found in CHARGE.

Methods

Consortium organization

The CHARGE Consortium[19] includes six cohort studies that completed genome-wide genotyping and had extensive data on multiple phenotypes including blood pressure. Each study adopted collaboration guidelines and established a consensus on phenotype harmonization, covariate selection and an analytical plan for within-study genome-wide association and prospective meta-analysis of results across studies. Each study received institutional review board approval of its consent procedures, examination and surveillance components, data security measures, and DNA collection and its use for genetic research. All participants in each study gave written informed consent for participation in the study and the conduct of genetic research.

Genotype imputation

For imputation of genotypes to the HapMap set of approximately 2.5 million SNPs, ARIC, FHS and RS used a hidden Markov model as implemented in MACH, and CHS used BIMBAM10 v0.99.[21] SNP imputation combined genotype data from each sample with the HapMap CEU samples and then inferred genotypes probabilistically according to shared haplotype stretches between study samples and HapMap release 22 build 36. Imputation results are summarized as an 'allele dosage' defined as the expected number of copies of the minor allele at that SNP (a fractional value between 0.0 and 2.0) for each genotype.

Statistical analyses

Cross-sectional analyses were conducted within each cohort using an additive genetic model, and within-study associations were combined by prospective meta-analysis. The phenotypes for meta-analysis were systolic and diastolic blood pressure and hypertension at the first examination attended. For participants who were taking antihypertensive medication we added 10 mmHg to observed SBP values and 5mm Hg to DBP values.[22] Hypertension was defined as SBP ≥ 140 or DBP ≥ 90 mmHg or drug treatment for hypertension at time of assessment. Within each cohort, regression models were fitted for systolic and diastolic blood pressure (separately) and allele dosage, adjusting for sex, age, age squared and BMI.

Meta-analysis of results was carried out using inverse-variance weighting. Before meta-analysis, results were filtered for minor allele frequency <0.005 and the genomic control parameter was calculated to adjust each study. After meta-analysis, the genomic control parameter was recalculated to adjust for between-study heterogeneity.[23] A predetermined threshold of 4 x 10-7 (stage 1) was used to indicate genome-wide significance within CHARGE. For 2.5 x 106 tests (the total number of imputed SNPs), this threshold means that the expected number of false-positive results is ≤1; the validity of this bound is not affected by correlation between test statistics.

Ten leading SNPs for SBP, ten for DBP and ten for hypertension were exchanged between CHARGE and Global BPgen, a consortium with a sample size of 34,433 individuals of European ancestry with analogous genome-wide data38. SNP selection was limited to one SNP per locus of interest, defined by an r2 ≤ 0.2. For rs880315, imputation in Global BPgen was suboptimal and this SNP was replaced with rs12046272. rs8096897 and rs10972206 were not selected for exchange owing to low minor allele frequencies (defined as < 0.01 for continuous traits, < 0.05 for hypertension). rs5761405 was selected for exchange, but was not available in the imputed results from Global BPgen, so the next most highly significant locus was selected in its place. For all 30 exchanged SNPs we carried out meta-analysis of CHARGE and Global BPgen results using inverse-variance weighting and considered a P value in the joint analysis (stage 2) significant at P = 5 x 10-8. Significant replication of a genome-wide significant SNP in CHARGE was defined as a P value < 0.008 for the same SNP in Global BPgen (0.05/6 genome-wide significant SNPs submitted for replication). One-sided tests were used to assess replication when the alignment of an allele and its directional effect were identical between CHARGE and Global BPgen.

Analysis of hypertension was conducted within each cohort, and the within-study associations were combined by meta-analysis. Within each cohort, regression models were fitted for hypertension, adjusting for sex, age, age squared and BMI. Meta-analysis of results was carried out using inverse-variance weighting. Before meta-analysis, results were filtered for low minor allele frequency < 0.01 and the genomic control parameter calculated to adjust each study. After meta-analysis, the genomic control parameter was calculated again to adjust for between-study heterogeneity. In the meta-analysis of CHARGE and Global BPgen, the analytical approach used in Global BPgen was different from that of CHARGE; in Global BPgen non-hypertensive controls were defined as individuals not taking any hypertensive medications and having a SBP ≤ 120 mmHg and a DBP ≤ 85 mmHg.

Blood pressure risk score was a weighted sum across ten top SNPs (separately for systolic and diastolic blood pressure) combining beta coefficients and doses of risk alleles, rounded to 1 mmHg for SBP (groups ≤6 to ≥ 15) and 0.5 mmHg for diastolic blood pressure (groups ≤2.5 to ≥ 7.5). Within a study, for each risk score group we calculated deviations of empirical blood pressure from the study mean. Across studies, we estimated mean deviation and standard error within risk score group, weighted by group and study-specific sample sizes. For hypertension, odds ratios (and standard errors) were the corresponding summary statistics, with the reference group being those with a weighted systolic risk score of 10 or a diastolic score of 5.

SNP associations with altered gene expression

To assess putative functional associations in our GWAS, we used bioinformatics tools to query existing GWAS databases of SNPs associated with cis-gene expression levels in immortalized liver (n=3,322)[25] and lymphoblastoid cell lines (n=10,823).[26] These expression-associated SNPs were then explored for association with blood pressure in the fully imputed HapMap blood pressure results for CHARGE. Statistical significance was defined by a P value of 1/n (where n is the number of tissue-specific cis eSNPs interrogated); this threshold will yield on average one false positive per tissue examined. The P-value thresholds for significance of eSNP associations for liver and lymphoblastoid cell lines were 3.0 x 10-4 and 9.2 x 10-5, respectively.

Results

Study samples

The total sample size for this analysis was 29,136 (AGES, n = 3,219; ARIC, n = 8,047; CHS, n = 3,277; FHS, n = 8,096; RS n = 4,737; RES, n = 1760). The mean age of the study participants at the initial examination varied from 38 years (FHS) to 72 years (CHS). The mean observed (and treatment corrected) SBP across the six cohorts ranged from 118 (120) mmHg (ARIC) to 143 (145) mmHg (RES); the mean DBP ranged from 72 (73) mmHg (ARIC) to 83(84) mmHg (AGES). The proportion of participants taking antihypertensive medication ranged from 5% (FHS) to 38% (CHS), and the proportion with hypertension ranged from 17% (FHS) to 60% (RES).

Meta-analysis of CHARGE cohort results

Within-cohort analyses were combined by meta-analysis and the results for all SNPs with P value < 1 x 10-6 are presented in Table 1 for SBP, Table 2 for DBP and Table 3 for hypertension; within each of these tables the results for the other two blood pressure phenotypes are also provided. The number of SNPs with P values <1 x 10-3 was 3,433 for systolic and 3,558 for diastolic blood pressure versus 2,540 expected. The proportion of SNPs with P < 1 x 10-3 that are intragenic was 47% for systolic and diastolic blood pressure versus an average of 37% for all imputed SNPs. For systolic blood pressure the meta-analysis identified 13 SNPs with P < 4 x 10-7 (stage 1 threshold). The strongest signal for systolic pressure was for rs2681492 (P = 3.0 x 10-11) in ATP2B1 on chromosome 12q21–23. A low minor allele frequency variant in C18orf1 (rs8096897; P = 3.2 x 10-8) showed evidence of association with SBP as did CASZ1 (rs880315; P = 2.1 x 10-7). A signal was identified on chromosome 12q24 for SH2B3 (rs3184504; P = 5.7 x 10-7) and for nearby ATXN2 (rs653178; P = 8.5 x 10-7). PLEKHA7 (chromosome 11p15.1, rs381815, P = 5.8 x 10-7) and a locus on chromosome 2q31–33adjacent to PMS1 and MSTN (rs7571613, P = 7.2 x 10-7) showed suggestive evidence of association. Of note, many of the top SBP-associated SNPs were also associated with other blood pressure phenotypes (Table 1).

For DBP (Table 2) there were 20 SNPs with P < 4 x 10-7. Significant association signals were detected in a large 1-Mb block of linkage disequilibrium on chromosome 12q24 that includes SH2B3 (rs3184504, P = 1.7 x 10-8), ATXN2 (rs653178, P = 2.0 x 10-8; r2 =1.0 with rs3184504), and TRAFD1 (rs17630235, P = 1.0 x 10-7; r2 =0.66 with rs3184504). In addition, ATP2B1 (chromosome 12q21, rs2681472, P = 3.7 x 10-8), TBX3-TBX5 (chromosome 12q24, rs2384550, P = 1.3 x 10-7) and PLEKHA7 (chromosome 11p15, rs11024074, P = 2.8 x 10-7) showed association with DBP. Suggestive evidence of association was found for loci in or adjacent to ULK4 (chromosome 3p22.1), CSK-ULK3 (chromosome 15q24) and CACNB2 (chromosome 10p12). Multiple DBP-associated SNPs were also associated with other blood pressure phenotypes (Table 2).

For the dichotomous trait of hypertension (Table 3), one significant association was detected for ATP2B1 (rs2681472, P = 1.7 x 10-8), with an odds ratio for hypertension of 1.17 per risk allele. Suggestive evidence of association was detected for ITGA9 (chromosome 3p22.2, rs7640747, P = 4.8 x 10-7) and CACNB2 (rs11014166, P = 7.8 x 10-7).

Independent replication and meta-analysis of top CHARGE SNPs

Thirty SNPs representing the top ten CHARGE Consortium loci for SBP, DBP and hypertension were exchanged for lookup within the Global BPgen Consortium GWAS results. One SNP for SBP, four for DBP and one for hypertension that attained stage 1 P < 4 x 10-7 in CHARGE were assessed for evidence of independent replication in Global BPgen (Table 4). Five of these six associations fulfilled criteria for external replication in Global BPgen of P < 0.008 (0.05/6, one-tailed test). The replicated loci included ATP2B1 (for SBP, DBP and hypertension), SH2B3 (DBP) and TBX3-TBX5 (DBP). PLEKHA7 did not replicate for DBP (rs11024074, P = 0.03 in Global BPgen); however, another SNP in PLEKHA7 was genome-wide significant (at P < 5 x 10-8, stage 2) for SBP (rs381815) in the joint meta-analysis of CHARGE and Global BPgen. Of note, for 29 of 30 CHARGE SNPs that were exchanged, the directional association (sign of beta) was identical in both consortia.

Table 4 provides results of joint meta-analysis of CHARGE and Global BPgen for the top ten CHARGE SNPs for SBP, DBP and hypertension. Four genome-wide significant (stage 2 P < 5 x 10-8) associations emerged for SBP (CYP17A1, PLEKHA7, ATP2B1 and SH2B3), six for DBP (ULK4, CACNB2, ATP2B1, SH2B3, TBX3-TBX5 and a locus adjacent to CSK-ULK3) and one for hypertension (ATP2B1). Three additional associations attained P < 4 x 10-7 (MDS1 for SBP; CACNB2 and a region near EDN3 for hypertension). Plots of association results across each of the genome-wide significant loci can be found online.[24]

Blood pressure risk score

Weighted risk scores, incorporating the top ten CHARGE loci for SBP and DBP, were applied to the study results to examine the influence of risk alleles in aggregate on deviation from mean blood pressure levels and odds ratios for hypertension. Figure 1 shows a continuous and graded relation of risk score on blood pressure levels and odds ratios for hypertension. Inverse-variance weighted regression estimates of slope (beta) and its standard error (s.e.) were obtained across risk score groups for deviation from mean blood pressure and odds ratios for hypertension. To summarize these findings, two-tailed P values (from Z = beta/s.e.(beta)) were obtained from testing the null hypothesis of a zero slope across risk score groups. The P values across risk score groups were as follows: 1.8 x 10-27 (SBP versus SBP risk score), 1.7 x 10-56 (DBP versus DBP risk score), 1.4 x 10-17 (hypertension versus SBP risk score) and 8.4 x 10-10 (hypertension versus DBP risk score).

Putative functional variation

A search for nonsynonymous SNPs among our blood pressure association results identified five such variants, including rs3184504 in SH2B3 (stage 2 P value for DBP = 2.6 x 10-14), rs267561 in ITGA9 (stage 1 P value for hypertension 2.6 x 10-6) and three linked nonsynonymous SNPs in ULK4 (rs2272007, rs3774372 and rs1716975; pairwise r2 = 0.82–1.0; lowest stage 1 P value = 1.5 x 10-6 for DBP). To further identify putative functional associations within our GWAS results, we culled from the 2.5 million HapMap SNPs in our analysis those that were previously reported from GWAS to be associated with altered gene expression in liver[25] (n = 3,322) or lymphoblastoid cell lines[26] (n = 10,823). These expression-associated SNPs (eSNPs or eQTLs) were then interrogated for association with blood pressure phenotypes within our GWAS results (Table 5). Of note, three of our genome-wide significant loci were captured through the analysis eSNPs, including rs739496 in SH2B3, which is associated with altered expression of nearby HSS00340376 in liver; rs6495126 near CSK-ULK3, which is associated with altered expression of ULK3 in liver; and nonsynonymous SNPs rs1716975 and rs2272007 in ULK4, which are associated with altered expression of ULK4 in lymphoblastoid cell lines. In addition, rs7571613 near PMS1 and MSTN is associated with altered expression of ORMDL1 and PMS1 in lymphoblastoid cell lines. Additional eSNPs with suggestive evidence of association with blood pressure phenotypes were rs7537765 near MTHFR-NPPA (expressed gene CLCN6), and several SNPs in KDM5A that are associated with expression of KDM5A, SLC6A12 and CCDC77.

Table 1: Genome-wide association results for SBP-associated SNPs with p value <1x10-6 sorted by SBP meta-analysis p value

SNP identifier	Chr	Position	Gene	MAF
rs2681492	12	88537220	ATP2B1	0.20
rs2681472	12	88533090	ATP2B1	0.18
rs11105354	12	88550654	ATP2B1	0.18
rs11105364	12	88593407		0.18
rs17249754	12	88584717		0.18
rs11105368	12	88598572		0.18
rs12579302	12	88574634		0.18
rs12230074	12	88614998		0.17
rs11105378	12	88614872		0.17
rs4842666	12	88465680		0.17
rs8096897	18	13428905	C18orf1	0.01
rs11105328	12	88466521		0.18
rs880315	1	10719453	CASZ1	0.35
rs3184504	12	110368991	SH2B3	0.48
rs381815	11	16858844	PLEKHA7	0.26
rs7926335	11	16874445	PLEKHA7	0.26
rs7571613	2	190513907	PMS1	0.18
rs11895934	2	190510498		0.18
rs7564968	2	190520217		0.18
rs653178	12	110492139	ATXN2	0.48
rs284277	1	10713384	CASZ1	0.35

Chr=chromosome; MAF=minor allele frequency;

Beta is the effect size on blood pressure, in mmHg, per allele based on the additive genetic model

CHARGE Meta-analysis SBP			CHARGE Meta-analysis DBP			CHARGE Meta-analysis Hypertension		
Beta	SE	P	Beta	SE	p	Beta	SE	p
-1.26	0.19	3.0E-11	-0.62	0.11	4.6E-08	-0.14	0.03	8.4E-08
-1.29	0.19	3.5E-11	-0.64	0.11	3.7E-08	-0.16	0.03	1.7E-08
-1.30	0.20	3.7E-11	-0.63	0.11	5.8E-08	-0.16	0.03	1.8E-08
-1.30	0.20	4.8E-11	-0.63	0.12	1.2E-07	-0.16	0.03	2.1E-08
-1.30	0.20	5.2E-11	-0.63	0.12	1.0E-07	-0.16	0.03	2.2E-08
-1.30	0.20	5.3E-11	-0.63	0.12	1.3E-07	-0.16	0.03	2.2E-08
-1.29	0.20	6.2E-11	-0.62	0.12	1.3E-07	-0.16	0.03	2.2E-08
-1.31	0.20	9.1E-11	-0.62	0.12	3.4E-07	-0.17	0.03	2.9E-08
-1.31	0.20	9.1E-11	-0.62	0.12	3.1E-07	-0.17	0.03	2.8E-08
-1.20	0.21	6.5E-09	-0.62	0.12	4.5E-07	-0.15	0.03	3.4E-07
-12.87	2.33	3.2E-08	-4.07	1.33	2.9E-03	-0.73	0.35	0.04
-1.11	0.20	4.2E-08	-0.61	0.12	5.1E-07	-0.15	0.03	7.1E-07
0.89	0.17	2.1E-07	0.30	0.10	2.9E-03	0.09	0.02	6.2E-05
0.75	0.15	5.7E-07	0.50	0.09	1.7E-08	0.07	0.02	7.4E-04
0.84	0.17	5.8E-07	0.51	0.10	4.3E-07	0.09	0.02	1.7E-04
0.85	0.17	5.8E-07	0.51	0.10	4.8E-07	0.09	0.02	1.9E-04
0.96	0.19	7.2E-07	0.55	0.11	2.2E-06	0.09	0.03	5.2E-04
0.96	0.19	7.3E-07	0.55	0.11	2.2E-06	0.09	0.03	5.5E-04
0.96	0.19	8.0E-07	0.55	0.11	2.3E-06	0.09	0.03	4.9E-04
0.74	0.15	8.5E-07	0.50	0.09	2.0E-08	0.07	0.02	7.8E-04
0.79	0.16	9.4E-07	0.24	0.09	0.01	0.09	0.02	6.9E-05

Table 2: Genome-wide association results for DBP SNPs with p value <1x10-6 sorted by DBP meta-analysis p value

SNP identifier	Chr	Position	Gene	MAF
rs3184504	12	110368991	SH2B3	0.48
rs653178	12	110492139	ATXN2	0.48
rs2681472	12	88533090	ATP2B1	0.17
rs4766578	12	110388754	ATXN2	0.49
rs10774625	12	110394602	ATXN2	0.49
rs2681492	12	88537220	ATP2B1	0.19
rs11105354	12	88550654	ATP2B1	0.17
rs17630235	12	111076069	TRAFD1	0.43
rs17249754	12	88584717		0.17
rs11066188	12	111095097		0.43
rs11105364	12	88593407		0.17
rs11105368	12	88598572		0.17
rs12579302	12	88574634		0.17
rs2384550	12	113837114	TBX3	0.35
rs1991391	12	113837049		0.35
rs6489992	12	113837152		0.37
rs11065987	12	110556807		0.42
rs11024074	11	16873795	PLEKHA7	0.28
rs11105378	12	88614872		0.17
rs12230074	12	88614998		0.17
rs7963771	12	113827875		0.31
rs381815	11	16858844	PLEKHA7	0.26
rs4842666	12	88465680		0.17
rs7926335	11	16874445	PLEKHA7	0.26
rs11105328	12	88466521		0.18
rs17696736	12	110971201	C12orf30	0.44
rs10744835	12	113838232		0.30
rs7977406	12	113843807		0.30
rs9815354	3	41887655	ULK4	0.17
rs6495122	15	72912698	CPLX3/ULK3	0.42
rs11014166	10	18748804	CACNB2	0.34
rs6768438	3	41840359	ULK4	0.16
rs9852991	3	41850459	ULK4	0.16
rs13401889	2	190618804	MSTN	0.21
rs9816772	3	41847881	ULK4	0.16

Chr=chromosome; MAF=minor allele frequency;

Beta is the effect size on blood pressure, in mmHg, per allele based on the additive genetic model

CHARGE Meta-analysis DBP			CHARGE Meta-analysis SBP			CHARGE Meta-analysis Hypertension		
Beta	SE	P	Beta	SE	p	Beta	SE	p
0.50	0.09	1.7E-08	0.75	0.15	5.7E-07	0.07	0.02	7.4E-04
0.50	0.09	2.0E-08	0.74	0.15	8.5E-07	0.07	0.02	7.7E-04
-0.64	0.12	3.7E-08	-1.29	0.19	3.5E-11	-0.16	0.03	1.7E-08
0.49	0.09	4.2E-08	0.73	0.15	1.2E-06	0.06	0.02	1.9E-03
0.49	0.09	4.2E-08	0.73	0.15	1.1E-06	0.06	0.02	1.8E-03
-0.62	0.11	4.6E-08	-1.26	0.18	3.0E-11	-0.14	0.03	8.4E-08
-0.63	0.12	5.8E-08	-1.30	0.19	3.7E-11	-0.16	0.03	1.8E-08
0.50	0.09	1.0E-07	0.69	0.15	1.1E-05	0.06	0.02	4.3E-03
-0.63	0.12	1.0E-07	-1.30	0.19	5.2E-11	-0.16	0.03	2.2E-08
0.50	0.09	1.1E-07	0.68	0.15	1.3E-05	0.06	0.02	4.2E-03
-0.63	0.12	1.2E-07	-1.30	0.19	4.8E-11	-0.16	0.03	2.1E-08
-0.63	0.12	1.2E-07	-1.30	0.19	5.3E-11	-0.16	0.03	2.2E-08
-0.62	0.12	1.2E-07	-1.29	0.19	6.2E-11	-0.16	0.03	2.2E-08
-0.48	0.09	1.3E-07	-0.71	0.15	4.3E-06	-0.08	0.02	5.6E-05
-0.48	0.09	1.4E-07	-0.71	0.15	3.8E-06	-0.09	0.02	5.6E-05
-0.48	0.09	2.0E-07	-0.71	0.15	4.7E-06	-0.08	0.02	1.9E-04
0.48	0.09	2.2E-07	0.70	0.15	9.4E-06	0.06	0.02	4.1E-03
0.50	0.10	2.8E-07	0.79	0.16	1.6E-06	0.09	0.02	5.2E-05
-0.62	0.12	3.1E-07	-1.31	0.20	9.1E-11	-0.17	0.03	2.8E-08
-0.62	0.12	3.4E-07	-1.31	0.20	9.1E-11	-0.17	0.03	2.9E-08
-0.53	0.10	4.3E-07	-0.73	0.17	4.7E-05	-0.07	0.02	3.8E-03
0.51	0.10	4.3E-07	0.84	0.16	5.8E-07	0.09	0.02	1.7E-04
-0.62	0.12	4.5E-07	-1.20	0.20	6.5E-09	-0.15	0.03	3.4E-07
0.51	0.10	4.8E-07	0.85	0.16	5.8E-07	0.09	0.02	1.9E-04
-0.61	0.12	5.1E-07	-1.11	0.20	4.2E-08	-0.15	0.03	7.1E-07
0.46	0.09	5.1E-07	0.64	0.15	3.5E-05	0.05	0.02	0.015
-0.49	0.10	7.1E-07	-0.68	0.16	3.9E-05	-0.07	0.02	1.5E-03
-0.49	0.10	7.6E-07	-0.69	0.16	2.9E-05	-0.08	0.02	1.2E-03
0.60	0.12	7.8E-07	0.08	0.20	6.9E-01	-0.01	0.03	0.83
0.45	0.09	8.0E-07	0.64	0.15	2.7E-05	0.07	0.02	4.0E-03
-0.46	0.09	8.7E-07	-0.74	0.15	2.1E-06	-0.11	0.02	7.8E-07
0.59	0.12	9.7E-07	0.11	0.20	5.9E-01	0.01	0.03	0.84
0.59	0.12	9.7E-07	0.11	0.20	5.9E-01	0.01	0.03	0.85
0.54	0.11	9.7E-07	0.88	0.18	2.7E-06	0.10	0.03	1.6E-04
0.59	0.12	9.7E-07	0.11	0.20	5.9E-01	0.01	0.03	0.85

Table 3: Genome-wide association results for hypertension SNPs with p value <1x10-6 sorted by hypertension meta-analysis p value

SNP identifier	Chr	Position	Gene	MAF
rs2681472	12	88533090	ATP2B1	0.17
rs11105354	12	88550654	ATP2B1	0.17
rs11105364	12	88593407		0.17
rs17249754	12	88584717		0.17
rs11105368	12	88598572		0.17
rs12579302	12	88574634		0.17
rs11105378	12	88614872		0.16
rs12230074	12	88614998		0.16
rs2681492	12	88537220	ATP2B1	0.19
rs4842666	12	88465680		0.17
rs7640747	3	37571809	ITGA9	0.38
rs11105328	12	88466521		0.18
rs743395	3	37573386	ITGA9	0.38
rs11014166	10	18748804	CACNB2	0.17

Chr=chromosome; MAF=minor allele frequency;

Beta is the effect size on blood pressure in mmHg, per allele based on the additive genetic model

CHARGE Meta-analysis Hypertension			CHARGE Meta-analysis SBP			CHARGE Meta-analysis DBP		
Beta	SE	p	Beta	SE	p	Beta	SE	p
-0.16	0.03	1.7E-08	-1.29	0.19	3.5E-11	-0.64	0.11	3.7E-08
-0.16	0.03	1.8E-08	-1.30	0.19	3.7E-11	-0.63	0.11	5.8E-08
-0.16	0.03	2.1E-08	-1.30	0.19	4.8E-11	-0.63	0.12	1.2E-07
-0.16	0.03	2.2E-08	-1.30	0.19	5.2E-11	-0.63	0.12	1.0E-07
-0.16	0.03	2.2E-08	-1.30	0.19	5.3E-11	-0.63	0.12	1.2E-07
-0.16	0.03	2.2E-08	-1.29	0.19	6.2E-11	-0.62	0.12	1.2E-07
-0.17	0.03	2.8E-08	-1.31	0.20	9.1E-11	-0.62	0.12	3.1E-07
-0.17	0.03	2.8E-08	-1.31	0.20	9.1E-11	-0.62	0.12	3.4E-07
-0.14	0.03	8.4E-08	-1.26	0.18	3.0E-11	-0.62	0.11	4.6E-08
-0.15	0.03	3.4E-07	-1.20	0.20	6.5E-09	-0.62	0.12	4.5E-07
0.12	0.02	4.8E-07	0.56	0.16	5.9E-04	0.32	0.09	9.5E-04
-0.15	0.03	7.1E-07	-1.11	0.20	4.2E-08	-0.61	0.12	5.1E-07
0.12	0.02	7.5E-07	0.58	0.16	4.4E-04	0.33	0.10	8.0E-04
-0.11	0.02	7.8E-07	-0.74	0.15	2.1E-06	-0.46	0.09	8.7E-07

Table 4: Meta-analysis in CHARGE and Global BPgen of top 10 loci for systolic and diastolic blood pressure and hypertension in CHARGE

SNP identifier	Chr	Position	Nearest Gene	Alleles (coded/other)	Freq. of coded allele
Systolic blood pressure					
rs12046278	1	10,722,164	CASZ1	T/C	0.64
rs7571613	2	190,513,907	PMS1	A/G	0.82
rs448378	3	170,583,593	MDS1	A/G	0.52
rs2736376	8	11,155,175	MTMR9	C/G	0.13
rs1910252	8	49,569,915	EFCAB1	T/C	0.18
rs11014166	10	18,748,804	CACNB2	A/T	0.66
rs1004467	10	104,584,497	CYP17A1	A/G	0.90
rs381815	11	16,858,844	PLEKHA7	T/C	0.26
rs2681492	12	88,537,220	ATP2B1	T/C	0.80
rs3184504	12	110,368,991	SH2B3	T/C	0.48
Diastolic blood pressure					
rs13423988	2	68,764,770	GPR73/ ARHGAP25	T/C	0.17
rs13401889	2	190,618,804	MSTN	T/C	0.79
rs9815354	3	41,887,655	ULK4	A/G	0.17
rs7016759	8	49,574,969	EFCAB1	T/C	0.83
rs11014166	10	18,748,804	CACNB2	A/T	0.66
rs11024074	11	16,873,795	PLEKHA7	T/C	0.72
rs2681472	12	88,533,090	ATP2B1	A/G	0.83
rs3184504	12	110,368,991	SH2B3	T/C	0.48
rs2384550	12	113,837,114	TBX3/TBX5	A/G	0.35
rs6495122	15	72,912,698	CSK/ULK3	A/C	0.42
Hypertension					
rs17806132	2	190,416,532	PMS1	A/G	0.16
rs305489	3	11,986,163	SYN2	A/G	0.55
rs7640747	3	37,571,809	ITGA9	C/G	0.62
rs11775334	8	10,109,030	MSRA	A/G	0.32
rs899364	8	11,366,954	FAM167A/BLK	T/G	0.32
rs11014166	10	18,748,804	CACNB2	A/T	0.66
rs2681472	12	88,533,090	ATP2B1	A/G	0.83
rs278126	12	118,620,100	CIT	T/G	0.28
rs11612893	12	129,290,572	FZD10/PIWIL1	T/G	0.10
rs16982520	20	57,192,115	ZNF831/ EDN3	A/G	0.88

CHARGE meta- analysis results			Global BPgen meta-analysis results			CHARGE + Global BPgen meta-analysis		
Beta	SE	p-value	Beta	SE	p-value	Beta	SE	p-value
-0.84	0.18	1.84E-06	-0.29	0.15	5.71E-02	-0.53	0.12	4.77E-06
-0.96	0.19	7.28E-07	-0.23	0.16	1.59E-01	-0.54	0.13	1.90E-05
-0.71	0.15	1.28E-06	-0.36	0.13	4.76E-03	-0.51	0.10	1.18E-07
-1.08	0.23	1.90E-06	-0.06	0.19	7.36E-01	-0.48	0.15	9.15E-04
-0.93	0.19	1.70E-06	-0.07	0.17	6.80E-01	-0.43	0.13	6.13E-04
0.74	0.16	2.11E-06	0.33	0.13	1.31E-02	0.50	0.10	7.03E-07
1.20	0.25	1.99E-06	0.94	0.21	1.08E-05	1.05	0.16	1.28E-10
0.84	0.17	5.76E-07	0.52	0.14	2.72E-04	0.65	0.11	1.89E-09
1.26	0.19	3.01E-11	0.50	0.17	4.07E-03	0.85	0.13	3.76E-11
0.75	0.15	5.73E-07	0.45	0.13	6.36E-04	0.58	0.10	4.52E-09
0.59	0.12	1.09E-06	0.11	0.11	3.22E-01	0.33	0.08	5.00E-05
-0.54	0.11	9.58E-07	-0.10	0.11	3.55E-01	-0.31	0.08	4.82E-05
0.60	0.12	7.88E-07	0.40	0.11	3.79E-04	0.49	0.08	2.54E-09
0.57	0.12	1.87E-06	0.06	0.11	5.79E-01	0.30	0.08	2.29E-04
0.46	0.09	8.82E-07	0.28	0.09	1.46E-03	0.37	0.06	1.24E-08
-0.50	0.10	2.83E-07	-0.17	0.09	6.82E-02	-0.33	0.07	1.20E-06
0.64	0.12	3.74E-08	0.36	0.12	2.43E-03	0.50	0.08	1.47E-09
0.50	0.09	1.68E-08	0.45	0.09	2.83E-07	0.48	0.06	2.58E-14
-0.48	0.09	1.32E-07	-0.23	0.09	1.06E-02	-0.35	0.06	3.75E-08
0.45	0.09	8.10E-07	0.35	0.09	3.98E-05	0.40	0.06	1.84E-10
0.14	0.03	1.14E-05	0.04	0.04	2.87E-01	0.10	0.02	4.70E-05
0.10	0.02	4.20E-06	0.01	0.03	7.75E-01	0.06	0.02	1.70E-04
-0.12	0.02	4.53E-07	-0.02	0.03	4.12E-01	-0.08	0.02	1.24E-05
0.10	0.02	1.03E-05	0.05	0.03	5.86E-02	0.08	0.02	4.05E-06
-0.10	0.02	6.95E-06	-0.04	0.03	1.32E-01	-0.08	0.02	1.01E-05
0.11	0.02	7.96E-07	0.07	0.03	1.06E-02	0.09	0.02	5.72E-08
0.16	0.03	1.65E-08	0.13	0.04	2.15E-04	0.15	0.02	1.75E-11
0.11	0.02	8.34E-06	-0.01	0.03	6.72E-01	0.06	0.02	1.74E-03
-0.19	0.04	7.62E-06	-0.04	0.06	4.19E-01	-0.14	0.03	5.50E-05
-0.14	0.03	4.95E-06	-0.11	0.04	7.44E-03	-0.13	0.02	1.59E-07

Table 5. SNPs association with blood pressure and altered tissue-specific gene expression

SNP	Chr	Position	Nearest gene(s)	SBP pval
Liver eSNPs				
rs7537765	1	11809889	NPPA; NPPB; CLCN6; MTHFR	1.8E-05
rs249209	5	79902964	DHFR; DP58; UNQ9217	8.5E-05
rs10963072	9	17368775	C9orf39	0.03
rs525381	12	255052	JARID1A; SLC6A13	9.3E-05
rs739496	12	110372041	SH2B3; ATXN2	2.9E-04
rs7312321	12	118520617	CCDC60	9.0E-04
rs6495126	15	72962078	CPLX3; ULK3; MPI; COX5A; LMAN1L; SCAMP2; C15orf17	3.0E-04
Lymphoblastoid cell line eSNPs				
rs1384883	1	74274065	FPGT	9.9E-03
rs12466395	2	190488943	PMS1	8.8E-04
rs7571613	2	190513907	PMS1	7.3E-07
rs1454301	2	190518307	PMS1	1.1E-06
rs2053163	2	190535268	PMS1	1.7E-05
rs6749643	2	190543718	MSTN	1.6E-06
rs7575810	2	190560410	MSTN	2.5E-05
rs1474359	2	190641251	MSTN	1.9E-05
rs1052501	3	41900402	ULK4	0.84
rs1716975	3	41935010	ULK4	0.94
rs2272007	3	41971140	ULK4	0.87
rs4572871	4	83979911	SEC31A; SCD5	2.3E-05
rs6601414	8	10014158	MSRA	3.0E-04
rs13254942	8	10295088	MSRA	5.6E-05
rs2898290	8	11471318	BLK	2.3E-05
rs4980878	12	297338	JARID1A; SLC6A13	4.8E-05
rs1860360	12	364161	JARID1A; CCDC77	6.2E-05

Highlighted p values are < 1/n, where n is the number of eSNPs interrogated.

‡ p value for association of eSNP with gene expression in liver or LCL.

DBP pval	HTN pval	Expressed Gene(s)	eSNP pval‡
9.2E-04	1.5E-04	CLCN6	1.4E-07
0.07	1.2E-03	HSS00169533	4.4E-05
0.11	6.3E-05	C9orf39	2.0E-06
0.14	5.3E-04	CCDC77; SLC6A12	2.1E-10
1.3E-05	0.01	HSS00340376	1.1E-06
0.03	6.0E-05	Contig30372_RC	1.1E-30
3.6E-05	1.2E-04	ULK3; AK001918; RPP25	8.6E-06
7.2E-05	2.5E-03	LRRC44;BC042056	1.4E-21
5.3E-05	8.8E-05	ORMDL1	2.4E-15
2.2E-06	5.3E-04	ORMDL1;PMS1	1.5E-08
2.2E-06	1.1E-03	PMS1;ORMDL1	2.5E-09
5.8E-06	5.8E-03	ORMDL1	2.1E-12
2.2E-06	7.3E-04	ORMDL1;PMS1	5.5E-09
2.7E-05	0.03	ORMDL1	3.6E-10
1.2E-05	1.0E-03	ORMDL1;PMS1	1.7E-11
4.2E-05	0.64	ULK4	3.9E-08
2.2E-06	0.96	ULK4	7.9E-08
1.5E-06	0.83	ULK4	2.7E-08
9.7E-03	3.5E-04	SCD5	6.4E-41
3.4E-05	4.9E-03	C8orf5	8.2E-09
1.9E-03	1.5E-04	C8orf5;FAM167A	1.0E-08
7.0E-03	7.9E-05	C8orf5;FAM167A;BLK	3.7E-12
0.12	1.6E-04	JARID1A	2.7E-09
0.10	1.6E-04	JARID1A	2.7E-09

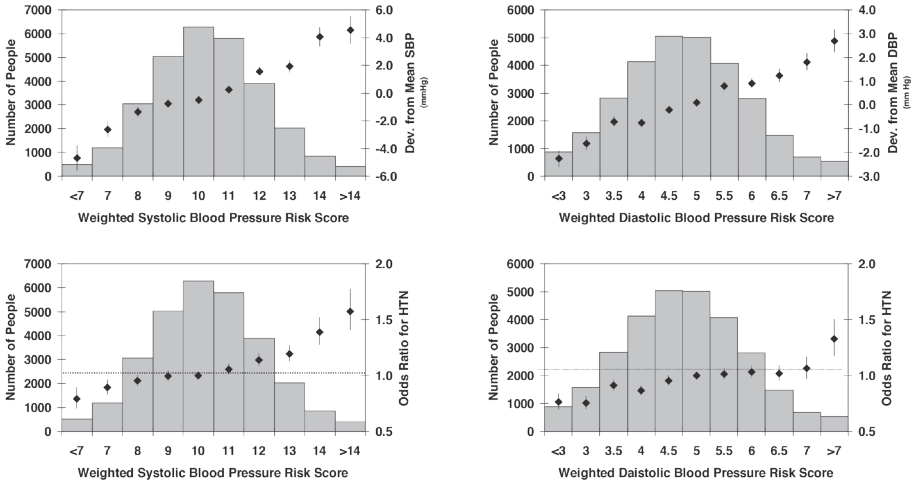


Figure 1 This figure shows deviation in blood pressure or odds ratio for hypertension as solid diamonds with whiskers extending to ±1 standard error. The top panels present deviation from mean systolic (left panel) and diastolic blood pressure (right panel) in mmHg according to weighted risk score. The bottom panels show odds ratios for hypertension in relation to systolic (left panel) and diastolic blood pressure (right panel) weighted risk score. DBP=diastolic blood pressure; HTN=hypertension; SBP=systolic blood pressure. The p values for slope across risk score groups were all highly significant: 1.8x10-27 (systolic blood pressure vs. systolic blood pressure risk score), 1.7x10-56 (diatolic blood pressure vs. diastolic blood pressure risk score), 1.4x10-17 (hypertension vs. systolic blood pressure risk score), and 8.4x10-10 (hypertension vs. diastolic blood pressure risk score).

Discussion

In this meta-analysis of results from 29,136 participants from six large prospective observational studies in the CHARGE Consortium, we identified multiple loci with evidence of association with levels of systolic and diastolic blood pressure and hypertension. We further replicated genome-wide significant SNPs in 34,433 independent subjects from the Global BPgen Consortium, and the joint analysis of results from the two consortia identified 11 genome-wide significant associations: four loci for SBP (ATP2B1, P = 3.8 x 10-11; CYP17A1, P =1.3 x 10-10; PLEKHA7, P = 1.9 x 10-9; SH2B3, P = 4.5 x 10-9), six loci for DBP (ATP2B1, P = 1.5 x 10-9; CACNB2, P = 1.2 x 10-8; CSK-ULK3 P = 1.8 x 10-10; SH2B3, P = 2.6 _ 10_14; TBX3-TBX5, P = 3.8 x 10-8; ULK4, P = 2.5 x 10-9) and one locus for hypertension (ATP2B1, P = 1.8 x 10-11). There was considerable concordance among top loci across all three phenotypes: ATP2B1 showed significant association with SBP, DBP and hypertension, CACNB2 showed strong evidence of association with all three traits and SH2B3 showed significant association with SBP and DBP. Of note, rs1004467, a common intronic variant in CYP17A1, a gene associated with a rare mendelian form of hypertension, emerged as a genome-wide significant locus in the meta-analysis of results from both consortia. Several additional loci showed suggestive association results, including MDS1, ITGA9, EDN3 and PMS1-MSTN. The top ten risk alleles for SBP and DBP within CHARGE were each associated with about a 1 and 0.5 mm Hg increase in SBP and DBP, respectively; there was a continuous and graded relation of the number of risk alleles to mean levels of SBP and DBP and odds ratios for hypertension. Last, analysis of gene expression associated SNPs within our GWAS provided additional promising blood pressure candidates (by virtue of the identified expressed genes), including KDM5A-SLC6A12-CCDC77, ORMDL1 and CLCN6.

We identified genome-wide significant association of ATP2B1 with SBP, DBP and hypertension (17% increase in odds per risk allele and 37% increase for two risk alleles). This gene encodes PMCA1, a plasma membrane calcium/calmodulin-dependent ATPase that is expressed in vascular endothelium and is involved in calcium pumping from the cytosol to the extracellular compartment.[27] An investigation of cultured rat aortic smooth muscle cells found elevated PMCA1 mRNA levels in spontaneously hypertensive rats compared to non-hypertensive controls, consistent with a role of ATP2B1 in blood pressure regulation.[28]

Genetic variation can contribute to altered blood pressure regulation by altering the structure of encoded proteins or by altering gene expression (that is, protein quantity). For SH2B3 we have strong evidence to support both mechanisms; a missense SNP (altered protein structure) and an eSNP (altered expression) were associated with blood pressure. Our most highly significant SNP for DBP (and our second strongest signal for SBP) was the nonsynonymous SNP rs3184504 in SH2B3 (Tables 1, 2 and 4), which introduces the amino acid substitution W262R in a plekstrin homology domain on exon 3. This coding variant is predicted by PolyPhen[29] to be probably damaging to the encoded protein. This SNP has recently also been found to be reproducibly associated with type 1 diabetes mellitus and celiac disease.[30,31] The association of this SNP with two autoimmune diseases suggests that immune response pathways may influence blood pressure by mechanisms

previously not appreciated. SH2B3 knockout mice are viable but show increased sensitivity to cytokines and abnormal growth factor signaling.[32] In addition, eSNP rs739496 (Table 5) was associated with blood pressure levels and with liver expression of a transcript adjacent to SH2B3. SH2B3 is located in a large block of linkage disequilibrium on chromosome 12 that contained multiple association signals across 700 kb from rs3184504 in SH2B3 to rs11066188 in C12orf51 for SBP and DBP, and that contains many genes (Fig. 1). Located near the midpoint between SH2B3 and C12orf51 is ALDH2, encoding acetaldehyde dehydrogenase 2, a critical enzyme in alcohol metabolism. A recent meta-analysis found that male homozygotes for the K671E variant (rs671) in ALDH2 had an increased odds of hypertension (odds ratio 2.42, P = 4.8 x 10-6) and 7 mm Hg higher mean SBP (P = 1.1 x 10-12) when compared with major allele homozygotes.[33] Although rs671 is absent in individuals of European descent in HapMap and was not included in our GWAS, our intriguing findings in the region encircling ALDH2 are consistent with a role of this gene in blood pressure regulation in people of European descent.

Another SNP (rs1004467) attaining genome-wide significance is in CYP17A1, encoding steroid 17-alpha-hydroxylase, an enzyme necessary for steroidogenesis. Mutations in CYP17A1 are found in individuals with 17a-hydroxylase deficiency, which is characterized by congenital adrenal hyperplasia with apparent mineralocorticoid excess, salt retention, hypokalemia and hypertension[34]; these mutations can lead to a spectrum of phenotypic severity.[35] Although mutations in CYP17A1 causing phenotypic 17a-hydroxylase deficiency are rare, our data suggest that common variants in CYP17A1 may also be associated with blood pressure by promoting mild forms of enzyme deficiency or dysfunction.

CACNB2, encoding the beta-2 subunit of a voltage-gated calcium channel, was associated with DBP and showed suggestive evidence of association with SBP and hypertension. The gene is expressed in the heart and a nonsynomymous variant in CACNB2 was identified in affected individuals with Brugada syndrome.[36] CACNB2 is one member of a family of voltage-gated calcium channel genes, several of which have effects on blood pressure regulation and serve as targets of calcium channel blockers. The beta-2 subunit interacts with alpha-1 calcium channels (Cav1.2) and this is a mechanism by which variation in CACNB2 may alter blood pressure.[37]

The joint meta-analysis of CHARGE and Global BPgen (Table 4) also identified PLEKHA7, ULK4, TBX3-TBX5 and a region adjacent to CSK-ULK3-CYP1A2 as genome-wide significant loci. Mutations in TBX5 (T-box transcription factor 5) cause structural cardiac malformations and can be associated with altered expression of NPPA[38], which also was a locus of interest in our eSNP analysis. CSK encodes cytoplasmic tyrosine kinase, which is involved in angiotensin II– dependent vascular smooth muscle cell proliferation.[39] Little is known about ULK3 or ULK4 and how variation in these genes might affect blood pressure. Three CHARGE loci that were identified as genome-wide significant in this analysis were also found to be genome-wide significant in the Global BPgen Consortium meta-analysis: CYP17A1 (rs1004467 in CYP17A1 in CHARGE versus rs11191548 in Global BPgen, respectively; r2 = 0.42), SH2B3-ATXN2 (rs3184504 versus rs653178; r2 = 1.0), and a locus containing CSK-ULK3-CYP1A2 (rs6495122 versus rs4886606; r2 = 0.56). In addition,

both consortia identified MDS1 as a locus of interest (rs448378 versus rs1918974; r 2 = 1.0). The region containing MTHFR-NPPA, which attained genome-wide significance in Global BPgen, was identified as a region of interest in the CHARGE analysis of eSNPs (Table 5; rs7537765 in CHARGE vs. rs17367504 in Global BPgen; r2 = 0.94). Other loci of interest (5 x 10-8 < P < 4 x 10-7) in the joint analysis of CHARGE and Global BPgen were MDS1 (rs448378, P = 1.2 x 10-7) and a region adjacent to EDN3 (rs16982520, P = 1.6 x 10-7). Endothelin-3 may have a role in renal-mediated hypertension in the rat.[40]

A search for putative functional variation within our GWAS identified five nonsynonymous SNPs. In addition to rs3184504 in SH2B3 (discussed above), rs267561 in ITGA9, which showed suggestive evidence of association with hypertension (P = 2.6 x 10-6), produces an E507G substitution that is predicted by PolyPhen to have possibly damaging effects[26]. Three linked nonsynonymous SNPs in ULK4 showed suggestive evidence of association with DBP (rs2272007, P = 1.5 < 10-6; rs3774372, P = 1.6 x 10-6; rs1716975, P = 2.2 x 10-6; pairwise r2 = 0.82–1.0); these amino-acid substitutions are predicted to be benign individually, but their conjoint effects on protein function is unknown. Interrogation of our GWAS results for SNPs that are associated with blood pressure phenotypes and altered gene expression confirmed SH2B3, ULK4 and ULK3 as loci of interest (Table 5). Another locus detected via eSNP associations with blood pressure was rs7537765 near NPPA, which encodes atrial natriuretic peptide and which was in linkage disequilibrium with rs198358 (r2 = 0.58), a SNP that has been shown to be associated with higher circulating natriuretic peptide levels and lower SBP.[41] Other promising candidates by virtue of the expressed genes in our eSNP analysis are KDM5A-SLC6A12-CCDC77, ORMDL1-PMS1 and CLCN6.

Although the conjoint effect of multiple risk alleles on blood pressure can be substantial, our findings underscore the small effect size of individual common allelic variants—about 1 mmHg each for systolic and 0.5 mmHg each for diastolic blood pressure per variant allele—and the necessity of very large sample sizes for detection of robust and significant results. The combined analysis of CHARGE and Global BPgen for our top SNPs reflects a sample size of 63,569 individuals and illustrates the advantage of large consortia for meta-analysis of underpinnings of common complex traits. Given the small effect sizes detected, it is not surprising that previous blood pressure GWAS failed to identify genome-wide significant results at P < 5 x 10-8.[13-18]

Understanding of allelic variation affecting blood pressure in the general population is in its infancy; until recently, there were few known genetic variants reproducibly associated with blood pressure variation. Our CHARGE findings, in conjunction with those of the Global BPgen Consortium,[20] establish the utility of genome-wide association approaches to identify common allelic variants pertaining to blood pressure physiology and pathophysiology. Our findings are consistent with the hypothesis that variation in scores, if not hundreds, of genes contribute to blood pressure variation. This hypothesis is supported by the excess number of SNPs showing association at P < 1 x 10-3 with blood pressure phenotypes. Future efforts to identify additional alleles associated with blood pressure will require complementary strategies, including larger genome-wide studies to identify additional common alleles and resequencing efforts in large samples to identify rare variants.

In aggregate, the proportion of blood pressure variation explained by the top ten CHARGE SBP- and DBP-associated SNPs across the six cohorts is 1% (increment in r2) after accounting for the major nongenetic determinants of blood pressure: age, age squared, sex and body mass index. The conjoint effect of multiple risk alleles on blood pressure levels, however, amounts to several mmHg (Fig. 1), which is sufficient to increase cardiovascular disease risk. Observational data indicate that a prolonged increase in DBP of 5 mmHg is associated with a 34% increase in risk for stroke and a 21% increase in risk of coronary events.[42]

Future analyses using larger samples can benefit from specific features of our study design. First, the vast majority of blood pressure values used in our analyses were obtained more than 15 years ago, when blood pressure treatment, which confounds genetic analyses, was less widely used; contemporary blood pressure data might be less likely to reveal genetic associations. Second, because allelic variation may affect both the low and high ends of the blood pressure distribution, we used the more powerful approach of analyzing blood pressure as a continuous trait, yet we also identified a genome-wide significant locus for hypertension. At the same time, one should recognize that our study results, based on participants of European descent, cannot necessarily be generalized to other populations. Although our analysis of eSNPs indicates that some of the genome-wide significant blood pressure loci we identified are associated with altered gene expression, the relevance of these findings to blood pressure is speculative. A similar approach, however, has been used to identify putative disease genes for childhood asthma[43], Crohn's disease[44] and a network of genes implicated in obesity.[45]

In conclusion, we have identified multiple genome-wide significant blood pressure loci that can be used to guide fine-mapping efforts to pinpoint causal variants and to understand how the implicated genes alter blood pressure physiology and contribute to hypertension. The characterization of new blood pressure–associated loci can serve as a basis for future approaches to early detection of high-risk individuals and for the development of novel therapies for the prevention or treatment of hypertension.

References

1. Fields LE, Burt VL, Cutler JA, Hughes J, Roccella EJ, Sorlie P. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. Hypertension. Oct 2004;44(4):398-404.
2. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. Lancet. May 3 2008;371(9623):1513-1518.
3. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. JAMA. Dec 11 1967;202(11):1028-1034.
4. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP Cooperative Research Group. JAMA. Jun 26 1991;265(24):3255-3264.
5. Levy D, DeStefano AL, Larson MG, et al. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. Hypertension. Oct 2000;36(4):477-483.
6. Chang YP, Liu X, Kim JD, et al. Multiple genes for essential-hypertension susceptibility on chromosome 1q. American journal of human genetics. Feb 2007;80(2):253-264.
7. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genetics in medicine : official journal of the American College of Medical Genetics. Mar-Apr 2002;4(2):45-61.
8. Koivukoski L, Fisher SA, Kanninen T, et al. Meta-analysis of genome-wide scans for hypertension and blood pressure in Caucasians shows evidence of susceptibility regions on chromosomes 2 and 3. Human molecular genetics. Oct 1 2004;13(19):2325-2332.
9. Lifton RP. Genetic determinants of human hypertension. Proceedings of the National Academy of Sciences of the United States of America. Sep 12 1995;92(19):8545-8551.
10. Tobin MD, Tomaszewski M, Braund PS, et al. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. Hypertension. Jun 2008;51(6):1658-1664.
11. Manolio TA, Brooks LD, Collins FS. A HapMap harvest of insights into the genetics of common disease. The Journal of clinical investigation. May 2008;118(5):1590-1605.
12. McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet. May 2008;9(5):356-369.
13. Levy D, Larson MG, Benjamin EJ, et al. Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. BMC medical genetics. 2007;8 Suppl 1:S3.
14. Diabetes Genetics Initiative of Broad Institute of H, Mit LU, Novartis Institutes of BioMedical R, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. Jun 1 2007;316(5829):1331-1336.
15. Kato N, Miyata T, Tabara Y, et al. High-density association study and nomination of susceptibility genes for hypertension in the Japanese National Project. Human molecular genetics. Feb 15 2008;17(4):617-627.
16. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. Jun 7 2007;447(7145):661-678.

17. Sabatti C, Service SK, Hartikainen AL, et al. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nature genetics. Jan 2009;41(1):35-46.
18. Wang Y, O'Connell JR, McArdle PF, et al. From the Cover: Whole-genome association study identifies STK39 as a hypertension susceptibility gene. Proceedings of the National Academy of Sciences of the United States of America. Jan 6 2009;106(1):226-231.
19. Psaty BM, O'Donnell CJ, Gudnason V, 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. Feb 2009;2(1):73-80.
20. Newton-Cheh C, Johnson T, Gateva V, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nature genetics. May 10 2009.
21. Servin B, Stephens M. Imputation-based analysis of association studies: candidate regions and quantitative traits. PLoS genetics. Jul 2007;3(7):e114.
22. Cui JS, Hopper JL, Harrap SB. Antihypertensive treatments obscure familial contributions to blood pressure variation. Hypertension. Feb 2003;41(2):207-210.
23. Senn S. Trying to be precise about vagueness. Stat Med. Mar 30 2007;26(7):1417-1430.
24. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. Dec 15 2008;24(24):2938-2939.
25. Schadt EE, Molony C, Chudin E, et al. Mapping the genetic architecture of gene expression in human liver. PLoS biology. May 6 2008;6(5):e107.
26. Dixon AL, Liang L, Moffatt MF, et al. A genome-wide association study of global gene expression. Nature genetics. Oct 2007;39(10):1202-1207.
27. Pande J, Mallhi KK, Sawh A, Szewczyk MM, Simpson F, Grover AK. Aortic smooth muscle and endothelial plasma membrane Ca2+ pump isoforms are inhibited differently by the extracellular inhibitor caloxin 1b1. American journal of physiology. Cell physiology. May 2006;290(5):C1341-1349.
28. Monteith GR, Kable EP, Kuo TH, Roufogalis BD. Elevated plasma membrane and sarcoplasmic reticulum Ca2+ pump mRNA levels in cultured aortic smooth muscle cells from spontaneously hypertensive rats. Biochemical and biophysical research communications. Jan 13 1997;230(2):344-346.
29. Sunyaev S, Ramensky V, Koch I, Lathe W, 3rd, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Human molecular genetics. Mar 15 2001;10(6):591-597.
30. Hunt KA, Zhernakova A, Turner G, et al. Newly identified genetic risk variants for celiac disease related to the immune response. Nature genetics. Apr 2008;40(4):395-402.
31. Smyth DJ, Plagnol V, Walker NM, et al. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med. Dec 25 2008;359(26):2767-2777.
32. Velazquez L, Cheng AM, Fleming HE, et al. Cytokine signaling and hematopoietic homeostasis are disrupted in Lnk-deficient mice. The Journal of experimental medicine. Jun 17 2002;195(12):1599-1611.
33. Chen L, Davey Smith G, Harbord RM, Lewis SJ. Alcohol intake and blood pressure: a systematic review implementing a Mendelian randomization approach. PLoS medicine. Mar 4 2008;5(3):e52.

34. Costa-Santos M, Kater CE, Auchus RJ, Brazilian Congenital Adrenal Hyperplasia Multicenter Study G. Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. The Journal of clinical endocrinology and metabolism. Jan 2004;89(1):49-60.
35. Yang J, Cui B, Sun S, et al. Phenotype-genotype correlation in eight Chinese 17alpha-hydroxylase/17,20 lyase-deficiency patients with five novel mutations of CYP17A1 gene. The Journal of clinical endocrinology and metabolism. Sep 2006;91(9):3619-3625.
36. Antzelevitch C, Pollevick GD, Cordeiro JM, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. Circulation. Jan 30 2007;115(4):442-449.
37. Lao QZ, Kobrinsky E, Harry JB, Ravindran A, Soldatov NM. New Determinant for the CaVbeta2 subunit modulation of the CaV1.2 calcium channel. The Journal of biological chemistry. Jun 6 2008;283(23):15577-15588.
38. Postma AV, van de Meerakker JB, Mathijssen IB, et al. A gain-of-function TBX5 mutation is associated with atypical Holt-Oram syndrome and paroxysmal atrial fibrillation. Circulation research. Jun 6 2008;102(11):1433-1442.
39. Sayeski PP, Ali MS. The critical role of c-Src and the Shc/Grb2/ERK2 signaling pathway in angiotensin II-dependent VSMC proliferation. Experimental cell research. Jul 15 2003;287(2):339-349.
40. Vogel V, Backer A, Heller J, Kramer HJ. The renal endothelin system in the Prague hypertensive rat, a new model of spontaneous hypertension. Clin Sci (Lond). Jul 1999;97(1):91-98.
41. Newton-Cheh C, Larson MG, Vasan RS, et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nature genetics. Mar 2009;41(3):348-353.
42. MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. Lancet. Mar 31 1990;335(8692):765-774.
43. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature. Jul 26 2007;448(7152):470-473.
44. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nature genetics. Aug 2008;40(8):955-962.
45. Emilsson V, Thorleifsson G, Zhang B, et al. Genetics of gene expression and its effect on disease. Nature. Mar 27 2008;452(7186):423-428.

Chapter 2.2

Genome-wide association of blood pressure: ICBP

Based on

Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature.

2011 Sep 11;478(7367):103-9.

Abstract

Background

Blood pressure is a heritable trait[1] influenced by several biological pathways and responsive to environmental stimuli. Over one billion people worldwide have hypertension (≥140 mmHg systolic blood pressure or ≥90 mmHg diastolic blood pressure)[2]. Even small increments in blood pressure are associated with an increased risk of cardiovascular events[3].

Methods

We conducted a genome-wide association study of systolic and diastolic blood pressure using a multi-staged design in up to 200,000 individuals of European descent.

Results

We identified sixteen novel loci: six of these loci contain genes previously known or suspected to regulate blood pressure (GUCY1A3–GUCY1B3, NPR3–c5orf23, ADM, FURIN–FES, GOSR2, GNAS–EDN3); the other ten provide new clues to blood pressure physiology. A genetic risk score based on 29 genome-wide significant variants was associated with hypertension, left ventricular wall thickness, stroke and coronary artery disease, but not kidney disease or kidney function. We also observed associations with blood pressure in East Asian, South Asian and African ancestry individuals.

Conclusions

Our findings provide new insights into the genetics and biology of blood pressure, and suggest potential novel therapeutic pathways for cardiovascular disease prevention.

Genetic approaches have advanced the understanding of biological pathways underlying inter-individual variation in blood pressure. For example, studies of rare Mendelian blood pressure disorders have identified multiple defects in renal sodium handling pathways[4]. More recently two genome-wide association studies (GWAS), each of >25,000 individuals of European ancestry, identified 13 loci associated with systolic blood pressure (SBP), diastolic blood pressure (DBP) and hypertension[5,6]. We now report results of a new meta-analysis of GWAS data that includes staged follow-up genotyping to identify additional blood pressure loci.

Primary analyses evaluated associations between 2.5 million genotyped or imputed single nucleotide polymorphisms (SNPs) and SBP and DBP in 69,395 individuals of European ancestry from 29 studies. Following GWAS meta-analysis, we conducted a three-stage validation experiment that made efficient use of available genotyping resources, to follow up top signals in up to 133,661 additional individuals of European descent.

Twenty-nine independent SNPs at 28 loci were significantly associated with SBP, DBP, or both in the meta-analysis combining discovery and follow-up data (Fig. 1, Table 1). All 29 SNPs attained association $P < 5 \times 10\text{-}9$, an order of magnitude beyond the standard genome-wide significance level for a single-stage experiment (Table 1).

Sixteen of these 29 associations were novel (Table 1). Two associations were near the FURIN and GOSR2 genes; prior targeted analyses of variants in these genes suggested they may be blood pressure loci[7,8]. At the CACNB2 locus we validated association for a previously reported 6 SNP, rs4373814, and detected a novel independent association for rs1813353 (pairwise r2 = 0.015 in HapMap CEU). Of our 13 previously reported associations[5,6], only the association at PLCD3 was not supported by the current results. Some of the associations are in or near genes involved in pathways known to influence blood pressure (NPR3, GUCY1A3–GUCY1B3, ADM, GNAS–EDN3, NPPA–NPPB and CYP17A1). Twenty-two of the 28 loci did not contain genes that were a priori strong biological candidates.

As expected from prior blood pressure GWAS results, the effects of the novel variants on SBP and DBP were small (Fig. 1 and Table 1). For all variants, the observed directions of effects were concordant for SBP, DBP and hypertension (Fig. 1, Table 1). Among the genes at the genome-wide significant loci, only CYP17A1, previously implicated in Mendelian congenital adrenal hyperplasia and hypertension, is known to harbour rare variants that have large effects on blood pressure[9].

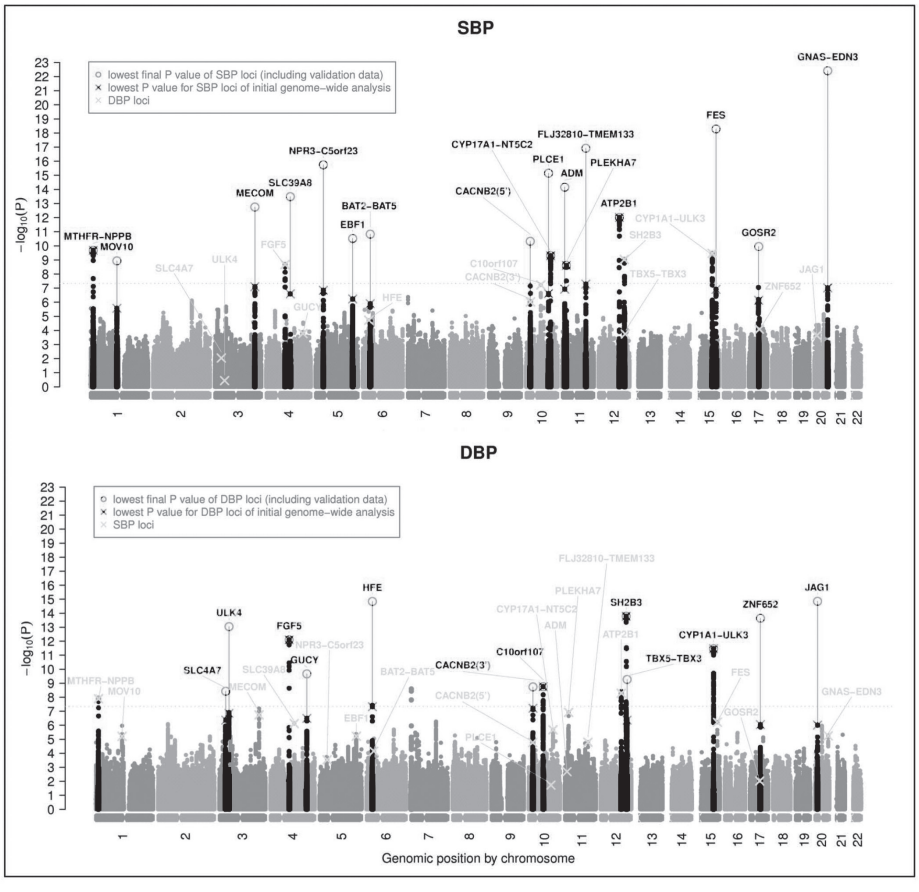


Figure 1. Genome-wide –log10 P-value plots.

Genome-wide –log10 P-value plots for systolic blood pressure (SBP: upper panel) and diastolic blood pressure (DBP: lower panel). SNPs within loci reaching genome wide significance are labeled in red and lowest P-values of the initial genome-wide analysis and analysis including the replication data are labeled separately. The dotted line indicates the genome-wide significance level adjusted for our sequential design (P=2.5 x 10-8).

We performed several analyses to identify potential causal alleles and mechanisms. First, we looked up the 29 genome-wide significant index SNPs and their close proxies (r2 > 0.8) among cis-acting expression SNP (eSNP) results from multiple tissues (Supplementary Materials section 5). For 13/29 index SNPs, we found an association between nearby eSNP variants and the expression levels of at least one gene transcript (10-4 > P > 10-51). In five cases, the index blood pressure SNP and the best eSNP from a genome-wide survey were identical, highlighting potential mediators of the SNP–blood pressure associations.

Second, because changes in protein sequence are a priori strong functional candidates, we sought non-synonymous coding SNPs that were in high linkage disequilibrium (r2 > 0.8) with the 29 index SNPs. We identified such SNPs at eight loci (Table 1). In addition we performed analyses testing for differences in genetic effect according to body mass index (BMI) or sex, and analyses of copy number variants, pathway enrichment and metabolomic data, but we did not find any statistically significant results.

We evaluated whether the blood pressure variants we identified in Europeans were associated with blood pressure in individuals of East Asian (N = 29,719), South Asian (N = 23,977) and African (N = 19,775) ancestries (Table 1). We found significant associations in individuals of East Asian ancestry for SNPs at nine loci and in individuals of South Asian ancestry for SNPs at six loci. The lack of significant association for individual SNPs may reflect small sample sizes, differences in allele frequencies or linkage disequilibrium patterns, imprecise imputation for some ancestries using existing reference samples, or a genuinely different underlying genetic architecture. Because of limited power to detect effects of individual variants in the smaller non-European samples, we created genetic risk scores for SBP and DBP incorporating all 29 blood pressure variants weighted according to effect sizes observed in the European samples. In each non-European ancestry group, risk scores were strongly associated with SBP (P = 1.1 × 10-40 in East Asian, P = 2.9 × 10-13 in South Asian, P = 9.8 × 10-4 in African ancestry individuals) and DBP (P = 2.9 × 10-48, P = 9.5 × 10-15 and P = 5.3 × 10-5, respectively).

We also created a genetic risk score to assess association of the variants in aggregate with hypertension and with clinical measures of hypertensive complications including left ventricular mass, left ventricular wall thickness, incident heart failure, incident and prevalent stroke, prevalent coronary artery disease (CAD), kidney disease and measures of kidney function, using results from other GWAS consortia (Table 2). The risk score was weighted using the average of SBP and DBP effects for the 29 SNPs. In an independent sample of 23,294 women[10], an increase of one standard deviation in the genetic risk score was associated with a 21% increase in the odds of hypertension (95% confidence interval 19–28%; Table 2). Among individuals in the top decile of the risk score, the prevalence of hypertension was 29% compared with 16% in the bottom decile (odds ratio 2.09, 95% confidence interval 1.86–2.36). Similar results were observed in an independent hypertension case-control sample (Table 2). In our study, individuals in the top compared to bottom quintiles of genetic risk score differed by 4.6 mmHg SBP and 3.0 mmHg DBP, differences that approach population-averaged blood pressure treatment effects for a single antihypertensive agent[11]. Epidemiological data have shown that differences in SBP and DBP of this magnitude, across the population range of blood pressure, are associated with an increase in cardiovascular disease risk[3]. Consistent with this and in line with findings from randomized trials of blood-pressure-lowering medication in hypertensive patients[12,13], the genetic risk score was positively associated with left ventricular wall thickness ($P = 6.0 \times 10\text{-}6$), occurrence of stroke ($P = 3.3 \times 10\text{-}5$) and CAD ($P = 8.1 \times 10\text{-}29$). The same genetic risk score was not, however, significantly associated with chronic kidney disease or measures of kidney function, even though these renal outcomes were available in a similar sample size as for the other outcomes (Table 2). The absence of association with kidney phenotypes could be explained by a weaker causal relationship between blood pressure and kidney phenotypes than with CAD and stroke. This finding is consistent with the mismatch between observational data that show a positive association of blood pressure with kidney disease, and clinical trial data that show inconsistent evidence of a benefit from blood pressure lowering on kidney disease prevention in patients with hypertension[14]. Thus, several lines of evidence converge to indicate that blood pressure elevation may in part be a consequence rather than a cause of sub-clinical kidney disease.

Table 1. Summary association results for 29 blood pressure SNPs

Locus	Index SNP	Chr	Position	Coded allele	Coded allele freq.
Novel genome-wide significant variants identified					
MOV10	rs2932538	1	113,018,066	G	0.75
SLC4A7	rs13082711	3	27,512,913	T	0.78
MECOM	rs419076	3	170,583,580	T	0.47
SLC39A8	rs13107325	4	103,407,732	T	0.50
GUCY1A3-GUCY1B3	rs13139571	4	156,864,963	C	0.76
NPR3-C5orf23	rs1173771	5	32,850,785	G	0.60
EBF1	rs11953630	5	157,777,980	T	0.37
HFE	rs1799945	6	26,199,158	G	0.14
BAT2-BAT5	rs805303	6	31,724,345	G	0.61
CACNB2(3')	rs1813353	10	18,747,454	T	0.68
PLCE1	rs932764	10	95,885,930	G	0.44
FLJ32810-TMEM133	rs633185	11	100,098,748	G	0.28
ADM	rs7129220	11	10,307,114	G	0.89
FES	rs2521501	15	89,238,392	T	0.31
GOSR2	rs17608766	17	42,368,270	T	0.86
JAG1	rs1327235	20	10,917,030	G	0.46
GNAS-EDN3	rs6015450	20	57,184,512	G	0.12
Validated loci that were reported previously					
MTHFR-NPPB	rs17367504	1	11,785,365	G	0.15
ULK4	rs3774372	3	41,852,418	T	0.83
FGF5	rs1458038	4	81,383,747	T	0.29
CACNB2(5')	rs4373814	10	18,459,978	G	0.55
C10orf107	rs4590817	10	63,137,559	G	0.84
CYP17A1-NT5C2	rs11191548	10	104,836,168	T	0.91
PLEKHA7	rs381815	11	16,858,844	T	0.26
ATP2B1	rs17249754	12	88,584,717	G	0.84
SH2B3	rs3184504	12	110,368,991	T	0.47
TBX5-TBX3	rs10850411	12	113,872,179	T	0.70
CYP1A1-ULK3	rs1378942	15	72,864,420	C	0.35
ZNF652	rs12940887	17	44,757,806	T	0.38

Estimates of SBP and DBP effects (beta) are in mmHg per coded allele; HTN effects (beta) are in ln (odds) units per coded allele.

SBP		DBP		HTN	
Beta	**P-value**	**Beta**	**P-value**	**Beta**	**P-value**
0.388	1.2*10-9	0.240	9.9*10-10	0.049	2.9*10-7
-0.315	1.5*10-6	-0.238	3.8*10-9	-0.035	3.6*10-4
0.409	1.8*10-13	0.241	2.1*10-12	0.031	3.1*10-4
-0.981	3.3*10-14	-0.684	2.3*10-17	-0.105	4.9*10-7
0.321	1.2*10-6	0.260	2.2*10-10	0.042	2.5*10-5
0.504	1.8*10-16	0.261	9.1*10-12	0.062	3.2*10-10
-0.412	3.0*10-11	-0.281	3.8*10-13	-0.052	1.7*10-7
0.627	7.7*10-12	0.457	1.5*10-15	0.095	1.8*10-10
0.376	1.5*10-11	0.228	3.0*10-11	0.054	1.1*10-10
0.569	2.6*10-12	0.415	2.3*10-15	0.078	6.2*10-10
0.484	7.1*10-16	0.185	8.1*10-7	0.055	9.4*10-9
-0.565	1.2*10-17	-0.328	2.0*10-15	-0.070	5.4*10-11
-0.619	3.0*10-12	-0.299	6.4*10-8	-0.045	1.1*10-3
0.650	5.2*10-19	0.359	1.9*10-15	0.059	7.0*10-7
-0.556	1.1*10-10	-0.129	0.017	-0.025	0.080
0.340	1.9*10-8	0.302	1.4*10-15	0.034	4.6*10-4
0.896	3.9*10-23	0.557	5.6*10-23	0.110	4.2*10-14
-0.547	3.6*10-19	-0.903	8.7*10-22	-0.103	2.3*10-10
-0.067	0.40	-0.367	9.0*10-14	-0.017	0.18
0.457	8.5*10-25	0.706	1.5*10-23	0.072	1.9*10-7
-0.373	4.8*10-11	-0.218	4.4*10-10	-0.046	8.5*10-8
0.419	1.3*10-12	0.646	4.0*10-12	0.096	9.8*10-9
0.464	9.4*10-13	1.095	6.9*10-26	0.097	1.4*10-5
0.349	5.3*10-10	0.575	5.3*10-11	0.062	3.4*10-6
0.522	1.2*10-14	0.928	1.8*10-18	0.126	1.1*10-14
0.448	3.6*10-25	0.598	3.8*10-18	0.056	2.6*10-6
0.354	5.4*10-8	0.253	5.4*10-10	0.045	5.2*10-6
0.416	2.7*10-26	0.613	5.7*10-23	0.073	1.0*10-8
0.362	1.8*10-10	0.271	2.3*10-14	0.046	1.2*10-7

Table 2. Genetic risk score and cardiovascular outcome association results

Phenotype	Source	Effect	SE		P-value
		(per SD of genetic risk score)			
Blood pressure phenotypes (for self reported BP in "healthy" female health professionals)					
SBP [mmHg]	WGHS	1.645	0.098	(a)	6.5x10-63
DBP [mmHg]	WGHS	1.057	0.067	(a)	8.4x10-57
prevalent hypertension	WGHS	0.211	0.018	(b)	3.1x10-33
Dichotomous endpoints					
Incident heart failure	CHARGE-HF	0.035	0.021	(c)	0.10
Incident stroke	NEURO-CHARGE	0.103	0.028	(c)	0.0002
Prevalent stroke	UK-US Stroke Collaborative Group(SCG)	0.075	0.037	(b)	0.05
Stroke (combined, incident and prevalent)	CHARGE & SCG	NA	NA	NA	3.3x10-5
Prevalent CAD	CARDIoGRAM	0.092	0.010	(b)	1.6x10-19
Prevalent CAD	C4D ProCARDIS	0.132	0.022	(b)	2.2x10-9
Prevalent CAD	C4D HPS	0.083	0.027	(b)	0.002
Prevalent CAD (combined)	CARDIoGRAM & C4D	0.100	0.009	(b)	8.1x10-29
Prevalent chronic kidney disease	CKDGen	0.014	0.015	(b)	0.35
Prevalent microalbuminuria	CKDGen	0.008	0.019	(b)	0.68
Continuous measures of target organ damage					
Left ventricular mass [g]	EchoGen	0.822	0.317	(a)	0.01
Left ventricular wall thickness[mm]	EchoGen	0.009	0.002	(a)	6.0x10-6
Serum creatinine	KidneyGen	-0.001	0.001	(d)	0.24
eGFR (4 parameter MDRD equation)	CKDGen	-0.0001	0.0009	(d)	0.93
Urinary albumin/creatinine ratio	CKDGen	0.005	0.007	(d)	0.43

(a) units are the unit of phenotypic measurement, either per SD of genetic risk score, or as a difference between top/bottom quintiles or deciles.

(b) units are ln(odds) per SD of genetic risk score, or odds ratio between top/bottom quintiles or deciles.

# SNPs	Contrast top vs bottom quintiles	deciles		N case/control	Reference (if available)
29	4.61	5.77	(a)	NA	1
29	2.96	3.71	(a)	NA	1
29	1.80	2.09	(b)	5,018/18,276	1
29	1.10	1.13	(c)	2,526/18,400	2
28	1.34	1.44	(c)	1,544/18,058	3
29	1.23	1.30	(b)	1,473/ 1,482	
NA	NA	NA	NA	3,017/19,540	
28	1.29	1.38	(b)	22,233/64,726	
29	1.45	1.59	(b)	5,720/ 4,381	
29	1.26	1.34	(b)	2,704/ 2,804	
29	1.32	1.42	(b)	30,657/71,911	
29	1.04	1.05	(b)	5,807/61,286	4
29	1.02	1.03	(b)	3,698/27,882	
29	2.30	2.89	(a)	NA	5
29	0.03	0.03	(a)	NA	5
29	1.00	1.00	(d)	NA	6
29	1.00	1.00	(d)	NA	4
29	1.01	1.02	(d)	NA	

(c) units are ln(hazard ratio) per SD of genetic risk score, or hazard ratio between top/bottom quintiles or deciles.

(d) units are ln(phenotype) per SD of genetic risk score, or phenotypic ratio between top/bottom quintiles or deciles.

Our discovery meta-analysis suggests an excess of modestly significant (10-5 < P < 10-2) associations probably arising from common blood pressure variants of small effect. By dividing our principal GWAS data set into non-overlapping discovery (N ≈ 56,000) and validation (N ≈ 14,000) subsets, we found robust evidence for the existence of such undetected common variants (Supplementary Fig. 5 and Supplementary Materials section 12). We estimate[15] that there are 116 (95% confidence interval 57–174) independent blood pressure variants with effect sizes similar to those reported here, which collectively can explain ~2.2% of the phenotypic variance for SBP and DBP, compared with 0.9% explained by the 29 associations discovered thus far.

Most of the 28 blood pressure loci harbour multiple genes and although substantial research is required to identify the specific genes and variants responsible for these associations, several loci contain highly plausible biological candidates. The NPPA and NPPB genes at the MTHFR–NPPB locus encode precursors for atrial- and B-type natriuretic peptides (ANP, BNP), and previous work has identified SNPs—modestly correlated with our index SNP at this locus—which are associated with plasma ANP, BNP and blood pressure[16]. We found the index SNP at this locus was associated with opposite effects on blood pressure and on ANP/BNP levels, consistent with a model in which the variants act through increased ANP/BNP production to lower blood pressure[16].

Two other loci identified in the current study harbour genes involved in natriuretic peptide and related nitric oxide signalling pathways[17,18], both of which act to regulate cyclic guanosine monophosphate. The first locus contains NPR3, which encodes the natriuretic peptide clearance receptor (NPR-C). NPR3 knockout mice exhibit reduced clearance of circulating natriuretic peptides and lower blood pressure[19]. The second locus includes GUCY1A3 and GUCY1B3, encoding the alpha and beta subunits of soluble guanylate cyclase; knockout of either gene in murine models results in hypertension[20].

Another locus contains ADM—encoding adrenomedullin—which has natriuretic, vasodilatory and blood-pressure-lowering properties[21]. At the GNAS–EDN3 locus, ZNF831 is closest to the index SNP, but GNAS and EDN3 are two nearby compelling biological candidates.

We identified two loci with plausible connections to blood pressure via genes implicated in renal physiology or kidney disease. At the first locus, SLC4A7 is an electro-neutral sodium bicarbonate co-transporter expressed in the nephron and in vascular smooth muscle[22]. At the second locus, PLCE1 (phospholipase-C-epsilon-1 isoform) is important for normal podocyte development in the glomerulus; sequence variation in PLCE1 has been implicated in familial nephrotic syndromes and end-stage kidney disease[23].

Missense variants in two genes involved in metal ion transport were associated with blood pressure in our study. The first encodes a His/Asp change at amino acid 63 (H63D) in HFE and is a low-penetrance allele for hereditary hemochromatosis[24]. The second is an Ala/Thr polymorphism located in exon 7 of SLC39A8, which encodes a zinc transporter that also transports cadmium and manganese[25]. The same allele of SLC39A8 associated with blood pressure in our study has recently been associated with high-density lipoprotein cholesterol levels[26] and BMI[27].

We have shown that 29 independent genetic variants influence blood pressure in people of European ancestry. The variants reside in 28 loci, 16 of which were novel, and we confirmed association of several of them in individuals of non-European ancestry. A risk score derived from the 29 variants was significantly associated with blood-pressure-related organ damage and clinical cardiovascular disease, but not kidney disease. These loci improve our understanding of the genetic architecture of blood pressure, provide new biological insights into blood pressure control and may identify novel targets for the treatment of hypertension and the prevention of cardiovascular disease.

References

1. Levy, D. et al. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham heart study. Hypertension 36, 477–483 (2000).
2. Kearney, P. M. et al. Global burden of hypertension: analysis of worldwide data. Lancet 365, 217–223 (2005).
3. Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 360, 1903–1913 (2002).
4. Lifton, R. P., Gharavi, A. G. & Geller, D. S. Molecular mechanisms of human hypertension. Cell 104, 545–556 (2001).
5. Newton-Cheh, C. et al. Genome-wide association study identifies eight loci associated with blood pressure. Nature Genet. 41, 666–676 (2009).
6. Levy, D. et al. Genome-wide association study of blood pressure and hypertension. Nature Genet. 41, 677–687 (2009).
7. Meyer, T. E. et al. GOSR2 Lys67Arg is associated with hypertension in whites. Am. J. Hypertens. 22, 163–168 (2009).
8. Li, N. et al. Associations between genetic variations in the FURIN gene and hypertension. BMC Med. Genet. 11, 124 (2010).
9. Mussig, K. et al. 17α-hydroxylase/17,20-lyase deficiency caused by a novel homozygous mutation (Y27Stop) in the cytochrome CYP17 gene. J. Clin. Endocrinol. Metab. 90, 4362–4365 (2005).
10. Ridker, P. M. et al. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. Clin. Chem. 54, 249–255 (2008).
11. Burt, V. L. et al. Trends in the prevalence, awareness, treatment, and control of hypertension in the adult US population. Data from the health examination surveys, 1960 to 1991. Hypertension 26, 60–69 (1995).
12. Blood Pressure Lowering Treatment Trialists' Collaboration Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. Br. Med. J. 336, 1121–1123 (2008).
13. Law, M. R., Morris, J. K. & Wald, N. J. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. Br. Med. J. 338, b1665 (2009).
14. Lewis, J. B. Blood pressure control in chronic kidney disease: is less really more? J. Am. Soc. Nephrol. 21, 1086–1092 (2010).
15. Park, J. H. et al. Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. Nature Genet. 42, 570–575 (2010).
16. Newton-Cheh, C. et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nature Genet. 41, 348–353 (2009).

17. Schenk, D. B. et al. Purification and subunit composition of atrial natriuretic peptide receptor. Proc. Natl Acad. Sci. USA 84, 1521–1525 (1987).
18. Schmidt, H. H. & Walter, U. NO at work. Cell 78, 919–925 (1994).
19. Matsukawa, N. et al. The natriuretic peptide clearance receptor locally modulates the physiological effects of the natriuretic peptide system. Proc. Natl Acad. Sci. USA 96, 7403–7408 (1999).
20. Friebe, A., Mergia, E., Dangel, O., Lange, A. & Koesling, D. Fatal gastrointestinal obstruction and hypertension in mice lacking nitric oxide-sensitive guanylyl cyclase. Proc. Natl Acad. Sci. USA 104, 7699–7704 (2007).
21. Ishimitsu, T., Ono, H., Minami, J. & Matsuoka, H. Pathophysiologic and therapeutic implications of adrenomedullin in cardiovascular disorders. Pharmacol. Ther. 111, 909–927 (2006).
22. Pushkin, A. et al. Cloning, tissue distribution, genomic organization, and functional characterization of NBC3, a new member of the sodium bicarbonate cotransporter family. J. Biol. Chem. 274, 16569–16575 (1999).
23. Hinkes, B. et al. Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. Nature Genet. 38, 1397–1405 (2006).
24. Feder, J. N. et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nature Genet. 13, 399–408 (1996).
25. He, L., Wang, B., Hay, E. B. & Nebert, D. W. Discovery of ZIP transporters that participate in cadmium damage to testis and kidney. Toxicol. Appl. Pharmacol. 238, 250–257 (2009).
26. Teslovich, T. M. et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466, 707–713 (2010).
27. Speliotes, E. K. et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nature Genet. 42, 937–948 (2010).

Chapter 2.3

HSD3B1 gene with aldosterone production and blood pressure

Based on

Expression and gene variation studies deny association of human 3ß-hydroxysteroid dehydrogenase type 1 gene (HSD3B1) with aldosterone production or blood pressure.
Submitted American Journal of Hypertension

Abstract

Background

Recent evidence suggests that the type I 3ß-hydroxysteroid dehydrogenase, a steroidogenic enzyme encoded by the HSD3B1 gene, could be involved in aldosterone production and that genetic variation in HSD3B1 is associated with blood pressure. These findings challenge the long-standing hypothesis that all adrenocortical steroidogenesis is executed by the type II iso-enzyme, encoded by HSD3B2.

Methods

Expression of HSD3B1 and HSD3B2 was investigated in various adrenocortical tissues (n=15) and in primary adrenal cell cultures (n=5) following stimulation with adrenocorticotropin and angiotensin II. Six tagging SNPs within the HSD3B1 gene were studied for association with blood pressure and hypertension in a meta-analysis of four Dutch cohorts (n=11,192).

Results

HSD3B1 expression was minimal or absent in adrenocortical tissues, including 6 aldosterone-producing adenomas. In contrast to the ubiquitously expressed HSD3B2 mRNA, HSD3B1 levels were not stimulated by adrenocorticotropin or angiotensin II. No variants in the HSD3B1 gene were associated with blood pressure or the occurrence of hypertension.

Conclusions

We found no evidence to support confirmation that HSD3B1 is involved in aldosterone synthesis in the human adrenal cortex or that genetic variation in HSD3B1 affects blood pressure or hypertension, favoring the hypothesis that all adrenocortical steroidogenesis is primarily dependent on the type II 3ß-hydroxysteroid dehydrogenase.

Introduction

The renin-angiotensin-aldosterone system (RAAS) is an important regulator of blood pressure. Angiotensin II (Ang II) binds to its type 1 receptor in the zona glomerulosa (ZG) of the adrenal cortex, leading to the stimulation of aldosterone production.[1] The mineralocorticoid aldosterone controls blood pressure primarily by increasing sodium reabsorption in the distal convoluted tube and collecting duct of the nephron.[2]

Similar to other steroid hormones, aldosterone is produced through sequential enzymatic reactions by several steroidogenic enzymes.[3] One of the essential conversions for all active steroid hormones is the formation of progesterone from pregnenolone, which is realized by the 3αhydroxysteroid dehydrogenase (3ß-HSD) / △5- △4 isomerase enzymes.

The human genome contains two 3ß-HSD enzymes that share 94% sequence homology: type I (HSD3B1) and type II (HSD3B2).[4] The type II enzyme was considered responsible for all adrenocortical and gonadal steroid production, whereas HSD3B1 was thought to be expressed in the placenta and in peripheral tissues, but not in the adrenal cortex.[5] However, recent developments have indicated that the type I 3ß-HSD might be the enzyme leading to aldosterone formation in the ZG. First, it was shown that increased expression of type VI 3ß-HSD caused hypertension in circadian clock-deficient Cry-null mice through stimulated production of aldosterone in the murine ZG.6 Murine Hsd3b6 was linked through sequence homology to human HSD3B1, the expression of which was subsequently shown to be localized in the human ZG. HSD3B2 expression on the other hand was relatively low in ZG cells.[6] These findings were underpinned by a recent study revealing enriched expression of both HSD3B1 and HSD3B2 in aldosterone-producing adenomas compared to non-tumorous sections and adrenal incidentalomas.[7]

Secondly, common variants and mutations in HSD3B1 have been associated with blood pressure increase, essential hypertension and primary hyperaldosteronism in humans.[7-10] Although these genetic studies were all performed in relatively small groups of hypertensive subjects, these findings suggested that HSD3B1 instead of HSD3B2 is responsible for mineralocorticoid production.

In order to investigate whether HSD3B1 plays an important role in aldosterone production in the human ZG, we studied expression levels of both 3ß-HSD enzymes in human adrenocortical tissues using specific assays designed for the two iso-enzymes as well as genetic associations between HSD3B1 and blood pressure in large study cohorts of Caucasian origin.

Methods

Study design

This study is a combination of data collected in vitro and population based data of four cohort studies, as described below. The in vitro experiments were used for the expression analyses of HSD3B1 and HSD3B2 in various adrenocortical tissues. The population-based data were used for genetic association analyses.

RNA analysis

Patient material

Tissue samples were collected from patients who underwent adrenalectomy at the Erasmus Medical Center, between 1994 and 2009. Samples from normal adrenal glands were obtained from radical nephrectomies due to renal cell carcinoma (n=9). Adrenocortical tumor samples were collected from patients after adrenalectomy because of Conn's syndrome (n=7), Cushing's syndrome (n=2) or suspicion of pheochromocytoma (n=1). For measurement of RNA representative tissue samples were snap-frozen and stored at -80 °C until further processing. For primary culture purposes, adrenal tissues were taken up in DMEM-F12 culture medium containing 5% fetal calf serum (FCS, Invitrogen, Carlsbad, CA, USA).

This study was approved by the Medical Ethical Committee of the Erasmus Medical Center and informed consent was obtained from all patients.

Primary culture

Isolated adrenocortical cells were obtained by treating the tissue samples with type I collagenase (Sigma-Aldrich, St. Louis, MO, USA) and removing debris by centrifugation through a Ficoll gradient.[11] Cell viability and type were checked by microscopical evaluation with trypan blue. Lipid-laden cells were identified as adrenocortical cells and plated at a density of 100.000 cells per well in DMEM-F12 containing 5% FCS and allowed to attach overnight. The next day medium was changed to serum free and 24 hours later cells were incubated with vehicle, 10 ng/mL ACTH1-24 (Novartis, Basel, Switzerland) or 100 nmol/L Ang II (Sigma) using 4 wells per treatment. After 48 hours of incubation the supernatant was removed from the cells and plates were frozen on dry ice and stored at -80°C.

Steroidogenic enzyme measurement

Total RNA was isolated from frozen tumor tissue and plated cells using Trizol reagent (Invitrogen). Subsequently, reverse transcription reactions were performed as previously described.[12] Twenty ng of RNA was used in duplicate in quantitative polymerase chain reaction (qPCR) for HPRT1, HSD3B1, HSD3B2, CYP11B1 and CYP11B2. Primer and dual-labeled probe sequences and qPCR have been reported previously.[13] Assays displayed no cross-reactivity with the homologous DNA sequences in related genes. One probe was used for both 3ß-HSD enzymes, whereas individual primers were manufactured for each gene giving rise to HSD3B1- and HSD3B2-specific PCR products of differing sizes (151 and 274 base pairs, respectively). Positive controls consisted of normal adrenal gland (CYP11B1 and HSD3B2), Conn adenoma (CYP11B2) and placenta (HSD3B1) and yielded threshold cycles (Ct) below 25.

Genetic analysis

Study populations

The Rotterdam Study I (RS-I), Rotterdam Study II (RS-II) and Rotterdam Study III (RS-III) are prospective population-based cohort studies. The RS-I comprises 7,983 subjects aged 55 years or older. Participants completed an interview at home and at the research center, where participants were subsequently examined. Baseline data were collected between 1990 and 1993. In 1999, inhabitants who turned 55 years of age or moved into the study district since the start of the study were invited to participate in an extension of the Rotterdam Study (RS-II), 3,011 participated. In 2006 a further extension of the cohort was initiated in which 3,932 subjects were included (RS-III), aged 45 years and older, living in the Ommoord district. The rationale and design of the RS have been described in detail elsewhere.[14]

The Erasmus Rucphen Family (ERF) Study is a large family-based cohort study, including over 3,000 participants descending from 22 couples living in the Rucphen region, the Netherlands, in the 19th century. The rationale and design of the ERF Study have been described in detail elsewhere.[15,16] All descendants were invited to visit the regional clinical research center where they were examined and a fasting blood sample was drawn. All participants filled out a questionnaire on risk factors. The participants included in these analyses consisted of the first series of participants.

The Medical Ethics Committee of Erasmus Medical Center approved the studies and written consent was obtained from all participants.

Genotyping

All RS participants with available DNA were genotyped using Illumina Infinium II HumanHap BeadChips (RS-I and RS-II) or using Illumina Human 610 Quad array (RS-III) at the Department of Internal Medicine, Erasmus Medical Center following manufacturer's protocols. Participants with call rate < 97.5%, excess autosomal heterozygosity, sex mismatch, or outlying identity-by-state clustering estimates were excluded. After quality control 5,974 RS-I participants, 2,157 RS-II participants and 2,082 RS-III participants were included. Of these, 4,742 RS-I participants, 1,760 RS-II participants and 2,072 RS-III participants had successful blood pressure measurements.

In ERF, all DNA samples were genotyped on four different platforms (Illumina 318K, Illumina 370K and Affymetrix 250K, Illumina610K), which were then merged and imputed to 2.5 million SNPs hapmap using build 36 HapMap (release 22) CEU populations as a reference cohort. After quality control, 2618 participants with genotyping and blood pressure measurements were included for these analyses.

TagSNP selection was based on linkage disequilibrium (r2>0.8) by using the international HapMap Project.[17] (http://www.hapmap.org).

Blood pressure measurements

Two seated blood pressure measurements were obtained of the right brachial artery with a random zero sphygmomanometer for RS-I and RS-II subjects and with an automated device (OMRON M17; OMRON healthcare Inc., Bannockburn, Illinois, USA) for RS-III and ERF subjects. The subject had been seated for at least five minutes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained and the averages of these two measurements were used for analysis. For participants who were taking anti-hypertensive medication we added 10 mmHg to observed SBP values and 5 mmHg to DBP values. Hypertension was defined as SBP≥140 or DBP≥90 mmHg or the use of antihypertensive medication at the time of assessment.

Statistical analyses

The mRNA levels were quantified by calculating expression relative to housekeeping gene HPRT1 using the delta-Ct method. Differences between groups of tissues were analyzed by Kruskal-Wallis test and post-hoc Dunn's multiple Comparison test. Effects of incubations were analyzed after log transformation using Student's t-test with Bonferroni correction. Statistical significance was assumed at P<0.05.

Individual SNP analyses were conducted within each cohort using an additive genetic model. Regression models were fitted for systolic, diastolic blood pressure (separately) and hypertension, in a raw model and a model adjusting for age, age2, sex and body mass index. Within study associations were combined by using an inverse-weighted variance meta-analysis. A threshold of P<0.008 was used to indicate statistical significance for genetic testing to correct for multiple testing with Bonferroni method (0.05/6). GenABEL was used for individual SNP analyses. METAL was used for meta-analyses.

Results

mRNA studies

HSD3B expression in adrenal tissues

Patient tissues were divided into 3 groups: normal whole adrenal glands (n=6), non-aldosterone secreting adenomas (1 non-functional, 2 cortisol-secreting) and aldosterone-secreting adenomas (n=6). Expression of both 3ß-HSD types, HSD3B1 and HSD3B2, as well as the enzymes responsible for the final conversion into cortisol (11ß-hydroxylase) and aldosterone (aldosterone synthase), encoded by CYP11B1 and CYP11B2 respectively, were studied (Figure 1). HSD3B1 expression was positive in 4 normal adrenal glands and one Conn adenoma, although at low levels (Ct range: 34.8-38.4). Moreover, expression of HSD3B1 was not increased in Conn adenomas compared to normal adrenals or non-aldosterone-producing adenomas. HSD3B2 mRNA was positive in all tissues except for one Conn adenoma, which was negative for both 3ß-HSD enzymes. CYP11B1 was highly expressed in all tissues studied, whereas CYP11B2 was most abundantly but not exclusively expressed in the Conn adenomas. Conn adenomas harbored a significantly higher expression of CYP11B2 compared to non-Conn adenomas (P=0.011). HSD3B1 expression levels were not associated with other steroidogenic enzyme levels, age, sex or tumor size.

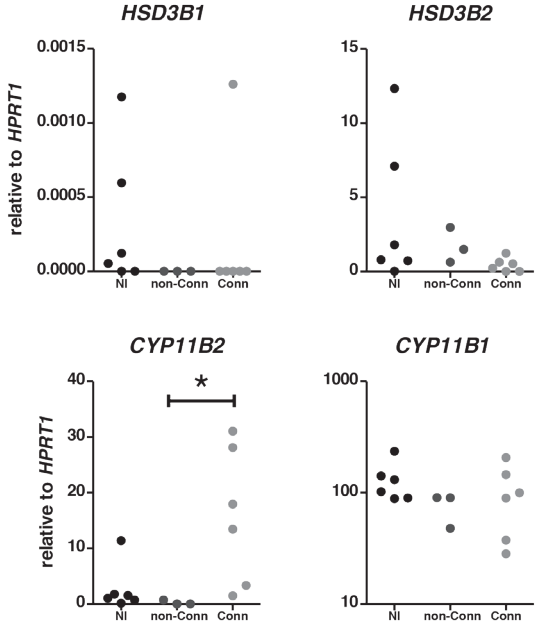


Figure 1: Quantitative analysis of enzymes deemed responsible for aldosterone (HSD3B1 and CYP11B2) and for cortisol production (HSD3B2 and CYP11B1) in normal adrenal glands (NI), non-aldosterone secreting adrenocortical adenomas (non-Conn) or Conn adenomas.

* P<0.05

Primary adrenal cell cultures

Basal expression of HSD3B1 was present in two out of three primary cultures of normal adrenals and in both cultures of Conn adenomas. HSD3B2 expression was positive in all samples studied and more abundant than HSD3B1 expression: 3231±2267-fold (mean±SEM) higher expression in normal adrenals and 79±38-fold higher in Conn adenomas. Incubation with ACTH or Ang II did not increase HSD3B1 expression in normal adrenal glands or Conn adenomas (P>0.05, Figure 2). In contrast, both ACTH and Ang II potently stimulated the expression of the steroidogenic enzymes HSD3B2 (71±43-fold, P=0.043 and 25±10-fold, P=0.020, respectively) and CYP11B1 (39±24-fold, P=0.032 and 6.5±2,1-fold, P=0.027, respectively)in primary cultures of adrenocortical cells (Figure 2). Ang II also potently increased the expression of CYP11B2 in cells derived from the Conn adenomas, although the interindividual responses were highly variable (58±34-fold, P=0.064, Figure 2).

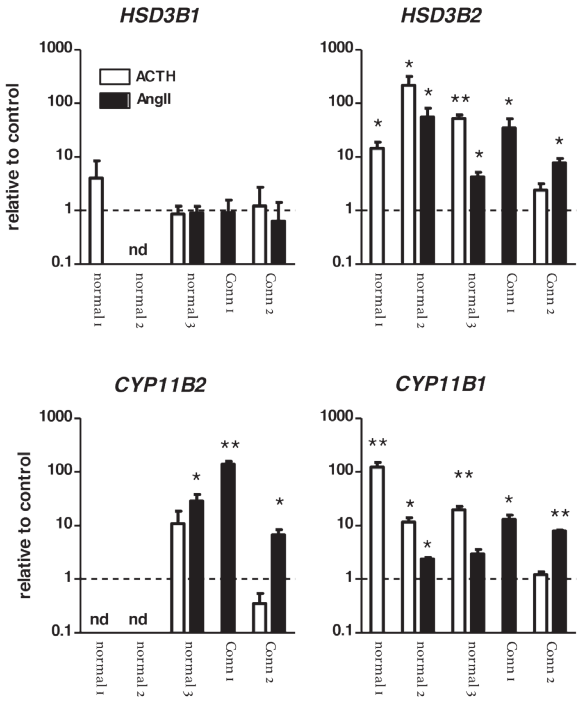


Figure 2: Effects of ACTH (10 ng/mL) and Ang II (100 nmol/L) incubation on steroidogenic enzyme mRNA levels of in primary cultures of three normal adrenal glands and two Conn adenomas. Data presented as mean ± SEM. nd: not detectable. * P<0.05, ** P<0.01, compared to control condition.

Genetic analysis

The total sample size for this analysis was 11,192 (RS-I, n=4,742; RS-II, n=1760; RS-III, n=2,072; ERF, n=2,618). Characteristics of the study sample are presented in Table 1. The mean age of study participants varied from 48.3 years to 67.6 years. For analysis we selected 6 tagSNPs in HSD3B1.

Table 1. Baseline characteristics of the study participants

	RS-I (n=4742)	RS-II (n=1760)	RS-III (n=2072)	ERF (n=2618)
Age, y	67.6	63.9	56.0	48.4
Gender male, %	39.6	44.5	43.8	45.0
Body mass index, kg/m2	26.2	27.2	27.7	26.7
Systolic blood pressure, mmHg	139	143	135	140
Diastolic blood pressure, mmHg	74	79	85	80
Use of antihypertensive medication, %	18.3	21.6	20.7	20
Hypertension, %	53	59.6	47.2	47

n=number

Within cohort analyses were combined by meta-analysis and results for all SNPs are presented in Table 2 for SBP, Table 3 for DBP and Table 4 for hypertension. The T allele of rs4986952 only increased systolic blood pressure in RS-II (effect 6.59 mmHg; SE 3.17; P=0.038), this effect diminished in the meta-analysis (effect 2.27 mmHg; SE 1.31; P=0.083, Table 2) and was also absent after Bonferroni correction. All other SNPs in HSD3B1 were not associated with systolic, diastolic blood pressure or hypertension (Tables 2, 3 and 4).

To confirm this result, we did a lookup in the ICBP project,[18] a large collaboration of GWA studies on blood pressure with publicly available p-values. In this data none of the available SNPs was associated with systolic or diastolic blood pressure, however rs4986952 and rs1047303 were not available due to low frequency (Table 5).

To investigate whether younger subjects would have higher blood pressure with T allele of rs4986952 we performed age interaction analyses. A borderline significant interaction with age was found for SBP (RS-I P=0.036; RS-II P=0.955; RS-III P=0.584; ERF P=0.128; meta-analyses p=0.019), and no interaction was found for DBP (RS-I P=0.010; RS-II P=0.125; RS-III P=0.431; ERF P=0.716; meta-analyses P=0.220).

Table 2. SNPs in the HSD3B1 gene associated with systolic blood pressure

SNPs	Coded allele	Allele frequency	Meta-analysis (RS-I, RS-II, RS-III, ERF)		
			Beta	SE	Pval
Unadjusted					
rs4986952	T	0.037	2.39	1.40	0.087
rs6428829	A	0.297	0.22	0.32	0.490
rs6203	T	0.427	-0.21	0.32	0.513
rs1047303	C	0.002	0.73	0.62	0.240
rs10754400	G	0.345	0.33	0.31	0.296
rs11581942	C	0.014	0.86	1.68	0.611
Adjusted *					
rs4986952	T	0.037	2.27	1.31	0.083
rs6428829	A	0.297	0.15	0.27	0.575
rs6203	T	0.427	-0.06	0.27	0.817
rs1047303	C	0.002	0.16	0.41	0.704
rs10754400	G	0.345	0.17	0.26	0.497
rs11581942	C	0.014	0.67	1.44	0.643

SE= standard error; Pval= p-value; * Adjusted for age, age2, sex and body mass index

Table 3. SNPs in HSD3B1 gene associated with diastolic blood pressure

SNPs	Coded allele	Allele frequency	Meta-analysis (RS-I, RS-II, RS-III, ERF)		
			Beta	SE	Pval
Unadjusted					
rs4986952	T	0.037	-0.50	0.73	0.492
rs6428829	A	0.297	-0.09	0.16	0.598
rs6203	T	0.427	-0.01	0.17	0.945
rs1047303	C	0.002	0.17	0.31	0.590
rs10754400	G	0.345	0.02	0.16	0.880
rs11581942	C	0.014	-0.02	0.87	0.984
Adjusted *					
rs4986952	T	0.037	-0.47	0.71	0.502
rs6428829	A	0.297	-0.07	0.15	0.638
rs6203	T	0.427	0.03	0.15	0.822
rs1047303	C	0.002	0.04	0.23	0.861
rs10754400	G	0.345	0.002	0.14	0.987
rs11581942	C	0.014	-0.10	0.79	0.897

SE= standard error; Pval= p-value; * Adjusted for age, age2, sex and body mass index

Table 4. SNPs in HSD3B1 gene associated with hypertension

SNPs	Coded allele	Allele frequency	Meta-analysis (RS-I, RS-II, RS-III)		
			OR	SE	Pval
Unadjusted					
rs4986952	T	0.037	1.10	0.13	0.476
rs6428829	A	0.297	0.97	0.04	0.336
rs6203	T	0.427	0.99	0.04	0.831
rs1047303	C	0.002	1.33	0.71	0.686
rs10754400	G	0.345	0.95	0.05	0.229
rs11581942	C	0.014	1.01	0.22	0.954
Adjusted *					
rs4986952	T	0.037	1.10	0.14	0.476
rs6428829	A	0.297	0.97	0.04	0.466
rs6203	T	0.427	0.97	0.03	0.467
rs1047303	C	0.002	1.68	0.75	0.488
rs10754400	G	0.345	0.99	0.04	0.740
rs11581942	C	0.014	1.04	0.19	0.835

OR= odds ratio; SE= standard error; Pval= p-value; * Adjusted for age, age2, sex and body mass index

Table 5. SNPS of the HSD3B1 gene in the ICBP project.

SNPs	Available ICBP project	P-value SBP	P-Value DBP
HSD3B1			
rs4986952	No	-	-
rs6428829	Yes	0.247	0.631
rs6203	Yes	0.387	0.921
rs1047303	No	-	-
rs10754400	Yes	0.251	0.931
rs11581942	Yes	0.812	0.462

SNPs= Single nucleotide polymorphisms; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; ICBP= The International Consortium for Blood Pressure Genome Wide Association Studies

- SNPs were not available due to low frequency.

Discussion

In the present study we found no or extremely low expression of HSD3B1 in the human adrenal cortex and no association between genetic variation in the HSD3B1 gene with systolic and diastolic blood pressure or hypertension in three large population-based studies and one family-based study. These findings plead against a significant role of HSD3B1 in the production of aldosterone in the ZG.

Since the discovery of two different 3ß-HSD enzymes that can both convert △5 steroids into △4 steroids,[4,19] it was commonly thought that the type II enzyme was responsible for all adrenocortical and gonadal steroidogenesis. The type I enzyme is mainly present in placenta and peripheral tissues, including liver, mammary gland and skin.[5] This theorem was supported by the discovery of patients with congenital adrenal hyperplasia (CAH) and male pseudohermaphroditism due to 3ß-HSD deficiency. Sequence analysis showed that these patients harbored mutations in HSD3B2, whereas the HSD3B1 gene was not affected.[20,21] This syndrome presents with a salt-wasting phenotype in case of HSD3B2 mutations leading to a complete abrogation of 3ß-HSD activity. Less severe forms are characterized by residual in vitro enzyme activity.[20,22] HSD3B2 thus appears the only 3ß-HSD in the human adrenal gland and gonads responsible for steroidogenesis as 3ß-HSD type 1 does not rescue 3ß-HSD activity in HSD3B2 mutant patients with a salt-wasting phenotype.

Therefore, the findings in a recent study in mice that showed that type VI 3ß-HSD contributes to hypertension in circadian clock-deficient Cry-null mice6 were unexpected. This observation was extrapolated to the presence of HSD3B1 in the ZG in two human adrenocortical tissues through micro-dissection. Moreover, genetic evidence on a possible link between type I 3ß-HSD and aldosterone has been reported, since several studies in hypertensive subjects showed an association between HSD3B1 and hypertension.[23-26]
In the present study, we could not confirm the findings by the two previous reports in a series of adrenocortical tissues, including aldosterone-producing adenomas. Similar to these previous studies[6,7] we used assays[13] that were specific for the two different iso-enzymes to prevent cross-reaction due to the high sequence homology. Human placenta, a tissue characterized by high HSD3B1 levels,[5] showed ample expression of HSD3B1 whereas the HSD3B2 expression was not detectable. In our study, HSD3B1 expression was low to absent in the adrenal cortex and was not regulated by the main tropic hormones that stimulate adrenocortical steroidogenesis, ACTH and Ang II. In contrast, aldosterone synthase (CYP11B2) was enriched in Conn adenomas and potently stimulated by Ang II. HSD3B2 mRNA was ubiquitously expressed, also in Conn adenomas, and was induced by both ACTH and Ang II. Although we did not microdissect our tissue samples, the lack of Ang II effects on HSD3B1 pleads against a pivotal role of this enzyme in the physiology of aldosterone production. The discrepancies between our findings and the report by Wu et al.[7] might be caused by the use of different assays and these prevent definite conclusions with regard to the effect of HSD3B1 in pathological states of the zona glomerulosa, such as in hypertension associated with changes in circadian rhythm[27] or primary hyperaldosteronism.

The previously reported presence of HSD3B1 expression in the human ZG6 of the normal adrenal cortex could also relate to the presence of other adrenocortical cells, such as adrenal stem cells and progenitor cells.[27] Aldosterone-producing cells were recently found to constitute only a small proportion of the ZG,[28] and thus 3ß-HSD type I could be localized in non-aldosterone-producing cells. This hypothesis is consistent with our findings that HSD3B1 is not induced by Ang II. Further determination of the role of type I 3ß-HSD in aldosterone production could be obtained by simultaneous immunostaining of CYP11B2 and HSD3B1 proteins, but due to the high sequence homology there are currently no specific antibodies that adequately distinguish between the two types of 3ß-HSD. This limitation is illustrated in the study by Wu et al. who used a non-specific antibody to 3ß-HSD and found enriched staining in aldosterone-producing adenomas.[7] Alternatively, specific knockdown of the minimal amounts of HSD3B1 in primary adrenocortical cells could provide conclusive proof on the role of this enzyme in aldosterone production.

Twin and family studies previously indicated that a substantial proportion of blood pressure variance is due to the effect of genes, with heritability estimates ranging from 30 to 60%.[29,30] Rosmond et al.[8] were the first who showed an association between HSD3B1 gene, blood pressure and hypertension. The T ➛ C Leu338 variant, rs6203, of HSD3B1 was shown to be associated with increased systolic and diastolic blood pressure in 263 men. In addition, the C allele was significantly more frequent in grade 1 hypertensive subjects (n=39). Shimodaira et al. subsequently demonstrated in 275 essential hypertension patients of Japanese origin that again rs6203 was associated with hypertension and that a second gain-of-stability and function SNP in HSD3B1, rs1047303,27 was also associated with hypertension.[9] Moreover, these two SNPs were associated with higher plasma aldosterone levels. Variants in the HSD3B1 gene were also associated with blood pressure, plasma aldosterone and potassium in a cohort of 729 newly discovered and never treated hypertensive patients.[10] In contrast, Speirs et al[31] published a study with 168 essential hypertensive patients and 312 normotensive controls that did not confirm the results of the previous studies; no association was demonstrated between rs6203 and hypertension. The recent Taiwanese study found an association between SNPs in HSD3B1 (rs6203) and HSD3B2 (rs12410453) and the occurrence of primary hyperaldosteronism. These findings do not reveal which of the iso-enzymes is responsible for aldosterone production in the human adrenal cortex, but might support a possible role of HSD3B1 in the pathophysiology of primary hyperaldosteronism.

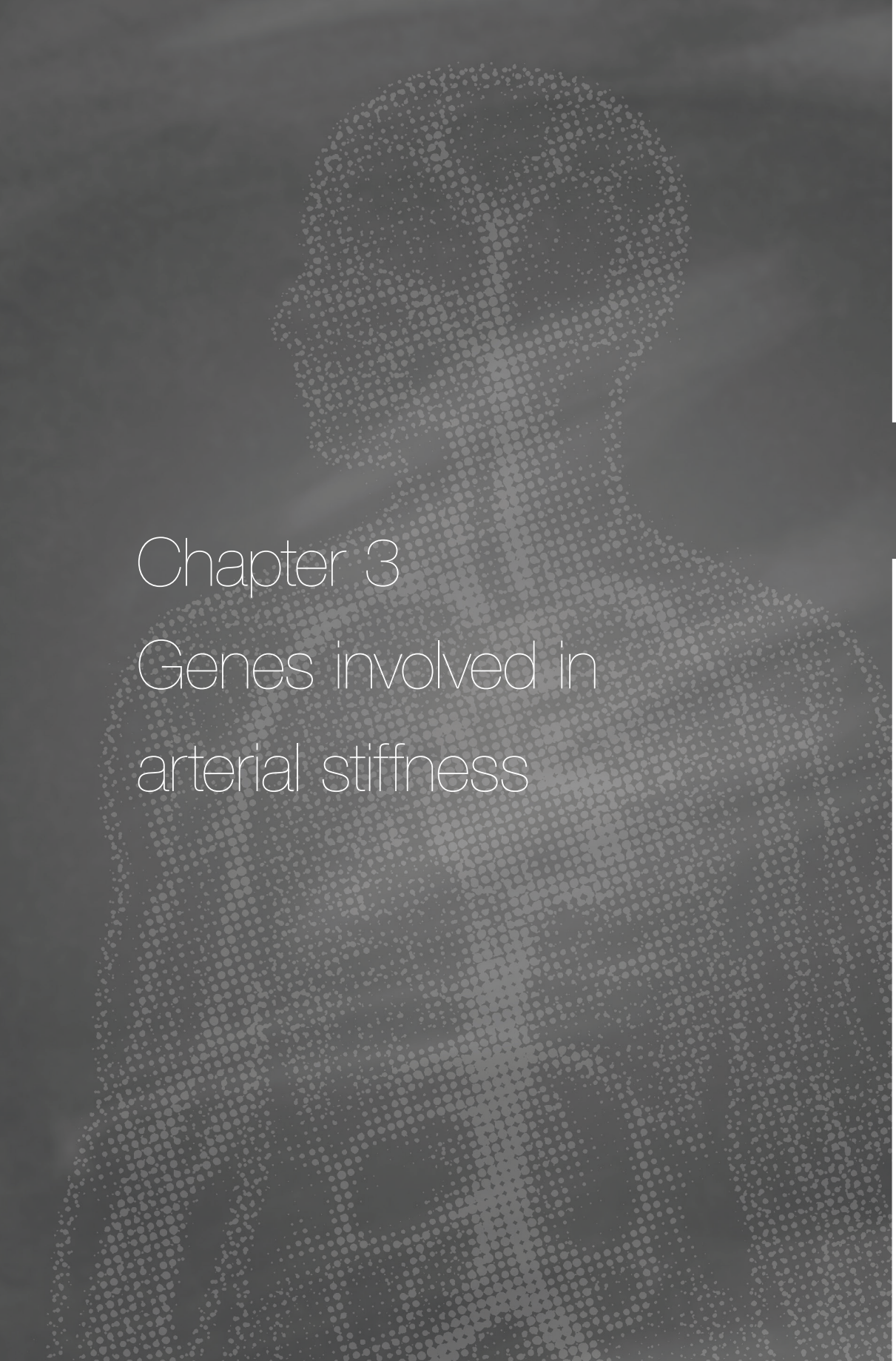
The present study, also including rs6203 and rs1047303, does not support the evidence of association between the HSD3B1 gene and blood pressure. Compared to previous studies that showed an association between HSD3B1 gene and blood pressure, our cohort size was more than ten times larger. On the other hand, the current results are obtained in a generally older, Caucasian population and therefore cannot be automatically extrapolated to other populations, although an older population is prone for hypertension. One SNP from the HSD3B1 gene, rs4986952, was borderline associated with SBP. The ERF study included relatively younger subjects from an isolated population, compared to the Rotterdam study populations. We hypothesized that on younger age there could be an effect of HSD3B1 on blood pressure and we performed therefore age-interaction analyses for rs4986952. No age interaction was found, implying that there is no age effect of HSD3B1 on blood pressure. We unfortunately have no data on aldosterone levels or the occurrence of primary hyperaldosteronism in the subjects of our cohort.

To conclude, through genetic and expression level analyses we found no relation between HSD3B1 and aldosterone production, blood pressure or hypertension. Therefore, it is unlikely that HSD3B1 plays a physiological role in human aldosterone synthesis. Consistent with the phenotype of HSD3B2 mutants, these studies support HSD3B2 as the pivotal enzyme responsible for all adrenocortical steroidogenesis. The role of HSD3B1 in primary hyperaldosteronism requires further investigation.

References

1. Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. Mol. Cell. Endocrinol. Aug 4 2011.
2. Funder JW. Minireview: Aldosterone and mineralocorticoid receptors: past, present, and future. Endocrinology. Nov 2010;151(11):5098-5102.
3. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine reviews. Feb 2011;32(1):81-151.
4. Rheaume E, Lachance Y, Zhao HF, et al. Structure and expression of a new complementary DNA encoding the almost exclusive 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase in human adrenals and gonads. Mol. Endocrinol. Aug 1991;5(8):1147-1157.
5. Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocr. Rev. Jun 2005;26(4):525-582.
6. Doi M, Takahashi Y, Komatsu R, et al. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. Nat. Med. Jan 2010;16(1):67-74.
7. Wu VC, Wu CK, Chang YC, et al. Association of the variations in the HSD3beta gene with primary aldosteronism. J Hypertens. Jul 2013;31(7):1396-1405; discussion 1405.
8. Rosmond R, Chagnon M, Bouchard C, Bjorntorp P. Polymorphism in exon 4 of the human 3 beta-hydroxysteroid dehydrogenase type I gene (HSD3B1) and blood pressure. Biochem. Biophys. Res. Commun. Apr 26 2002;293(1):629-632.
9. Shimodaira M, Nakayama T, Sato N, et al. Association of HSD3B1 and HSD3B2 gene polymorphisms with essential hypertension, aldosterone level, and left ventricular structure. Eur. J. Endocrinol. Oct 2010;163(4):671-680.
10. Tripodi G, Citterio L, Kouznetsova T, et al. Steroid biosynthesis and renal excretion in human essential hypertension: association with blood pressure and endogenous ouabain. Am. J. Hypertens. Apr 2009;22(4):357-363.
11. Lamberts SW, Bons EG, Bruining HA, de Jong FH. Differential effects of the imidazole derivatives etomidate, ketoconazole and miconazole and of metyrapone on the secretion of cortisol and its precursors by human adrenocortical cells. J. Pharmacol. Exp. Ther. Jan 1987;240(1):259-264.
12. Hofland J, Timmerman MA, de Herder WW, van Schaik RH, de Krijger RR, de Jong FH. Expression of activin and inhibin subunits, receptors and binding proteins in human adrenocortical neoplasms. Clin Endocrinol (Oxf). Dec 2006;65(6):792-799.
13. Chai W, Hofland J, Jansen PM, et al. Steroidogenesis vs. steroid uptake in the heart: do corticosteroids mediate effects via cardiac mineralocorticoid receptors? Journal of hypertension. May 2010;28(5):1044-1053.
14. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol. 2009;24(9):553-572.
15. Aulchenko YS, Heutink P, Mackay I, et al. Linkage disequilibrium in young genetically isolated Dutch population. Eur J Hum Genet. Jul 2004;12(7):527-534.

16. Pardo LM, MacKay I, Oostra B, van Duijn CM, Aulchenko YS. The effect of genetic drift in a young genetically isolated population. Annals of human genetics. May 2005;69(Pt 3):288-295.
17. Pettersson FH, Anderson CA, Clarke GM, et al. Marker selection for genetic case-control association studies. Nature protocols. 2009;4(5):743-752.
18. Ehret GB, Munroe PB, Rice KM, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. Oct 6 2011;478(7367):103-109.
19. Luu The V, Lachance Y, Labrie C, et al. Full length cDNA structure and deduced amino acid sequence of human 3 beta-hydroxy-5-ene steroid dehydrogenase. Mol. Endocrinol. Aug 1989;3(8):1310-1312.
20. Moisan AM, Ricketts ML, Tardy V, et al. New insight into the molecular basis of 3beta-hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. J. Clin. Endocrinol. Metab. Dec 1999;84(12):4410-4425.
21. Rheaume E, Simard J, Morel Y, et al. Congenital adrenal hyperplasia due to point mutations in the type II 3 beta-hydroxysteroid dehydrogenase gene. Nat. Genet. Jul 1992;1(4):239-245.
22. Simard J, Rheaume E, Mebarki F, et al. Molecular basis of human 3 beta-hydroxysteroid dehydrogenase deficiency. J. Steroid Biochem. Mol. Biol. Jun 1995;53(1-6):127-138.
23. Rosmond R, Chagnon M, Bouchard C, Bjorntorp P. Polymorphism in exon 4 of the human 3 beta-hydroxysteroid dehydrogenase type I gene (HSD3B1) and blood pressure. Biochemical and biophysical research communications. Apr 26 2002;293(1):629-632.
24. Shimodaira M, Nakayama T, Sato N, et al. Association of HSD3B1 and HSD3B2 gene polymorphisms with essential hypertension, aldosterone level, and left ventricular structure. European journal of endocrinology / European Federation of Endocrine Societies. Oct;163(4):671-680.
25. Speirs HJ, Katyk K, Kumar NN, Benjafield AV, Wang WY, Morris BJ. Association of G-protein-coupled receptor kinase 4 haplotypes, but not HSD3B1 or PTP1B polymorphisms, with essential hypertension. Journal of hypertension. May 2004;22(5):931-936.
26. Tripodi G, Citterio L, Kouznetsova T, et al. Steroid biosynthesis and renal excretion in human essential hypertension: association with blood pressure and endogenous ouabain. Am J Hypertens. Apr 2009;22(4):357-363.
27. Kim AC, Barlaskar FM, Heaton JH, et al. In search of adrenocortical stem and progenitor cells. Endocr. Rev. May 2009;30(3):241-263.
28. Nishimoto K, Nakagawa K, Li D, et al. Adrenocortical zonation in humans under normal and pathological conditions. J. Clin. Endocrinol. Metab. May 2010;95(5):2296-2305.
29. Snieder H, Harshfield GA, Treiber FA. Heritability of blood pressure and hemodynamics in African- and European-American youth. Hypertension. Jun 2003;41(6):1196-1201.
30. van Rijn MJ, Schut AF, Aulchenko YS, et al. Heritability of blood pressure traits and the genetic contribution to blood pressure variance explained by four blood-pressure-related genes. J. Hypertens. Mar 2007;25(3):565-570.
31. Speirs HJ, Katyk K, Kumar NN, Benjafield AV, Wang WY, Morris BJ. Association of G-protein-coupled receptor kinase 4 haplotypes, but not HSD3B1 or PTP1B polymorphisms, with essential hypertension. J. Hypertens. May 2004;22(5):931-936.



Chapter 3
Genes involved in arterial stiffness

Chapter 3.1
Genome-wide association study of pulse pressure and mean arterial pressure: ICBP

Based on

Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet. 2011 Sep 11;43(10):1005-11.

Abstract

Background

Numerous genetic loci have been associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) in Europeans. We undertook a genome-wide association study of two further blood pressure phenotypes, pulse pressure (PP) and mean arterial pressure (MAP).

Methods

We conducted a meta-analysis of genome-wide association study of PP and MAP in the setting of the International Consortium of Blood Pressure Genome-Wide Association Studies (ICBP-GWAS). Discovery analyses were performed in 76,064 subjects of 35 studies from European ancestry. Independent follow-up analysis were performed in 48,607 subjects from European ancestry.

Results

We identified at genome-wide significance (P= 2.7x10-8 to P=2.3x10-13) four novel PP loci (at 4q12 near CHIC2/PDGFRAI, 7q22.3 near PIK3CG, 8q24.12 in NOV, 11q24.3 near ADAMTS-8), two novel MAP loci (3p21.31 in MAP4, 10q25.3 near ADRB1) and one novel locus associated with both traits (2q24.3 near FIGN). For three of the novel PP signals, the estimated effect for SBP was opposite to that for DBP, in contrast to the majority of common SBP- and DBP-associated variants which show concordant effects on both traits.

Conclusions

These findings indicate novel genetic mechanisms underlying blood pressure variation, including pathways that may differentially influence SBP and DBP.

Introduction

High blood pressure is a major risk factor for coronary heart disease and stroke[1]. Large genome-wide association studies in Europeans have reported 29 novel loci for systolic and diastolic blood pressure (SBP and DBP) where alleles have effect sizes of up to 0.5-1mm Hg[2-4]. Even small increments in blood pressure levels have important effects on cardiovascular morbidity and mortality at the population level[5]. We undertook a genome-wide association study of two further blood pressure phenotypes, pulse pressure (PP, the difference between SBP and DBP), a measure of stiffness of the main arteries, and mean arterial pressure (MAP), a weighted average of SBP and DBP. Both PP and MAP are predictive of hypertension[6] and cardiovascular disease[7-11].

Methods

This study was undertaken by the International Consortium of Blood Pressure Genome-Wide Association Studies (ICBP-GWAS), which aims to further the understanding of the genetic architecture underlying blood pressure.

We first conducted a genome-wide association meta-analysis of PP and MAP in 74,064 individuals of European ancestry from 35 studies. Genotypes were imputed using HapMap. To account for effects of anti-hypertensive treatments, we imputed underlying SBP and DBP by adding a constant to each[3,4]. Associations were adjusted for age, age2, sex and body mass index. We combined results across studies using an inverse variance weighted meta-analysis and, to correct for residual test statistic inflation, applied genomic control (GC) both to study-level association statistics and to the meta-analysis (λ_{GC}=1.08 for PP, λ_{GC}=1.12 for MAP)[12]. Independent follow-up analyses were performed in 48,607 individuals of European ancestry.

Results

SNPs in 12 regions showed genome-wide significant association (P<5x10-8) with either PP or MAP in our discovery data (Stage 1), including two novel regions for PP (7q22.3 near PIK3CG, P=1.2x10-10 and 11q24.3 near ADAMTS8, P=8.5x10-11; Table 1) and 10 regions previously associated with SBP and DBP2-4. For follow-up in a series of independent cohorts we selected 99 SNPs comprising those with P<1x10-5 for either PP or MAP and SNPs reported in recent large genome-wide association studies of SBP and DBP2-4 to evaluate their effects on PP and MAP (Stage 2).

After meta-analysis of the Stage 1 and Stage 2 data, the two novel regions showing genome-wide association with PP after Stage 1 (near PIK3CG and near ADAMTS8) remained genome-wide significant. In addition, we found genome-wide significant associations for SNPs at two further novel loci for PP (at 4q12 near CHIC2/PDGFRA and 8q24.12 in NOV), two novel loci for MAP (3p21.31 in MAP4, 10q25.3 near ADRB1), and one novel locus for both traits (2q24.3 near FIGN) (Table 1). The novel signals for MAP were strongly associated with both SBP and DBP (P=7.7x10-7 to P=1.8x10-12), reflecting the high inter-correlations among these three blood pressure traits[10,13]. For the sentinel SNPs in three of the novel PP loci, the estimated effects on SBP were in the opposite direction to the effects on DBP (Table 1, Figure 1). Our findings show that analyses of PP and MAP reveal loci influencing blood pressure phenotypes which may not always be detectable by studying SBP and DBP separately.

Five additional loci for PP and 19 loci for MAP reaching genome-wide significance (P<5x10-8, Stage 1 and Stage 2 combined) were recently shown to be associated with SBP/DBP2-4. We used sentinel SNPs from both the novel and known regions showing genome-wide significant associations with PP or MAP in the combined Stage 1 and 2 data to create weighted risk scores for: i) PP (10 independent SNPs) and; ii) MAP (22 SNPs). We studied the associations of both risk scores with hypertension and blood pressure related outcomes including coronary heart disease, heart failure, stroke, echocardiographic measures of left ventricular structure, pulse wave velocity, renal function and renal failure. Adjusting for multiple testing for the 12 traits evaluated (P=0.05/12=4.1x10-3), the PP SNP risk score was associated with prevalent hypertension (P=7.9x10-6), incident stroke (P=4.9x10-4) and coronary heart disease (P=4.3x10-4), and the MAP SNP risk score was associated with hypertension (P=5.1x10-16), coronary heart disease (P=4.0 x10-20), stroke (P=0.0019) and left ventricular wall thickness (P=2.1x10-4), highlighting the clinical relevance of these alternative measures of blood pressure phenotype[9,11].

Table 1. Top genome-wide association results for PP and MAP

Locus	Coded allele & freq	Stage 1			Stage 2		
		N eff	Beta (Se)	P	N eff	Beta (Se)	P
Pulse Pressure							
rs13002573 near FIGN	G 0.203	73043	-0.320 (0.07)	5.43x10-6	43955	-0.296 (0.089)	8.58x10-4
rs871606 near CHIC2	T 0.85	71444	0.428 (0.096)	9.28x10-06	44082	0.431 (0.121)	3.75x10-4
rs17477177 near PIK3CG	T 0.717	72997	-0.460 (0.071)	1.19x10-10	39999	-0.344 (0.094)	2.72x10-4
rs2071518 NOV (3' UTR)	T 0.167	73252	0.304 (0.067)	5.72x10-6	45804	0.323 (0.086)	1.60x10-4
rs11222084 near ADAMTS-8	T 0.375	67704	0.415 (0.064)	8.45x10-11	40391	0.211 (0.081)	9.17x10-3
Mean Arterial Pressure							
rs1446468 near FIGN	T 0.534	69264	-0.291 (0.061)	1.68x10-6	39650	-0.418 (0.082)	3.80x10-7
rs319690 MAP4 (intron)	T 0.51	59137	0.306 (0.066)	3.88x10-6	34359	0.280 (0.09)	1.89x10-3
rs2782980 near ADRB1	T 0.198	61284	-0.345 (0.071)	1.14x10-6	37788	-0.326 (0.094)	5.55x10-4

Table 1 – Summary of Pulse Pressure (PP) and Mean Arterial Pressure (MAP) association results from Stages 1 and 2 and the combined analysis for all SNPs that showed genome-wide significant (P<5x10-8) association with PP and/or MAP on combined analysis and which had not previously been reported for Systolic (SBP) or Diastolic Blood Pressure (DBP). SBP and DBP combined Stage 1 and Stage 2 association results, based on the same sample set as for PP and MAP are also shown.

Stage 1+ 2			SBP Stage 1+2		DBP Stage 1+2	
N eff	**Beta (Se)**	**P**	**Beta (Se)**	**P**	**Beta (Se)**	**P**
116998	-0.310 (0.055)	1.76x10-8	-0.416 (0.081)	3.25x10-7	-0.107 (0.052)	4.02x20-2
115525	0.429 (0.075)	1.32x10-8	0.403 (0.112)	3.04x10-4	-0.010 (0.072)	8.85x10-1
112996	-0.418 (0.057)	2.27x10-13	-0.552 (0.084)	5.67x10-11	-0.081 (0.055)	1.40x10-1
119056	0.312 (0.053)	3.66x10-9	0.181 (0.078)	2.08x10-2	-0.145 (0.050)	3.89x10-3
108095	0.337 (0.05)	1.90x10-11	0.263 (.074)	4.00x10-4	-0.101 (0.048)	3.44x10-2
108914	-0.336 (0.049)	6.46x10-12	-0.499 (0.071)	1.82x10-12	-0.265 (0.046)	6.88x10-9
93496	0.297 (0.053)	2.69x10-8	0.423 (0.077)	4.74x10-8	0.282 (0.05)	1.84x10-8
99072	-0.338 (0.057)	2.46x10-9	-0.406 (0.082)	7.66x10-7	-0.283 (0.053)	9.60x10-8

Discussion

None of the genes in the identified novel regions is a strong candidate for blood pressure regulation, although several are implicated in mechanisms that may influence blood pressure. The most significant association with PP is within a putative mRNA clone (AF086203) spanning ~13.7kb at 7q22.3, 94kb upstream of PIK3CG (rs17477177, P=2.3x10-13, Table 1). PIK3CG encodes the phosphoinositide-3-kinase, catalytic, gamma polypeptide protein which phosphorylates phosphoinositides and modulates extracellular signals. This region was earlier associated with mean platelet volume, platelet count, and platelet aggregation[14-16], but the sentinel SNPs reported in those studies are independent of SNP rs17477177 reported here (r2<0.01). Mice lacking the catalytic subunit of PI3Kgamma have shown resistance to SBP-lowering effects of beta-adrenergic receptor agonists[17]; PI3Kgamma activity is increased in the failing human heart and associated with down-regulation of beta-adrenergic receptors in the plasma membrane[18]. The second locus for PP located at 11q24.3 spans 35.5kb with the top-ranking SNP (rs11222084, P=1.9x10-11) lying 1.6kb downstream of ADAMTS-8. This gene is highly expressed in macrophage-rich areas of human atherosclerotic plaques and may affect extracellular matrix remodeling[19]. The third locus for PP spans 28.5kb at 8q24.12 with the sentinel SNP (rs2071518, P=3.7x10-9) located in the 3'UTR of NOV which encodes the nephroblastoma overexpressed (CCN3) protein, associated with angiogenesis, proliferation, and inhibition of vascular smooth muscle cell growth and migration[20], and with reduced neointimal thickening in mice null for CCN3[21]. Mice with mutations in NOV that truncate the NOV protein exhibit abnormal cardiac development[22]. Of the genes evaluated for expression in human aortic samples at the novel PP loci, NOV showed by far the highest expression levels. The fourth locus for PP is 4q12 with the top-ranking SNP (rs871606, P=1.3x10-8) located 76.7kb downstream of CHIC2 which encodes a cysteine-rich hydrophobic domain containing protein associated with acute myeloid leukaemia[23]. This SNP is located 296kb upstream of PDGFRA which encodes platelet-derived growth factor receptor alpha, a cell surface receptor for members of the platelet-derived growth factor family involved in kidney development. Variants in PDGFRA have been associated with red blood cell count and other haematological indices[24] but are independent (r2<0.3) of rs871606.

For MAP we identified two novel loci. The first locus for MAP is at 10q25.3, 22.3kb upstream of ADRB1 (rs2782980, P=2.5x10-9). ADRB1 encodes the beta-1-adrenergic receptor, which mediates the effects of the stimulatory G protein and cAMP/protein kinase A pathway to increase heart rate and myocardial contraction. Polymorphisms in this gene have been associated with resting heart rate, response to beta-blockers[25], and hypertension[26]. ADRB1 knockout mice have no difference in heart rate or blood pressure compared with the wild type but do exhibit a significant reduction in the response of both phenotypes to catecholamines[27]. SNP rs2782980 is associated with expression of an ADRB1 transcript in brain tissue.The second locus for MAP spans over 300kb at 3p21.31 with the top-ranking SNP (rs319690, P=2.7x10-8) lying within an intron of the microtubule associated protein 4 gene, MAP4. Coating of microtubules by MAP4 may inhibit beta adrenergic receptor recycling and number, as seen in cardiac hypertrophy and failure[28]. MAP4 was detectably expressed in human aortic samples.

The locus associated both with PP (SNP rs13002573, P=1.8x10-8) and MAP (rs1446468, P= 6.5x10-12) is in an intergenic region spanning ~280kb at 2q24.3, although the two signals are ~50kb apart and statistically independent (r2=0.075). The top PP SNP lies ~320kb upstream of FIGN and ~430kb downstream of GRB14 (growth factor receptor-bound protein 14). Relatively little is known regarding FIGN (fidgetin).

We report seven novel loci associated with PP and MAP based on genome-wide discovery and follow-up among a total of ~125,000 individuals. Our results expand knowledge of the genetic architecture of blood pressure and PP regulation and may give clues as to possible novel targets for blood pressure therapies.

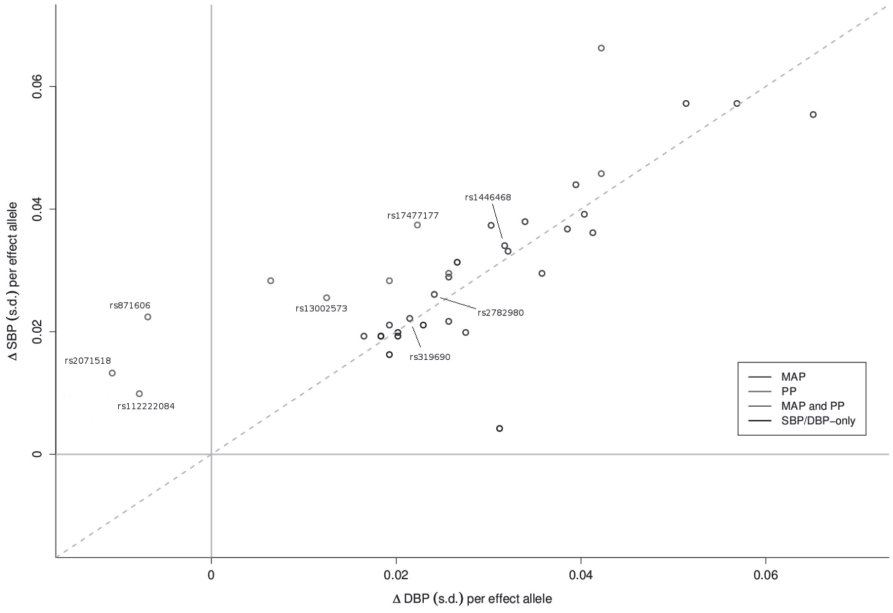


Figure 1 - Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) effect sizes (beta coefficients) for all BP SNPs identified in the present study, obtained from follow-up samples only. Beta coefficients are shown as standard deviation (s.d.) differences so that SBP and DBP are measured on comparable scales. The novel SNPs found in the present study are labelled with their rs-numbers. For illustration purposes the effect allele for each SNP is defined such that the direction of the SBP effect is always positive.

References

1. Lawes CM, Vander Hoorn S, Law MR, Elliott P, MacMahon S, Rodgers A. Blood pressure and the global burden of disease 2000. Part II: estimates of attributable burden. J Hypertens. Mar 2006;24(3):423-430.
2. ICBP. Identification of multiple novel loci influencing blood pressure and cardiovascular disease. 2010.
3. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. Nature genetics. May 10 2009.
4. Newton-Cheh C, Johnson T, Gateva V, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nature genetics. May 10 2009.
5. Rose G. Strategies of prevention: the individual and the population. In: Marmot M EP, ed. Coronary heart disease epidemiology: From aetiology to Public health. 2nd ed. Oxford: Oxford University Press; 2005:631-641.
6. Domanski MJ, Mitchell GF, Norman JE, Exner DV, Pitt B, Pfeffer MA. Independent prognostic information provided by sphygmomanometrically determined pulse pressure and mean arterial pressure in patients with left ventricular dysfunction. J Am Coll Cardiol. Mar 15 1999;33(4):951-958.
7. Antikainen RL, Jousilahti P, Vanhanen H, Tuomilehto J. Excess mortality associated with increased pulse pressure among middle-aged men and women is explained by high systolic blood pressure. J Hypertens. Apr 2000;18(4):417-423.
8. Domanski M, Mitchell G, Pfeffer M, et al. Pulse pressure and cardiovascular disease-related mortality: follow-up study of the Multiple Risk Factor Intervention Trial (MRFIT). Jama. May 22-29 2002;287(20):2677-2683.
9. Franklin SS, Lopez VA, Wong ND, et al. Single versus combined blood pressure components and risk for cardiovascular disease: the Framingham Heart Study. Circulation. Jan 20 2009;119(2):243-250.
10. Sesso HD, Stampfer MJ, Rosner B, et al. Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in Men. Hypertension. Nov 2000;36(5):801-807.
11. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. Dec 14 2002;360(9349):1903-1913.
12. Devlin B, Roeder K. Genomic control for association studies. Biometrics. Dec 1999;55(4):997-1004.
13. Darne B, Girerd X, Safar M, Cambien F, Guize L. Pulsatile versus steady component of blood pressure: a cross-sectional analysis and a prospective analysis on cardiovascular mortality. Hypertension. Apr 1989;13(4):392-400.
14. Johnson AD, Yanek LR, Chen MH, et al. Genome-wide meta-analyses identifies seven loci associated with platelet aggregation in response to agonists. Nat Genet. Jul 2010;42(7):608-613.

15. Soranzo N, Rendon A, Gieger C, et al. A novel variant on chromosome 7q22.3 associated with mean platelet volume, counts, and function. Blood. Apr 16 2009;113(16):3831-3837.
16. Soranzo N, Spector TD, Mangino M, et al. A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. Nat Genet. Nov 2009;41(11):1182-1190.
17. Oudit GY, Crackower MA, Eriksson U, et al. Phosphoinositide 3-kinase gamma-deficient mice are protected from isoproterenol-induced heart failure. Circulation. Oct 28 2003;108(17):2147-2152.
18. Perrino C, Schroder JN, Lima B, et al. Dynamic regulation of phosphoinositide 3-kinase-gamma activity and beta-adrenergic receptor trafficking in end-stage human heart failure. Circulation. Nov 27 2007;116(22):2571-2579.
19. Wagsater D, Bjork H, Zhu C, et al. ADAMTS-4 and -8 are inflammatory regulated enzymes expressed in macrophage-rich areas of human atherosclerotic plaques. Atherosclerosis. Feb 2008;196(2):514-522.
20. Ellis PD, Chen Q, Barker PJ, Metcalfe JC, Kemp PR. Nov gene encodes adhesion factor for vascular smooth muscle cells and is dynamically regulated in response to vascular injury. Arterioscler Thromb Vasc Biol. Aug 2000;20(8):1912-1919.
21. Shimoyama T, Hiraoka S, Takemoto M, et al. CCN3 inhibits neointimal hyperplasia through modulation of smooth muscle cell growth and migration. Arterioscler Thromb Vasc Biol. Apr 2010;30(4):675-682.
22. Heath E, Tahri D, Andermarcher E, Schofield P, Fleming S, Boulter CA. Abnormal skeletal and cardiac development, cardiomyopathy, muscle atrophy and cataracts in mice with a targeted disruption of the Nov (Ccn3) gene. BMC Dev Biol. 2008;8(18):18.
23. Cools J, Bilhou-Nabera C, Wlodarska I, et al. Fusion of a novel gene, BTL, to ETV6 in acute myeloid leukemias with a t(4;12)(q11-q12;p13). Blood. Sep 1 1999;94(5):1820-1824.
24. Kamatani Y, Matsuda K, Okada Y, et al. Genome-wide association study of hematological and biochemical traits in a Japanese population. Nat Genet. Mar 2010;42(3):210-215.
25. Dorn GW, 2nd. Adrenergic signaling polymorphisms and their impact on cardiovascular disease. Physiol Rev. Jul 2010;90(3):1013-1062.
26. Kitsios GD, Zintzaras E. Synopsis and data synthesis of genetic association studies in hypertension for the adrenergic receptor family genes: the CUMAGAS-HYPERT database. Am J Hypertens. Mar 2010;23(3):305-313.
27. Rohrer DK, Chruscinski A, Schauble EH, Bernstein D, Kobilka BK. Cardiovascular and metabolic alterations in mice lacking both beta1- and beta2-adrenergic receptors. J Biol Chem. Jun 11 1999;274(24):16701-16708.
28. Cheng G, Qiao F, Gallien TN, Kuppuswamy D, Cooper Gt. Inhibition of beta-adrenergic receptor trafficking in adult cardiocytes by MAP4 decoration of microtubules. Am J Physiol Heart Circ Physiol. Mar 2005;288(3):H1193-1202.

Chapter 3.2
Genome-wide association study of arterial stiffness: the AortaGen consortium

Based on

Common genetic variation in the remote 3'BCL11B enhancer is associated with carotid-femoral pulse-wave velocity in a meta-analysis of 11 community based cohorts: the AortaGen Consortium. Circ Cardiovasc Genet. 2012 Feb 1;5(1):81-90.

Abstract

Background

Carotid-femoral pulse wave velocity (CFPWV) is a heritable, direct measure of aortic stiffness that is strongly associated with increased risk for major cardiovascular disease events.

Methods

We conducted a meta-analysis of genome-wide association data in 9 community-based European ancestry cohorts consisting of 20,634 participants. Results were replicated in 2 additional European ancestry cohorts involving 5,306 participants.

Results

We identified a locus on chromosome 14 in the conserved core of the BCL11B gene enhancer that is associated with CFPWV (rs7152623, minor allele frequency = 0.42, P = 7.4 x 10-11; replication P = 1.4 x 10-6; meta-analysis P = 3.1 x 10-15). The association persisted when adjusted for mean arterial pressure (P = 1.0 x 10-11). Results were consistent in younger (<55 years, 6 cohorts, N=13,914, P = 2.3 x 10-9) and older (9 cohorts, N=12,026, P = 9.4 x 10-6) participants. In separate meta-analyses, the locus was associated with increased risk for coronary artery disease (hazard ratio [HR]=1.05, confidence interval [CI]=1.02 to 1.08, P=0.0013) and heart failure (HR=1.10, CI=1.03 to 1.16, P=0.004).

Conclusions

Common genetic variation in the BCL11B gene enhancer is associated with higher CFPWV and increased risk for cardiovascular disease. BCL11B codes for a transcription factor interacting protein and transcriptional repressor that modulates several pathways relevant to aortic function, including cardiovascular development, T-cell differentiation, cellular senescence, and matrix protein expression. Elucidation of the role this novel locus plays in aortic stiffness may facilitate development of therapeutic interventions that limit aortic stiffening and related cardiovascular disease events.

Introduction

Several recent studies have demonstrated that carotid-femoral pulse wave velocity (CFPWV), a direct measure of stiffness of the wall of the thoracic and abdominal aorta, is associated with increased risk for major cardiovascular disease (CVD) events in high risk[1-4] and community-based samples[5-8]. Various risk factors for abnormal CFPWV have been identified, including standard CVD risk factors such as age, glucose intolerance, lipid disorders, and hypertension[9]. In addition, CFPWV is a moderately heritable trait,[10;11] although molecular mechanisms contributing to aortic stiffness remain largely undefined. In light of evidence of a genetic component of aortic stiffness, we performed a meta-analysis of genome-wide association study (GWAS) data from 9 community based cohorts, with replication genotyping in 2 additional cohorts, in order to evaluate associations of common genetic variation with CFPWV. In addition, we hypothesized that in light of the association between CFPWV and CVD risk, genetic variants that affect CFPWV should have a proportional effect on CVD risk. Therefore, we interrogated existing clinical endpoint GWAS data to determine whether variants associated with CFPWV were associated with CVD risk.

Methods

Consortium Organization

The AortaGen Consortium includes 9 cohort studies that completed genome-wide genotyping and had measured CFPWV, plus 2 cohort studies that had measured CFPWV and collected DNA for replication genotyping. Each study adopted collaboration guidelines and the consortium established a consensus on phenotype harmonization, covariate selection, and an analytical plan for within-study genome-wide association and prospective meta-analysis of results across studies. Each study received institutional review board approval of its consent procedures, examination and surveillance components, data security measures, and DNA collection and its use for genetic research. All participants in each study gave written informed consent for participation in the study and the conduct of genetic research. (Table 1)

Genotyping and Imputation

For genome-wide SNP sets, genotyping was carried out using commercially available arrays. Prior to imputation, quality control measures were applied as outlined in Supplementary Table S1. MACH was used by all cohorts for imputation of genotypes to the HapMap set of approximately 2.5 million SNPs.

Expression Methods

Human Tissues and Cell Lines

Commercially available cultured human aortic smooth muscle cells, adult human cardiac fibroblasts and human umbilical vein endothelial cells (HUVEC) were purchased and cultured according to the protocol recommended by the manufacturer (Cell Application Inc); CD3+ enriched cells from a healthy donor were provided by Dr. P. Olkhanud (NIA, Baltimore, USA).

Aortic Tissue Collection and Conservation

The aortic tissue samples were obtained from cadaveric donors or beating heart donors through transplant coordinators from the Addenbrooke's Hospital, Cambridge. Fresh thoracic and abdominal aorta removed by the surgical team at the time of organ donation was immediately placed in tissue medium and transported to the Addenbrooke's Hospital, where it was processed immediately. Each specimen was trimmed free of blood vessels, fat and any surrounding deposits. A sample of tissue from the ascending aortic rings was chopped into small pieces and preserved overnight at 4°C in a tube containing RNAlater solution. Solution was removed the following day and sample stored at -80°C for RNA extraction. All samples and patient data were handled in accordance with the policies and procedures of the Human Tissue Act, and the study was approved by the Local and Regional Ethics Committees. Informed consent was also obtained from the relatives.

RNA Extraction, cDNA Preparation, PCR Amplification and Sequencing

Total RNA was extracted using RNeasy Mini Kit (Qiagen Inc) with an additional on-column DNAse digestion step, according to the protocol recommended by the manufacturer. Commercially available total RNA samples extracted from human heart, human skeletal muscles, human kidney and human brain were obtained from Cell Application Inc. For cDNA synthesis, 2 μg of total RNA were used with the cDNA Archive Kit (Applied Biosystems Inc) using oligo (dT) primers in 25 μg of final volume. A control sample lacking reverse transcriptase was processed along with each cDNA synthesis in order to detect genomic DNA contamination. For subsequent PCR reactions, 1 μl of cDNA mixture was used together with Platinum Taq-Polymerase (Invitrogen Inc) or KOD-polymerase (Novagen Inc) in a final volume of 25 μl. Primers were designed with Vector NTI 11.0 software. Amplification products of appropriate size were excised from agarose gel and purified with QIAquick Gel Extraction Kit (Qiagen Inc); recovered DNA fragments were cloned with TOPO TA Cloning® Kit (Invitrogen Inc). Three independent clones for each sample were selected for follow-up sequencing to avoid possible reading errors. We used BigDye® Terminator v1.1 kit (Applied Biosystems Inc) for sequencing reaction and samples were analyzed on a 3130xl Genetic Analyzer (Applied Biosystems Inc).

Total RNA was extracted from human aortic tissue using the TRIzol® Plus RNA Purification System (Invitrogen). Extraction of RNA was conducted according to the manufacturer's protocol and was further purified using PureLink™ silica-gel spin columns followed by DNase I digestion to minimize genomic DNA contamination (Invitrogen, PureLink™ RNA Mini Kit). First strand cDNA synthesis was performed on 1 μg total RNA using AMV reverse transcriptase according to manufacturer instructions (Reverse Transcription System, Promega). Reverse transcription was initiated using random hexamer primers and the reaction carried out at 42°C for 60 min, followed by heat inactivation at 95°C for 5 min. PCR primers were designed to target BP432414, DB129663, BCL11B and VRK1 (Supplementary Table S2). A 5 μl aliquot of cDNA was used as template DNA in a 25 μl PCR reaction. Each reaction contained 5 pmol of each primer, 0.1 mM dNTPs, 1 U AGSGold™ DNA polymerase, 2.5 mM MgCl2, 75 mmol/L Tris-HCL (pH 9.0), 20 mM (NH4)2SO2, and 0.01% TWEEN-20. The PCR protocol consisted of 10 min at 95°C, followed by a touchdown

procedure of 15 cycles of 95°C for 15 s, 68°C for 15 s, and 72°C for 15 s, decreasing annealing by 1°C per cycle. Following the initial 15 cycles the method consisted of 30 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 15 s, with a final extension of 72°C for 10 min. To verify that the amplified product was the targeted gene, the product from one sample was sequenced at Geneservice (www.geneservice.co.uk) using Sanger sequencing followed by analysis on Applied Biosystems 3730 DNA Analyzer.

Statistical Analyses

The phenotype for meta-analysis was a sex-specific (in Framingham, cohort- and sex-specific) standardized regression residual for 1000/CFPWV, adjusted for age, age2, height and weight. Genome-wide association analyses were conducted within each cohort using an additive gene-dose model. Linear mixed effects models were fitted to account for relatedness in pedigrees. Within-study associations were combined by prospective meta-analysis using inverse-variance weighting. During meta-analysis, results were filtered for weighted mean minor allele frequency <0.01 and the genomic control parameter was calculated to adjust each study. After meta-analysis, the genomic control parameter was recalculated to adjust for among-study heterogeneity. For the initial meta-analysis, a pre-determined threshold of 4.0 x 10-7 (stage 1) was used to select SNPs for attempted replication.12 Based on a preliminary analysis of 6 cohorts, we selected SNPs from 2 loci (the SNP with the lowest P and 1 or 2 proxy SNPs to accommodate differing genotyping platforms) for attempted replication. SNPs were genotyped in 2 additional cohorts and analyzed within cohort using a similar analysis plan except that observed rather than imputed genotypes were used in the analyses. Results from the 2 replication cohorts were then combined by meta-analysis. We considered a P<0.025 (0.05/2) and same direction of effect for the replication meta-analysis as indicative of successful replication. To assess possible effect modification by age, we performed an age-stratified analysis based on the approximate overall median age of 55 years. For cohorts that spanned this age cutoff (FHS, ERF, Sardinia, ACCT), analyses were repeated in subgroups <55 and ≥55 years of age. Cohorts with predominantly older (AGES, BLSA, HABC, RS-I, RS-II) or younger (HAPI, Asklepios) participants were included in the older or younger group in their entirety to preserve adequate sample size. These groupings resulted in 9 sets of data consisting of predominantly older participants and 6 sets of data consisting of predominantly younger participants.

Results

Characteristics of participants at the time of CFPWV measurement in the 11 (9 discovery, 2 replication) AortaGen Consortium cohorts are presented in Table 1. Cohort mean age varied from 34 to 75 years whereas cohort mean CFPWV varied from 5.5 to 13.6 m/s, corresponding to inverse CFPWV of 193 to 77 ms/m, respectively. Sample sizes varied from 618 to 6,033 participants, with an aggregate of 20,634 and 5,306 participants in the discovery and replication phases, respectively.

GWAS meta-analysis results from 9 cohorts are summarized in Figure 1. The quantile-quantile (Q-Q) plot shows minimal evidence of test statistic inflation (λ_{gc} = 1.03) and a sharp divergence from a slope near unity at a P-value of approximately 1 x 10-4. The negative log P (Manhattan) plot reveals a region of genome-wide significant association on the distal long arm of chromosome 14 (14q32.2, rs1381289, beta=-0.073±0.011 SD/allele, P = 5.6 x 10-11). In addition, there is a suggestive region of association on the short arm of chromosome 10 (10p12.32, rs10764094, beta=-0.057±0.011 SD/allele, P=2.4x10-7). A listing of top SNPs from the 9-cohort meta-analysis with a P < 1 x 10-5 is presented in Table 2. The table provides results for the top SNP from separate loci defined by LD structure (r2<0.80).

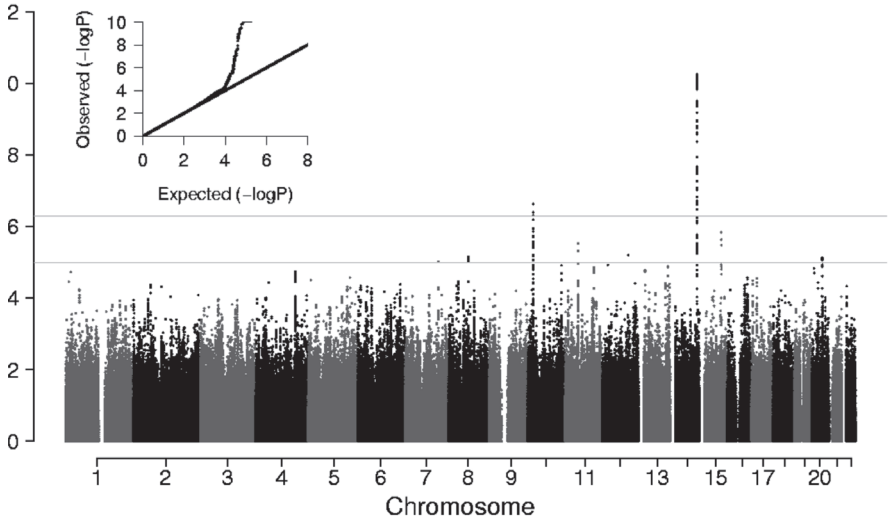


Figure 1. Q-Q and signal intensity (Manhattan) plots of genome-wide association data for CFPWV. The upper horizontal line corresponds to P = 4.0 x 10-7 whereas the lower line corresponds to P = 1.0 x 10-5.

Based on a preliminary meta-analysis of early GWAS results from 6 cohorts (Table 1; AGES, FHS, ERF, RS-I, RS-II, and Sardinia; 17,854 participants), we selected rs7152623 on chromosome 14 and rs17729837 on chromosome 10 for attempted replication. We successfully replicated the association with rs7152623 on chromosome 14 (replication beta=-0.086±0.020 SD/allele, P=1.4x10-6, combined beta=-0.076±0.010 SD/allele, P=3.1x10-15, Figure 2). The effect was attenuated modestly and remained significant when we further adjusted for mean arterial pressure at the time of measurement of CFPWV (beta=-0.060±0.009 SD/allele, P=1.0 x 10-11). In addition, results were consistent when evaluated separately in subgroups defined by median age, remaining associated in both younger (<55 years of age, 6 cohorts, N=13,914, beta=-0.081±0.014 SD/allele, P=2.3 x 10-9) and older (≥55 years of age, 9 cohorts, N=12,026, beta=-0.061±0.014 SD/allele, P=9.4x10-6) participants. The association with rs17729837 on chromosome 10 did not replicate (P = 0.97).

Details of the region of significant association on chromosome 14 are presented online.

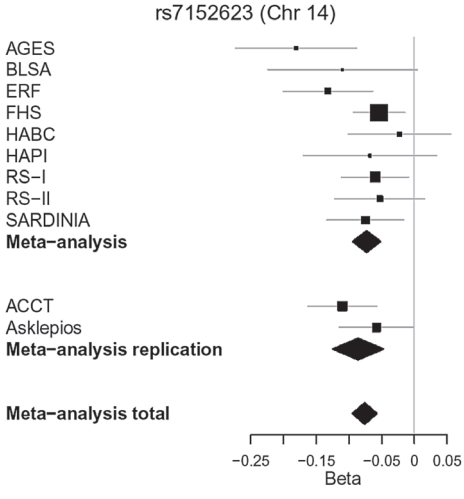


Figure 2. Forest plot of association results for rs7152623 on chromosome 14. Results for individual cohorts are plotted against the cohort effect size (beta coefficient). The size of the box is proportional to the study's weight in the meta-analysis (inversely proportional to estimated variance of the effect-size estimator). Horizontal lines are the 95% confidence intervals. Diamonds represent the results of meta-analyses; the center denotes overall estimate and the width denotes 95% confidence interval.

To assess potential functional implications of our findings, we used reverse transcriptase polymerase chain reaction (RT-PCR) to evaluate expression of DB129663 and BP432414 in human aortic samples and various cell lines. In light of the putative role of this region as a remote enhancer of BCL11B14 and relative proximity to VRK1, we also probed for expression of these genes. DB129663, BP432414, BCL11B and VRK1 were detected in whole aortic rings. VRK1 and DB129663 were expressed in cultured aortic smooth muscle cells, human umbilical vein endothelial cells (HUVECs) and adult cardiac fibroblasts. BCL11B was expressed in the same samples except cardiac fibroblasts. All transcripts were expressed in CD3+ cells.

To assess the potential clinical relevance of our finding of a locus on Chr14 strongly associated with CFPWV, we performed a lookup in GWAS results for various clinical endpoints thought to be related to arterial stiffness. We looked up the SNP with the lowest P-value in the meta-analysis of 9 cohorts (rs1381289, Table 2) and found an association with increased risk for coronary artery disease (hazard ratio [HR]=1.05, confidence interval [CI]=1.02 to 1.08 per allele, P=0.0013) and heart failure (HR=1.10, CI=1.03 to 1.16 per allele, P=0.004). In the Framingham Heart Study, after adjusting for age and sex, CFPWV was associated with an excess cardiovascular disease risk corresponding to a HR=1.65 per SD.[15] Based on this estimate, and given that each minor allele of rs1381289 was associated with a 0.073 SD increase in CFPWV, we would anticipate that the association between rs1381289 and CFPWV would result in an excess risk for cardiovascular disease events corresponding to HR=1.047 per allele, which is comparable to the findings from GWAS results. In addition, rs1381289 was associated with higher pulse pressure (beta=0.18±0.06 mm Hg/allele, P=0.002), indicating that the increase in aortic stiffness associated with this SNP is detectable as a modest but significant increase in pressure pulsatility. In contrast, the SNP was not associated with stroke (P>0.7), glomerular filtration rate estimated by using serum creatinine (P>0.6) or cystatin (P>0.5) or prevalent chronic kidney disease (P>0.6).

We also sought to replicate a previously reported association between CFPWV and a SNP (rs3742207) in COL4A1.16 After excluding 2 cohorts involved in the original report (Sardinia, HAPI), we found modest evidence of association for this SNP (rs3742207, beta=-0.025±0.011 SD/allele, P=0.017).

Table 1. Clinical characteristics of study participants.

Cohort	N	Percent women	Age (yrs)	Percent <55 yrs of age	Height (cm)
AGES	967	58	75±5	0	168±9
BLSA	618	49	62±18	34	170±10
ERF	1970	57	48±14	55	167±9
FHS	6033	54	49±15	68	169±10
HABC	1354	49	74±3	0	166±9
HAPI	808	46	46±15	67	167±9
RS-I	3011	57	72±7	0	167±9
RS-II	1657	54	64±8	0	169±9
SARDINIA	4216	56	43±17	78	160±9
Replication Cohorts					
ACCT	2932	52	34±19	77	171±10
Asklepios	2374	52	46±6	93	169±9

Mean values ± SD except as noted; *Original cohort; †Offspring cohort; ‡Third Generation cohort; AGES, Age, Gene/Environment Susceptibility-Reykjavik Study; BLSA, Baltimore Longitudinal Study of Aging; ERF, Erasmus Rucphen Family Study; FHS, Framingham Heart Study; HABC, Health, Aging and Body Composition; HAPI, Heredity and Phenotype Intervention; RS, Rotterdam Study; ACCT, Anglo Cardiff Collaborative Trial.

Weight (kg)	CFPWV (m/s)	Inverse CFPWV (ms/m)	Years of CFPWV assessment	Years of DNA collection
75±14	13.1±4.3	83±24	2005	2002-2006
75±16	7.2±2.5	154±49	1989-2008	1995-2006
75±15	9.5±2.1	110±21	2002-2005	2002-2005
77±17	8.5±3.5	131±35	1999-2001* 1998-2001† 2002-2005‡	1996-1999* 1996-1999† 2002-2005‡
74±14	8.8±3.7	131±47	1997-1998	1997-1998
74±13	5.5±1.4	193±42	2003-2008	2000-2008
74±12	13.6±3.0	77±17	1997-1999	1990-1993
77±13	12.6±3.2	84±18	2000-2001	2000-2001
65±13	6.7±2.1	163±44	2001-2004	2001-2004
72±14	6.7±2.2	161±40	2001-2009	2001-2009
74±14	6.6±1.5	157±29	2002-2004	2002-2004

Table 2. Genome wide association results for CFPWV in 9 cohorts.

SNP	Chromosome Number	Position	Allele Coded	Freq
rs1381289	14	97,662,117	T	0.436
rs987514	14	97,698,696	T	0.436
rs10782490	14	97,619,136	C	0.471
rs22225442	14	97,692,347	C	0.323
rs17773233	14	97,652,412	T	0.225
rs1461587	14	97,673,604	G	0.256
rs1381273	14	97,718,813	T	0.469
rs10764094	10	19,950,544	C	0.473
rs8015529	14	97,571,972	G	0.359
rs4778983	15	80,290,133	C	0.301
rs7161307	14	97,677,436	T	0.215
rs6485690	11	46,755,207	A	0.308
rs10740923	10	19,907,637	G	0.464
rs7959220	12	95,117,079	G	0.027
rs6472483	8	70,791,920	T	0.452
rs6101837	20	38,155,981	C	0.416
rs10827649	10	19,949,776	G	0.436
rs6947805	7	121,844,471	T	0.050

*Individual analyses were adjusted for age, age2, sex, height and weight.

†LD block includes ARHGAP1, ZNF408, F2, CKAP5 and LRP4.

Meta-analysis*			Closest Gene
Beta	**SE**	**P**	
-0.073	0.011	5.6 x 10-11	C14orf64
-0.069	0.011	4.5 x 10-10	C14orf64
-0.066	0.011	2.7 x 10-9	C14orf64
-0.071	0.012	1.2 x 10-8	C14orf64
-0.074	0.013	2.1 x 10-8	C14orf64
-0.070	0.013	1.5 x 10-7	C14orf64
-0.059	0.011	1.9 x 10-7	C14orf64
0.057	0.011	2.4 x 10-7	C10orf112
-0.066	0.013	2.5 x 10-7	C14orf64
0.057	0.012	1.5 x 10-6	EFTUD1
-0.065	0.013	1.7 x 10-6	C14orf64
-0.056	0.012	3.0 x 10-6	CKAP5†
-0.052	0.011	3.9 x 10-6	C10orf112
0.266	0.059	6.3 x 10-6	ELK3
-0.050	0.011	7.1 x 10-6	SLCO5A1
-0.050	0.011	7.5 x 10-6	MAFB
-0.049	0.011	8.6 x 10-6	C10orf112
0.117	0.026	9.5 x 10-6	CADPS2

Discussion

We performed a meta-analysis of GWAS results for CFPWV from 9 community-based cohorts involving 20,634 participants spanning a broad age range and identified a locus of genome-wide significant association in an apparent gene desert on 14q32.2. This finding was replicated in 2 additional cohorts involving 5,306 participants. We identified a conserved sequence within the region of significant association surrounded by a cluster of primate-specific, noncoding RNAs (ncRNAs). We evaluated 2 of these ncRNAs, which have at least one associated SNP within an exon, and demonstrate that they are expressed in relevant human cardiac and vascular tissues and cell lines, including full thickness aortic rings, aortic smooth muscle cells, cardiac fibroblasts and HUVECs. In light of the putative role of the region of significant association as a gene enhancer,[17-19] we also assayed for and demonstrated expression of flanking known genes, BCL11B and VRK1, in the same tissues and cell lines. Our findings indicate that the VRK1-BCL11B gene desert harbors a regulatory locus that modulates aortic stiffness. The association was consistent in younger and older participants, suggesting that the effects on CFPWV of genetic variation at this locus manifest early in life, prior to the marked increase in CFPWV that occurs from midlife onward. In addition, we demonstrated that the locus is associated with increased risk for cardiovascular disease, consistent with the hypothesis that increased aortic stiffness, as assessed by CFPWV, plays a causal role in the pathogenesis of cardiovascular disease. Further elucidation of potential mechanisms of aortic stiffening mediated through this locus may provide novel insights into the pathogenesis of aortic stiffening and could potentially offer insights into currently unavailable targeted interventions that prevent or attenuate aortic stiffening with advancing age and reduce the associated excess risk for major CVD events.

Prior studies provide strong evidence that the region of association with CFPWV that we have identified on 14q32.2 represents the core of a gene enhancer. The region encompasses various regulatory features, including several DNAse-I hypersensitive sites and transcription factor binding sites and high levels of nuclear matrix attachment.[20-22] Chromatin modifications in the region, including high levels of acetylation of histone 3 at lysine 27 (H3K27) and monomethylation at lysine 4 (H3K4) assessed in a lymphoblastoid cell line, are consistent with enhancer function (http://genome.ucsc.edu).[23] Despite considerable genomic separation from the enhancer core (~1 MB telomeric), BCL11B is thought to be the primary target of the enhancer.[24-26] BCL11B is located on the minus strand, positioning the enhancer in the remote 3' region of the gene. The closest known gene in the opposite direction, VRK1, is ~1.1 MB centromeric to the enhancer core and is on the plus strand, again positioning the enhancer in the remote 3' region of VRK1, suggesting that one or both genes could potentially be a target of the enhancer, although a CTCF binding site just to the VRK1-side of the enhancer region may represent an insulator that renders specificity to BCL11B (http://genome.ucsc.edu).

In support of BCL11B as a target, numerous translocations have been described that insert fragments of 5q35 at various positions in a breakpoint cluster region that falls between the enhancer core and 3' BCL11B. These translocations interpose the homeobox genes TLX3 or NKX2-5 between the enhancer and 3' BCL11B and result in ectopic activation of the homeobox gene, dysregulated T-cell proliferation and acute T-cell lymphoblastic leukemia.[27-29] T-cell regulatory signals directed at BCL11B may interact with the enhancer to drive ectopic activation of the interposed homeobox gene, leading to cell-specific malignant transformation. Su et al. used translocation data to map enhancer function to a 58 kB segment of the genome that corresponds to the telomeric shoulder of our locus of significant association with CFPWV.[30] Relative proximity of the enhancer to BCL11B, the important role that BCL11B plays in T-cell development and the T-cell specificity of malignant transformations involving translocations into the region support the hypothesis that BCL11B is a primary target of this enhancer.

BCL11B codes for chicken ovalbumin upstream promoter transcription factor (COUP-TF) interacting protein 2 (CTIP2), which is a cofactor in the COUP-TF family of transcription factors[31] and a direct transcriptional repressor.[32] There are several potential mechanisms for an effect of BCL11B on aortic stiffness. COUP-TFII (NR2F2) modulates the angiopoietin-1 and vascular endothelial growth factor pathways and plays a critical role in the development of the heart and great vessels.[33] In addition, BCL11B is a C2H2 zinc finger protein that can directly bind DNA in a sequence specific manner and effect transcriptional repression independent of COUP-TF family members.[34] Direct transcriptional repression mediated by BCL11B involves trichostatin-A insensitive deacetylation of histones in repressed genes. A key binding partner in this BCL11B-mediated gene silencing is the class III histone deacetylase SIRT1, the mammalian ortholog of the yeast SIR2 gene product, a key regulator of aging at the cellular and whole organism level in diverse species.[35,36] Acting directly and in concert with SIRT1, BCL11B mediates antiapoptotic modulation of gene expression at several levels. With SIRT1 as a cofactor, BCL11B represses the expression of the proapoptotic cyclin-dependent protein kinase inhibitors p21 (CDKN1A) and p57 (CDKN1C).[37,38] In addition, BCL11B represses heme oxygenase-1 (HMOX1),[39] which is a stress inducible gene that enhances proapoptotic effects of the cell cycle regulator p53 (TP53) in vascular smooth muscle cells.[40] BCL11B also represses expression of the matrix protein fibronectin (FN1) and the adhesion molecule cadherin 10 (CDH10).[41] Furthermore, BCL11B appears to be expressed in the aorta at day 14.5 in developing mouse embryos (although this observation was not discussed).[42] Our findings suggest that variants in the region of association that we have identified may interfere with transcription factor binding in the enhancer region or may alter the function or expression of regulatory ncRNAs in the region. If these variants reduce expression of BCL11B, altered aortic development, reduced activity of SIRT1, increased expression of proapoptotic factors, fibronectin or cadherin or alterations in additional as yet unidentified targets may contribute to increased aortic stiffness.

In addition to direct effects of BCL11B on aortic function, there are potential indirect effects mediated through the known role that BCL11B plays in immune cell function. T-cell specific deletion of BCL11B at the CD4+ single positive stage is associated with increased numbers of proinflammatory TCRαα double negative T-cells.[43] Periaortic fat is infiltrated with this same class of proinflammatory T-cells in angiotensin induced models of hypertension.44 Thus, a genetic variant that attenuates expression of BCL11B could potentially enhance proliferation of and tissue infiltration by proinflammatory TCRαα DN T-cells, leading to abnormal aortic stiffness.

Similarly, there are mechanisms whereby VRK1, which we have shown is expressed in aortic tissue, could potentially modulate aortic properties. In distinct contrast to BCL11B, VRK1 has primarily proapoptotic and prosenescence effects, mediated in large part through phosphorylation of threonine 18 of p53, leading to reduced ubiquination and increased nuclear levels of p53.[45] Recent work has shown that exercise training, which is an effective intervention to reduce aortic stiffness,[46] is a potent inhibitor of aortic p53 activity and is associated with improved endothelial function and resistance to stress-induced endothelial cell senescence and apoptosis.[47] Thus, enhanced activity of VRK1, leading to stabilization of p53 levels, could have an adverse effect on aortic stiffness.

The contrasting effects of BCL11B and VRK1 on cell cycle regulation and nearly equidistant location from the enhancer region located in the intervening gene desert raise the possibility that this regulatory region may modulate the balance between cell survival or senescence and apoptosis. The entire segment spanning from VRK1 to BCL11B, including the intervening 2 MB gene desert is duplicated on chromosome 2 as VRK2 and BCL11A, suggesting that the genomic architecture between these 2 gene pairs may have an important effect on function of one or both genes. SNPs in both gene deserts have been associated with Type I and Type II diabetes.[48,49] Thus, transcription factors or other signaling molecules acting at a single enhancer locus between VRK1 and BCL11B could potentially negatively or positively modulate cell survival and thereby alter aortic stiffness and other aging phenotypes, such as diabetes or coronary artery disease.[50]

We demonstrated that two overlapping ESTs that fall completely within the region of highly significant association with CFPWV are expressed in aortic tissue and cell lines. These primate-specific, potentially regulatory ncRNAs overlap the conserved sequence and are expressed in cDNA extracts from full thickness human aortic rings and various human cell lines, including aortic smooth muscle cells, HUVECs and cardiac fibroblasts. One of the highly associated SNPs in the region (rs710285) is located in an exon of DB129663, suggesting a possible functional effect. The enhancer core region mapped by Su et al. corresponds to the putative promoter region of DB129663. Thus, enhancer function at our chromosome 14 locus may specifically target DB129663, which appears to be a ncRNA of unknown function, rather than BCL11B. Additional work will be required to test this hypothesis and further define the function of DB129663.

To assess the potential importance of the many borderline associations that we found with CFPWV, we compared our results to published results for echocardiographic measures51 and stroke,[52] which are related to arterial stiffness. Published results included several of the cohorts in our consortium (AGES, FHS, RS-I, RS-II). The strongest evidence for potential overlap was found in a long LD block spanning from approximately 46.68 to 47.20 MB on chromosome 11. This genomic region encompasses numerous SNPs that showed moderate evidence of association with stroke and CFPWV. There are 5 missense SNPs in LRP4 and one in the thrombin gene (F2) in this region. The SNP in this region with the lowest P value for association with stroke (rs10734548) is an intronic SNP in CKAP5 that also showed moderate association with CFPWV (P = 1.25 x 10-4). This SNP is in high LD with a nonsynonymous SNP in LRP4 (rs3816614, r2 = 0.93, CEU HapMap release 22). Similarly, the SNP in this region with the lowest P value for association with CFPWV (rs6485690, Table 2) is an intronic SNP in CKAP5, which is in high LD with another nonsynonymous SNP in LRP4 (rs6485702, r2 = 0.95, CEU HapMap release 22). Two additional nonsynonymous SNPs in LRP4 (rs2306029 and rs2306033) were associated with CFPWV. In each case, the minor allele was associated with higher CFPWV and increased risk for stroke, consistent with the known relation between arterial stiffness and stroke.3

Several additional SNPs with suggestive associations to CFPWV (10-8 < P < 10-5) may merit further consideration and additional replication genotyping. The locus on chromosome 10 with the second lowest P-value in our GWAS meta-analysis lies in the vicinity of a putative protein coding gene that may represent a novel member of the low density lipoprotein receptor-related protein (LRP) family,[53] which is interesting in light of the additional moderate evidence of association that we found with LRP4 in the present analysis and prior reports of association with stroke[52] and bone mineral density.[54;55] The CFPWV association with LRP4 includes a nonsynonymous SNP (rs6485702) that has been related to bone mineral density,56 although a separate report involving several of our cohorts positioned the region of highest association with bone mineral density in the promoter region of ARHGAP1.[57] Bone density and arterial stiffness are related phenotypes that share many common pathways.[58] The recently observed inhibitory role that LRP4 plays in Wnt signaling in bone[59] coupled with the adverse effects of Wnt signaling in the aorta[60] suggests that a mutation that impairs the ability of LRP4 to modulate the Wnt signaling cascade could simultaneously contribute to osteopenia and aortic stiffening. The chromosome 11 locus that encompasses LRP4 and additional potential candidates, including ARHGAP1 and F2, represents a long LD block that was also associated with stroke in a prior meta-analysis that included several of the cohorts in our study. The direction of effect in the prior study (higher risk for the minor allele) and ours (stiffer aorta with the minor allele) was consistent with the known association between increased CFPWV and increased risk for stroke.3 In addition, a prior family-based linkage analysis for myocardial infarction found a single significant linkage peak in the vicinity of our association peak on chromosome 14.[61] These regions of overlap with prior results involving separate but related phenotypes support the clinical relevance of our associations and suggest that several genetic variants that impact CFPWV may eventually manifest as age-related morbidity and major cardiovascular events.

We also attempted to replicate a previously reported association of CFPWV with a SNP in the COL4A1 gene in the only published GWAS that has evaluated CFPWV.[62] The present results found modest evidence of association with some heterogeneity of effect, suggesting that additional work will be required to determine whether variation in LD patterns or other factors could potentially account for heterogeneous effects at this locus.

There are limitations of our study that should be considered. The cohorts comprised exclusively white participants of European descent. Thus, our findings may not generalize to other populations. Slightly different methods were used to assess CFPWV in the various cohorts. However, our use of standardized residuals generated within each cohort should have minimized the effects of these technical differences between studies. A major strength of our study is the use of data from 11 large community-based cohorts that routinely ascertained CFPWV, which should enhance generalizability of our findings.

In conclusion, we performed the first large scale GWAS of CFPWV, which is a moderately heritable measure of aortic stiffness and important risk factor for cardiovascular events. We identified a highly significant locus of association at 14q32.2 in the VRK1-BCL11B gene desert in an LD block that corresponds to the core of a gene enhancer that is thought to target BCL11B. We have also shown that genetic variation at this locus is associated with increased risk for major CVD, providing strong support for the hypothesis that increased CFPWV contributes to the pathogenesis of CVD. We have shown that 2 potentially regulatory ncRNAs surrounding a conserved sequence, as well as flanking genes, BCL11B and VRK1, are expressed in human aorta. Downstream targets of BCL11B and VRK1, including FN1, HMOX1 and p53, are known to be involved in aortic function. Further work will be required to define precise mechanisms mediating the association between CFPWV and genetic variation in the VRK1-BCL11B gene desert. Elucidation of pathways affected by this locus will provide new insights into the process of aortic stiffening in humans and could yield potential targets for specific interventions that reverse or attenuate aortic stiffening and prevent the associated morbidity and mortality.

References

1. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P, Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37:1236-1241.
2. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. 2002;39:10-15.
3. Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, Boutouyrie P. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. Stroke. 2003;34:1203-1206.
4. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol. 2001;21:2046-2050.
5. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgeon H, Kritchevsky S, Pahor M, Bauer D, Newman A. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation. 2005;111:3384-3390.
6. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. 2006;113:657-663.
7. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation. 2006;113:664-670.
8. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the framingham heart study. Circulation. 2010;121:505-511.
9. Mitchell GF, Guo CY, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D. Cross-sectional correlates of increased aortic stiffness in the community: the Framingham Heart Study. Circulation. 2007;115:2628-2636.
10. Mitchell GF, DeStefano AL, Larson MG, Benjamin EJ, Chen MH, Vasan RS, Vita JA, Levy D. Heritability and a genome-wide linkage scan for arterial stiffness, wave reflection, and mean arterial pressure: the Framingham Heart Study. Circulation. 2005;112:194-199.
11. Pilia G, Chen WM, Scuteri A, Orru M, Albai G, Dei M, Lai S, Usala G, Lai M, Loi P, Mameli C, Vacca L, Deiana M, Olla N, Masala M, Cao A, Najjar SS, Terracciano A, Nedorezov T, Sharov A, Zonderman AB, Abecasis GR, Costa P, Lakatta E, Schlessinger D. Heritability of cardiovascular and personality traits in 6,148 Sardinians. PLoS Genet. 2006;2:e132.

12. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Kottgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009;41:677-687.

13. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Magi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparso T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proenca C, Kumari M, Qi L, Timpson NJ, Gieger C, Zabena C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccasecca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Bottcher Y, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllensten U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanali N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD, Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jorgensen T, Jula A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martinez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orru M, Pakyz R, Palmer CN, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvanen AC, Tanaka T, Thorand B, Tichet J, Tonjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Rios M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010;42:105-116.

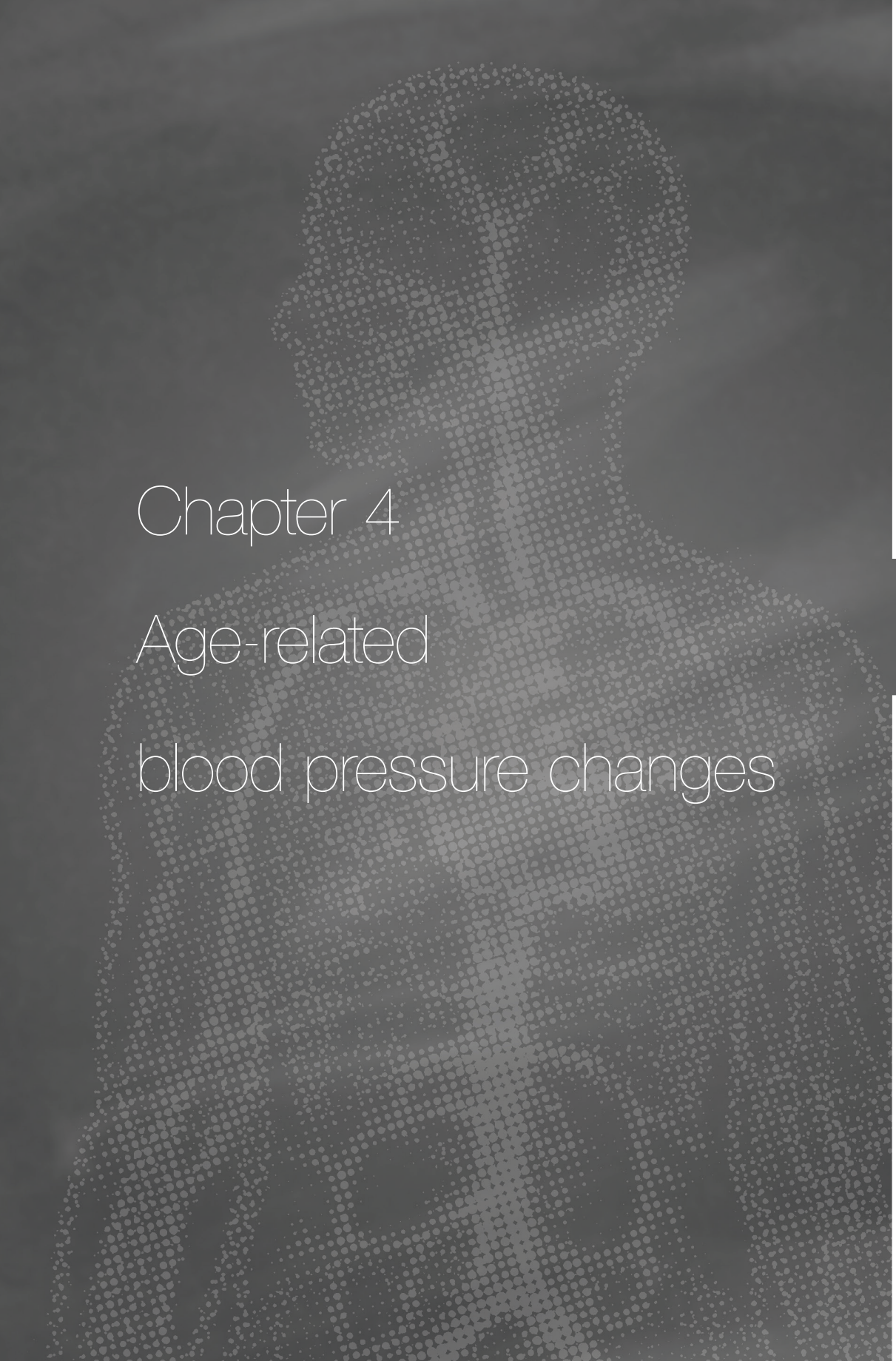
14. Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, MacLeod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. Cancer Res. 2007;67:1461-1471.
15. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. 2010;121:505-511.
16. Tarasov KV, Sanna S, Scuteri A, Strait JB, Orru M, Parsa A, Lin PI, Maschio A, Lai S, Piras MG, Masala M, Tanaka T, Post W, O'Connell JR, Schlessinger D, Cao A, Nagaraja R, Mitchell BD, Abecasis GR, Shuldiner AR, Uda M, Lakatta EG, Najjar SS. COL4A1 is associated with arterial stiffness by genome-wide association scan. Circ Cardiovasc Genet. 2009;2:151-158.
17. Nagel S, Kaufmann M, Drexler HG, MacLeod RA. The cardiac homeobox gene NKX2-5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). Cancer Res. 2003;63:5329-5334.
18. Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, MacLeod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. Cancer Res. 2007;67:1461-1471.
19. Su XY, Della-Valle V, Andre-Schmutz I, Lemercier C, Radford-Weiss I, Ballerini P, Lessard M, Lafage-Pochitaloff M, Mugneret F, Berger R, Romana SP, Bernard OA, Penard-Lacronique V. HOX11L2/TLX3 is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32). Blood. 2006;108:4198-4201.
20. Nagel S, Kaufmann M, Drexler HG, MacLeod RA. The cardiac homeobox gene NKX2-5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). Cancer Res. 2003;63:5329-5334.
21. Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, MacLeod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. Cancer Res. 2007;67:1461-1471.
22. Su XY, Della-Valle V, Andre-Schmutz I, Lemercier C, Radford-Weiss I, Ballerini P, Lessard M, Lafage-Pochitaloff M, Mugneret F, Berger R, Romana SP, Bernard OA, Penard-Lacronique V. HOX11L2/TLX3 is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32). Blood. 2006;108:4198-4201.
23. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. Cell. 2007;129:823-837.
24. Nagel S, Kaufmann M, Drexler HG, MacLeod RA. The cardiac homeobox gene NKX2-5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). Cancer Res. 2003;63:5329-5334.

25. Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, MacLeod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. Cancer Res. 2007;67:1461-1471.
26. Su XY, Della-Valle V, Andre-Schmutz I, Lemercier C, Radford-Weiss I, Ballerini P, Lessard M, Lafage-Pochitaloff M, Mugneret F, Berger R, Romana SP, Bernard OA, Penard-Lacronique V. HOX11L2/TLX3 is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32). Blood. 2006;108:4198-4201.
27. Nagel S, Kaufmann M, Drexler HG, MacLeod RA. The cardiac homeobox gene NKX2-5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). Cancer Res. 2003;63:5329-5334.
28. Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, MacLeod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. Cancer Res. 2007;67:1461-1471.
29. Su XY, Della-Valle V, Andre-Schmutz I, Lemercier C, Radford-Weiss I, Ballerini P, Lessard M, Lafage-Pochitaloff M, Mugneret F, Berger R, Romana SP, Bernard OA, Penard-Lacronique V. HOX11L2/TLX3 is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32). Blood. 2006;108:4198-4201.
30. Su XY, Della-Valle V, Andre-Schmutz I, Lemercier C, Radford-Weiss I, Ballerini P, Lessard M, Lafage-Pochitaloff M, Mugneret F, Berger R, Romana SP, Bernard OA, Penard-Lacronique V. HOX11L2/TLX3 is transcriptionally activated through T-cell regulatory elements downstream of BCL11B as a result of the t(5;14)(q35;q32). Blood. 2006;108:4198-4201.
31. Avram D, Fields A, Pretty On Top, Nevrivy DJ, Ishmael JE, Leid M. Isolation of a novel family of C(2)H(2) zinc finger proteins implicated in transcriptional repression mediated by chicken ovalbumin upstream promoter transcription factor (COUP-TF) orphan nuclear receptors. J Biol Chem. 2000;275:10315-10322.
32. Avram D, Fields A, Senawong T, Topark-Ngarm A, Leid M. COUP-TF (chicken ovalbumin upstream promoter transcription factor)-interacting protein 1 (CTIP1) is a sequence-specific DNA binding protein. Biochem J. 2002;368:555-563.
33. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. Genes Dev. 1999;13:1037-1049.
34. Avram D, Fields A, Senawong T, Topark-Ngarm A, Leid M. COUP-TF (chicken ovalbumin upstream promoter transcription factor)-interacting protein 1 (CTIP1) is a sequence-specific DNA binding protein. Biochem J. 2002;368:555-563.
35. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science. 2000;289:2126-2128.
36. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature. 2001;410:227-230.
37. Cherrier T, Suzanne S, Redel L, Calao M, Marban C, Samah B, Mukerjee R, Schwartz C, Gras G, Sawaya BE, Zeichner SL, Aunis D, Van Lint C, Rohr O. p21(WAF1) gene promoter is epigenetically silenced by CTIP2 and SUV39H1. Oncogene. 2009;28:3380-3389.

38. Topark-Ngarm A, Golonzhka O, Peterson VJ, Barrett B, Jr., Martinez B, Crofoot K, Filtz TM, Leid M. CTIP2 associates with the NuRD complex on the promoter of p57KIP2, a newly identified CTIP2 target gene. J Biol Chem. 2006;281:32272-32283.
39. Topark-Ngarm A, Golonzhka O, Peterson VJ, Barrett B, Jr., Martinez B, Crofoot K, Filtz TM, Leid M. CTIP2 associates with the NuRD complex on the promoter of p57KIP2, a newly identified CTIP2 target gene. J Biol Chem. 2006;281:32272-32283.
40. Liu XM, Chapman GB, Wang H, Durante W. Adenovirus-mediated heme oxygenase-1 gene expression stimulates apoptosis in vascular smooth muscle cells. Circulation. 2002;105:79-84.
41. Topark-Ngarm A, Golonzhka O, Peterson VJ, Barrett B, Jr., Martinez B, Crofoot K, Filtz TM, Leid M. CTIP2 associates with the NuRD complex on the promoter of p57KIP2, a newly identified CTIP2 target gene. J Biol Chem. 2006;281:32272-32283.
42. Leid M, Ishmael JE, Avram D, Shepherd D, Fraulob V, Dolle P. CTIP1 and CTIP2 are differentially expressed during mouse embryogenesis. Gene Expr Patterns. 2004;4:733-739.
43. Albu DI, Feng D, Bhattacharya D, Jenkins NA, Copeland NG, Liu P, Avram D. BCL11B is required for positive selection and survival of double-positive thymocytes. J Exp Med. 2007;204:3003-3015.
44. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. J Exp Med. 2007;204:2449-2460.
45. Vega FM, Sevilla A, Lazo PA. p53 Stabilization and accumulation induced by human vaccinia-related kinase 1. Mol Cell Biol. 2004;24:10366-10380.
46. Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR. Aging, habitual exercise, and dynamic arterial compliance. Circulation. 2000;102:1270-1275.
47. Werner C, Furster T, Widmann T, Poss J, Roggia C, Hanhoun M, Scharhag J, Buchner N, Meyer T, Kindermann W, Haendeler J, Bohm M, Laufs U. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. Circulation. 2009;120:2438-2447.
48. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, Julier C, Morahan G, Nerup J, Nierras C, Plagnol V, Pociot F, Schuilenburg H, Smyth DJ, Stevens H, Todd JA, Walker NM, Rich SS. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009;41:703-707.
49. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, Manning AK, Florez JC, Wilson PW, D'Agostino RB, Sr., Cupples LA. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008;359:2208-2219.
50. Broeckel U, Hengstenberg C, Mayer B, Holmer S, Martin LJ, Comuzzie AG, Blangero J, Nurnberg P, Reis A, Riegger GA, Jacob HJ, Schunkert H. A comprehensive linkage analysis for myocardial infarction and its related risk factors. Nat Genet. 2002;30:210-214.

51. Vasan RS, Glazer NL, Felix JF, Lieb W, Wild PS, Felix SB, Watzinger N, Larson MG, Smith NL, Dehghan A, Grosshennig A, Schillert A, Teumer A, Schmidt R, Kathiresan S, Lumley T, Aulchenko YS, Konig IR, Zeller T, Homuth G, Struchalin M, Aragam J, Bis JC, Rivadeneira F, Erdmann J, Schnabel RB, Dorr M, Zweiker R, Lind L, Rodeheffer RJ, Greiser KH, Levy D, Haritunians T, Deckers JW, Stritzke J, Lackner KJ, Volker U, Ingelsson E, Kullo I, Haerting J, O'Donnell CJ, Heckbert SR, Stricker BH, Ziegler A, Reffelmann T, Redfield MM, Werdan K, Mitchell GF, Rice K, Arnett DK, Hofman A, Gottdiener JS, Uitterlinden AG, Meitinger T, Blettner M, Friedrich N, Wang TJ, Psaty BM, van Duijn CM, Wichmann HE, Munzel TF, Kroemer HK, Benjamin EJ, Rotter JI, Witteman JC, Schunkert H, Schmidt H, Volzke H, Blankenberg S. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. JAMA. 2009;302:168-178.
52. Ikram MA, Seshadri S, Bis JC, Fornage M, DeStefano AL, Aulchenko YS, Debette S, Lumley T, Folsom AR, van den Herik EG, Bos MJ, Beiser A, Cushman M, Launer LJ, Shahar E, Struchalin M, Du Y, Glazer NL, Rosamond WD, Rivadeneira F, Kelly-Hayes M, Lopez OL, Coresh J, Hofman A, DeCarli C, Heckbert SR, Koudstaal PJ, Yang Q, Smith NL, Kase CS, Rice K, Haritunians T, Roks G, de Kort PL, Taylor KD, de Lau LM, Oostra BA, Uitterlinden AG, Rotter JI, Boerwinkle E, Psaty BM, Mosley TH, van Duijn CM, Breteler MM, Longstreth WT, Jr., Wolf PA. Genomewide association studies of stroke. N Engl J Med. 2009;360:1718-1728.
53. Zubenko GS, Hughes HB, III, Zubenko WN. D10S1423 identifies a susceptibility locus for Alzheimer's disease (AD7) in a prospective, longitudinal, double-blind study of asymptomatic individuals: Results at 14 years. Am J Med Genet B Neuropsychiatr Genet. 2010;153B:359-364.
54. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Snorradottir S, Center JR, Nguyen TV, Alexandersen P, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K. New sequence variants associated with bone mineral density. Nat Genet. 2009;41:15-17.
55. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, Zillikens MC, Kavvoura FK, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Grundberg E, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra B, Pastinen T, Pols HA, Sigurdsson G, Soranzo N, Thorleifsson G, Thorsteinsdottir U, Williams FM, Wilson SG, Zhou Y, Ralston SH, van Duijn CM, Spector T, Kiel DP, Stefansson K, Ioannidis JP, Uitterlinden AG. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet. 2009;41:1199-1206.
56. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Snorradottir S, Center JR, Nguyen TV, Alexandersen P, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K. New sequence variants associated with bone mineral density. Nat Genet. 2009;41:15-17.

57. Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, Hsu YH, Richards JB, Zillikens MC, Kavvoura FK, Amin N, Aulchenko YS, Cupples LA, Deloukas P, Demissie S, Grundberg E, Hofman A, Kong A, Karasik D, van Meurs JB, Oostra B, Pastinen T, Pols HA, Sigurdsson G, Soranzo N, Thorleifsson G, Thorsteinsdottir U, Williams FM, Wilson SG, Zhou Y, Ralston SH, van Duijn CM, Spector T, Kiel DP, Stefansson K, Ioannidis JP, Uitterlinden AG. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet. 2009;41:1199-1206.
58. Sabit R, Bolton CE, Edwards PH, Pettit RJ, Evans WD, McEniery CM, Wilkinson IB, Cockcroft JR, Shale DJ. Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2007;175:1259-1265.
59. Choi HY, Dieckmann M, Herz J, Niemeier A. Lrp4, a novel receptor for Dickkopf 1 and sclerostin, is expressed by osteoblasts and regulates bone growth and turnover in vivo. PLoS One. 2009;4:e7930.
60. Al Aly Z, Shao JS, Lai CF, Huang E, Cai J, Behrmann A, Cheng SL, Towler DA. Aortic Msx2-Wnt calcification cascade is regulated by TNF-alpha-dependent signals in diabetic Ldlr-/- mice. Arterioscler Thromb Vasc Biol. 2007;27:2589-2596.
61. Broeckel U, Hengstenberg C, Mayer B, Holmer S, Martin LJ, Comuzzie AG, Blangero J, Nurnberg P, Reis A, Riegger GA, Jacob HJ, Schunkert H. A comprehensive linkage analysis for myocardial infarction and its related risk factors. Nat Genet. 2002;30:210-214.
62. Tarasov KV, Sanna S, Scuteri A, Strait JB, Orru M, Parsa A, Lin PI, Maschio A, Lai S, Piras MG, Masala M, Tanaka T, Post W, O'Connell JR, Schlessinger D, Cao A, Nagaraja R, Mitchell BD, Abecasis GR, Shuldiner AR, Uda M, Lakatta EG, Najjar SS. COL4A1 is associated with arterial stiffness by genome-wide association scan. Circ Cardiovasc Genet. 2009;2:151-158.



Chapter 4

Age-related blood pressure changes

Chapter 4.1
Arterial stiffness and hypertension

Based on:
Arterial stiffness and hypertension in a large population of untreated subjects.
The Rotterdam Study.
Accepted Journal of Hypertension

Abstract

Background

We studied whether arterial stiffness measured as aortic pulse wave velocity (aPWV) and carotid distensibility, was associated with different subtypes of hypertension in a large population of untreated middle-aged and elderly men and women.

Methods

The study was conducted within the framework of the population-based Rotterdam Study. We included 4088 subjects with information on aPWV, with among these 3554 subjects with carotid distensibility measurements without use of anti-hypertensive medication. Isolated systolic hypertension (ISH) was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure <90 mmHg. Combined systolic and diastolic hypertension (Sys/Dia HT) was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg. ANCOVA was used to compare means of arterial stiffness for the different subtypes of hypertension. Multinomial logistic regression analysis was performed to investigate the association of arterial stiffness and the subtypes of hypertension in models adjusted for age, sex, mean arterial pressure, heart rate and cardiovascular risk factors.

Results

The mean age of the subjects was 68 years, 45.3% was men, 1597 subjects had ISH and 441 subjects had Sys/Dia HT. Aortic PWV was higher (13.2 m/s vs. 12.9 m/s; P=0.008) in subjects with ISH compared to subjects with Sys/Dia HT. Multivariate odds ratio's (OR) and corresponding 95% CI of aPWV, for ISH were 1.53 (1.38-1.71) and 1.28(1.09-1.53) for Sys/Dia HT. Corresponding ORs associated with carotid distensibility were 0.84 (0.75-0.94) and 0.66 (0.54-0.81), respectively. Age significantly modified the association of aPWV with subtypes of hypertension (p<0.001).

Conclusions

In a large untreated population, we found significant associations of both aPWV and carotid distensibility with ISH and Sys/Dia HT. Subjects with ISH had higher values of aortic stiffness when compared to subjects with Sys/Dia HT, a difference that was most pronounced at older age. The results suggest that aortic stiffness contributes to ISH in older subjects without treatment for hypertension.

Introduction

Hypertension is a common and well established risk factor for cardiovascular disease[1]. The prevalence of hypertension increases with advancing age. Isolated systolic hypertension (ISH) is the most frequent type of hypertension in the elderly[2]. This is due to the continuous increase in systolic blood pressure with advancing age whereas diastolic blood pressure tends to remain constant or declines with advancing age, indicating a patent regulation of mean arterial pressure3. The most likely explanation for the age related rise in systolic blood pressure and fall in diastolic blood pressure is large artery stiffening[3]. Indeed, several studies have shown that a specific measure of arterial stiffness, i.e. aortic pulse wave velocity (aPWV), is increased in subjects with ISH compared to controls[4,5], while carotid distensibility shows a decline. This relation has been confirmed in young adults[6] and elderly women[7]. Furthermore, aPWV has been shown to be an independent predictor of the longitudinal increase in systolic blood pressure[8] and is associated with subsequent cardiovascular morbidity and mortality[9,10].

Previous studies included relatively small groups of subjects and there is limited information on the relation of arterial stiffness with ISH in different age categories of healthy untreated subjects. Furthermore, the relation of arterial stiffness with the different types of hypertension, including ISH and combined systolic and diastolic hypertension (Sys/Dia HT), has not been fully elucidated. Therefore, we studied whether two measures of arterial stiffness, aPWV and carotid distensibility, were associated with ISH and Sys/Dia HT in a large population of untreated subject. We additionally investigated whether the association was different in categories for age.

Methods

Study population

The present study was performed within the framework of the Rotterdam Study (RS), a large population-based prospective cohort study. From 1990 to 1993, 7983 subjects aged 55 and over living in Ommoord, a suburb of Rotterdam, the Netherlands participated in the Rotterdam Study (RS-1). In 1999, inhabitants who turned 55 years of age or moved into the study district since the start of the study were invited to participate in an extension of the Rotterdam Study (RS-II) of whom 3011 participated. The overall aim of the Rotterdam Study is to access the occurrence of risk factors for chronic diseases in the elderly. The study design and objectives of the Rotterdam Study are described elsewhere[11]. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.

Measures of arterial stiffness

Aortic pulse wave velocity

The aPWV was obtained with subjects in supine position. Before the aPWV measurement, blood pressure was measured twice with a sphygmomanometer after five minutes of rest and the mean was taken. The aPWV was assessed with an automatic device (Complior® Artech Medical, Pantin – France)[12] that measures the time delay between the rapid early upstroke of the pulse pressure waves recorded simultaneously in the carotid artery and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape over the surface of the body. The aPWV was calculated as the ratio between distance and the foot-foot time delay and was expressed in meter per second.

Carotid distensibility

Common carotid distensibility was assessed with the subjects in supine position, with the head tilted slightly to the contralateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery was measured by means of a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. The details of this technique have been described elsewhere[13]. After 5 minutes of rest, a region at 1.5 cm proximal to the origin of the bulb of the carotid artery was identified with the use of B-mode ultrasound, where after the system was switched to M-mode. The displacement of the arterial walls was obtained by processing the radiofrequency signals originating from two selected sample volumes positioned over the anterior and posterior walls. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of 4 cardiac cycles of 3 successive recordings. The cross-sectional arterial wall distensibility coefficient, expressed in MPa-1, was calculated according to the following equation: distensibility coefficient=2ΔD/ (D × pulse pressure)[14] with pulse pressure defined as the difference between systolic and diastolic blood pressure.

In a reproducibility study performed among 47 subjects, the intraclass correlation coefficient was 0.80 for both the aPWV and the carotid distensibility coefficient[15].

Blood pressure measurements

Two blood pressure measurements were obtained at the right brachial artery with a random zero sphygmomanometer after the subject had been seated for at least five minutes. Systolic blood pressure, first Korotkoff phase and diastolic blood pressure, fifth Korotkoff phase, were obtained and the mean of the two blood pressure values was used in the analyses. Normotensive was defined as systolic blood pressure ≤140 mmHg and diastolic blood pressure ≤90 mmHg. ISH was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≤ 90 mmHg[16]. Sys/Dia HT was defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg. Mean arterial pressure was estimated as MAP= DBP + PP/3.

Cardiovascular risk factors

Information on medical history, smoking habits and medication use was obtained during a home interview. Smoking was divided into three categories: current, former and never smokers. During the research center visit, height and weight were measured, and the body mass index (BMI) was computed (kg/m2). Diabetes mellitus was defined as a history of diabetes mellitus and/or the use of blood glucose lowering medication and/or a fasting serum glucose level ≥7.0 mmol/l[17]. Serum total cholesterol and high-density lipoproteins (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System, Mannheim, Germany)[18].

Prevalent coronary heart disease

A history of coronary heart disease (CHD) was obtained through direct questioning and was considered positive when confirmed according to hospital discharge date or written information from the subject's general practitioner, as described previously[19]. CHD was defined myocardial infarction (MI), a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft. A history of MI was considered present in case of self-report of MI confirmed by electrocardiogram or additional clinical information or the presence of an electrocardiogram characteristic of prior MI.

Population for analysis

During the third examination phase of RS-I (1997-1999) and during the first examination phase of RS-II (2000-2001), a computerized questionnaire was completed and cardiovascular risk factors and arterial stiffness were assessed. In total 6938 subjects visited the research center. Measures of both blood pressure levels and aortic pulse wave velocity (aPWV) were obtained in 5773 subjects, and among these, 4640 subjects had a measurement for carotid distensibility.

We excluded subjects with use of anti-hypertensive medication (n=1309) and subjects with missing covariate information (n=376), resulting in 4088 subjects for analyses with aPWV. Among these 3554 subjects were available for carotid distensibility analyses. Missing information was primarily due to logistic reasons.

Statistical analysis

Mean values of systolic, diastolic, mean arterial and pulse pressure, aPWV and carotid distensibility in different age categories were plotted in figures. ANCOVA was used to compare age, gender, aPWV and carotid distensibility between subtypes of hypertension categories, i.e. normotensive, ISH and Sys/Dia HT. Multinomial logistic regression models were performed to investigate the association of standardized cardiovascular risk factors and standardized values of aPWV and carotid distensibility with subtypes of hypertension. Standardized values were obtained by dividing each measure by its standard deviation. The models were adjusted for age, gender, cohort, body mass-index, total cholesterol, high-density cholesterol, smoking, diabetes mellitus, and for mean arterial pressure and heart rate, when appropriate. We tested for interaction by age, gender, diabetes mellitus and prevalent cardiovascular disease. If the interaction term was significant we performed stratified analyses. ANCOVA was used to compare means of arterial stiffness in subtypes of hypertension by age categories, adjusted for age, gender, cohort, mean arterial pressure, heart rate and cardiovascular risk factors, when appropriate. For descriptive purposes we generated figures with mean values of measures of arterial stiffness in the age categories for subjects in the different hypertension categories. P-values less than 0.05 were considered statistically significant. All analyses were performed using SPSS 15.0 statistical package for Windows 2003 (SPSS, INC., Chicago, Illinois, USA).

Results

The baseline characteristics of the study population are shown in table 1. The mean age of the study population was 68.1 years and the percentage men were 45.3%.

Table 1: Baseline characteristics of the study participants in untreated subjects (n=4088)

Characteristics	Total (n=4088) Mean ± SD or percentage
Age (years)	68.1 ± 8.1
Men (%)	45.3
Systolic blood pressure (mmHg)	141 ± 20
Diastolic blood pressure (mmHg)	76 ± 11
Mean arterial pressure (mmHg)	98 ± 12
Pulse pressure (mmHg)	65 ± 17
Heart rate (bpm)	74 ± 13
Body mass index (kg/m2)	26.5 ± 3.8
Total cholesterol (mmol/l)	5.83 ± 1.00
High-density lipoprotein cholesterol (mmol/l)	1.41 ± 0.39
Current smokers (%)	18.7
Diabetes mellitus (%)	11.1
Aortic pulse wave velocity (m/sec)	12.8 ± 3.0
Carotid distensibility coefficient (MPa-1)*	12.0 ± 4.8
Prevalent CHD (%)	8.9

SD: standard deviation; n: number

* available for 3554 subjects

With increasing age there is an increase in systolic blood pressure and pulse pressure, a decrease in diastolic blood pressure and no change in mean arterial pressure (Figure 1a). There is an increase in aPWV and decrease in carotid distensibility with age (Figure 1b). Of the 4088 subjects in this study, 1597 (39.1%) subjects had ISH and 414 (10.1%) subjects had Sys/Dia HT. Subjects with ISH were older compared to normotensives and subjects with Sys/Dia HT ($p < 0.001$).

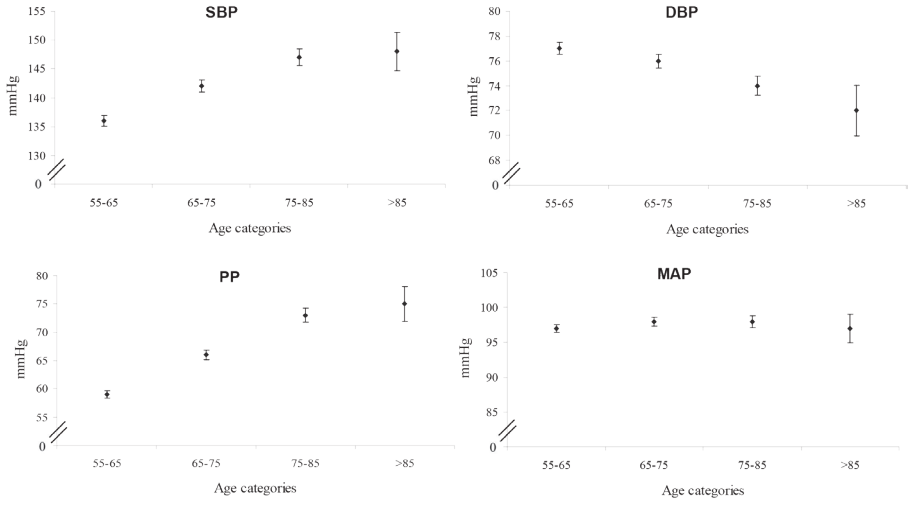


Figure 1a. Blood pressure components in age categories

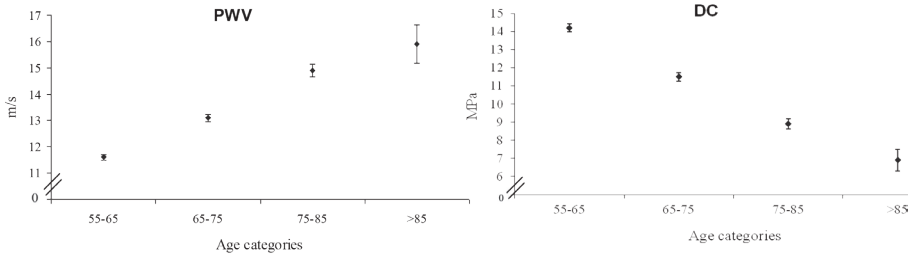


Figure 1b. Pulse wave velocity and carotid distensibility in categories of age

SBP Systolic blood pressure; DBP diastolic blood pressure; MAP mean arterial pressure; PP pulse pressure; aPWV aortic pulse wave velocity; DC; Carotid distensibility coefficient

Of the traditional cardiovascular risk factors, age (per standard deviation; OR 1.78; 95% CI 1.64-1.94), body mass index (per standard deviation; OR 1.26; 95% CI 1.17-1.35) and diabetes mellitus (OR 1.54; 95% CI 1.23-1.93) were strongly associated with ISH. Male gender (OR 1.68; 95% CI 1.33-2.13), body mass index (per standard deviation; OR 1.45; 95% CI 1.30-1.61) and diabetes mellitus (OR 1.53; 95% CI 1.10-2.14) were strongly associated with Sys/Dia HT. Age was only weakly associated with Sys/Dia HT (per standard deviation; OR 1.14; 95% CI 0.99-1.30) (Table 2).

APWV and carotid distensibility were strongly associated with both ISH and Sys/Dia HT. OR's and corresponding 95% CI of aPWV, after adjustment for cardiovascular risk factors, were 1.53 (1.38-1.71) for ISH and 1.28 (1.09-1.53) for Sys/Dia HT. OR's for carotid distensibility were 0.84 (0.75-0.94) for ISH and 0.66 (0.54-0.81) for Sys/Dia HT (Table 2).

Table 2: Odds ratio's for ISH and Sys/Dia HT associated with cardiovascular risk factors and measures of arterial stiffnessrs (per 1-sd

	Odds Ratio, 95% CI ISH (n= 1597)	Odds Ratio, 95% CI Sys/Dia HT (n=414)
Age	1.78 (1.64-1.94)	1.14 (0.99-1.30)
Male gender	1.11 (0.96-1.30)	1.68 (1.33-2.13)
Body mass index	1.26 (1.17-1.35)	1.45 (1.30-1.61)
Total cholesterol	1.06 (0.98-1.13)	1.04 (0.93-1.17)
High-density cholesterol	0.97 (0.90-1.05)	1.03 (0.91-1.16)
Current smoking	0.82 (0.69-0.98)	0.72 (0.54-0.97)
Diabetes mellitus	1.54 (1.23-1.93)	1.53 (1.10-2.14)
Aortic pulse wave velocity*	1.53 (1.38-1.71)	1.28 (1.09-1.53)
Carotid distensibility*	0.84 (0.75-0.94)	0.66 (0.54-0.81)

Multinomial logistic regression with normotensives as reference outcome category
All models are adjusted for age, gender, cohort, body mass-index, total cholesterol, high-density cholesterol, smoking and diabetes mellitus, when appropiate
* additionally adjusted for mean arterial pressure and heart rate
CI confidence interval; ISH isolated systolic hypertension; Sys/Dia HT=combined systolic and diastolic hypertension

After adjustment for age, gender, mean arterial pressure and heart rate, subjects with ISH had higher aPWV values, compared to normotensives ($p<0.001$) and subjects with Sys/Dia HT ($p=0.008$). Subsequently, had subjects with ISH lower carotid distensibility values compared to normotensives, but compared to subjects with Sys/Dia HT there was no difference ($p = 0.071$) (Table 3).

Table 3: Measures of arterial stiffness in subtypes of hypertension.

	Normotensive	ISH	Sys/Dia HT
N (%)	2077 (50.8)	1597 (39.1)	414 (10.1)
Age (years)	66.6	70.5 *‡	66.7
Male gender (%)	43.6	45.1‡	54.6 *
aPWV (m/s)	12.5	13.2 *‡	12.9 †
DC (1/MPa)	12.4	11.8 *	11.3 *

Models are adjusted for age, gender, mean arterial pressure and heart rate, when appropriate.

* significantly different from normotensive at p<0.001

† significantly different from normotensive at p<0.05

‡ significantly different from Sys/Dia HT at p<0.05

aPWV aortic pulse wave velocity; DC carotid distensibility coefficient; Sys/Dia HT combined systolic and diastolic hypertension; ISH isolated systolic hypertension

Age significantly modified the relation between aPWV and subtypes of hypertension (p=0.001), this was not the case for the relation between carotid distensibility and subtypes of hypertension (Table 4). Aortic PWV increased with increasing age for all subtypes of hypertension (Figure 2), this increase was stronger among normotensives and among subjects with ISH compared to subjects with Sys/Dia HT resulting in higher mean values in subjects with ISH compared to subjects with Sys/Dia HT.

There was no significant interaction between arterial stiffness and subtypes of hypertension according to categories of gender, diabetes mellitus and previous CHD.

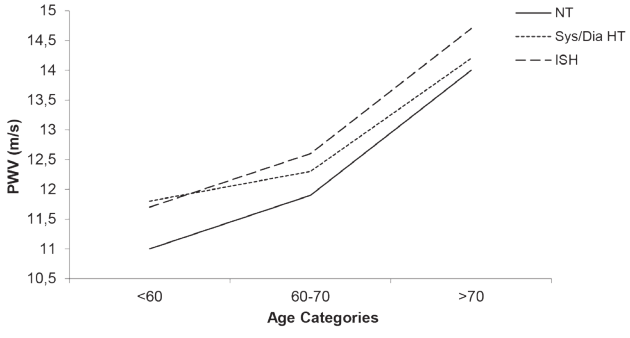


Figure 2.

Figure 2. Pulse wave velocity by age in different subtypes of hypertension.

aPWV: aortic pulse wave velocity; NT normotension; ; ISH isolated systolic hypertension; Sys/Dia HT=combined systolic and diastolic hypertension

Discussion

In the present study performed in a large middle-aged and elderly untreated population, we found significant associations of both aPWV and carotid distensibility with ISH and Sys/Dia HT. Subjects with ISH had higher values of aortic stiffness when compared to subjects with Sys/Dia HT. The difference was largest among older subjects.

The main finding of this paper was that subjects with ISH have a stiffer aorta, as measure with aPWV, than subjects with Sys/Dia HT. Franklin et al[3] showed already two decades ago that there is a reduction of diastolic blood pressure after age 60 and an increase in systolic blood pressure, resulting in a steep increase of pulse pressure. The most likely explanation is the age related stiffening of the aorta. This has been confirmed in several cross sectional studies, showing that subjects with ISH have higher values of arterial stiffness compared to normotensive subjects[4,5] and has also been confirmed in young adults and elderly women[7]. Furthermore, it is traditionally believed that arterial stiffening is accelerated by higher mean and systolic blood pressures because of structural and functional alterations in the walls of the central elastic arteries in response to the chronically elevated distending pressures[20]. Recently, the Baltimore Longitudinal Study of Aging (BLSA) showed prospectively that arterial stiffening precedes and predisposes to accelerated longitudinal increases in systolic blood pressure and to future hypertension, suggesting that arterial stiffening is an underlying pathophysiological cause of the increase in pressure[8].

Interestingly, we found a higher value of carotid distensibility in subjects with ISH than compared with subjects with Sys/Dia HT, indicating more elastic carotid arteries in subjects with ISH compared to subjects with Sys/Dia HT. You might speculate about this difference in carotid stiffness compared to aortic stiffness. The arterial tree is not a homogenous system and there are differences in the structure and function of various arteries. Carotid distensibility is a local measure of carotid stiffness, whereas aPWV is a more regional measure of arterial stiffness, combining the central elastic aorta and more muscular illiaca and femoral arteries[21]. Although carotid femoral pulse wave velocity and carotid stiffness provide similar information on the impact of aging on large artery stiffness in normal subjects, this is not the case for subjects with cardiovascular risk factors, such as hypertension and diabetes mellitus. The influence of hypertension on the different parts of the arterial tree has been shown in a paper by Laurent et al, in which there is a reduced distensibility in the proximal large arteries compared to the medium-sized distal arteries[22]. In subjects with type 1 diabetes mellitus, there is an alteration of aortic distensibility earlier in disease process, compared to carotid distensibility[23]. In addition, it has been shown that the correlation between aortic stiffness and carotid stiffness becomes weaker as the number of cardiovascular risk factors increase. The discrepancies between aortic stiffness and carotid stiffness result from different influences of cardiovascular risk factors on both parameters[24].

Table 4: Mean values of aPWV and carotid distensibility in subtypes of hypertension according to age

	n	Normotensive aPWV*	ISH aPWV*	Sys/Dia HT aPWV*
Age				
<60	422/186/84	11.0 ± 0.10	11.7 ± 0.14†	11.8 ± 0.24†
60-70	1035/601/203	11.9 ± 0.07	12.6 ± 0.09†	12.3 ± 0.16
>70	620/810/127	14.0 ± 0.12	14.7 ± 0.10†	14.2 ± 0.26

	n	Normotensive DC*	ISH DC*	Sys/Dia HT DC*
Age				
<60	369/161/76	15.4 ± 0.24	14.5 ± 0.34†	13.8 ± 0.57†
60-70	927/508/173	13.2 ± 0.13	13.0 ± 0.17	12.5 ± 0.31
>70	552/676/112	9.9 ± 0.13	9.2 ± 0.12†	9.2 ± 0.29†

*Adjusted mean ± standard error. Model is adjusted for age, gender, cohort, body mass-index,
total cholesterol, high-density cholesterol, smoking and diabetes mellitus
† significantly different from normotensive at $p<0.05$
ISH: isolated systolic hypertension; Sys/Dia HT: combined systolic and diastolic hypertension;
aPWV: aortic pulse wave velocity; DC: Carotid distensibility coefficient;
n: number of subjects, normotensives, subjects with ISH, subjects with Sys/Dia HT,
respectively per age categories

We confirmed previous observations that with increasing age there is a rise of systolic blood pressure and pulse pressure and a reduction of diastolic blood pressure, implying that ISH is the most prevalent form of hypertension in elderly[3]. Increasing age is also associated with increased arterial stiffness even in healthy normotensive individuals, confirming previous observations that ISH is primarily associated with stiffening of the central arteries[4,5,7]. The association remained after correction for mean arterial pressure, suggesting an increase in isobaric stiffness rather than a passive rise because of an increase in mean pressure[25,26]. Proposed mechanisms underlying arterial stiffening include degeneration of elastin, endothelial wall dysfunction and calcium deposition in the vascular wall[27]. Enzymes degrading elastin, including MMP-9, MMP-2 and various serine proteases, were all correlated with aortic stiffness[28], suggesting that extracellular matrix remodeling contributes to stiffening of the aorta. Wallace et al. showed that endothelial function was independently associated with aortic stiffness[4] and several studies have shown that calcification was correlated with stiffer central arteries[29,30].

We found an age-dependency of the relation of aortic stiffness with ISH and Sys/Dia HT. We found a stronger age-related increase of aortic stiffness in subjects with ISH when compared with subjects with Sys/Dia HT. Interestingly, the aorta of the very old subjects with Sys/Dia HT was as stiff as the aorta of normotensive subjects. The main physiological abnormality in the Sys/Dia HT is an increased MAP concomitant with a higher peripheral vascular resistance. Although, previous studies have shown that central 'elastic' arteries are also stiffer in subjects with Sys/Dia HT [31,32], it is less clear whether this increased aortic stiffness is simply due to the higher operating pressure of hypertensive arteries. Studies, in which mean pressure was reduced, showed a normalization of aortic stiffness values, suggesting that isobaric stiffness was normal in hypertensive subjects[33,34]. The finding of this paper reinforces the hypothesis that Sys/Dia HT is not a pathophysiological result of stiff arteries.

Several issues regarding the methods of the present study need to be discussed. First, the measures of stiffness were not available for all the participants; it might be that information was missing mostly in those subjects with a higher cardiovascular risk load, though missing information was mostly due to logistic reasons and therefore mostly random. Second, the measurements of aortic and carotid stiffness were performed only once; it is likely that the use of multiple measurements would have improved accuracy and precision. Third, in computing the carotid distensibility coefficient, we used the brachial pulse pressure rather than the carotid pulse pressure. Information on comparisons between carotid and brachial pulse pressures indicates that the difference between these pressures is 8 mmHg in a presumed healthy population and 2.6 mmHg in patients with severe coronary artery disease[35]. These findings indicate that using brachial artery pulse pressure instead of carotid artery pulse pressure may have led to an underestimation of the distensibility, different in subjects with and without cardiovascular disease. This may lead to an underestimation of the association with disease. It has been suggested to derive carotid artery pulse pressure using brachial artery pulse pressure[36]. However, to perform this procedure brachial mean pressure should be directly measured, while in our study this was computed from blood pressure components. Fourth, we conducted an observational cross-sectional study; therefore we cannot determine the directionally of the observed associations. Finally, the present results are obtained in a Caucasian population and therefore cannot be extrapolated to other populations.

In summary, in this population of untreated subjects, we found significant associations of both aPWV and carotid distensibility with ISH and Sys/Dia HT. Subjects with ISH had higher values of aortic stiffness when compared with subjects with Sys/Dia HT, which was primarily present in older subjects without treatment for hypertension.

References

1. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. Lancet. May 3 2008;371(9623):1513-1518.
2. Franklin SS, Jacobs MJ, Wong ND, L'Italien GJ, Lapuerta P. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. Hypertension. Mar 2001;37(3):869-874.
3. Franklin SS, Gustin Wt, Wong ND, et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation. Jul 1 1997;96(1):308-315.
4. Wallace SM, Yasmin, McEniery CM, et al. Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. Hypertension. Jul 2007;50(1):228-233.
5. Yasmin, McEniery CM, O'Shaughnessy KM, et al. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. Arterioscler Thromb Vasc Biol. Aug 2006;26(8):1799-1805.
6. McEniery CM, Yasmin, Wallace S, et al. Increased stroke volume and aortic stiffness contribute to isolated systolic hypertension in young adults. Hypertension. Jul 2005;46(1):221-226.
7. Berry KL, Cameron JD, Dart AM, et al. Large-artery stiffness contributes to the greater prevalence of systolic hypertension in elderly women. Journal of the American Geriatrics Society. Mar 2004;52(3):368-373.
8. Najjar SS, Scuteri A, Shetty V, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. Journal of the American College of Cardiology. Apr 8 2008;51(14):1377-1383.
9. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. Feb 7 2006;113(5):657-663.
10. Verwoert GC, Elias-Smale SE, Rizopoulos D, et al. Does aortic stiffness improve the prediction of coronary heart disease in elderly? The Rotterdam Study. Journal of human hypertension. Jan 13 2011.
11. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. Aug 2011;26(8):657-686.
12. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. Sep 1995;26(3):485-490.
13. Hoeks AP, Brands PJ, Smeets FA, Reneman RS. Assessment of the distensibility of superficial arteries. Ultrasound Med Biol. 1990;16(2):121-128.
14. Reneman RS, van Merode T, Hick P, Muytjens AM, Hoeks AP. Age-related changes in carotid artery wall properties in men. Ultrasound Med Biol. Jun 1986;12(6):465-471.
15. van Popele NM, Elizabeth Hak A, Mattace-Raso FU, et al. Impaired fasting glucose is associated with increased arterial stiffness in elderly people without diabetes mellitus: the Rotterdam Study. Journal of the American Geriatrics Society. Mar 2006;54(3):397-404.

16. Mancia G, De Backer G, Dominiczak A, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension. Jun 2007;25(6):1105-1187.
17. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. Jul 1997;20(7):1183-1197.
18. Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral arterial disease in the elderly: The Rotterdam Study. Arterioscler Thromb Vasc Biol. Feb 1998;18(2):185-192.
19. Leening MJG, Kavousi M, Heeringa J, et al. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. European journal of epidemiology. Mar 2012;27(3):173-185.
20. O'Rourke MF, Nichols WW. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. Hypertension. Apr 2005;45(4):652-658.
21. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. Nov 2006;27(21):2588-2605.
22. Laurent S, Hayoz D, Trazzi S, et al. Isobaric compliance of the radial artery is increased in patients with essential hypertension. Journal of hypertension. Jan 1993;11(1):89-98.
23. Giannattasio C, Failla M, Grappiolo A, Gamba PL, Paleari F, Mancia G. Progression of large artery structural and functional alterations in Type I diabetes. Diabetologia. Feb 2001;44(2):203-208.
24. Paini A, Boutouyrie P, Calvet D, Tropeano AI, Laloux B, Laurent S. Carotid and aortic stiffness: determinants of discrepancies. Hypertension. Mar 2006;47(3):371-376.
25. Nichols WW, Nicolini FA, Pepine CJ. Determinants of isolated systolic hypertension in the elderly. J Hypertens Suppl. Aug 1992;10(6):S73-77.
26. Meaney E, Soltero E, Samaniego V, et al. Vascular dynamics in isolated systolic arterial hypertension. Clinical cardiology. Dec 1995;18(12):721-725.
27. McEniery CM, Wilkinson IB, Avolio AP. Age, hypertension and arterial function. Clinical and experimental pharmacology & physiology. Jul 2007;34(7):665-671.
28. Yasmin, McEniery CM, Wallace S, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler Thromb Vasc Biol. Feb 2005;25(2):372.
29. Odink AE, Mattace-Raso FU, van der Lugt A, et al. The association of arterial stiffness and arterial calcification: the Rotterdam study. Journal of human hypertension. Mar 2008;22(3):205-207.
30. McEniery CM, McDonnell BJ, So A, et al. Aortic calcification is associated with aortic stiffness and isolated systolic hypertension in healthy individuals. Hypertension. Mar 2009;53(3):524-531.
31. Asmar R, Benetos A, London G, et al. Aortic distensibility in normotensive, untreated and treated hypertensive patients. Blood Press. Jan 1995;4(1):48-54.
32. Safar ME, Laurent S. Behaviour of conduit arteries in hypertension. Clin Exp Hypertens. Nov 1993;15(6):1033-1045.

33. Ting CT, Brin KP, Lin SJ, et al. Arterial hemodynamics in human hypertension. The Journal of clinical investigation. Dec 1986;78(6):1462-1471.
34. Merillon JP, Fontenier GJ, Lerallut JF, et al. Aortic input impedance in normal man and arterial hypertension: its modification during changes in aortic pressure. Cardiovascular research. Nov 1982;16(11):646-656.
35. Waddell TK, Dart AM, Medley TL, Cameron JD, Kingwell BA. Carotid pressure is a better predictor of coronary artery disease severity than brachial pressure. Hypertension. Oct 2001;38(4):927-931.
36. Van Bortel LM, Balkestein EJ, van der Heijden-Spek JJ, et al. Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. Journal of hypertension. Jun 2001;19(6):1037-1044.

Chapter 4.2
Orthostatic hypotension and the risk of cardiovascular disease

Based on

Orthostatic hypotension and risk of cardiovascular disease in elderly people: the Rotterdam study. Journal of the American Geriatric Society 2008 Oct; 56:1816-20.

Abstract

Background

Orthostatic hypotension is common in the elderly and has been associated with cardiovascular morbidity and mortality in studies performed in younger subjects. Aim of the present study was to investigate the prognostic role of orthostatic hypotension in the elderly.

Methods

The risk of coronary heart disease, stroke, heart failure and all-cause mortality associated with orthostatic hypotension, was investigated with Cox proportional hazard models in 5064 subjects of the Rotterdam study, a large ongoing population-based study, performed in subjects aged 55 years and older.

Results

At baseline, 901 subjects had orthostatic hypotension. During follow-up, 668 subjects had coronary heart disease (CHD) (mean follow-up 6.0 ± 3.5 years), and 1,835 subjects died (mean follow-up period 7.8 ± 3.8 years). Orthostatic hypotension increased the risk of CHD (hazard ratio (HR) 1.31, 95% confidence interval (CI) 1.08–1.57) and all-cause mortality (HR1.22, 95% CI 1.09–1.36), in models adjusted for age and sex. The risk was slightly lower after additional adjustment for cardiovascular risk factors. In analyses stratified for age, the HRs for all-cause mortality were 1.80 (95% CI 1.25–2.60), 1.13(0.89–1.42), and 1.27 (95% CI 1.11–1.44), in the first, second, and third tertile of age, respectively.

Conclusions

Orthostatic hypotension increases the risk of CHD and all-cause mortality in elderly people. The risk of CVD and mortality is strongest in younger and very old subjects.

Introduction

Orthostatic hypotension is common in the elderly[1-4] and is associated with syncope[5,6], falls[7], fractures and potential morbidity, leading to functional impairment and increased hospitalization[8]. Several studies have investigated the relation between orthostatic hypotension and cardiovascular diseases. Well-designed population-based prospective large cohort studies, have found that orthostatic hypotension increases the risk of stroke[9], coronary heart disease[10] (CHD) and mortality[11] in middle-aged subjects. Other studies have investigated the prognostic role of orthostatic hypotension in the elderly; however, results were contradictory[3,12-15] or performed in specific categories of patients[16-18]. No previous study has investigated the relation between orthostatic hypotension and the risk of heart failure.

The aim of the present study is to investigate whether orthostatic hypotension increases the risk of primary cardiovascular disease and all-cause mortality in the elderly. The present study is performed within the framework of the Rotterdam study, a large population-based prospective ongoing study, which has included subjects aged 55 years and over at baseline.

Methods

Study Population

The Rotterdam study is a population-based prospective cohort study comprising 7,983 subjects aged 55 and over living in Ommoord, a suburb of Rotterdam, The Netherlands. Overall aim of the Rotterdam study is assessing the occurrence of and risk factors for chronic diseases in the elderly. The study design and objectives of the Rotterdam study are described elsewhere[19]. At baseline, a trained interviewer visited the 7983 subjects at home for a computerized questionnaire. Participants visited the research center for the baseline examination from March 1990 to July 1993. During these visits at the research center, blood pressure measurements were obtained and established cardiovascular risk factors were measured. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.

Previous Cardiovascular Disease and Cardiovascular Risk Factors

A history of CHD, stroke and heart failure was obtained through direct questioning and considered positive when confirmed by hospital discharge date or written information from the subjects general practitioner, as described previously[20-22]. CHD was defined as fatal or non-fatal myocardial infarction, a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft, sudden cardiac death and death due to ventricular fibrillation and congestive heart failure. A history of myocardial infarction was considered present in case of self-report of myocardial infarction confirmed by electrocardiogram or additional clinical information, or the presence of an electrocardiogram characteristic of prior myocardial infarction. Information on smoking habits and the use of anti-hypertensive medication were obtained during the interview. Smoking was divided in three categories namely current, former and non-smokers. Height and weight were measured,

and the body mass index (BMI) was computed (Kg/m2). Diabetes mellitus was defined as the use of blood glucose lowering medication or a random or post-load serum glucose level 11.1 mmol/l.[23] Serum total cholesterol and high-density lipoproteins (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System, Mannheim, Germany). Two seated blood pressure measurements are obtained at the right brachial artery with a random zero sphygmomanometer. The mean of two consecutive measurements was used. Hypertension was defined as systolic blood pressure≥ 140 mmHg and/or diastolic blood pressure≥ 90 mmHg and/or the use of anti-hypertensive medication. Orthostatic hypotension was measured with a Dinamap automatic blood pressure recorder (Dinamap R, Tampa, USA). The baseline reading is the mean of two blood pressure measurements on the right upper arm with the subject in supine position after five minutes of rest. Measurements were repeated in the standing position after one, two and three minutes. Orthostatic hypotension is defined as a decline in systolic blood pressure of ≥20 mmHg and/or decline in diastolic blood pressure of ≥10 mmHg from supine to standing position at any of the three measurements.

Incident Cardiovascular Events and All-cause Mortality

Information on fatal and non-fatal cardiovascular outcomes was obtained through automated linkage with the files from general practitioners and letters and discharge reports from medical specialists. When a cardiovascular event was reported, the research assistants collected additional information from medical records of the general practitioner and in addition, obtained information from the hospital discharge records or nursing home records including letters from medical specialists. For the diagnosis of cardiac events, two research physicians independently coded all reported events. In case of disagreement, a medical expert in the field made a decision. In case of stroke, two research physicians and an experienced neurologist coded the events. Codes were assigned according to the International Classification of Diseases (ICD-10), 10th edition[24]. Coronary heart disease was defined as the occurrence of a fatal or non-fatal myocardial infarction (ICD-10 code I21), a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft, other forms of acute (I24) or chronic ischemic heart disease (I25), sudden cardiac death (I46 and R96), and death due to ventricular fibrillation (I49) and congestive heart failure (I50). ICD-10 codes used for coding stroke were I61, I63 and I64. Heart failure was determined by using a validated score, which is similar with the definition of heart failure of the European Society of Cardiology[25]. Information on vital status was acquired at regular intervals from the municipal authorities of Rotterdam. For the present study, follow up was completed for stroke and CHD till January 2005, for heart failure and all-cause mortality till October 2006.

Population for Analysis

In total 7,983 subjects (response rate 78%) agreed to participate and were interviewed at home, and 7,129 subjects visited the research center for physical examinations. Blood pressure measurements both in supine and standing position were available in 6,455 participants. Subjects with CHD (n=743), stroke (n=164) or heart failure (n=194) at baseline and subjects with missing data on smoking (n=357), use of anti-hypertensive medication (n=2,191), BMI (n=1,073), blood pressure (n=975), diabetes mellitus (n=202), total cholesterol (n=944) and HDL cholesterol (n=973) were

excluded from the analyses. The number of missing is overlapping, leaving a total number of 5,064 subjects available for this analysis.

Data Analysis

The baseline characteristics of the subjects with and without orthostatic hypotension are compared with Student t tests for continuous variables and with the chi-square test for categorical variables. A power calculation was performed, this showed a power of 96% to reach a hazard ratio of 2 in a population of 5064 subjects. We used Cox-proportional hazard models to estimate hazard ratios with corresponding 95% confidence intervals for CHD, stroke, heart failure and all-cause mortality by orthostatic hypotension status. The first model was adjusted for age and gender. Model two was additionally adjusted for smoking, use of anti-hypertensive medication, BMI, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol and HDL cholesterol. To investigate whether the prognostic role of orthostatic hypotension varied with aging together with the age-related changes of cardiovascular risk load, we performed analyses in tertiles of age, gender, hypertension status and diabetes mellitus status. We considered statistical significance when the p-value was below 0.05. All analyses are performed using SPSS 11.0 statistical package for Windows 2000 (SPSS Inc., Chicago, Illinois, USA).

Table 1. Baseline Characteristics of the Study Subjects (n=5064)

Characteristics	Total subjects (n= 5,064)	Subjects without OH (n=4,163)	Subjects with OH (n=901)	P value*
Men (%)	38.4 %	40.1 %	30.9 %	< .001
Age (years)	68.1 ± 8.5	67.3 ± 8.2	71.8 ± 8.8	< .001
Smoking (%)				
Current smoker	22.4 %	22.3 %	23 %	0.667
Former smoker	41.6 %	42.6 %	37 %	0.002
Never smoker	36.0 %	35.1 %	40 %	0.005
Anti-hypertensive medication (%)	26.9 %	25.3 %	34.3 %	< .001
Body mass index (kg/m2)	26.2 ± 3.6	26.2 ± 3.5	26.5 ± 4.0	.035
Systolic blood pressure (mmHg)	139 ± 22	138 ± 22	144 ± 23	< .001
Diastolic blood pressure (mmHg)	74 ± 11	74 ± 11	74 ± 12	.935
Diabetes mellitus (%)	8.9 %	8.3 %	11.5 %	.002
Total cholesterol (mmol/L)	6.66 ± 1.22	6.65 ± 1.20	6.69 ± 1.33	.349
HDL cholesterol (mmol/L)	1.37 ± 0.38	1.37 ± 0.37	1.35 ± 0.40	.075

Values with a ± sign are the mean ± SD or percentage

* Test of difference between means was performed using Student t test, and test of differences between proportions was performed with chi-square test.

OH= orthostatic hypotension, HDL= High-density lipoprotein

Results

The baseline characteristics of the 5,064 subjects are shown in table 1. Thirty-eight percent of the subjects were men and the mean age was 68 years. Of the 5,064 subjects in this study, 901 (17.8%) subjects had orthostatic hypotension; the prevalence of orthostatic hypotension was higher in women than in men (P< .001). Subjects with orthostatic hypotension were older (P< .001), used more often anti-hypertensive medication (P< .001), had higher BMI (P= .035), had higher systolic blood pressure levels (P< .001) and had higher prevalence of diabetes mellitus (P= .002). During follow-up had 668 subjects CHD (mean follow-up period 6.0±3.5 years), 503 subjects had a stroke (mean follow-up period 6.7±3.6 years), 571 subjects developed heart failure (mean follow-up period 6.6±3.9 years) and 1,835 subjects died (mean follow-up period 7.8±3.8 years). Of the 901 subjects with orthostatic hypotension at baseline 152 subjects had CHD, 119 subjects had stroke, 140 subjects had heart failure and 465 subjects died, during follow-up. In models adjusted for age and gender, orthostatic hypotension predicted CHD (HR 1.31; 95% CI 1.08-1.57), heart failure (HR 1.21; 95% CI 1.00-1.48) and all-cause mortality (HR 1.22; 95% CI 1.09-1.36) (table 2). The association between orthostatic hypotension and heart failure was less consistent in models adjusted for cardiovascular risk factors. To study whether the association between orthostatic hypotension and incident events varied by age, gender, hypertension status and diabetes mellitus status, stratified analyses were performed. Mean age was for age-specific tertiles 59.3 (range 55-63), 67.0 (range 63.1-71.3), 78.0 (range 71.4-98.7), in the first, second and third tertile, respectively. Hypertension was present in 57,6 % and diabetes mellitus was present in 8,9% of the participants. Among the younger subjects (first tertile), orthostatic hypotension predicted all-cause mortality (HR 1.80; 95% CI 1.25-2.60) and showed a trend for CHD (HR 1.56; 95% CI 0.99-2.46). In the older subjects (third tertile) orthostatic hypotension predicted stroke (HR 1.35; 95% CI 1.04-1.75), heart failure (HR 1.32; 95% CI 1.04-1.67) and all-cause mortality (HR 1.27; 95% CI 1.11-1.44) (table 3). In hypertensive participants, orthostatic hypotension increased the risk of CHD (HR 1.27; 95% CI 1.03-1.57) and all-cause mortality (HR 1.20; 95% CI 1.06-1.36). In subjects with diabetes mellitus, orthostatic hypotension gave a twofold increase risk of heart failure (HR 1.97; 95% CI 1.22-3.19).

Table 2: Cox Proportional Hazards Regression Models of Orthostatic Hypotension and Risk of CHD, Stroke, Heart Failure and All-cause Mortality.

	CHD	P value	Stroke	P value
	(668/5,064) HR (95% CI)		(503/5,064) HR (95% CI)	
Model 1	1.31 (1.08-1.57)	0.005	1.17 (0.95-1.45)	0.13
Model 2	1.20 (1.00-1.45)	0.05	1.10 (0.89-1.36)	0.35

Model 1 adjusted for age and gender

Model 2 adjusted for model 1 + smoking, use of anti-hypertensive medication, body mass index, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol

CHD = Coronary heart disease, HR = Hazard ratio, CI = Confidence interval

Table 3: Stratified Proportional Hazard Models of Orthostatic Hypotension and Risk of CHD, Stroke, Heart Failure and All-cause Mortality

	CHD Events/ subjects	HR (95% CI)	Stroke Events/ subjects	HR (95% CI)
Gender				
Male	343/1,946	1.18 (0.88-1.57)	198/1,946	1.09 (0.76-1.58)
Female	325/3,118	1.22 (0.95-1.56)	305/3,118	1.10 (0.85-1.42)
Age				
Tertile 1	154/1,688	1.56 (0.99-2.46)	61/1,688	1.56 (0.76-3.20)
Tertile 2	230/1,689	1.14 (0.81-1.62)	169/1,689	0.76 (0.49-1.19)
Tertile 3	284/1,687	1.21 (0.94-1.57)	273/1,687	1.35 (1.04-1.75)
Hypertension				
No	198/2,146	0.93 (0.61-1.40)	129/2,146	0.90 (0.55-1.46)
Yes	470/2,918	1.27 (1.03-1.57)	374/2,918	1.14 (0.90-1.44)
Diabetes Mellitus				
No	576/4,615	1.15 (0.94-1.41)	422/4,615	1.16 (0.92-1.46)
Yes	92/449	1.36 (0.84-2.21)	81/449	0.91 (0.52-1.61)

Model adjusted for age, gender, smoking, use of anti-hypertensive medication, body mass index, systolic blood pressure, diastolic blood pressure, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol.(When appropriate) CHD = Coronary heart disease, HR = Hazard ratio, CI = Confidence interval

Heart failure	P value	All-cause mortality	P value
(571/5,064)		(1835/5,064)	
HR (95% CI)		HR (95% CI)	
1.21 (1.00-1.48)	0.05	1.22 (1.09-1.36)	<0.001
1.12 (0.92-1.36)	0.27	1.16 (1.04-1.29)	0.007

Heart failure		All-cause mortality	
Events/ subjects	HR (95% CI)	Events/ subjects	HR (95% CI)
249/1,946	0.95 (0.67-1.33)	802/1,946	1.27 (1.07-1.51)
322/3,118	1.22 (0.96-1.56)	1033/3,118	1.10 (0.95-1.26)
64/1,688	1.13 (0.53-2.41)	211/1,688	1.80 (1.25-2.60)
183/1,689	0.91 (0.61-1.37)	501/1,689	1.13 (0.89-1.42)
324/1,687	1.32 (1.04-1.67)	1123/1,687	1.27 (1.11-1.44)
126/2,146	0.99 (0.61-1.60)	564/2,146	1.05 (0.84-1.30)
445/2,918	1.14 (0.92-1.42)	1271/2,918	1.20 (1.06-1.36)
485/4,615	1.00 (0.80-1.24)	1579/4,615	1.15 (1.02-1.29)
86/449	1.97 (1.22-3.19)	256/449	1.29 (0.97-1.72)

Discussion

In the present study orthostatic hypotension increased slightly the risk of CHD and all-cause mortality in apparently healthy elderly. Associations between orthostatic hypotension and cardiovascular risk were strongest in the relatively younger and very old participants.

Previous studies have investigated the relation between orthostatic hypotension and the risk of CHD, stroke and mortality. The atherosclerosis risk in communities (ARIC) study, a large population-based prospective cohort study which included middle-aged participants (45-65 years old), found strong associations between orthostatic hypotension and stroke9, CHD10 and mortality[11]. Other studies were performed in the older subjects. The Honolulu Heart program[17], a prospective study of 3,741 Japanese men aged 71 to 93 years, found that orthostatic hypotension increased the risk of all-cause mortality in elderly Japanese men. Other authors have shown that orthostatic hypotension increased the risk of myocardial infarction and cardiovascular death in 700 home-dwelling Finnish elderly aged 70 years and older[12,14]. On the contrary, in a study including 500 acute geriatric ward patients[15], and in a study including 350 subjects aged 65 years and over[3], orthostatic hypotension did not increase the risk of vascular death.

As previously reported by others, also in this large population-based study, orthostatic hypotension increased the risk of cardiovascular disease and all-cause mortality in the elderly. According to previous studies[10,18,26], subjects with orthostatic hypotension were older, used more often anti-hypertensive medication, had a higher mean BMI, higher mean levels of systolic blood pressure and more often had diabetes mellitus.

The association between orthostatic hypotension and cardiovascular disease may have several explanations. Orthostatic challenge gives a displacement of blood to the lower body. This could give a decrease in thoracic blood volume from 25 to 30%[27]. The secondary reduction in the coronary and cerebral flow during a strong postural blood pressure drop may cause myocardial and cerebral ischeamia[3]. Orthostatic hypotension could also be the expression of underlying cardiovascular disease. Cerebral infarcts, myocardial infarcts and heart failure[28,29] could cause orthostatic hypotension[3]. Also diabetes mellitus[30] and hypertension[10] may be associated with orthostatic hypotension. Therefore, one possible question could be whether the association between orthostatic hypotension and cardiovascular disease might be explained by these conditions. For this reason, subjects with previous cardiovascular disease were excluded at baseline and analyses adjusted for diabetes mellitus, hypertension and other cardiovascular risk factors were performed. Nevertheless, associations between orthostatic hypotension and CHD and all-cause mortality remained statistically significant after adjustments, suggesting an independent prognostic role of orthostatic hypotension. The predictive role of orthostatic hypotension was strongest in the relatively younger and the very old subjects. Some studies have suggested that orthostatic hypotension is a marker of frailty in the very old[17], whereas the prevalence of orthostatic hypotension is much lower in the relatively younger subjects. Therefore, it might be speculated that the presence of orthostatic hypotension in younger adults might be the expression of an underlying silent cardiovascular disease.

It is known that hypertension is associated with orthostatic hypotension[4,26], particularly in the elderly, and that both might be the expression of blood pressure dysfunction; in the present study we found that in hypertensives orthostatic hypotension increased the risk of CHD and all-cause mortality.

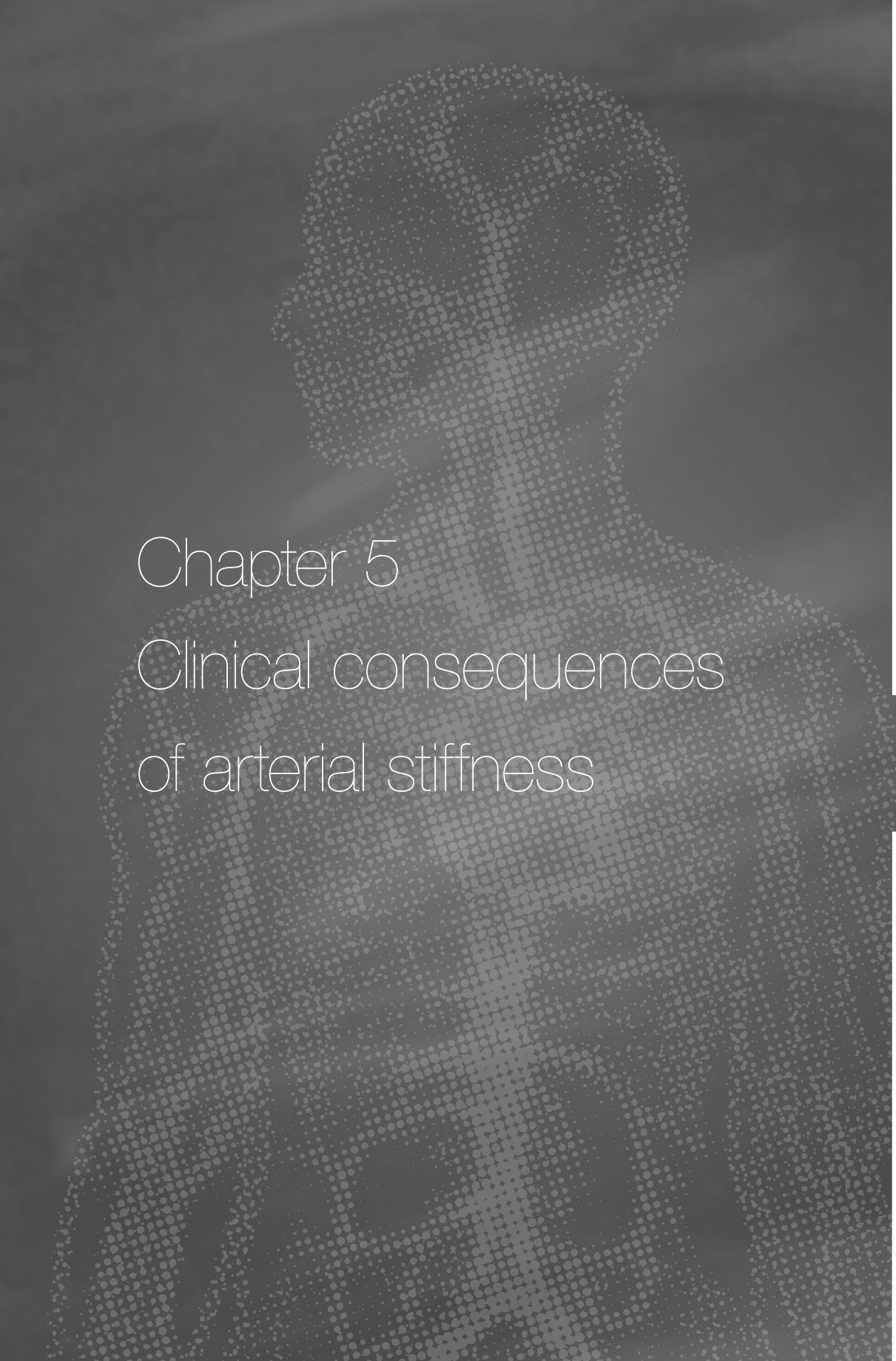
The present study has some limitations. First, blood pressure measurements were performed once, which could bias our results because of the day variability of blood pressure. The use of multiple blood pressure measurements might have improved accuracy and precision. Second, measures of orthostatic hypotension and blood pressure were not available for all participants. However, in our opinion, this will not have biased our results, since this was almost entirely due to logistic reasons and therefore random. Third, the study subjects were Caucasian, therefore generalizability of the results might be difficult. Fourth, there could be some residual confounding. Orthostatic hypotension increases the risk of falls and consequent hip fractures and these are associated with high mortality rate. However, since we did not adjust for these variables we cannot assess this relation. Finally, no correction for multiple comparisons was performed; however, we do not think that this might have biased our results.

In conclusion, orthostatic hypotension increases the risk of CHD and all-cause mortality in apparently healthy elderly subjects. These findings support the call[11] to understand the mechanisms responsible for cardiovascular morbidity and mortality in subjects with orthostatic hypotension. There is much evidence that orthostatic hypotension increases cardiovascular risk in the elderly[9-12,14,17], future studies are needed to investigate whether identification and treatment of orthostatic hypotension can improve cardiovascular prognosis.

References

1. Caird FI, Andrews GR, Kennedy RD. Effect of posture on blood pressure in the elderly. Br Heart J. May 1973;35(5):527-530.
2. Mader SL, Josephson KR, Rubenstein LZ. Low prevalence of postural hypotension among community-dwelling elderly. Jama. Sep 18 1987;258(11):1511-1514.
3. Raiha I, Luutonen S, Piha J, Seppanen A, Toikka T, Sourander L. Prevalence, predisposing factors, and prognostic importance of postural hypotension. Arch Intern Med. May 8 1995;155(9):930-935.
4. Mattace-Raso FU, van der Cammen TJ, Knetsch AM, et al. Arterial stiffness as the candidate underlying mechanism for postural blood pressure changes and orthostatic hypotension in older adults: the Rotterdam Study. Journal of hypertension. Feb 2006;24(2):339-344.
5. Lipsitz LA. Syncope in the elderly patient. Hosp Pract (Off Ed). Oct 30 1986;21(10A):33-44.
6. Lipsitz LA, Pluchino FC, Wei JY, Rowe JW. Syncope in institutionalized elderly: the impact of multiple pathological conditions and situational stress. J Chronic Dis. 1986;39(8):619-630.
7. Tinetti ME, Williams TF, Mayewski R. Fall risk index for elderly patients based on number of chronic disabilities. Am J Med. Mar 1986;80(3):429-434.
8. Shibao C, Grijalva CG, Raj SR, Biaggioni I, Griffin MR. Orthostatic hypotension-related hospitalizations in the United States. Am J Med. Nov 2007;120(11):975-980.
9. Eigenbrodt ML, Rose KM, Couper DJ, Arnett DK, Smith R, Jones D. Orthostatic hypotension as a risk factor for stroke: the atherosclerosis risk in communities (ARIC) study, 1987-1996. Stroke. 2000;31(10):2307-2313.
10. Rose KM, Tyroler HA, Nardo CJ, et al. Orthostatic hypotension and the incidence of coronary heart disease: the Atherosclerosis Risk in Communities study. Am J Hypertens. Jun 2000;13(6 Pt 1):571-578.
11. Rose KM, Eigenbrodt ML, Biga RL, et al. Orthostatic hypotension predicts mortality in middle-aged adults: the Atherosclerosis Risk In Communities (ARIC) Study. Circulation. Aug 15 2006;114(7):630-636.
12. Luukinen H, Koski K, Laippala P, Airaksinen KE. Orthostatic hypotension and the risk of myocardial infarction in the home-dwelling elderly. J Intern Med. Apr 2004;255(4):486-493.
13. Hossain M, Ooi WL, Lipsitz LA. Intra-individual postural blood pressure variability and stroke in elderly nursing home residents. J Clin Epidemiol. May 2001;54(5):488-494.
14. Luukinen H, Koski K, Laippala P, Kivela SL. Prognosis of diastolic and systolic orthostatic hypotension in older persons. Arch Intern Med. Feb 8 1999;159(3):273-280.
15. Weiss A, Beloosesky Y, Kornowski R, Yalov A, Grinblat J, Grossman E. Influence of orthostatic hypotension on mortality among patients discharged from an acute geriatric ward. J Gen Intern Med. Jun 2006;21(6):602-606.
16. Luukinen H, Airaksinen KE. Orthostatic hypotension predicts vascular death in older diabetic patients. Diabetes Res Clin Pract. Feb 2005;67(2):163-166.
17. Masaki KH, Schatz IJ, Burchfiel CM, et al. Orthostatic hypotension predicts mortality in elderly men: the Honolulu Heart Program. Circulation. Nov 24 1998;98(21):2290-2295.

18. Davis BR, Langford HG, Blaufox MD, Curb JD, Polk BF, Shulman NB. The association of postural changes in systolic blood pressure and mortality in persons with hypertension: the Hypertension Detection and Follow-up Program experience. Circulation. Feb 1987;75(2):340-346.
19. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. Eur J Epidemiol. 2007;22(11):819-829.
20. Bots ML, Looman SJ, Koudstaal PJ, Hofman A, Hoes AW, Grobbee DE. Prevalence of stroke in the general population. The Rotterdam Study. Stroke. Sep 1996;27(9):1499-1501.
21. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. Mar 9 2004;109(9):1089-1094.
22. Bleumink GS, Knetsch AM, Sturkenboom MC, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J. Sep 2004;25(18):1614-1619.
23. Diabetes mellitus. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1985;727:1-113.
24. WHO. International statistical classification of diseases and related health problems. 10 ed. Geneva: WHO. 1992.
25. Remme WJ, Swedberg K. Guidelines for the diagnosis and treatment of chronic heart failure. Eur Heart J. Sep 2001;22(17):1527-1560.
26. Rutan GH, Hermanson B, Bild DE, Kittner SJ, LaBaw F, Tell GS. Orthostatic hypotension in older adults. The Cardiovascular Health Study. CHS Collaborative Research Group. Hypertension. Jun 1992;19(6 Pt 1):508-519.
27. Smith JJ, Porth CM, Erickson M. Hemodynamic response to the upright posture. J Clin Pharmacol. May 1994;34(5):375-386.
28. Robertson D, DesJardin JA, Lichtenstein MJ. Distribution and observed associations of orthostatic blood pressure changes in elderly general medicine outpatients. Am J Med Sci. May 1998;315(5):287-295.
29. Kubo SH, Cody RJ. Circulatory autoregulation in chronic congestive heart failure: responses to head-up tilt in 41 patients. Am J Cardiol. Sep 1 1983;52(5):512-518.
30. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care. May 2003;26(5):1553-1579.



Chapter 5
Clinical consequences of arterial stiffness

Chapter 5.1

Aortic stiffness and heart failure

Based on:

Elevated aortic stiffness predicts heart failure in an aging population. The Rotterdam Study. Submitted

Abstract

Background

Hypertension is a well-known risk factor for heart failure. Accumulating evidence suggests that arterial stiffness could also be an important predictor of heart failure.

Methods

Within the framework of the Rotterdam Study, we investigated the role of different blood pressure components and aortic stiffness in predicting incident heart failure in community dwelling elderly. In the present study, we included 5104 subjects with both blood pressure measurements and information on aortic pulse wave velocity. Cox proportional hazard models, adjusted for cardiovascular risk factors, were performed.

Results

The mean age of the subjects was 68.3 years, 44.2% was male. After a mean follow-up of 7.9 years 393 subjects developed heart failure. Systolic blood pressure (hazard ratio 1.22; 95% confidence interval 1.11-1.34), pulse pressure (hazard ratio 1.28; 95% confidence interval 1.16-1.40) and aortic pulse-wave velocity (hazard ratio 1.16; 95% confidence interval 1.05-1.27) were significantly associated with incident heart failure after adjustment for age and gender.

Conclusions

The pulsatile components of blood pressure and aortic stiffness are associated with the risk of heart failure in community dwelling elderly.

Introduction

Heart failure is one of the most prevalent cardiovascular disorders in industrialized countries[1-4], representing a major health problem in aging populations[2,4,5]. Hypertension is a well-established risk factor of heart failure[6]; the prognostic significance of systolic blood pressure has extensively been reported[7,8] and clinical trials have demonstrated a reduction in the incidence of heart failure with lowering of elevated blood pressure[9]. There is evidence that the pulse pressure, an indirect measure of arterial stiffening, is a stronger predictor of cardiovascular morbidity and mortality in the elderly, when compared with other blood pressure components[10]. Pulse pressure has been associated with heart failure in several large studies in middle-aged subjects[8], elderly[11,12] and in subjects with isolated systolic hypertension[13]. However, arterial stiffness can also be directly assessed by measuring the carotid femoral pulse wave velocity representing aortic stiffness[14]. To the best of our knowledge, only one study has investigated the value of aortic pulse wave velocity (aPWV) in predicting heart failure in elderly subjects[15], in which an association between aPWV and heart failure was not found.

The Rotterdam Study, a large ongoing longitudinal population-based study, provides an opportunity to obtain knowledge on long term associations with incident disease in an aging population. We investigated the role of different blood pressure components and aortic stiffness in predicting incident heart failure in community dwelling elderly.

Methods

Study population

The present study was performed within the framework of the Rotterdam Study (RS-I), a population-based prospective cohort study comprising 7983 participants aged 55 and over living in Ommoord, a suburb of Rotterdam, the Netherlands. In 1999, inhabitants who turned 55 years of age or moved into the study district since the start of the study were invited to participate in an extension of the Rotterdam Study (RS-II) of whom 3011 participated (67% response rate).The overall aim of the Rotterdam study is to access the occurrence of and risk factors for chronic diseases in the elderly. The study design and objectives of the Rotterdam study are described elsewhere[16]. During the third examination phase of RS-I (1997-1999) and during the first examination phase of RS-II (2000-2001), a computerized questionnaire was completed and cardiovascular risk factors and arterial stiffness were assessed. Overall, 6938 subjects visited the research center, of those, measures of both blood pressure levels and aPWV were obtained in 5773 subjects. We excluded subjects with prevalent heart failure at baseline (n=216), and subjects loss to follow-up (n=30) or missing informed consent (n=25) or missing covariate information (n=452), resulting in a sample of 5050 subjects. Missing information was primarily due to logistic reasons. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.

Blood pressure measurements

Two blood pressure measurements were obtained at the right brachial artery with a random zero sphygmomanometer after the subject had been seated for at least five minutes. Systolic blood pressure (SBP), first Korotkoff phase and diastolic blood pressure (DBP), fifth Korotkoff phase, were obtained and the mean of the two blood pressure values was used in the analyses. Pulse pressure (PP) was calculated as SBP minus DBP. Mean arterial pressure was calculated as DBP + 1/3 PP.

Aortic stiffness

Carotid-femoral pulse wave velocity (PWV), a measure of aortic stiffness, was obtained with subjects in supine position. Before the aPWV measurement, the blood pressure was measured twice with a sphygmomanometer after five minutes of rest and the mean was taken. The aPWV was assessed with an automatic device (Complior® Artech Medical, Pantin – France)[17] that measures the time delay between the rapid upstroke of the feet of simultaneous recorded pulse waves in the carotid artery and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape over the surface of the body. The aPWV was calculated as the ratio between distance and the foot-foot time delay and was expressed in meter per second.

Cardiovascular risk factors

Information on medical history, smoking habits and the use of anti-hypertensive medication was obtained during the interview. Smoking was divided into three categories: current, former and non-smokers. Height and weight were measured, and the body mass index (BMI) was computed (kg/m2). Diabetes mellitus was defined as a history of diabetes mellitus and/or the use of blood glucose lowering medication and/or a fasting serum glucose level equal to or greater than 7.0 mmol/l[18]. Serum total cholesterol and high-density lipoproteins (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System).[19]

Heart failure assessment

Assessment of prevalent and incident heart failure has been described in detail elsewhere.[4,5,20] Briefly, prevalent heart failure was determined by using a validated score which is similar with the definition of heart failure of the European Society of Cardiology[3]. This score is based on the presence of at least two signs or symptoms which are suggestive for heart failure or use of heart failure medication and objective evidence of cardiovascular disease.

Cases of incident heart failure were obtained by continuously monitoring subjects in the Rotterdam study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners. All available data on these events were copied from the medical records. Incident heart failure was adjudicated in accordance with the criteria of the European Society of Cardiology[3] on the basis of combination of signs and symptoms, and objective evidence of cardiac dysfunction, including chest radiographs or echocardiography. Two independent research physicians adjudicated all potential heart failure cases. In case of disagreement the judgment of a cardiologist was sought and considered decisive. Only definite and probable cases of heart failure were included in the analyses. The date of incident heart failure was defined as the day of the first occurrence of symptoms suggestive of heart failure, or the day of receipt of a first prescription for a loop diuretic or angiotensin-converting enzyme inhibitor for heart failure. Subjects were followed from baseline until the first of one of the following: a diagnosis of incident heart failure, death, loss to follow-up (<1%) or date of last collection of information for determination of heart failure, January 1st, 2009.

Statistical analysis

First, we examined the association between standardized blood pressure components (systolic, diastolic, mean arterial and pulse pressure), standardized aPWV and the risk of incident heart failure using Cox Proportional Hazards regression. Hazard ratios (HR) and corresponding 95% confidence intervals (95% CI) were calculated. Standardized values were obtained by dividing each measure by its standard deviation. Covariates selected for adjustments were based on prior reports[21]. The first model included age, sex and cohort. In the second model we additionally adjusted for smoking, BMI, use of anti-hypertensive medication, total cholesterol, HDL cholesterol and diabetes mellitus; in models including aPWV we additionally adjusted for mean arterial pressure and heart rate. To investigate whether the effect of pulse pressure on the risk of heart failure was independent of systolic blood pressure, we performed analyses where both systolic pressure and pulse pressure were included in the model. To assess whether the associations were mediated by myocardial infarction, we performed analyses additionally adjusted for prevalent myocardial infarction. The Kaplan-Meier method was used to estimate survival curves of heart failure associated with tertiles of PWV. Finally, we tested for interaction by age. When this interaction term was significant we performed age-stratified analyses. P-values less than 0.05 were considered statistically significant. All analyses were performed using SPSS 21.0 statistical package for Windows 2012 (SPSS, INC., Chicago, Illinois, USA).

Table 1: Baseline Characteristics of the study participants (n=5050).

Characteristics	Mean ± SD or percentage
Age (years)	68.3 ± 7.9
Men (%)	44.2
Systolic blood pressure (mmHg)	143 ± 21
Diastolic blood pressure (mmHg)	77 ± 11
Mean arterial pressure (mmHg)	99 ± 13
Pulse pressure (mmHg)	66 ± 17
Pulse wave velocity (m/sec)	13.1 ± 3.1
Heart rate (bpm)	74 ± 13
Body mass index (kg/m2)	26.9 ± 4.0
Total cholesterol (mmol/L)	5.8 ± 1.0
High density lipoprotein cholesterol (mmol/l)	1.4 ± 0.4
Current smokers (%)	17.4
Diabetes mellitus (%)	12.4
Use of antihypertensive medication (%)	23.1

SD= standard deviation

Results

The baseline characteristics of the study population are shown in table 1. The mean age of the study population was 68.3 years and the percentage men were 44.2%. During a mean of 7.9 years follow-up, 393 subjects developed heart failure with a median time to event of 8.4 years.

SBP (HR 1.22; 95% CI 1.11-1.34) and pulse pressure (HR 1.28; 95% CI 1.16-1.40) predicted heart failure in the age, gender and cohort adjusted model (table 2). In the model adjusted for cardiovascular risk factors and myocardial infarction, the associations between systolic blood pressure, pulse pressure and heart failure were slightly decreased, however remained statistically significant. DBP (HR 1.00; 95% CI 0.91-1.10) showed no association with heart failure and mean arterial pressure (HR 1.12; 95% CI 1.02-1.23) a very weak association and became non-significant after adjustment for cardiovascular risk factors and myocardial infarction. When both SBP and pulse pressure were included in the same model, pulse pressure (HR 1.28; 95% CI 1.06-1.54) remained a predictor of heart failure, whereas SBP (HR 0.95; 95% CI 0.79-1.15) did not.

Table 2: Hazard ratios of heart failure associated with a 1- standard deviation increment of blood pressure components

Cases/Subjects 393/5050	Systolic blood pressure HR (95% CI)	Diastolic blood pressure HR (95% CI)	Pulse pressure HR (95% CI)	Mean arterial pressure HR (95% CI)
Model 1	1.22 (1.11-1.34)	1.00 (0.91-1.10)	1.28 (1.16-1.40)	1.12 (1.02-1.23)
Model 2	1.16 (1.06-1.28)	0.97 (0.88-1.07)	1.22 (1.11-1.34)	1.07 (0.97-1.18)
Model 3	1.18 (1.07-1.30)	0.99 (0.89-1.09)	1.22 (1.11-1.34)	1.09 (0.99-1.21)

Model 1: Adjusted for age, gender and cohort

Model 2: Model 1 + smoking, body mass index, use of anti-hypertensive medication, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol

Model 3: Model 2 + prevalent myocardial infarction

HR: Hazard ratio; CI: Confidence interval

Higher PWV was positively associated with heart failure. In the model adjusted for age, gender and cohort, aortic pulse wave velocity was associated with heart failure (HR 1.16; 95% CI 1.05-1.27) (table 3). This association was borderline significant after adjustment for cardiovascular risk factors and prevalent myocardial infarction.

Table 3: Hazard ratios of heart failure associated with a 1- standard deviation increment of aPWV

	aPWV
Cases/Subjects	**HR (95% CI)**
393/5050	
Model 1	1.16 (1.05-1.27)
Model 2	1.11 (1.00-1.22)
Model 3	1.10 (1.00-1.22)

Model 1: Adjusted for age, gender and cohort

Model 2: Model 1 + mean arterial pressure, heart rate, smoking, use of anti-hypertensive medication, body mass index, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol

Model 3: Model 2 + prevalent myocardial infarction

HR: Hazard ratio; CI: Confidence interval; aPWV: aortic pulse wave velocity

There was significant effect modification by age with aPWV (P=0.008), therefore we performed analysis for subjects younger and older than 70 years of age. Systolic and pulse pressure are in the oldest group predictive for heart failure (HR 1.19; 95% CI 1.07-1.35 and HR 1.23; 95% CI 1.11-1.37, respectively) with the same magnitude as in the overall analysis (table 4). Aortic pulse wave velocity is associated with heart failure in the youngest group (HR1.29 ; 95% CI 1.03-1.62).

Table 4: Stratified proportional hazard models of systolic blood pressure, pulse pressure, aPWV and risk of heart failure

	Cases/ subjects	Systolic blood pressure HR (95% CI)	P for interaction	Pulse pressure HR (95% CI)	P for interaction	aPWV HR (95% CI)	P for interaction
Age							
< 70	109/3063	1.11 (0.91-1.35)	0.635	1.15 (0.94-1.42)	0.792	1.29 (1.03-1.62)	0.008
> 70	284/1987	1.19 (1.07-1.33)		1.23 (1.11-1.37)		1.07 (0.96-1.20)	

Adjusted for age, gender, cohort, smoking, use of anti-hypertensive medication, body mass index, diabetes mellitus, total cholesterol, high-density lipoprotein cholesterol, prevalent myocardial infarction and additionally mean arterial pressure and heart rate, when appropriate

HR: Hazard ratio; CI: Confidence interval; aPWV: aortic pulse wave velocity

Discussion

In this population-based prospective cohort study which included more than 5000 participants we found that aortic stiffness predicted heart failure in the an apparently healthy aging population.

Previous population-based studies have reported on the association of blood pressure with incident heart failure. Several studies demonstrated the role of pulse pressure in predicting heart failure in the elderly[11-13]. The Framingham Heart Study has demonstrated that each component of blood pressure was associated with the risk of heart failure, but systolic blood pressure and pulse pressure is associated with the highest risk[8]. The magnitude of the association between pulse pressure and heart failure reported in our study is comparable with that reported in previous studies in the elderly. However, in the present study the diastolic blood pressure was not predictive of heart failure as previously found in large studies which included relatively young subjects[8]. This is the first large population-based on healthy middle aged and elderly subjects, which showed a consistent relation between elevated aortic stiffness and incident heart failure in the elderly and remained significant after adjustment for cardiovascular risk factors.

Heart failure is a growing health problem in the aging population with a high morbidity and mortality. To improve treatment of heart failure, mechanism regarding development of heart failure should be unraveled. Hypertension is one of the well-known risk factors for heart failure and the prognostic significance of systolic blood pressure has extensively been reported[7,22,23]. The Framingham Heart Study showed that with advancing age from age 50 years onwards there is a shift from diastolic pressure to systolic pressure and then to pulse pressure, a proxy of aortic stiffness, as predictors of cardiovascular risk[10] and increasing evidence suggests a link between stiffness of the conduit vessels and cardiovascular disease morbidity and mortality[15,24-26]. Both pulse pressure and aortic pulse wave velocity are suggested to be a risk factor for development and progression of heart failure. Aortic stiffness is involved in the pathogenesis of hypertension[27,28] and is also related to myocardial infarction and diabetes mellitus[29], which are all risk factors for developing heart failure. Secondly, increased aortic stiffness increases the pulsatile load on the left ventricle and could lead to left ventricular hypertrophy[30] and concentric remodeling[31], which is one of the major determinants of cardiac diastolic dysfunction. Finally, abnormal shear stress associated with increased aortic stiffness stimulates hypertrophy and atherogenesis in central arteries, including the coronary arteries[32]. A heart with a normal coronary circulation is capable of regulation coronary blood flow by means of vasodilatation to secure the metabolic needs of the myocardium even when the diastolic perfusion pressure declines[33]. However, in the presence of coronary artery disease this regulation mechanism can be exhausted and a decline in aortic diastolic blood pressure could lead to subendocardial ischemia and extensive ventricular damage[34].

Both systolic and pulse pressure increased the risk of heart failure. When systolic blood pressure and pulse pressure are included both in one model, only pulse pressure predicted incident heart failure. This suggests that pulse pressure is not only a reflection of the separate effects of high systolic blood pressure and low diastolic blood pressure, but has an additional independent predictive role. Increased pulse pressure is supposed to be an expression of stiffer central arteries. This was confirmed by our results of increasing levels of aPWV and incident heart failure in the present study.

Finally, we found a stronger predictive role of aortic stiffness in the relatively young subjects, compared to the very old subjects. It is suggested that the prognostic value of several predictors of cardiovascular disease, as aortic stiffness, might decrease with age, due to selective survival and the influence of co-morbidity on risk factor levels. Conversely, the predictive role of both systolic and pulse pressure was strongest in the very old subjects. Brachial systolic and pulse pressure can overestimate central systolic and pulse pressure especially in younger subjects[14], leading to an underestimation of the predicted risk. However, the pressure amplification might be attenuated and even lost during aging, which leads to better correlated peripheral and central pressures in the elderly and a more accurate prediction of cardiovascular risk with brachial pulse pressure in older subjects.

Several issues regarding our study methods should be discussed. First, information on aPWV was not available for all participants, however this was most due to logistic reasons and therefore random. Secondly, the measurements of aortic stiffness were performed only once; it might be speculated that the use of multiple measurements of aortic stiffness could improve accuracy and precision. Finally, the present results are obtained in a Caucasian population and therefore cannot be extrapolated to other populations. The present study has also several strengths. The Rotterdam Study is a large population-based prospective cohort study in the elderly with an extensive follow-up. Besides a baseline screening in the majority of participants using a validated score, we applied clinical criteria for heart failure throughout the Rotterdam Study, based upon guidelines of the European Society of Cardiology. Apart from hospital discharge letters, medical records from general practitioners were available for assessment of cases. Consequently, less severe cases were also included in our study.

In conclusion, we found that elevated aortic stiffness increases the risk of heart failure in apparently healthy elderly.

References

1. Ho KK, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure: the Framingham Study. Journal of the American College of Cardiology. Oct 1993;22(4 Suppl A):6A-13A.
2. Bonneux L, Barendregt JJ, Meeter K, Bonsel GJ, van der Maas PJ. Estimating clinical morbidity due to ischemic heart disease and congestive heart failure: the future rise of heart failure. American journal of public health. Jan 1994;84(1):20-28.
3. Swedberg K, Cleland J, Dargie H, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. Eur Heart J. Jun 2005;26(11):1115-1140.
4. Bleumink GS, Knetsch AM, Sturkenboom MC, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J. Sep 2004;25(18):1614-1619.
5. Mosterd A, Hoes AW, de Bruyne MC, et al. Prevalence of heart failure and left ventricular dysfunction in the general population; The Rotterdam Study. Eur Heart J. Mar 1999;20(6):447-455.
6. Kannel WB, Castelli WP, McNamara PM, McKee PA, Feinleib M. Role of blood pressure in the development of congestive heart failure. The Framingham study. N Engl J Med. Oct 19 1972;287(16):781-787.
7. Vasan RS, Levy D. The role of hypertension in the pathogenesis of heart failure. A clinical mechanistic overview. Arch Intern Med. Sep 9 1996;156(16):1789-1796.
8. Haider AW, Larson MG, Franklin SS, Levy D. Systolic blood pressure, diastolic blood pressure, and pulse pressure as predictors of risk for congestive heart failure in the Framingham Heart Study. Ann Intern Med. Jan 7 2003;138(1):10-16.
9. Kostis JB, Davis BR, Cutler J, et al. Prevention of heart failure by antihypertensive drug treatment in older persons with isolated systolic hypertension. SHEP Cooperative Research Group. Jama. Jul 16 1997;278(3):212-216.
10. Franklin SS, Larson MG, Khan SA, et al. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. Circulation. Mar 6 2001;103(9):1245-1249.
11. Vaccarino V, Berger AK, Abramson J, et al. Pulse pressure and risk of cardiovascular events in the systolic hypertension in the elderly program. Am J Cardiol. Nov 1 2001;88(9):980-986.
12. Chae CU, Pfeffer MA, Glynn RJ, Mitchell GF, Taylor JO, Hennekens CH. Increased pulse pressure and risk of heart failure in the elderly. Jama. Feb 17 1999;281(7):634-639.
13. Kostis JB, Lawrence-Nelson J, Ranjan R, Wilson AC, Kostis WJ, Lacy CR. Association of increased pulse pressure with the development of heart failure in SHEP. Systolic Hypertension in the Elderly (SHEP) Cooperative Research Group. Am J Hypertens. Aug 2001;14(8 Pt 1):798-803.
14. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. Nov 2006;27(21):2588-2605.

15. Sutton-Tyrrell K, Najjar SS, Boudreau RM, et al. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation. Jun 28 2005;111(25):3384-3390.
16. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. Eur J Epidemiol. Aug 2011;26(8):657-686.
17. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. Sep 1995;26(3):485-490.
18. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. Jul 1997;20(7):1183-1197.
19. Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral arterial disease in the elderly: The Rotterdam Study. Arterioscler Thromb Vasc Biol. Feb 1998;18(2):185-192.
20. Leening MJG, Kavousi M, Heeringa J, et al. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. European journal of epidemiology. Mar 2012;27(3):173-185.
21. Mitchell GF, Guo CY, Benjamin EJ, et al. Cross-sectional correlates of increased aortic stiffness in the community: the Framingham Heart Study. Circulation. May 22 2007;115(20):2628-2636.
22. Psaty BM, Furberg CD, Kuller LH, et al. Association between blood pressure level and the risk of myocardial infarction, stroke, and total mortality: the cardiovascular health study. Arch Intern Med. May 14 2001;161(9):1183-1192.
23. Mattace-Raso FU, van der Cammen TJ, van Popele NM, et al. Blood pressure components and cardiovascular events in older adults: the Rotterdam study. Journal of the American Geriatrics Society. Sep 2004;52(9):1538-1542.
24. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. Feb 7 2006;113(5):657-663.
25. Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. May 2001;37(5):1236-1241.
26. Jankowski P, Kawecka-Jaszcz K, Czarnecka D, et al. Pulsatile but not steady component of blood pressure predicts cardiovascular events in coronary patients. Hypertension. Apr 2008;51(4):848-855.
27. Najjar SS, Scuteri A, Shetty V, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. Journal of the American College of Cardiology. Apr 8 2008;51(14):1377-1383.
28. London GM. Role of arterial wall properties in the pathogenesis of systolic hypertension. Am J Hypertens. Jan 2005;18(1 Pt 2):19S-22S.
29. van Popele NM, Elizabeth Hak A, Mattace-Raso FU, et al. Impaired fasting glucose is associated with increased arterial stiffness in elderly people without diabetes mellitus: the Rotterdam Study. Journal of the American Geriatrics Society. Mar 2006;54(3):397-404.

30. Girerd X, Laurent S, Pannier B, Asmar R, Safar M. Arterial distensibility and left ventricular hypertrophy in patients with sustained essential hypertension. American heart journal. Oct 1991;122(4 Pt 2):1210-1214.
31. Palmieri V, Bella JN, Roman MJ, et al. Pulse pressure/stroke index and left ventricular geometry and function: the LIFE Study. Journal of hypertension. Apr 2003;21(4):781-787.
32. van Popele NM, Mattace-Raso FU, Vliegenthart R, et al. Aortic stiffness is associated with atherosclerosis of the coronary arteries in older adults: the Rotterdam Study. Journal of hypertension. Dec 2006;24(12):2371-2376.
33. Mosher P, Ross J, Jr., McFate PA, Shaw RF. Control of Coronary Blood Flow by an Autoregulatory Mechanism. Circulation research. Mar 1964;14:250-259.
34. Kass DA, Saeki A, Tunin RS, Recchia FA. Adverse influence of systemic vascular stiffening on cardiac dysfunction and adaptation to acute coronary occlusion. Circulation. Apr 15 1996;93(8):1533-1541.

Chapter 5.2
Aortic stiffness and the prediction of cardiovascular disease

Based on:
Does aortic stiffness improve the prediction of coronary heart disease in elderly? The Rotterdam Study.
J Hum Hypertens. 2012 Jan;26(1):28-34.

Abstract

Background

It has been demonstrated that aortic stiffness is an independent predictor of cardiovascular disease. We investigated whether this measure is of use in cardiovascular risk stratification in clinical practice for elderly subjects (mean age 71.5 years).

Methods

Within the framework of the Rotterdam Study we stratified subjects free of coronary heart disease at baseline into categories of low (<10%), intermediate (10-20%) and high (>20%) 10-year risk of coronary heart disease based on Framingham risk factors. Within each risk category, we determined the percentages of subjects moving into a higher or lower risk category when adding aortic stiffness to the Framingham risk factors.

Results

Among 2849 participants, 223 coronary heart disease events occurred during a median follow-up of 7.9 years. In the low risk group 5% of the subjects could be reclassified and in the high-risk group 6% of the subjects could be reclassified to the intermediate risk group. In the intermediate risk group 3% could be reclassified to the high-risk group and 6% to the low risk group.

Conclusion

In a population of elderly subjects, aortic stiffness measurement in addition to Framingham risk factors leads to a limited reclassification of subjects in 10-year cardiovascular disease risk categories. Therefore, aortic stiffness is associated with the risk of coronary heart disease in elderly, but provides no additional value in cardiovascular risk stratification.

Introduction

Primary prevention of coronary heart disease (CHD) is based on the assessment of traditional cardiovascular risk factors, including older age, male gender, hypercholesterolaemia, low levels of high-density lipoprotein cholesterol, smoking, hypertension and diabetes mellitus. Treatment of these risk factors has shown to reduce cardiovascular morbidity and mortality. Current guidelines recommend algorithms such as SCORE[1] risk chart or Framingham Risk Score[2] for predicting the 10 year absolute risk for CHD. Non-invasive measures of arterial stiffness, as the aortic pulse wave velocity (aPWV), have shown to be independent risk factors of CHD[3,4] and, therefore, are proposed as addition to traditional risk factor for predicting incident CHD[5]. The recent European guidelines for the diagnosis and treatment of hypertension suggest that an aPWV higher than 12 m/s is a marker of target organ damage[6]. However, whether measures of arterial stiffness are of use in cardiovascular risk prediction in clinical practice needs to be determined.

We have investigated the predictive value of aPWV beyond traditional risk factor assessment in an ongoing population-based prospective study in the elderly. Subjects were stratified in three risk categories, namely low (<10%), intermediate (10-20%) and high (>20%), based on the Framingham risk factors. We determined the percentages of subjects moving into a higher or lower risk category when adding aPWV to the Framingham risk factors.

Methods

Study population

The present study is performed within the framework of the Rotterdam Study, a population-based prospective cohort study in Ommoord, a suburb of Rotterdam, The Netherlands. Overall aim of the Rotterdam Study is assessing the occurrence of and risk factors for chronic diseases. The study design and objectives of the Rotterdam Study are described elsewhere[7]. Briefly, the baseline visit started between 1990 and 1993. All inhabitants of Ommoord, aged 55 years and older were invited to participate (n=10,275). Of them, 7983 (78%) gave their written informed consent and took part in the baseline examination. Since the start of the study, follow-up visits took place in the period 1993 through 1996 for the second visit, in the period 1997 through 1999 for the third visit, and in the period 2002 through 2004 for the fourth visit. A flow diagram of the Rotterdam Study is provided in Figure 1. During the third examination phase, which took place from 1997 until 1999, a computerized questionnaire was completed and assessment of cardiovascular risk factors and arterial stiffness were performed. A total of 4024 participants visited the research center, of these 3445 persons had an aPWV measurement. Missing information on aPWV was primarily due to logistic reasons, including illness of the cardiovascular research assistants, maintenance service of the Complior device and temporary technical problems with the Complior device, and is therefore likely to be randomly distributed. Prevalent CHD was defined as a history of clinically manifest myocardial infarction,

coronary artery bypass grafting or percutaneous transluminal coronary angioplasty. We excluded subjects with prevalent CHD and stroke at the third examination (n=581) and subjects lost to follow-up (n=15). The current analysis was carried out in 2849 subjects. The Medical Ethics Committee of Erasmus Medical Center approved the study and written consent was obtained from all participants.

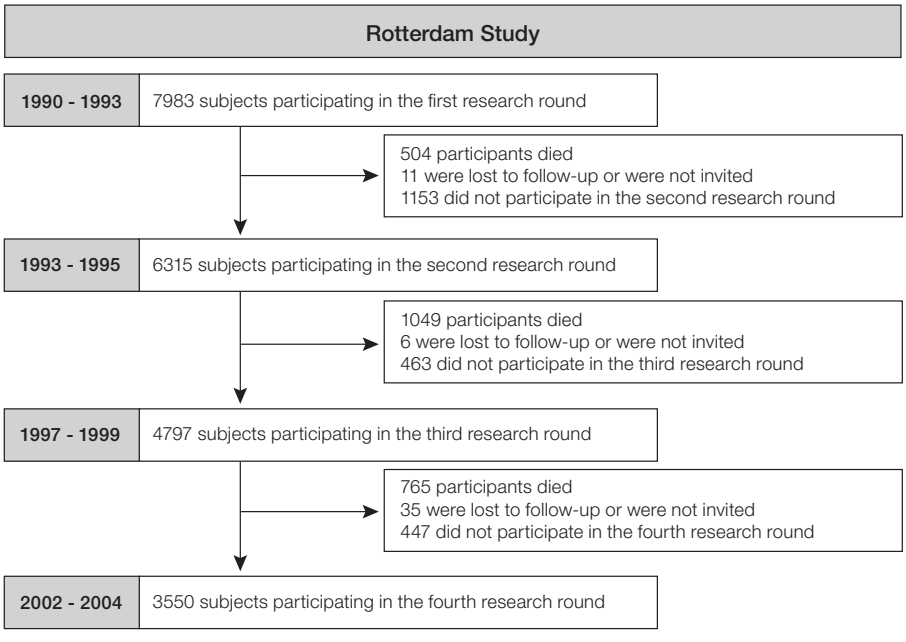


Figure 1. Diagram of the study population of the Rotterdam Study

Aortic stiffness

Carotid-femoral pulse wave velocity, a measure of aortic stiffness, was obtained with subjects in supine position. Before the aPWV measurement, blood pressure was measured twice in supine position with a sphygmomanometer after five minutes of rest and the mean value was taken. Measurements of aPWV were performed during the morning or afternoon (no specific time) and the subjects were non-fasting. The aPWV was assessed with an automatic device (Complior® Artech Medical, Pantin – France)[8] that measures the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid artery and the femoral artery. The distance between the recording sites in the carotid and the femoral artery was measured with a tape over the surface of the body. The aPWV was calculated as the ratio between distance and the foot-to-foot time delay and was expressed in meters per second.

Cardiovascular risk factors

Information on medical history, smoking habits and the use of anti-hypertensive medication was obtained during the interview. Two seated blood pressure measurements were obtained at the right brachial artery with a random zero sphygmomanometer. Diabetes mellitus was defined as a history of diabetes mellitus and/or the use of blood glucose lowering medication and/or a fasting serum glucose level equal to or higher than 7.0 mmol/l[9]. Serum total cholesterol and high-density lipoproteins (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System, Mannheim, Germany).[10]

Clinical outcomes

The follow-up procedure has previously been described in detail[11]. Briefly, information on fatal and nonfatal coronary endpoints was obtained through automated linkage with files from general practitioners and letters and discharge reports from medical specialists. Two research physicians independently coded all reported events according to the International Classification of disease, 10th edition (ICD-10)[12]. In case of disagreement, consensus was reached. A medical expert in cardiovascular disease, whose judgment was considered final, reviewed all events. CHD was defined as the occurrence of fatal or nonfatal myocardial infarction (MI) (ICD 10 code I21), percutaneous transluminal coronary angioplasty, coronary artery bypass grafting and CHD mortality. In identifying myocardial infarctions, all available information, which included ECG, cardiac enzyme levels, and the clinical judgment of the treating specialist, was used. If a non-fatal event occurred within 28 days prior to CHD death, the event was contributed to CHD mortality.

Statistical analysis

Mean values with standard deviation (SD) and percentages were calculated for continuous and categorical baseline variables, respectively. The baseline characteristics of males and females were compared using Student t-test for continuous variables and chi-square test for categorical variables.

aPWV and risk of CHD

Cox proportional hazard analysis was performed to estimate the hazard ratios (HRs) with corresponding 95% confidence interval (CI) for CHD associated with tertiles of aPWV. Analyses were adjusted for age and gender, and additionally for current smoking, total cholesterol, HDL cholesterol, systolic blood pressure, use of anti-hypertensive medication and diabetes mellitus.

Prediction Model

We used a Weibull survival model to predict individual 10-year CHD risk probabilities and to classify persons into a priori defined risk categories[2]. These categories were low (<10%), intermediate (10-20%) and high (>20%) 10-year risk of total CHD events. We restricted the predictors of the model to components of the Framingham Risk Score, in accordance with the ATP III guidelines[2]. The model included age, gender, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, HDL cholesterol, diabetes mellitus and current smoking (yes/no). Additionally, we extended the first model with aPWV (model 2). To allow for possible nonlinear effects we used restricted cubic splines with three knots for continuous variables. We examined global model fit using Nagelkerke's generalized model R^2. This is a measure of the fraction of -2 log likelihood explained by the predictors, analogous to the percentage of variance explained in a linear model. Higher percentages indicate better model fit[13,14]. Furthermore, as a measure of discrimination we used the c-index that is defined as the proportion of all pairs of patients whose survival time can ordered such that he patient who survived longer has the higher predicted survival.[15-17] For internal validation of the model, we used bootstrap sampling (150 samples) to correct for over-optimism and calculated the bootstrap adjusted Nagelkerke's R^2. To determine how well the model predicts disease, the calibration of the model was evaluated by comparing the predicted 10-year risk probabilities with observed 10-year risk in the three risk categories (low, intermediate, high-risk). We computed reclassification percentages to study the incremental ability of aPWV to classify subjects in risk categories according to commonly used categories of 10-year CHD risk (low, intermediate, high-risk). We used the reclassification approach comparable to the method previously used by Cook et al[18]. To evaluate true improvement in classification by addition of PWV to the "Framingham model" we calculated the net reclassification improvement (NRI) by the method of Steyerberg and Pencina, which is specially designed for survival data.[19]

In case of missing values for the Framingham predictors, values were imputed using the Expectation Maximization method. Analyses were performed using SPSS version 15 (SPSS, INC., Chicago, Illinois) and R version 2.7.2 software (R Foundation for Statistical Computing, Vienna, Austria).

Results

The baseline characteristics of the 2849 participants are shown in table 1. The population of the present study consisted of 1097 men (38.5%) and 1752 women. Mean age at baseline was 70.9 years for men and 71.9 years for women (p<0.001). More men than women were smokers (17.8% vs. 14.2%, p<0.001) and women used more frequently anti-hypertensive medication (25.3% vs. 19.9%, p=0.001) and serum lipid reducing agents (11.0% vs. 7.4%, p=0.002). The mean aPWV in men was slightly higher than in women (13.7 vs 13.1 m/s; p<0.001). The median follow-up duration (inter-quartile range) was 7.9 (7.3-8.7) years. During follow-up, 223 events occurred, of which 122 in men and 101 in women.

Table 1. Baseline characteristics of the population

Variable	All n=2849	Men n=1097	Women n=1752	P value
Age, y	71.5±6.7	70.9±6.4	71.9±6.9	<0.001
Men, %	38.5	-	-	
Current smoking, %	15.5	17.8	14.2	<0.001
Systolic blood pressure, mmHg	143±21	143±21	143±22	0.925
Diastolic blood pressure, mmHg	76±11	77±11	75±11	<0.001
Anti-hypertensive medications, %	23.2	19.9	25.3	0.001
Total cholesterol, mmol/l	5.9±1.0	5.6±0.9	6.1±0.9	<0.001
HDL cholesterol, mmol/l	1.4±0.4	1.3±0.3	1.5±0.4	<0.001
Serum lipid reducing agents, %	9.6	7.4	11.0	0.002
Diabetes mellitus, %	12.2	13.6	11.4	0.078
aPWV, m/s	13.3±3.0	13.7±3.1	13.1±2.9	<0.001

Values are mean ± sd for continuous variables and percentages for dichotomous variable

HDL cholesterol: high-density lipoprotein cholesterol; aPWV: aortic pulse wave velocity; n: number

The risk of CHD increased with increasing aPWV up to an age and gender adjusted hazard ratio (HR) of 1.94 (95% CI 1.32-2.85, p = 0.001) for subjects in the highest aPWV tertile compared with subjects in the lowest tertile (p for trend = 0.001) (Table 2, Figure 2). We divided the subjects into three risk categories (low, intermediate and high 10-year risk of CHD) based on the Framingham covariates. We found a modest model performance (adjusted R2 5.8%, bootstrap corrected c-index 0.690). Addition of aPWV measurement to the Framingham covariates did not improve the model performance (adjusted R2 5.6 %, bootstrap corrected c-index 0.685) (Table 3). In the low and high-risk groups, aPWV measurements hardly led to reclassification of subjects into a higher or lower risk category. In the low risk group, 5% (81) of the subjects could be reclassified and in the high-risk group, 6% (20) of the subjects could be reclassified to the intermediate risk group when adding aPWV measurement. In the intermediate risk group additional measurement of aPWV resulted in slight reclassification. In this category, 3% (29) of the subjects could be reclassified to the high-risk group and 6% (50) of the subjects could be reclassified to the low risk group (table 4). Generally, the observed 10-year risks agreed with the corresponding categories of predicted risk, indicating adequate calibration of the model. In other words, using the model we were able to predict correctly absolute 10-year risk of CHD (table 4). However, the observed 10-year risks in the intermediate risk groups to the low and high-risk groups agreed less. This suggests that the reclassification in these groups is not totally correct and might not predict the correct 10-year risks. Addition of PWV to the Framingham model did not improve risk classification as indicated by an NRI of 0.02% (p=0.5478). In analysis stratified for gender we found no differences between men and women (data not shown).

Table 2. Hazard ratios of coronary heart disease associated with tertiles of aPWV.

		HR (95% CI)	
	Cases/subjects	**Model 1**	**Model 2**
aPWV			
Tertile 1	41/950	1.0 (reference)	1.0 (reference)
Tertile 2	78/950	1.63 (1.11-2.39)	1.41 (0.96-2.09)
Tertile 3	104/949	1.94 (1.32-2.85)	1.46 (0.98-2.19)
		P for trend = 0.001	P for trend = 0.092

Model 1: age and gender adjusted

Model 2: additionally adjusted for systolic blood pressure, use of anti-hypertensive medication, smoking, total cholesterol, high-density cholesterol and diabetes mellitus

aPWV: aortic pulse wave velocity; HR: hazard ratio; 95% CI: 95% confidence interval

Discussion

In the present study performed in a large population of elderly subjects, we found that measurements of aortic stiffness in addition to traditional cardiovascular risk factors led to minor reclassification of subjects within 10-year cardiovascular disease risk categories.

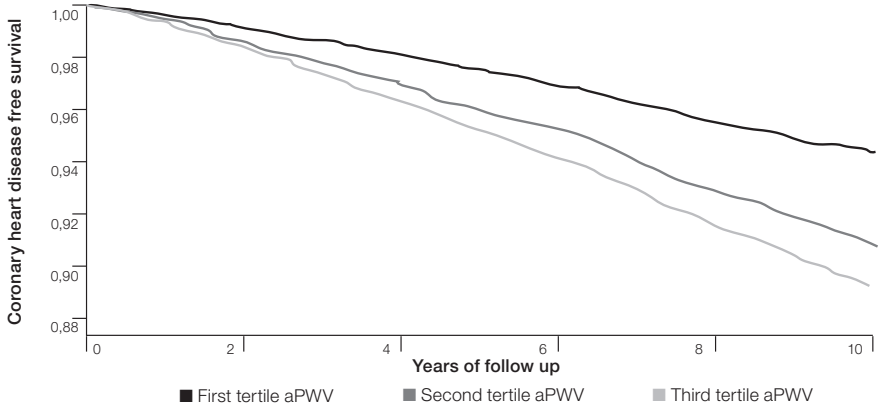


Figure 2. Coronary heart disease free survival by tertiles of aPWV in models adjusted for age and gender.
aPWV: aortic pulse wave velocity

Table 3. Measures of model fit and discrimination for coronary heart disease models with and without aPWV

	Model 1	Model 2
Model fit		
Likelihood ratio	120.49	122.69
Nagelkerke R2	0.058	0.056
Discrimination		
C statistic	0.690	0.685

Model 1: age, gender, total cholesterol, high-density cholesterol, systolic blood pressure, smoking, diabetes mellitus and use of anti-hypertensive medication
Model 2: add aPWV
aPWV: aortic pulse wave velocity

Aortic stiffness has been associated with cardiovascular morbidity and mortality in hypertensive subjects[20], patients with end-stage renal disease[21], in patients with diabetes mellitus[22] and in the general population[3,4,23,24]. Consequently, aortic stiffness has been proposed as an addition to the traditional risk factors for the prediction of cardiovascular disease. Previous findings of the Rotterdam Study[3] reported a slight increase of the c-index when aortic stiffness was added to prediction models containing conventional cardiovascular risk factors. Also in hypertensive subjects, an increase in the area under the curve (AUC) for cardiovascular disease was shown when aPWV was added to the Framingham Risk Score[20]. The recent guidelines for diagnosis and treatment of hypertension[6] have suggested that an aPWV higher than 12m/s might be considered as a marker of target organ damage and a very recent multicentric study has suggested normal and reference values for PWV in large European populations[25]. However, it is still not completely clear whether adding aortic stiffness measurement to the daily practice is of clinical value for the individual patient. Therefore, we investigated the possible clinical predictive value of aortic stiffness, beyond traditional risk factors through reclassification of subjects within the Framingham risk categories, determining the percentages of subjects moving into a higher or lower risk category.

Table 4: Coronary heart disease risk reclassification comparing the Framingham risk model with the model additionally including aPWV

		10-Year Risk categories for FRS + aPWV			Total n reclassified (%)
10- Year risk for FRS		**0-10% (low)**	**10-20% (intermediate)**	**>20% (high)**	
0-10% (low)	Total n	1565	81	0	81
	(%)	(95)	(5)	-	(5)
	Observed risk (95% CI)	0.05 (0.04-0.07)	0.12 (0.06-0.23)	NA	
10-20% (intermediate)	Total n	50	765	29	79
	(%)	(6)	(91)	(3)	(9)
	Observed risk (95% CI)	0.18 (0.09-0.35)	0.13 (0.11-0.17)	0.19 (0.08-0.44)	
>20% (high)	Total n	0	20	339	20
	(%)	-	(6)	(94)	(6)
	Observed risk (95% CI)	NA	0.07 (0.01-0.41)	0.30 (0.24-0.36)	

aPWV: aortic pulse wave velocity; n: number; FRS: Framingham Risk Score; NA: not applicable

We confirmed the association between aortic stiffness and the risk of cardiovascular disease in a population of elderly. However, we found a minor reclassification when adding aortic stiffness to the Framingham risk covariates, suggesting low additional value of aortic stiffness in the clinical management of CHD in the elderly. The minor reclassification showed in this study could have several explanations. First, the prognostic value of several predictors of cardiovascular disease, as aortic stiffness, might decrease with age, due to selective survival and the influence of co-morbidity on risk factor levels. Therefore, we cannot exclude that aortic stiffness could be used for the clinical management of CVD in younger subjects. This has been suggested in a recent study performed within the framework of the Framingham Heart Study[26], which showed that the addition of aortic stiffness to standard CVD risk factors improved model fit and resulted in a well-calibrated model with improved risk discrimination and risk classification in middle aged subjects. Second, it has been suggested that the association between a single risk factor and the outcome must be much higher than we traditionally find in observational studies to be useful in predicting absolute risk for individual patients[27]. Therefore, the measurement of aortic stiffness alone might not be adequate to improve cardiovascular risk prediction above traditional factors, but could still be adequate in combination with other additional risk factors and/or non-invasive tests. In accordance with this, the Atherosclerosis Risk in Communities (ARIC) Study[28] added multiple non-traditional risk factors and markers of subclinical disease to traditional risk factors. No single risk marker alone could be identified that improved risk prediction, while addition of a set of markers was able to improve prediction. Third, the present study is an extension of a previous report of the Rotterdam Study[3], however the association of aortic stiffness with cardiovascular disease was somewhat smaller in the present study as compared to the previous report, possibly due to a longer follow-up.

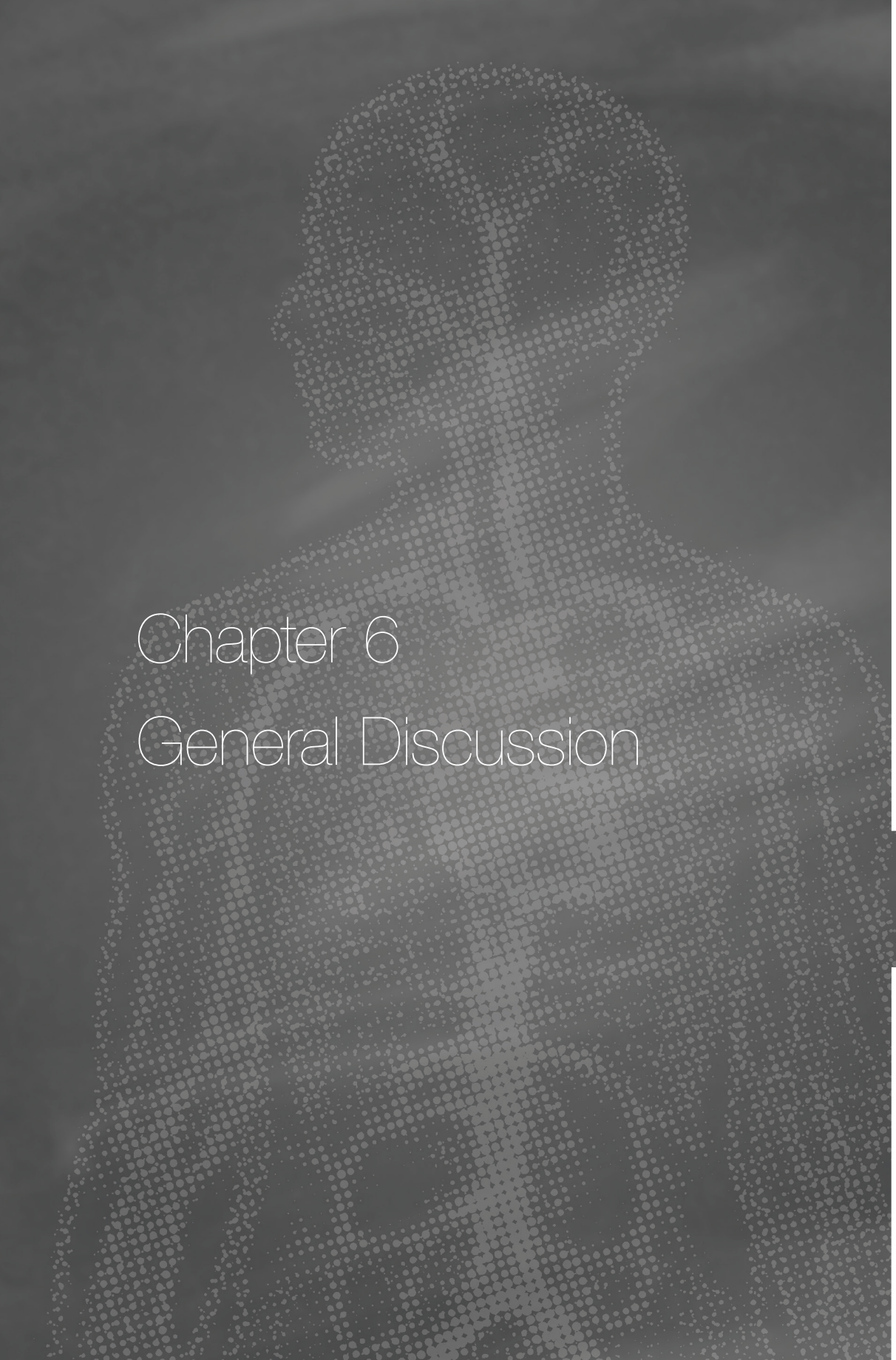
Moreover, some methodological limitations need to be considered. First, we choose to refit a model based on Framingham risk factors to stratify our population in the well known risk categories, because previous research within the Rotterdam study showed that application of the original Framingham Risk Score led to systematic overestimation of CHD risk in men[29]. Second, we restricted the predictors in the model to components of this Framingham Risk Score and included, age, gender, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, high-density (HDL) cholesterol, diabetes mellitus and current smoking (yes/no). For this reason we did not include other risk factors for coronary heart disease, including kidney function, body mass index, diastolic blood pressure or measures of atherosclerosis as the carotid intima media thickness. Third, information on measures of stiffness was not available for all subjects who visited the research center. It might be that information was missing mostly in those subjects with a higher cardiovascular risk load. Fourth, the measurements of aortic stiffness were performed only once; it might be speculated that the use of multiple measurements of aortic stiffness could improve accuracy and precision. Finally, these results are obtained in a Caucasian population and therefore the results cannot be extrapolated to other populations.

In the present study, we found a limited additional prognostic role of aortic stiffness beyond the traditional cardiovascular risk factors in a population of elderly subjects. Therefore, aortic stiffness has no additional value in cardiovascular risk stratification. Considering the increasing interest for functional arterial measurements further studies in larger cohorts, including also younger subjects are needed to determine the role of aortic stiffness in cardiovascular disease prediction.

References

1. Conroy RM, Pyorala K, Fitzgerald AP, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. Jun 2003;24(11):987-1003.
2. Expert Panel on Detection EaToHBCiA. 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. May 16 2001;285(19):2486-2497.
3. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. Feb 7 2006;113(5):657-663.
4. Sutton-Tyrrell K, Najjar SS, Boudreau RM, et al. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation. Jun 28 2005;111(25):3384-3390.
5. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. Nov 2006;27(21):2588-2605.
6. Mancia G, De Backer G, Dominiczak A, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension. Jun 2007;25(6):1105-1187.
7. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: 2010 objectives and design update. Eur J Epidemiol. 2009;24(9):553-572.
8. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. Hypertension. Sep 1995;26(3):485-490.
9. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. Jul 1997;20(7):1183-1197.
10. Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral arterial disease in the elderly: The Rotterdam Study. Arterioscler Thromb Vasc Biol. Feb 1998;18(2):185-192.
11. Kardys I, Kors JA, van der Meer IM, Hofman A, van der Kuip DA, Witteman JC. Spatial QRS-T angle predicts cardiac death in a general population. Eur Heart J. Jul 2003;24(14):1357-1364.
12. WHO. International statistical classification of diseases and related health problems. 10 ed. Geneva: WHO. 1992.
13. Nagelkerke N. A note on a general definition of the coefficient of determination. Biometrika. 1991;78(3):2.
14. Ash A, Shwartz M. R2: a useful measure of model performance when predicting a dichotomous outcome. Stat Med. Feb 28 1999;18(4):375-384.
15. Harrell F. Regression modeling with application to linear models, logistic regression and survival analysis. New York, NY: Springer-Verslag; 2001.
16. Efron B TR. An introdution to bootstrap. New York: Chapman & Hall; 1993.
17. Steyerberg EW. Clinical Prediction Models. New york, NY: Springer; 2009.
18. Cook NR, Buring JE, Ridker PM. The Effect of Including C-Reactive Protein in Cardiovascular Risk Prediction Models for Women. Ann Intern Med. Jun 5 2006.

19. Steyerberg EW, Pencina MJ. Reclassification calculations for persons with incomplete follow-up. Ann Intern Med. Feb 2;152(3):195-196; author reply 196-197.
20. Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. Jan 2002;39(1):10-15.
21. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. Kidney Int. May 2003;63(5):1852-1860.
22. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. Oct 15 2002;106(16):2085-2090.
23. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol. Dec 2001;21(12):2046-2050.
24. van Popele NM, Mattace-Raso FU, Vliegenthart R, et al. Aortic stiffness is associated with atherosclerosis of the coronary arteries in older adults: the Rotterdam Study. Journal of hypertension. Dec 2006;24(12):2371-2376.
25. Mosterd A, Hoes AW, de Bruyne MC, et al. Prevalence of heart failure and left ventricular dysfunction in the general population; The Rotterdam Study. Eur Heart J. Mar 1999;20(6):447-455.
26. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. Feb 2;121(4):505-511.
27. Ware JH. The limitations of risk factors as prognostic tools. N Engl J Med. Dec 21 2006;355(25):2615-2617.
28. Chambless LE, Folsom AR, Sharrett AR, et al. Coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC) study. J Clin Epidemiol. Sep 2003;56(9):880-890.
29. Koller MT, Steyerberg EW, Wolbers M, et al. Validity of the Framingham point scores in the elderly: results from the Rotterdam study. American heart journal. Jul 2007;154(1):87-93.



Chapter 6
General Discussion

Cardiovascular morbidity and mortality is increasing due to aging populations, consequently cardiovascular disease remains the single leading cause of death worldwide.[1,2] The main risk factors for cardiovascular disease are tobacco use, obesity, high blood pressure, diabetes mellitus and raised lipids, with an attributable risk of 80% for these risk factors. A large proportion of cardiovascular disease is preventable by addressing these risk factors.[3]

The leading cardiovascular risk factor is raised blood pressure, to which 16.5% of global deaths can be attributed.[4] The relationship of blood pressure components to cardiovascular disease is more complex than initially thought. Not only the two extremes, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the arterial pulse wave but also the pulsatile component, pulse pressure defined as the difference between SBP and DBP, play a role in predicting cardiovascular disease, mostly in aging populations.[5] Pulse pressure is regarded as surrogate measure of arterial stiffness.[6]

This thesis is focused on arterial hemodynamics, including blood pressure and arterial stiffness. First we attempt to unravel the genetic architecture of blood pressure and arterial stiffness through genome-wide association (GWA) studies. Second, we describe the relationship between blood pressure and arterial stiffness and finally we focus on the clinical consequences.

This discussion puts the results of our research in perspective of the literature, addresses potential clinical implications, elaborates on methodological considerations and provides directions for future research.

Part I: Genetic association studies on arterial hemodynamics

Blood pressure regulation and hypertension

Hypertension is a common disease with an increasing prevalence with aging: the estimated prevalence is 50-70% in the elderly.[7] Despite extensive physiologic investigation, the primary determinants of this trait as well as the factors which determine specific clinical outcomes, remain partly understood. For most common diseases, the risk of an individual is influenced by multiple genetic and environmental factors. Although many mutations are known, blood pressure does not typically segregate in families consistent with mendelian transmission and a variety of factors such as salt intake, age, gender and body mass index can chronically influence blood pressure.[8] (Figure 1)

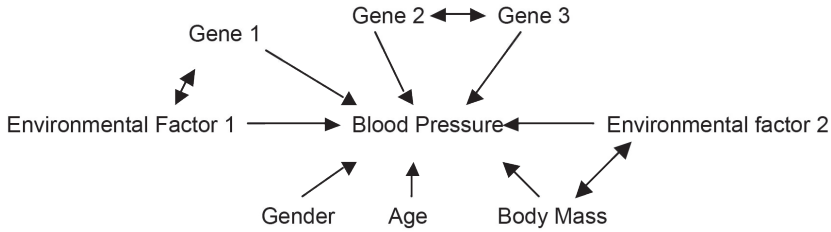


Figure 1. Multifactorial model of blood pressure determination

The substantial heritability of blood pressure (30-60%) has prompted extensive efforts to identify its genetic architecture to unravel the pathogenesis of hypertension.[9,10] Several approaches have been used to search for genes associated with individual blood pressure variation in the general population, but provided limited consistent evidence of loci.[11]

Family based studies with rare high or low blood pressure syndromes have identified mutations with gain or loss of function in several renal sodium handling regulatory genes and steroid hormone metabolism (CYP11B1[12], CYP11B2[13], WNK1/4[14], KLHL3[15,16], CUL3[15], SCNN1B[17], CYP17A1[18], HSD11B2[19], NR3C2[20], KCNJ5[21]), providing important insights into the mechanisms of blood pressure regulation and in particular a central role of the kidney.[8] Two of these renal sodium handling genes (KCNJ1[22], coding for potassium channel and WNK1[23], member of WNK subfamily of serine/threonine protein kinase controlling transport of sodium and chloride ions) have been found to be associated with blood pressure levels in the general population.

Several candidate genes have been investigated for blood pressure variation in the general population[24,25] but failed to replicate.[11] Therefore, the vast majority of the genetic contribution to variation in blood pressure in the general population remained unexplained. Several reasons for the limited replication have been proposed, including population stratification, variable linkage disequilibrium between polymorphism being studied and the true causal variant, gene-gene and gene-environment interactions, weak genetic effects and lack of power.[11]

With the introduction of GWA studies, a powerful approach for identifying genetic loci for common diseases and quantitative traits became available.[26] Large-scale GWA studies in which hundreds of thousands of common genetic variants are genotyped and analyzed for disease association have shown great success in identifying genes associated with common diseases and traits.

In **chapter 2** we describe GWA studies for blood pressure and hypertension. In **chapter 2.1** we used the framework of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium, comprised of the Atherosclerosis Risk in Elderly study (ARIC), Age, Gene/Environment Susceptibility-Reykjavik study (AGES), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS) and the Rotterdam Study (RS). In **chapter 2.2** we extend this to a bigger collaboration, the International Collaboration on Blood Pressure (ICBP) consortium.

We identified several novel loci for SBP, DBP and hypertension in a GWA meta-analysis of more than 29,000 subjects in the framework of CHARGE, with replication in an independent consortium, Global BPgen consortium[27], with almost 35,000 subjects. We showed genome-wide significant associations for four novel loci for SBP, six loci for DBP and one locus for hypertension. Table 1 provides a brief overview of the loci for blood pressure and hypertension, including the results from Global BPgen consortium. The top ten risk alleles were associated with about 1 and 0.5 mmHg increase per risk allele in SBP and DBP, respectively. Although we presented multiple variants with a substantial joined effect on blood pressure, the effect sizes of the individual common alleles were small and explained a small proportion of the blood pressure variation. Further efforts to identify additional alleles associated with blood pressure will require larger GWA studies.

To increase the sample size, a new collaboration between CHARGE and Global BPgen was formed, the ICBP consortium. **Chapter 2.2** described this GWA analyses of SBP and DBP, which used a multi-stage design in 200,000 individuals of European descent and identified 16 novel loci and brought the total number of identified variants with blood pressure up to 29. (Table 1) Nine loci of these 29 were confirmed in individuals from East-Asian ancestry and six loci in individuals from South-Asian ancestry. A risk score derived from these 29 associations was significantly associated with hypertension, blood pressure related organ damage including left ventricular wall thickness and clinical cardiovascular disease including stroke and coronary artery disease. These 29 SNPs explained 0,9% of the phenotypic variation. Calculations estimated that there are up to 116 independent blood pressure variants with effect sizes similar to those reported in this paper, which can explain up to 2.2% of the phenotypic variance for SBP and DBP.

These studies demonstrated that GWA analyses are successful in identifying variants for complex traits and identified variants have clinical relevance, however we also demonstrated that there is still an amount of work necessary in identifying variants related to blood pressure and even if we identify those variants the explained variance is limited.

In **chapter 2.3** we describe a candidate gene analyses of the human 3beta-hydroxysteroid dehydrogenase type 1 (HSD3B1) gene, which was suggested as gene of interest for blood pressure regulation with circadian clock malfunction.[28] We showed that HSD3B1 expression was minimal or absent in adrenocortical tissues, and was not stimulated by adrenocorticotropin or angiotensin II. Subsequently, we selected six tagging SNPs in the HSD3B1 gene and performed analyses in the RS and Erasmus Rucphen family study (ERF). No variants were associated with blood pressure or the occurrence of hypertension. These results were confirmed with a lookup in the ICBP consortium. To conclude, we deny an association of human 3beta-hydroxysteroid dehydrogenase type 1 (HSD3B1) gene with aldosterone production or blood pressure.

Table 1. Genes implicated to be associated with blood pressure

Consortium	SBP	DBP
CHARGE[29]	ATP2B1, CYP17A1, PLEKHA7, SH2B3	ATP2B1, CACNB2, CSK-ULK3, SH2B3, TBX3-TBX-5, ULK4
Global BP gen[27]	MTHFR, CYP17A1, PLCD3	FGF5, c10orf107, SH2B3, CYP1A2, ZNF652
ICBP[30]	SLC39A8, ATP2B1, GNAS-EDN3, CYP17A1-NT5C2, MTHFR-NPPB, HFE, C10orf107, FGF5, CYP1A1-ULK3, CACNB2(3´), SH2B3, FURIN-FES, FLJ32810-TMEM133, PLEKHA7, ADM, NPR3-C5orf23, EBF1, PLCE1, BAT2-BAT5, MOV10, ZNF652, CACNB2(5´), MECOM, GOSR2	SLC39A8, ATP2B1, GNAS-EDN3, CYP17A1-NT5C2, MTHFR-NPPB, HFE, C10orf107, FGF5, CYP1A1-ULK3, CACNB2(3´), SH2B3, FURIN-FES, FLJ32810-TMEM133, PLEKHA7, NPR3-C5orf23, EBF1, BAT2-BAT5, MOV10, ZNF652, TBX5-TBX3, CACNB2(5´), JAG1, GUCY1A3-GUCY1B3, MECOM, SLC4A7, ULK4

Arterial stiffness

There is an increasing interest in the genetic background of arterial stiffness. This is challenging job due to the heterogeneity of the arterial tree resulting in phenotypic variation between central and distal arteries, but also between aortic compartments.[31] Arterial stiffness is a heritable trait with estimations of 25-40% heritability,[32,33] largely independent of the influence of blood pressure, age and other cardiovascular risk factors. Genetic determinants of blood pressure have been investigated, but steady and pulsatile components of blood pressure have separate physical determinants and it is reasonable to expect different genetic background as well. Studies on the association between genetic polymorphisms and arterial stiffness could provide significant information about the mechanism of arterial stiffness and the role of genetics in accelerated aging. Genetic factors may directly influence the structure of the arterial wall or act indirectly through age, blood pressure, smoking, cholesterol levels, glycaemia and other classical risk factors.[34]

Several studies investigated genetic variants for involvement in the pathophysiology of arterial stiffness.[35] The renin angiotensin aldosterone system (RAAS), which is involved in the blood pressure control, cell proliferation, matrix production and vascular hypertrophy, has been thought to have a major role in arterial stiffening.[36,37] Polymorphisms in the angiotensin converting enzyme (ACE) gene[38-42], angiotensinogen (AGT) gene[43,44], the angiotensin II type 1 receptor(AGTR1) gene[39,45-47] and the aldosterone synthase (CYP11B2) gene[47-50], have been investigated, however the results are inconsistent, as both positive and negative associations have been reported.
Other non-matrix related genetic variants, genes assumed to be involved in cell proliferation, vascular hypertrophy and affecting functional properties as blood pressure regulation, have been studied with arterial stiffness and included nitric oxide synthase (NOS)[51-53], beta 2-adrenoreceptor (ß2-AR)[54,55], endothelin (ET)[56,57], endothelin receptors (ETaR and ETbR)[57,58] and G-proteins.[59]

Genetic variations in extracellular matrix proteins or genes that modulated structural changes in proteins have been studied for evidence that they affect the arterial wall remodeling process. Elastin and collagen are the main extracellular matrix proteins of the vessel wall, they can be synthesized the novo, but are also susceptible to enzymatic degradation by enzymes including elastases and matrix metalloproteinases (MMPs). The elastin gene (ELN)[60] and gene encoding collagen type 1A (COL1A)[61] have both been associated with arterial stiffness in the general population. A variant in the COL4A1 has been identified with one of the first GWA analyses.[62] The gene coding fibrillin 1 (FBN1)[63,64], the disease gene for Marfan's syndrome, has also been associated with arterial stiffness in healthy subjects. Matrix homeostasis is a critical determinant of the mechanical properties of the blood vessels, the mechanisms whereby matrix proteins are deposited and turned over in the vessel wall are likely to play a role in the process of arterial stiffening. The genetic variation of the activity of MMPs, and then especially MMP3[65] and MMP9[66,67] has been extensively investigated and shown to be associated with increased age-related arterial stiffening.

However, most of the mechanisms linking these above mentioned gene polymorphisms to arterial wall properties have remained unclear or the association is waiting to be confirmed in much larger populations. Therefore, we performed GWA studies for arterial stiffness, which are described in **chapter 3**.

In **chapter 3.1** we performed a GWA study of pulse pressure, a surrogate measure of arterial stiffness of the main arteries and MAP, a weighted average of SBP and DBP, in the framework of the ICBP consortium. We identified four novel loci for pulse pressure, two novel loci for mean arterial pressure (MAP) and one novel locus for both traits, and 21 loci for PP and MAP previously associated with SBP and DBP. (Table 2) The novel loci for MAP were strongly associated with SBP and DBP, reflecting the high intercorrelations among these blood pressure traits. On the other hand for three of the novel loci found for PP, the estimated effects on SBP were in the opposite direction to the effects on DBP, suggesting new genetic pathways underlying blood pressure variation. The risk score containing the 10 independent SNPs (novel and already known from SBP and DBP associations), was associated with prevalent hypertension, left ventricular wall thickness, incident stroke and coronary heart disease, confirming the clinical relevance of pulse pressure.

In **chapter 3.2** we described GWA study for aortic stiffness, measured by the carotid femoral pulse wave velocity PWV (cfPWV), the golden standard measurement of arterial stiffness in 9 community-based cohorts (AGES, Baltimore Longitudinal Study of Aging (BLSA), ERF, FHS, Health Aging and Body Composition (HABC), Heredity and Phenotype Intervention (HAPI), RS-I, RS-II, SARDINIA) and replicated in two cohorts (Anglo Cardiff Collaborative Trial (ACCT), Asklepios Study (AS)). We identified common genetic variation in a locus in the BCL11B gene desert, which is associated with higher cfPWV and related cardiovascular disease events, including coronary artery disease and heart failure. We performed sequencing of this region and two of this non-coding RNAs are expressed in relevant human cardiac and vascular tissues and cell lines, including full thickness aortic rings, aortic smooth muscle cells, cardiac fibroblasts and HUVECs. We indicated that the gene desert of VRK1-BCL11B harbors a regulatory locus that modulated aortic stiffness.

Table 2. Genes implicated to be associated with arterial stiffness or MAP

Trait	Novel loci	Previous association SBP/DBP
Pulse pressure[68]	PIK3CG, ADAMTS8, CHIC2, NOV, FIGN	NPR3-C5orf23, PLCE1, CUP17A1-NT5C, ATP2B1, GOSR2
MAP[68]	MAP, ADRB1, FIGN	MTHFR-NPPB, SLC4A7, MECOM, FGF5, SLC39A8, NPR3-C5orf23, EBF1, HFE, CACNB2(3'),C10orf107, CYP17A1-NT5C2,PLEKHA7, FJ32810-TMEM33, ATP2B1, ATXN2, TBX5-TBX3, CSK, FES, JAG1, GNAS-EDN3
PWV[69]	BCL11B	-

MAP: mean arterial pressure; SBP systolic blood pressure; DBP diastolic blood pressure; PWV pulse wave velocity

We have demonstrated that specific components of blood pressure have different genetic determinants, that the pulsatile component is modulated by in part a different set of genes. Genetic variation of pulse pressure could provide clues for the pathophysiology of arterial stiffness. Although it has been shown that arterial stiffness is a hereditable trait, it remains difficult to link it consistently with specific genetic variants. This could have diverse reasons. First, the heterogeneity of the vascular tree, reflects different phenotypes and is therefore dependent of the place of measurement. The GWA study on pulse pressure used the blood pressure measurements of the brachial artery, whereas the GWA study on arterial stiffness used cfPWV and therefore combines the thoracic part of the aorta with the abdominal part en even included part of the iliac arteries. However, the pulse pressure loci were also associated with cfPWV and the other way around, suggesting that the place of measurement did not have a big influence. Second, the polygenic nature of arterial stiffness and the gene-gene and gene environment interactions, make it difficult to identify the contribution of one specific gene and can explain the inconsistencies between single gene studies. Finally, in the light of the large consortia, the GWA of arterial stiffness has been performed in a relatively small sample size and an increase in sample size could help to identify more genetic loci.

The success of genome wide association studies for common diseases

The rare high and low blood pressure syndromes have identified mutations with gain or loss of function in several renal sodium handling regulatory genes and steroid hormone metabolism. It has been suggested that similar mechanisms also contribute to the genetic origin of hypertension in the general population, however those mutations are very unlikely to explain much of the BP variation in the general population based on their low frequency in the general population.[70] Interestingly, anti-hypertensive medication which is used in clinical practice, is partly based on these pathways identified in these rare syndromes and have shown to be useful in the treatment of hypertension in the general population

For common diseases, multiple genetic and environmental factors influence an individuals risk of being affected. The past years GWA analyses have dominated the search for new genes for complex diseases overtaking approaches of gene finding such as candidate gene and linkage analyses. Hypertension, blood pressure and arterial stiffness have been considered as complex (or polygenic) genetic traits[71], where linkage studies and candidate gene studies were underpowered to find variants with modest effects for these traits.[72] A model for the genetics of complex traits has been the common disease-common variant hypotheses, which implies that common disease is due to allelic variants with a frequency greater than 5% in the general population and a small individual effect size.[73,74] GWA studies are designed to identify common variants through hypothesis-free association analyses of hundreds of thousands of single nucleotide polymorphisms (SNPs). (Figure 2)

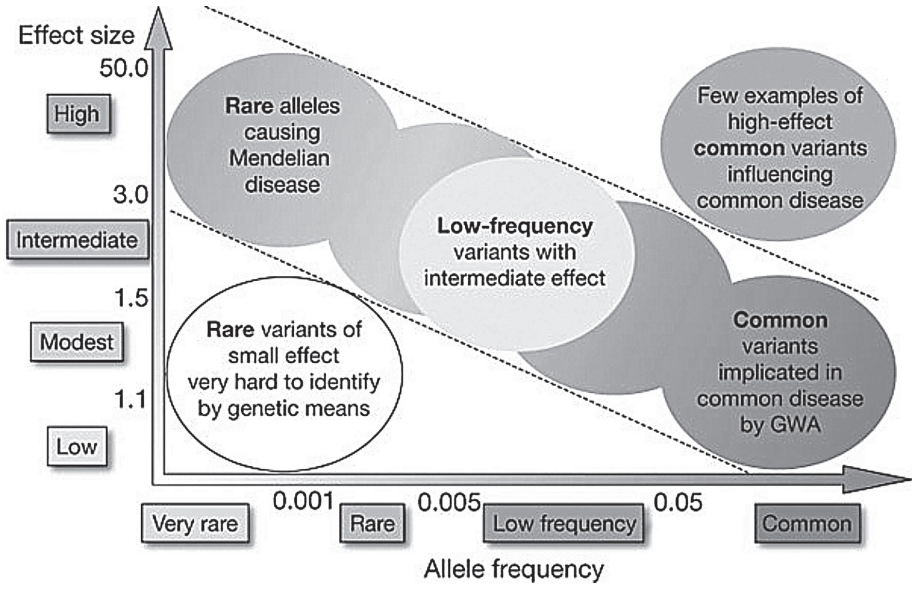


Figure 2. Spectrum of allele frequency and effect size in genetic disease

GWA studies has been successful in identifying common variants for hypertension and blood pressure traits.[70] The first large-scale attempt to identify genetic variants for hypertension was carried out by the Welcome Trust Case Control Consortium, but did not identify genome/wide significant variants.[75] The need for larger sample sizes was recognized and large consortia were formed to share and pool data sets and decrease costs of genotyping. Up to date 43 variants associated with SBP, DBP and hypertension have been replicated in independent samples[70] including the genetic variants described in this thesis.[29,30]

While increasing sample size will assist in finding new variants with smaller effects, there is still only a small fraction of the heritability explained.[29,30]; This could have several reasons. First, there will be true positives that remained undetected due to the use of the stringent threshold levels of statistical significance imposed in GWA studies ($P < 5x10-8$) to adjust for multiple testing. This has been shown with genetic scoring method in which genetic risk scores, including also many nongenome-wide significant SNPs, explained more of the variance than scores based only on very significant SNPs.[76] Second, it is possible that common variants act on common disease at many loci, explaining little individually but explaining a much larger share of the trait or disease collectively. The complex genetic interactions between multiple loci rather than single genes make up the complex genetic basis of common diseases. However, hardly any genome-wide significant interaction has been reported. Lack of robust findings in gene-gene and gene-environment interactions are usually attributed to lack of power. Third, the focus of the ´missing´ heritability has shifted to the possible contribution of rare variants, where next generation sequencing comes in place. It has been thought that rare variants (minor allele frequency < 5%) have substantial effect sizes.[77] Moreover, with sequencing it is more likely to find the functional variants of the known loci. Next generation sequencing has recently been performed and results are underway.

Most discovery efforts were carried out using samples of European origin. Hypertension is more common among people of African descent compared with European and consequently have African descents have a higher risk of hypertensive end-organ damage.[78,79] Prior research has reported considerable difference in genetic patterns for blood pressure across ethnic groups. Identification of potential genetic loci implicated in hypertension provides an opportunity for new treatment and management strategies for this high-risk population, but also provides a new strategy in identifying genes and the underlying pathophysiology. Efforts have been made to improve the involvement of non-European samples, where using participants of African origin in the Candidate-gene Association Resource (CARe) has been a nice example.[80]

Risk scores based on the novel loci for blood pressure, were all associated with incident hypertension, blood pressure related organ damage and clinical cardiovascular disease. With this in mind the question arises if we can improve individual cardiovascular risk prediction with individual genetic profile. However, up to now, the predictive ability of genetic risk scores remains poor for cardiovascular disease[81] (and most other common disease), because the variants found so far only accounted for a small proportion of genetic variance. The biggest gains from the GWAs strategy include our improved understanding of pathophysiology of genetic pathways involved in blood pressure regulation and not a better prediction. Genes and regions identified are novel and fill critical gaps in our current knowledge. Whether increasing sample size, complex modeling with interactions terms or sequencing will be successful in unraveling the missing heritability remains to be determined. Therefore, it is difficult to predict the clinical future of genetic cardiovascular risk prediction.[82]

Part II: Consequences of arterial hemodynamics

Age-related blood pressure changes

Hypertension is a common disease among the general population. The prevalence of hypertension increases with advancing age.[7,83] Before 1990, only diastolic blood pressure was considered relevant in clinical practice. Elevated systolic pressure was considered normal and even an index of cardiac strength. From 1990 onwards research focused on elevated systolic pressure. Isolated systolic hypertension (ISH) is the most frequent form of hypertension in the elderly[84], due to continuous increase in systolic blood pressure with advancing age, whereas diastolic blood pressure remains constant or declines, resulting in a steep increase of pulse pressure.[85] The most likely explanation for these changes is the age-related stiffening of the aorta.[86-89] The association between arterial stiffness and hypertension is particular of interest, because the functional relationship is likely bidirectional and can be best described as feed forward in vicious cycle.[90] It is traditionally believed that arterial stiffening is accelerated by higher mean and systolic blood pressure because of structural and functional alterations in the walls of central elastic arteries in response to chronically elevated distending pressures.[91] Research from the last years has shifted the paradigm to the other direction supporting that elevated arterial stiffness increases systolic blood pressure. Two large population based studies showed prospectively that arterial stiffening precedes and predisposes to longitudinal increase in systolic blood pressure and to future hypertension.[92,93] In **chapter 4.1** we confirmed the association of arterial stiffness with ISH and showed that subjects with ISH have a stiffer aorta compared to normotensive subjects, suggesting within the scope of the literature that arterial stiffening is an underlying pathophysiological cause of the increase in pressure.

We also found an association between aortic and carotid stiffness with combined systolic and diastolic hypertension (Sys/Dia HT), but the aorta was less stiff compared with subject with ISH and this difference was most pronounced at older age. Previous studies have shown that central 'elastic' arteries are also stiffer in subjects with Sys/Dia HT[94,95], it is less clear whether this increase of aortic stiffness is simply due to the higher operating pressure of hypertensive arteries. Studies, in which mean pressure was reduced, showed a normalization of aortic stiffness values, suggesting that isobaric stiffness was normal in Sys/Dia HT subjects.[96,97] This reinforces the hypothesis that Sys/Dia HT is not a pathophysiological result of stiff arteries and supports the idea that different pathophysiologies underly ISH and Sys/Dia HT.

Orthostatic hypotension is common in the elderly[98-101] and among hypertensive subjects.[102,103] Orthostatic hypotension has been associated with syncope[104,105] and falls[106], functional impairment and frequent cause of hospitalization.[107] Orthostatic challenge is responsible for distribution of the blood to lower parts of the body, thereby decreasing cardiac filling and cardiac output.[108] The arterial baroreflex has an essential role in the short-term regulation of blood pressure by adapting heart rate, stroke volume and vascular tone to changes in pressure.[109,110] If baroreflex response does not properly function, blood pressure levels will fall. Baroreflex sensitivity decreases with advancing age and conditions as hypertension and diabetes mellitus may contribute as well. Arterial stiffness has been suggested to be involved in the pathophysiology of decreased baroreflex sensitivity,[111-115] leading to orthostatic hypotension.[101] There is a higher pressure threshold in stiff arteries due to the more intense pressure change that is needed to distend the arterial wall.

In **chapter 4.2** we elaborate on the relationship between orthostatic hypotension and cardiovascular diseases and all-cause mortality. Orthostatic hypotension increased the risk of coronary heart disease (CHD) and all-cause mortality. Several explanations are proposed, including orthostatic hypotension as an expression of the underlying cardiovascular disease. Second, the displacement of blood to the lower body with orthostatic challenge, gives a reduction in coronary and cerebral flow and may cause ischemia.[100]

Arterial stiffness in cardiovascular disease

Heart failure is a growing health problem in the aging population with a substantial morbidity and mortality.[116-119] To improve treatment of heart failure, the mechanisms regarding development of heart failure should be unraveled. In **chapter 5.1** we confirmed the relation between SBP and heart failure and demonstrated that both pulse pressure and aPWV are associated with the development of heart failure. Several studies demonstrated the role of pulse pressure in predicting heart failure in the elderly.[120-122] Several mechanisms have been proposed. First, aortic stiffness is involved in the pathogenesis of hypertension[93,123] and is also related to myocardial infarction and diabetes mellitus[124], which are all risk factors for developing heart failure. Secondly, increased aortic stiffness increases the pulsatile load on the left ventricle and could lead to left ventricular hypertrophy[125] and concentric remodeling[126], which is one of the major determinants of cardiac diastolic dysfunction. Finally, a decline in in aortic diastolic blood pressure could lead to subendocardial ischemia and extensive ventricular damage[127] in the presence of coronary artery disease. Pulse pressure predicted incident heart failure independently of SBP, suggesting additional predictive capacity of pulse pressure.

Population-wide primary prevention and individual health-care intervention strategies have contributed to declining mortality trends. If people at risk for developing cardiovascular disease can be identified and measures taken to reduce their cardiovascular risk, a vast majority of fatal and non/fatal cardiovascular events can be prevented. Arterial stiffness measurements have been suggested for use in the clinical practice to improve cardiovascular disease prediction for individual patients. Several studies have demonstrated that increased arterial stiffness is associated with elevated risk of cardiovascular disease, including CHD, stroke, cardiovascular mortality and heart failure (this thesis) in high risk samples, including patients with hypertension[128], end-stage renal disease[129] and diabetes mellitus[130], but also in community based samples.[131-134] In addition, the guidelines for diagnosis and treatment of hypertension have suggested that an aPWV higher than 12m/s might be considered as a marker of target organ damage.[135] A multicentric study has suggested normal and reference values for PWV in a general population from European descent.[136] In **chapter 5.2** we have added aortic stiffness to the Framingham risk factors and determined if the risk classification for CHD improved. However, the addition of aortic stiffness led to minor reclassification of subjects within 10-year cardiovascular disease risk categories, suggesting low additional value of aortic stiffness in the clinical management of CHD in the elderly.

Clinical practice

As described in **chapter 5.2** we were not able to show an additive value of aortic stiffness in individual cardiovascular disease prediction. This does not mean that arterial stiffness is of no use in the clinical practice.

First, the study described above was performed in elderly subjects of general population. It has been suggested that the prognostic value of predictors of cardiovascular disease might decrease with age, due to selective survival and the influence of co-morbidity on risk factor levels. Therefore, it is well possible that aortic stiffness is of value for the prediction of cardiovascular disease in younger subjects. This has been suggested in two recent studies.[137,138] They showed that the addition of aortic stiffness to standard cardiovascular disease risk factors improved the model fit and resulted in improved risk classification in younger subjects. Further, it has been suggested that arterial stiffness is of use for primary prevention of special risk groups, including asymptomatic hypertensive subjects.[128,139]

Second, the measurement of aortic stiffness alone might not be adequate to improve cardiovascular risk prediction above traditional factors, but could still be adequate in combination with other additional risk factors and/or non-invasive tests. In accordance with this, the Atherosclerosis Risk in Communities (ARIC) Study[140] added multiple non-traditional risk factors and markers of subclinical disease to traditional risk factors. A single risk marker that improved risk prediction was not identified, but addition of a set of markers improved prediction.

Third, increased arterial stiffness is, at least in part determined by risk factors, which are combined in the Framingham risk score, including smoking, high cholesterol, hypertension and diabetes mellitus. We could speculate to replace these factors in the risk score, with a single measurement easy to perform, such as aPWV measurement. Furthermore, reduction of blood pressure, hyperglycemia and lipids may not reflect the true reduction in arterial wall damage. Normalization of the these standard cardiovascular risk factors can be achieved in weeks and thereby leading to a strong reduction in cardiovascular risk scores, but without any improvement on arterial stiffness, which may require a long-lasting correction. This temporal dissociation between the expected cardiovascular risk and true cardiovascular risk, can be improved by measurement of arterial stiffness.[141]

Fourth, aortic stiffness may have clinical value beyond cardiovascular disease prediction. Arterial stiffness can be a suitable target for novel risk reduction strategies. Previous studies have suggested that aPWV attenuation improves survival.[142] Non pharmacological treatment, which were able to reduce arterial stiffness, include exercise and dietary changes including weight loss, low salt diet, moderate alcohol consumption, dark chocolate and fish oil. The majority of existing drugs do not appear to lower aPWV in a blood pressure independent manner, however long-term blockade of the renin-angiotensin system may show some benefit.

Future research

An important limitation of GWAs is that genome wide significant SNPs often merely tag but do not provide direct information on the causal variants. To translate those signals to biological function, follow-up studies are necessary, also referred as ´post-GWA´ analyses. Figure 3 displays a diagram on which future research can be build.

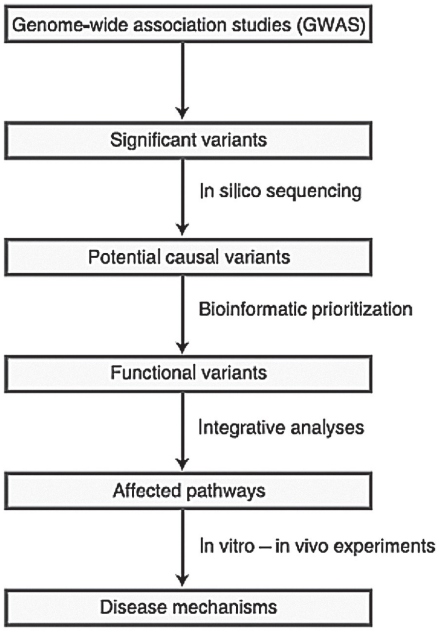


Figure 3. Translation of significant GWAs signals into disease mechanisms[143]

Directions for future research for the clinical use of arterial stiffness with the use of predictive models and follow-up of patients with preventive treatment.

Concluding remarks

The identification of genetic determinants underlying the heritability of arterial hemodynamics is far from complete. However, it has been more powerful than the genetic case-control analyses. We have identified several novel loci associated with arterial hemodynamics, which could improve our understanding on the pathogenesis of arterial hemodynamics.

We provided evidence supporting the hypothesis that arterial stiffness has his own genetic background, suggesting an at least partly independent pathophysiology from hypertension.

Arterial stiffness increases the risk of cardiovascular disease and there are several potential clinical uses of arterial stiffness, but it did not improve risk prediction in the study described in this thesis.

References

1. Alwan A, Maclean DR, Riley LM, et al. Monitoring and surveillance of chronic non-communicable diseases: progress and capacity in high-burden countries. Lancet. Nov 27 2010;376(9755):1861-1868.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS medicine. Nov 2006;3(11):e442.
3. Beaglehole R, Bonita R, Horton R, et al. Priority actions for the non-communicable disease crisis. Lancet. Apr 23 2011;377(9775):1438-1447.
4. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. Dec 15 2012;380(9859):2224-2260.
5. Dart AM, Kingwell BA. Pulse pressure--a review of mechanisms and clinical relevance. Journal of the American College of Cardiology. Mar 15 2001;37(4):975-984.
6. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. Nov 2006;27(21):2588-2605.
7. Mancia G, Fagard R, Narkiewicz K, et al. 2013 Practice guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC): ESH/ESC Task Force for the Management of Arterial Hypertension. Journal of hypertension. Oct 2013;31(10):1925-1938.
8. Lifton RP. Genetic determinants of human hypertension. Proceedings of the National Academy of Sciences of the United States of America. Sep 12 1995;92(19):8545-8551.
9. Levy D, DeStefano AL, Larson MG, et al. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. Hypertension. Oct 2000;36(4):477-483.
10. van Rijn MJ, Schut AF, Aulchenko YS, et al. Heritability of blood pressure traits and the genetic contribution to blood pressure variance explained by four blood-pressure-related genes. Journal of hypertension. Mar 2007;25(3):565-570.
11. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. Genetics in medicine : official journal of the American College of Medical Genetics. Mar-Apr 2002;4(2):45-61.
12. White PC, Dupont J, New MI, Leiberman E, Hochberg Z, Rosler A. A mutation in CYP11B1 (Arg-448----His) associated with steroid 11 beta-hydroxylase deficiency in Jews of Moroccan origin. The Journal of clinical investigation. May 1991;87(5):1664-1667.
13. Lifton RP, Dluhy RG, Powers M, et al. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature. Jan 16 1992;355(6357):262-265.
14. Wilson FH, Disse-Nicodeme S, Choate KA, et al. Human hypertension caused by mutations in WNK kinases. Science. Aug 10 2001;293(5532):1107-1112.
15. Boyden LM, Choi M, Choate KA, et al. Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. Nature. Feb 2 2012;482(7383):98-102.

16. Louis-Dit-Picard H, Barc J, Trujillano D, et al. KLHL3 mutations cause familial hyperkalemic hypertension by impairing ion transport in the distal nephron. Nature genetics. Apr 2012;44(4):456-460, S451-453.
17. Shimkets RA, Warnock DG, Bositis CM, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell. Nov 4 1994;79(3):407-414.
18. Goldsmith O, Solomon DH, Horton R. Hypogonadism and mineralocorticoid excess. The 17-hydroxylase deficiency syndrome. N Engl J Med. Sep 28 1967;277(13):673-677.
19. Mune T, Rogerson FM, Nikkila H, Agarwal AK, White PC. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. Nature genetics. Aug 1995;10(4):394-399.
20. Geller DS, Farhi A, Pinkerton N, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. Science. Jul 7 2000;289(5476):119-123.
21. Choi M, Scholl UI, Yue P, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Science. Feb 11 2011;331(6018):768-772.
22. Tobin MD, Tomaszewski M, Braund PS, et al. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. Hypertension. Jun 2008;51(6):1658-1664.
23. Tobin MD, Raleigh SM, Newhouse S, et al. Association of WNK1 gene polymorphisms and haplotypes with ambulatory blood pressure in the general population. Circulation. Nov 29 2005;112(22):3423-3429.
24. Cowley AW, Jr. The genetic dissection of essential hypertension. Nat Rev Genet. Nov 2006;7(11):829-840.
25. Sober S, Org E, Kepp K, et al. Targeting 160 candidate genes for blood pressure regulation with a genome-wide genotyping array. PLoS One. 2009;4(6):e6034.
26. Manolio TA, Brooks LD, Collins FS. A HapMap harvest of insights into the genetics of common disease. The Journal of clinical investigation. May 2008;118(5):1590-1605.
27. Newton-Cheh C, Johnson T, Gateva V, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nature genetics. May 10 2009.
28. Doi M, Takahashi Y, Komatsu R, et al. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. Nature medicine. Jan;16(1):67-74.
29. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. Nature genetics. May 10 2009.
30. International Consortium for Blood Pressure Genome-Wide Association S, Ehret GB, Munroe PB, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. Oct 6 2011;478(7367):103-109.
31. Safar ME, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. Circulation. Jun 10 2003;107(22):2864-2869.
32. North KE, MacCluer JW, Devereux RB, et al. Heritability of carotid artery structure and function: the Strong Heart Family Study. Arterioscler Thromb Vasc Biol. Oct 1 2002;22(10):1698-1703.

33. Mitchell GF, DeStefano AL, Larson MG, et al. Heritability and a genome-wide linkage scan for arterial stiffness, wave reflection, and mean arterial pressure: the Framingham Heart Study. Circulation. Jul 12 2005;112(2):194-199.
34. Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. Hypertension. Jun 2005;45(6):1050-1055.
35. Yasmin, O'Shaughnessy KM. Genetics of arterial structure and function: towards new biomarkers for aortic stiffness? Clin Sci (Lond). Jun 2008;114(11):661-677.
36. Johnston CI. Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling. Hypertension. Feb 1994;23(2):258-268.
37. Morishita R, Gibbons GH, Ellison KE, et al. Evidence for direct local effect of angiotensin in vascular hypertrophy. In vivo gene transfer of angiotensin converting enzyme. The Journal of clinical investigation. Sep 1994;94(3):978-984.
38. Balkestein EJ, Staessen JA, Wang JG, et al. Carotid and femoral artery stiffness in relation to three candidate genes in a white population. Hypertension. Nov 2001;38(5):1190-1197.
39. Benetos A, Gautier S, Ricard S, et al. Influence of angiotensin-converting enzyme and angiotensin II type 1 receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients. Circulation. Aug 15 1996;94(4):698-703.
40. Mattace-Raso FU, van der Cammen TJ, Sayed-Tabatabaei FA, et al. Angiotensin-converting enzyme gene polymorphism and common carotid stiffness. The Rotterdam study. Atherosclerosis. May 2004;174(1):121-126.
41. Sie MP, Yazdanpanah M, Mattace-Raso FU, et al. Genetic variation in the renin-angiotensin system and arterial stiffness. The Rotterdam Study. Clin Exp Hypertens. Jul 2009;31(5):389-399.
42. Taniwaki H, Kawagishi T, Emoto M, et al. Association of ACE gene polymorphism with arterial stiffness in patients with type 2 diabetes. Diabetes Care. Nov 1999;22(11):1858-1864.
43. Bozec E, Fassot C, Tropeano AI, et al. Angiotensinogen gene M235T polymorphism and reduction in wall thickness in response to antihypertensive treatment. Clin Sci (Lond). Nov 2003;105(5):637-644.
44. Bozec E, Lacolley P, Bergaya S, et al. Arterial stiffness and angiotensinogen gene in hypertensive patients and mutant mice. Journal of hypertension. Jul 2004;22(7):1299-1307.
45. Benetos A, Topouchian J, Ricard S, et al. Influence of angiotensin II type 1 receptor polymorphism on aortic stiffness in never-treated hypertensive patients. Hypertension. Jul 1995;26(1):44-47.
46. Gardier S, Vincent M, Lantelme P, Rial MO, Bricca G, Milon H. A1166C polymorphism of angiotensin II type 1 receptor, blood pressure and arterial stiffness in hypertension. Journal of hypertension. Nov 2004;22(11):2135-2142.
47. Lajemi M, Labat C, Gautier S, et al. Angiotensin II type 1 receptor-153A/G and 1166A/C gene polymorphisms and increase in aortic stiffness with age in hypertensive subjects. Journal of hypertension. Mar 2001;19(3):407-413.
48. Pojoga L, Gautier S, Blanc H, et al. Genetic determination of plasma aldosterone levels in essential hypertension. Am J Hypertens. Jul 1998;11(7):856-860.

49. Safar ME, Cattan V, Lacolley P, et al. Aldosterone synthase gene polymorphism, stroke volume and age-related changes in aortic pulse wave velocity in subjects with hypertension. Journal of hypertension. Jun 2005;23(6):1159-1166.
50. Wojciechowska W, Staessen JA, Stolarz K, et al. Association of peripheral and central arterial wave reflections with the CYP11B2 -344C allele and sodium excretion. Journal of hypertension. Dec 2004;22(12):2311-2319.
51. Chen W, Srinivasan SR, Bond MG, et al. Nitric oxide synthase gene polymorphism (G894T) influences arterial stiffness in adults: The Bogalusa Heart Study. Am J Hypertens. Jul 2004;17(7):553-559.
52. Lacolley P, Gautier S, Poirier O, Pannier B, Cambien F, Benetos A. Nitric oxide synthase gene polymorphisms, blood pressure and aortic stiffness in normotensive and hypertensive subjects. Journal of hypertension. Jan 1998;16(1):31-35.
53. Wilkinson IB, MacCallum H, Cockcroft JR, Webb DJ. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. Br J Clin Pharmacol. Feb 2002;53(2):189-192.
54. Snieder H, Dong Y, Barbeau P, et al. Beta2-adrenergic receptor gene and resting hemodynamics in European and African American youth. Am J Hypertens. Nov 2002;15(11):973-979.
55. Yuan M, Ohishi M, Ito N, et al. Genetic influences of beta-adrenoceptor polymorphisms on arterial functional changes and cardiac remodeling in hypertensive patients. Hypertens Res. Nov 2006;29(11):875-881.
56. Jin JJ, Nakura J, Wu Z, et al. Association of endothelin-1 gene variant with hypertension. Hypertension. Jan 2003;41(1):163-167.
57. Lajemi M, Gautier S, Poirier O, et al. Endothelin gene variants and aortic and cardiac structure in never-treated hypertensives. Am J Hypertens. Aug 2001;14(8 Pt 1):755-760.
58. Nicaud V, Poirier O, Behague I, et al. Polymorphisms of the endothelin-A and -B receptor genes in relation to blood pressure and myocardial infarction: the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) Study. Am J Hypertens. Mar 1999;12(3):304-310.
59. Nurnberger J, Opazo Saez A, Mitchell A, et al. The T-allele of the C825T polymorphism is associated with higher arterial stiffness in young healthy males. Journal of human hypertension. Apr 2004;18(4):267-271.
60. Hanon O, Luong V, Mourad JJ, Bortolotto LA, Jeunemaitre X, Girerd X. Aging, carotid artery distensibility, and the Ser422Gly elastin gene polymorphism in humans. Hypertension. Nov 2001;38(5):1185-1189.
61. Brull DJ, Murray LJ, Boreham CA, et al. Effect of a COL1A1 Sp1 binding site polymorphism on arterial pulse wave velocity: an index of compliance. Hypertension. Sep 2001;38(3):444-448.
62. Tarasov KV, Sanna S, Scuteri A, et al. COL4A1 is associated with arterial stiffness by genome-wide association scan. Circ Cardiovasc Genet. Apr 2009;2(2):151-158.
63. Medley TL, Cole TJ, Gatzka CD, Wang WY, Dart AM, Kingwell BA. Fibrillin-1 genotype is associated with aortic stiffness and disease severity in patients with coronary artery disease. Circulation. Feb 19 2002;105(7):810-815.

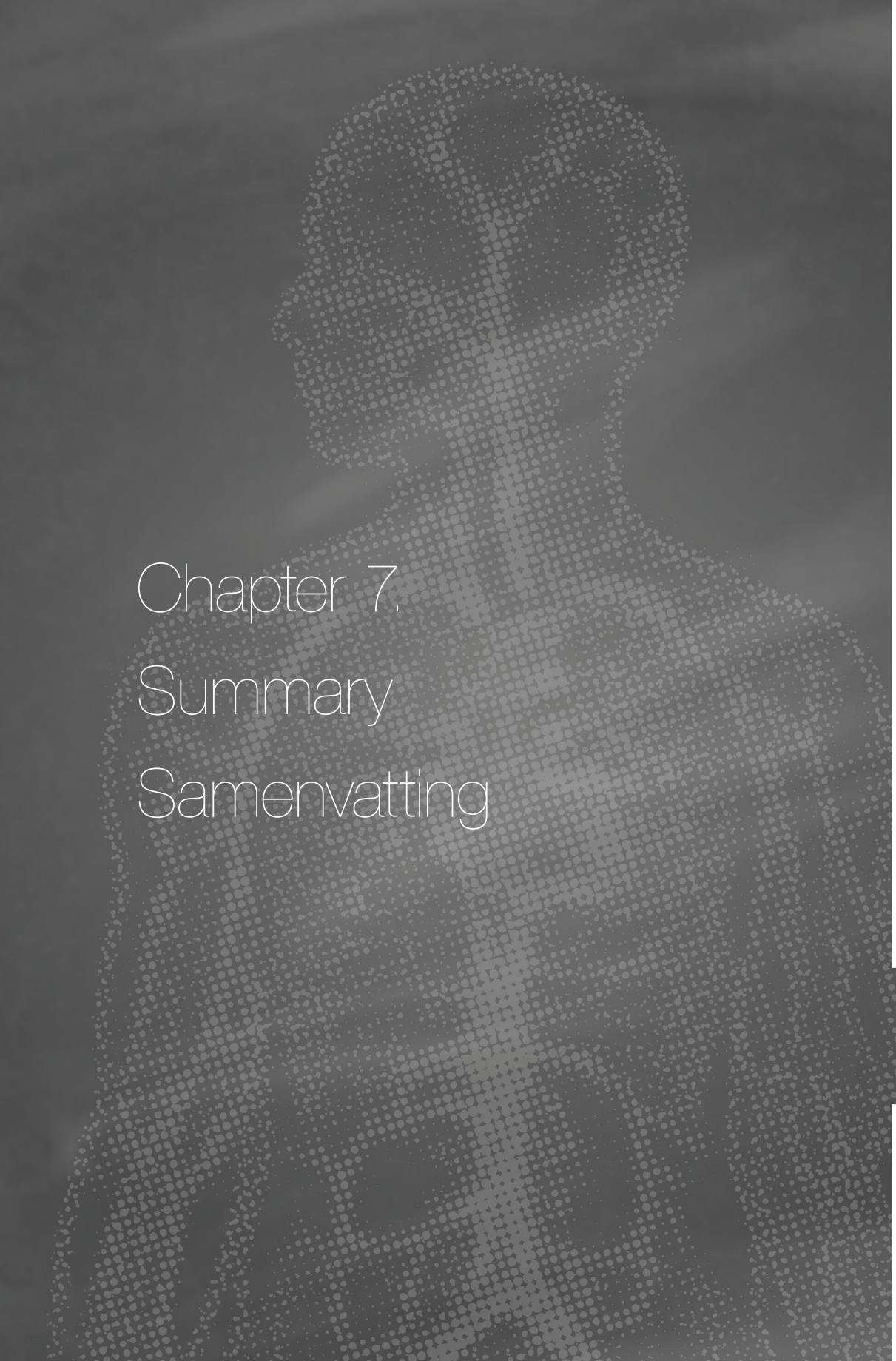
64. Powell JT, Turner RJ, Sian M, Debasso R, Lanne T. Influence of fibrillin-1 genotype on the aortic stiffness in men. J Appl Physiol (1985). Sep 2005;99(3):1036-1040.
65. Medley TL, Kingwell BA, Gatzka CD, Pillay P, Cole TJ. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. Circulation research. Jun 13 2003;92(11):1254-1261.
66. Medley TL, Cole TJ, Dart AM, Gatzka CD, Kingwell BA. Matrix metalloproteinase-9 genotype influences large artery stiffness through effects on aortic gene and protein expression. Arterioscler Thromb Vasc Biol. Aug 2004;24(8):1479-1484.
67. Yasmin, McEniery CM, Wallace S, et al. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler Thromb Vasc Biol. Feb 2005;25(2):372.
68. Wain LV, Verwoert GC, O'Reilly PF, et al. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nature genetics. Oct 2011;43(10):1005-1011.
69. Mitchell GF, Verwoert GC, Tarasov KV, et al. Common genetic variation in the 3'-BCL11B gene desert is associated with carotid-femoral pulse wave velocity and excess cardiovascular disease risk: the AortaGen Consortium. Circ Cardiovasc Genet. Feb 1 2012;5(1):81-90.
70. Ehret GB, Caulfield MJ. Genes for blood pressure: an opportunity to understand hypertension. Eur Heart J. Apr 2013;34(13):951-961.
71. The NATURE of essential hypertension. Lancet. Dec 5 1959;2(7110):1027-1030.
72. Risch NJ. Searching for genetic determinants in the new millennium. Nature. Jun 15 2000;405(6788):847-856.
73. Chakravarti A. Population genetics--making sense out of sequence. Nature genetics. Jan 1999;21(1 Suppl):56-60.
74. Lander ES, Schork NJ. Genetic dissection of complex traits. Science. Sep 30 1994;265(5181):2037-2048.
75. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. Jun 7 2007;447(7145):661-678.
76. Taal HR, Verwoert GC, Demirkan A, et al. Genome-wide profiling of blood pressure in adults and children. Hypertension. Feb 2012;59(2):241-247.
77. Hindorff LA, Sethupathy P, Junkins HA, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences of the United States of America. Jun 9 2009;106(23):9362-9367.
78. Collins R, Winkleby MA. African American women and men at high and low risk for hypertension: a signal detection analysis of NHANES III, 1988-1994. Preventive medicine. Oct 2002;35(4):303-312.
79. Hertz RP, Unger AN, Cornell JA, Saunders E. Racial disparities in hypertension prevalence, awareness, and management. Arch Intern Med. Oct 10 2005;165(18):2098-2104.
80. Fox ER, Young JH, Li Y, et al. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Human molecular genetics. Jun 1 2011;20(11):2273-2284.

81. Thanassoulis G, Vasan RS. Genetic cardiovascular risk prediction: will we get there? Circulation. Nov 30 2010;122(22):2323-2334.
82. Janssens AC, van Duijn CM. Genome-based prediction of common diseases: methodological considerations for future research. Genome medicine. 2009;1(2):20.
83. Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. Lancet. May 3 2008;371(9623):1513-1518.
84. Franklin SS, Jacobs MJ, Wong ND, L'Italien GJ, Lapuerta P. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. Hypertension. Mar 2001;37(3):869-874.
85. Franklin SS, Gustin Wt, Wong ND, et al. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation. Jul 1 1997;96(1):308-315.
86. Berry KL, Cameron JD, Dart AM, et al. Large-artery stiffness contributes to the greater prevalence of systolic hypertension in elderly women. Journal of the American Geriatrics Society. Mar 2004;52(3):368-373.
87. McEniery CM, Yasmin, Wallace S, et al. Increased stroke volume and aortic stiffness contribute to isolated systolic hypertension in young adults. Hypertension. Jul 2005;46(1):221-226.
88. Wallace SM, Yasmin, McEniery CM, et al. Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. Hypertension. Jul 2007;50(1):228-233.
89. Yasmin, McEniery CM, O'Shaughnessy KM, et al. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. Arterioscler Thromb Vasc Biol. Aug 2006;26(8):1799-1805.
90. Alghatrif M, Strait JB, Morrell CH, et al. Longitudinal trajectories of arterial stiffness and the role of blood pressure: the Baltimore longitudinal study of aging. Hypertension. Nov 2013;62(5):934-941.
91. O'Rourke MF, Nichols WW. Aortic diameter, aortic stiffness, and wave reflection increase with age and isolated systolic hypertension. Hypertension. Apr 2005;45(4):652-658.
92. Kaess BM, Rong J, Larson MG, et al. Aortic stiffness, blood pressure progression, and incident hypertension. JAMA. Sep 5 2012;308(9):875-881.
93. Najjar SS, Scuteri A, Shetty V, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. Journal of the American College of Cardiology. Apr 8 2008;51(14):1377-1383.
94. Asmar R, Benetos A, London G, et al. Aortic distensibility in normotensive, untreated and treated hypertensive patients. Blood Press. Jan 1995;4(1):48-54.
95. Safar ME, Laurent S. Behaviour of conduit arteries in hypertension. Clin Exp Hypertens. Nov 1993;15(6):1033-1045.
96. Merillon JP, Fontenier GJ, Lerallut JF, et al. Aortic input impedance in normal man and arterial hypertension: its modification during changes in aortic pressure. Cardiovascular research. Nov 1982;16(11):646-656.

97. Ting CT, Brin KP, Lin SJ, et al. Arterial hemodynamics in human hypertension. The Journal of clinical investigation. Dec 1986;78(6):1462-1471.
98. Caird FI, Andrews GR, Kennedy RD. Effect of posture on blood pressure in the elderly. Br Heart J. May 1973;35(5):527-530.
99. Mader SL, Josephson KR, Rubenstein LZ. Low prevalence of postural hypotension among community-dwelling elderly. Jama. Sep 18 1987;258(11):1511-1514.
100. Raiha I, Luutonen S, Piha J, Seppanen A, Toikka T, Sourander L. Prevalence, predisposing factors, and prognostic importance of postural hypotension. Arch Intern Med. May 8 1995;155(9):930-935.
101. Mattace-Raso FU, van der Cammen TJ, Knetsch AM, et al. Arterial stiffness as the candidate underlying mechanism for postural blood pressure changes and orthostatic hypotension in older adults: the Rotterdam Study. Journal of hypertension. Feb 2006;24(2):339-344.
102. Hiitola P, Enlund H, Kettunen R, Sulkava R, Hartikainen S. Postural changes in blood pressure and the prevalence of orthostatic hypotension among home-dwelling elderly aged 75 years or older. Journal of human hypertension. Jan 2009;23(1):33-39.
103. Judd E, Calhoun DA. Hypertension and orthostatic hypotension in older patients. Journal of hypertension. Jan 2012;30(1):38-39.
104. Lipsitz LA. Syncope in the elderly patient. Hosp Pract (Off Ed). Oct 30 1986;21(10A):33-44.
105. Lipsitz LA, Pluchino FC, Wei JY, Rowe JW. Syncope in institutionalized elderly: the impact of multiple pathological conditions and situational stress. J Chronic Dis. 1986;39(8):619-630.
106. Tinetti ME, Williams TF, Mayewski R. Fall risk index for elderly patients based on number of chronic disabilities. Am J Med. Mar 1986;80(3):429-434.
107. Shibao C, Grijalva CG, Raj SR, Biaggioni I, Griffin MR. Orthostatic hypotension-related hospitalizations in the United States. Am J Med. Nov 2007;120(11):975-980.
108. Smith JJ, Porth CM, Erickson M. Hemodynamic response to the upright posture. J Clin Pharmacol. May 1994;34(5):375-386.
109. Brown AM. Receptors under pressure. An update on baroreceptors. Circulation research. Jan 1980;46(1):1-10.
110. Billman GE, Schwartz PJ, Stone HL. Baroreceptor reflex control of heart rate: a predictor of sudden cardiac death. Circulation. Oct 1982;66(4):874-880.
111. Mattace-Raso FU, van den Meiracker AH, Bos WJ, et al. Arterial stiffness, cardiovagal baroreflex sensitivity and postural blood pressure changes in older adults: the Rotterdam Study. Journal of hypertension. Jul 2007;25(7):1421-1426.
112. Eveson DJ, Robinson TG, Shah NS, Panerai RB, Paul SK, Potter JF. Abnormalities in cardiac baroreceptor sensitivity in acute ischaemic stroke patients are related to aortic stiffness. Clin Sci (Lond). May 2005;108(5):441-447.
113. Chesterton LJ, Sigrist MK, Bennett T, Taal MW, McIntyre CW. Reduced baroreflex sensitivity is associated with increased vascular calcification and arterial stiffness. Nephrol Dial Transplant. Jun 2005;20(6):1140-1147.
114. Monahan KD, Dinenno FA, Seals DR, Clevenger CM, Desouza CA, Tanaka H. Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. Am J Physiol Heart Circ Physiol. Jul 2001;281(1):H284-289.

115. Monahan KD, Tanaka H, Dinenno FA, Seals DR. Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. Circulation. Oct 2 2001;104(14):1627-1632.
116. Ho KK, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure: the Framingham Study. Journal of the American College of Cardiology. Oct 1993;22(4 Suppl A):6A-13A.
117. Bonneux L, Barendregt JJ, Meeter K, Bonsel GJ, van der Maas PJ. Estimating clinical morbidity due to ischemic heart disease and congestive heart failure: the future rise of heart failure. American journal of public health. Jan 1994;84(1):20-28.
118. Swedberg K, Cleland J, Dargie H, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. Eur Heart J. Jun 2005;26(11):1115-1140.
119. Bleumink GS, Knetsch AM, Sturkenboom MC, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J. Sep 2004;25(18):1614-1619.
120. Vaccarino V, Berger AK, Abramson J, et al. Pulse pressure and risk of cardiovascular events in the systolic hypertension in the elderly program. Am J Cardiol. Nov 1 2001;88(9):980-986.
121. Chae CU, Pfeffer MA, Glynn RJ, Mitchell GF, Taylor JO, Hennekens CH. Increased pulse pressure and risk of heart failure in the elderly. Jama. Feb 17 1999;281(7):634-639.
122. Kostis JB, Lawrence-Nelson J, Ranjan R, Wilson AC, Kostis WJ, Lacy CR. Association of increased pulse pressure with the development of heart failure in SHEP. Systolic Hypertension in the Elderly (SHEP) Cooperative Research Group. Am J Hypertens. Aug 2001;14(8 Pt 1):798-803.
123. London GM. Role of arterial wall properties in the pathogenesis of systolic hypertension. Am J Hypertens. Jan 2005;18(1 Pt 2):19S-22S.
124. van Popele NM, Elizabeth Hak A, Mattace-Raso FU, et al. Impaired fasting glucose is associated with increased arterial stiffness in elderly people without diabetes mellitus: the Rotterdam Study. Journal of the American Geriatrics Society. Mar 2006;54(3):397-404.
125. Girerd X, Laurent S, Pannier B, Asmar R, Safar M. Arterial distensibility and left ventricular hypertrophy in patients with sustained essential hypertension. American heart journal. Oct 1991;122(4 Pt 2):1210-1214.
126. Palmieri V, Bella JN, Roman MJ, et al. Pulse pressure/stroke index and left ventricular geometry and function: the LIFE Study. Journal of hypertension. Apr 2003;21(4):781-787.
127. Kass DA, Saeki A, Tunin RS, Recchia FA. Adverse influence of systemic vascular stiffening on cardiac dysfunction and adaptation to acute coronary occlusion. Circulation. Apr 15 1996;93(8):1533-1541.
128. Boutouyrie P, Tropeano AI, Asmar R, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. Jan 2002;39(1):10-15.
129. Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. Kidney Int. May 2003;63(5):1852-1860.

130. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. Oct 15 2002;106(16):2085-2090.
131. Mattace-Raso FU, van der Cammen TJ, Hofman A, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. Feb 7 2006;113(5):657-663.
132. Sutton-Tyrrell K, Najjar SS, Boudreau RM, et al. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation. Jun 28 2005;111(25):3384-3390.
133. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol. Dec 2001;21(12):2046-2050.
134. van Popele NM, Mattace-Raso FU, Vliegenthart R, et al. Aortic stiffness is associated with atherosclerosis of the coronary arteries in older adults: the Rotterdam Study. Journal of hypertension. Dec 2006;24(12):2371-2376.
135. Mancia G, De Backer G, Dominiczak A, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension. Jun 2007;25(6):1105-1187.
136. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. Eur Heart J. Jun 7.
137. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. Circulation. Feb 2;121(4):505-511.
138. Ben-Shlomo Y, Spears M, Boustred C, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. Journal of the American College of Cardiology. Nov 4 2013.
139. Laurent S, Briet M, Boutouyrie P. Arterial stiffness as surrogate end point: needed clinical trials. Hypertension. Aug 2012;60(2):518-522.
140. Chambless LE, Folsom AR, Sharrett AR, et al. Coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC) study. J Clin Epidemiol. Sep 2003;56(9):880-890.
141. Boutouyrie P, Laurent S, Briet M. Importance of arterial stiffness as cardiovascular risk factor for future development of new type of drugs. Fundam Clin Pharmacol. Jun 2008;22(3):241-246.
142. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. Circulation. Feb 20 2001;103(7):987-992.
143. Wang X, Prins BP, Sober S, Laan M, Snieder H. Beyond genome-wide association studies: new strategies for identifying genetic determinants of hypertension. Curr Hypertens Rep. Dec 2011;13(6):442-451.



Chapter 7.
Summary
Samenvatting

Summary

Cardiovascular disease is the number one leading cause of morbidity and mortality worldwide. A large proportion of cardiovascular diseases can be prevented by addressing risk factors and early assessment of target organ damage. The leading cardiovascular risk factor is raised blood pressure, however this relationship is more complex than only the two extremes, systolic and diastolic blood pressure. The pulsatile component of blood pressure plays a role in predicting cardiovascular disease.

Chapter 1 gives an overview of the general principles of the hemodynamic system, the age-related changes and the clinical consequences of age related vascular changes and genetic analysis of arterial hemodynamics.

Part 1 of this thesis is focused on the genetic risk factors for age related changes of the vascular system.

In **chapter 2.1** we identified several novel loci for SBP, DBP and hypertension in a GWA meta-analysis of more than 29,000 subjects in the framework of CHARGE, with replication in an independent consortium, Global BPgen consortium, with almost 35,000 subjects. We showed genome-wide significant associations for four novel loci for SBP, six loci for DBP and one locus for hypertension. The top ten risk alleles were associated with about 1 and 0.5 mmHg increase per risk allele in SBP and DBP, respectively. Although we presented multiple variants with a substantial joined effect on blood pressure, the effect sizes of the individual common alleles were small and explained a small proportion of the blood pressure variation.

In **chapter 2.2** we increased the sample size and extended the work to a bigger collaboration, which used a multi-stage design in 200,000 individuals of European descent in the framework of the International Collaboration on Blood Pressure (ICBP) consortium. We identified 16 novel loci and brought the total number of identified variants with blood pressure up to 29. Nine loci of these 29 were confirmed in individuals from East-Asian ancestry and six loci in individuals from South-Asian ancestry. A risk score derived from these 29 associations was significantly associated with hypertension, blood pressure related organ damage including left ventricular wall thickness and clinical cardiovascular disease including stroke and coronary artery disease. These 29 SNPs explained 0,9% of the phenotypic variation. Calculations estimated that there are up to 116 independent blood pressure variants with effect sizes similar to those reported in this paper, which can explain up to 2.2% of the phenotypic variance for SBP and DBP.

In **chapter 2.3** we describe a candidate gene analyses of the human 3beta-hydroxysteroid dehydrogenase type 1 (HSD3B1) gene, which was suggested as gene of interest for blood pressure regulation in mouse with circadian clock malfunction. We showed that HSD3B1 expression was minimal or absent in adrenocortical tissues, and was not stimulated by adrenocorticotropin or angiotensin II. Subsequently, we selected six tagging SNPs in the HSD3B1 gene and performed analyses in the RS and Erasmus Rucphen family study (ERF). No variants were associated with systolic or diastolic blood pressure or the occurrence of hypertension. These results were confirmed with a lookup in the ICBP consortium. To conclude, we deny an association of human 3beta-hydroxysteroid dehydrogenase type 1 (HSD3B1) gene with aldosterone production or blood pressure.

In **chapter 3.1** we performed a GWA study of pulse pressure, a component of blood pressure which reflects arterial stiffness of the main arteries and mean arterial pressure (MAP), the steady component of blood pressure, within the framework of the ICBP consortium. We identified four novel loci for pulse pressure, two novel loci for MAP and one novel locus for both traits, and 21 loci for PP and MAP previously associated with SBP and DBP. The novel loci for MAP were strongly associated with SBP and DBP, reflecting the high intercorrelations among these blood pressure traits. On the other hand for three of the novel loci found for PP, the estimated effects on SBP were in the opposite direction to the effects on DBP, suggesting new genetic pathways underlying blood pressure variation. The risk score containing the 10 independent SNPs (novel and already known from SBP and DBP associations), was associated with prevalent hypertension, left ventricular wall thickness , incident stroke and coronary heart disease, confirming the clinical relevance of pulse pressure.

In **chapter 3.2** we described GWA study for aortic stiffness, measured by the carotid femoral pulse wave velocity PWV (cfPWV), the golden standard measurement of arterial stiffness in 9 community-based cohorts (AGES, Baltimore Longitudinal Study of Aging (BLSA), ERF, FHS, Health Aging and Body Composition (HABC), Heredity and Phenotype Intervention (HAPI), RS-I, RS-II, SARDINIA) and replicated in two cohorts (Anglo Cardiff Collaborative Trial (ACCT), Asklepios Study (AS)). We identified common genetic variation in a locus in the BCL11B gene desert, which is associated with higher cfPWV and related cardiovascular disease events, including coronary artery disease and heart failure. We performed sequencing of this region and two of this non-coding RNAs are expressed in relevant human cardiac and vascular tissues and cell lines, including full thickness aortic rings, aortic smooth muscle cells, cardiac fibroblasts and HUVECs. We indicated that the gene desert of VRK1-BCL11B harbors a regulatory locus that modulated aortic stiffness.

Part 2 of this thesis is focused on the cardiovascular consequences of age related changes of the vascular system.

The association between arterial stiffness and hypertension is of interest, because the functional relationship is likely bidirectional and can be best described as feed forward in vicious cycle. In **chapter 4.1** we confirmed the association of arterial stiffness with isolated systolic hypertension (ISH) and showed that subjects with ISH have a stiffer aorta compared to normotensive subjects and subjects with combined systolic and diastolic hypertension. This difference was most pronounced at older age.

Since orthostatic hypotension is common in elderly and among hypertensive subjects, we elaborate in **chapter 4.2** on the relationship between orthostatic hypotension and cardiovascular diseases and all-cause mortality. Orthostatic hypotension increased the risk of coronary heart disease (CHD) and all-cause mortality.

Heart failure is a growing health problem in the aging population. To improve treatment of heart failure, the mechanisms regarding development of heart failure should be unraveled. In **chapter 5.1** we confirmed the relation between SBP and heart failure and demonstrated that both pulse pressure and aPWV are associated with the development of heart failure.

Population-wide primary prevention and individual health-care intervention strategies for cardiovascular disease have contributed to declining mortality trends. If people at risk for developing cardiovascular disease can be identified and measures taken to reduce their cardiovascular risk, a vast majority of fatal and non/fatal cardiovascular events can be prevented. In **chapter 5.2** we have added aortic stiffness to the Framingham risk factors and determined if the risk classification for CHD improved. However, the addition of aortic stiffness led to minor reclassification of subjects within 10-year cardiovascular disease risk categories, suggesting low additional value of aortic stiffness in the clinical management of CHD in the elderly.

In **chapter 6** we discussed the main findings of the studies presented in this thesis, we puts the results of our research in perspective of the literature, addresses potential clinical implications, elaborates on methodological considerations and provides directions for future research.

Samenvatting

Hart- en vaatziekte is wereldwijd doodsoorzaak nummer één. Een groot gedeelte hiervan is te voorkomen door het behandelen van risicofactoren en het vroeg herkennen van schade aan doelorganen. Hoge bloeddruk is de belangrijkste risicofactor; deze relatie bestaat uit meer dan alleen de systolische en diastolische bloeddruk. De pulsatiele component van bloeddruk speelt ook een rol in het voorspellen van hart- en vaatziekten.

Hoofdstuk 1 geeft een overzicht van de algemene principes van arteriële hemodynamiek met hierbij de leeftijdsafhankelijke veranderingen en de klinische consequenties hiervan en daarnaast uitleg over de genetische analyses. Het hoofdstuk eindigt met de doelen van dit proefschrift.

Deel 1 van dit poefschrift beschrijft de genetische risicofactoren voor leeftijdsafhankelijke veranderingen van het vasculaire stelsel.

In **hoofdstuk 2.1** beschrijven we genoom brede meta-analyses bij 29,000 deelnemers van het CHARGE consortium en met replicatie in een onafhankelijk consortium, Global BPgen, met bijna 35,000 deelnemers. We hebben meerdere nieuwe gen loci gevonden voor systolische en diastolische bloeddruk en hypertensie. Er waren vier loci significant geassocieerd met systolische bloeddruk, zes loci met diastolische bloeddruk en een locus met hypertensie. De top tien risico allelen gaven een toename van 1 en 0.5mmHg per allel in respectievelijk systolische en diastolische bloeddruk. De effecten van de individuele allelen waren klein en verklaarden een klein gedeelte van de bloeddrukvariatie, maar tezamen hebben de risico allelen een substantieel effect.

In **hoofdstuk 2.2** beschrijven we de uitbreiding van de genoom brede meta-analyses naar een meerdere stappen analyse in bijna 200,000 deelnemers van Europese afkomst van het internationale samenwerkingsconsortium voor bloeddruk. We hebben 16 nieuwe genetische loci gevonden, waarmee het totaal aantal gevonden varianten op 29 voor bloeddruk kwam. Negen van deze loci hebben we bevestigd in deelnemers van Oost-Azië afkomst en zes loci in deelnemers van Zuid-Afrikaanse afkomst. Een risico score van de 29 in totaal gevonden varianten is significant geassocieerd met hypertensie, bloeddruk gerelateerde orgaanschademarkers zoals linker ventrikel wanddikte en hart- en vaatziekten zoals beroerte en coronairlijden. Deze 29 varianten verklaren ongeveer 0.9% van de bloeddrukvariatie. Volgens berekeningen zijn er 116 onafhankelijke bloeddrukvarianten met dezelfde effect grootte als gevonden in deze paper, die samen in totaal 2.2% van de systolische en diastolische bloeddrukvariatie verklaren.

In **hoofdstuk 2.3** beschrijven we een kandidaat gen analyse van HSD3B1 gen, welke werd gesuggereerd als interessant gen voor bloeddruk regulatie in muizen met stoornis in regulatie van dagritme. We laten zien dat HSD3B1 expressie in bijnierweefsel minimaal tot afwezig is en dat dit niet gestimuleerd werd door adrenocorticotrofine of angiotensine II. Daarnaast selecteerden we zes SNPs uit de HSD3B1 gen en analyseerden deze SNPs in de Rotterdam en Erasmus Rucphen familie studie. Geen van de varianten waren geassocieerd met systolische of diastolische bloeddruk of hypertensie. Deze resultaten werden bevestigd in het internationale samenwerkingsconsortium voor bloeddruk. We vonden geen bewijs voor een verband tussen HSD3B1 gen met aldosteron productie of bloeddruk.

In **hoofdstuk 3.1** beschrijven we een genoom brede analyse van polsdruk, een component van bloeddruk die maat is voor arteriële vaatwandstijfheid van de grote vaten, en het gewogen gemiddelde van systolische en diastolische bloeddruk, de constante component van bloeddruk, in deelnemers van het internationaal samenwerkingsconsortium voor bloeddruk. We hebben vier nieuwe loci voor polsdruk, twee nieuwe loci voor het gewogen gemiddelde van systolische en diastolische bloeddruk en één locus voor beide gevonden. Tevens vonden we 21 loci voor polsdruk en gewogen gemiddelde voor systolische en diastolische bloeddruk die eerder waren gevonden voor systolische en diastolische bloeddruk. De nieuw gevonden loci voor gewogen gemiddelde van systolische en diastolische bloeddruk waren sterk geassocieerd met systolische en diastolische bloeddruk, waarmee de sterke correlatie tussen deze metingen werd bevestigt. Daarentegen, de drie nieuw gevonden loci voor polsdruk hadden een tegengesteld effect in systolische bloeddruk ten opzichte van diastolische bloeddruk, waarmee nieuwe genetische paden onderliggende bloeddruk werden gesuggereerd. De risico score met de tien onafhankelijke loci (de nieuw gevonden en al bekende voor systolische en diastolische bloeddruk) waren geassocieerd met hypertensie, linker ventrikel wanddikte, beroerte en coronairlijden en bevestigd de klinische relevantie van polsdruk metingen.

In **hoofdstuk 3.2** beschrijven we een genoom brede meta-analyse van stijfheid van aorta, gemeten via pols golf snelheid van de carotis naar femoralis slagader, de gouden standaardmeting van arteriële vaatwandstijfheid, in negen populatie studies (AGES, Baltimore Longitudinal Study of Aging (BLSA), ERF, FHS, Health Aging and Body Composition (HABC), Heredity and Phenotype Intervention (HAPI), RS-I, RS-II, SARDINIA). We vonden een verband tussen genetische variatie in het niet-coderende deel van BCL11B gen met hogere vaatwandstijfheid en daarbij gerelateerde hart- en vaatziekten als coronairlijden en hartfalen. Sequencen van de BCL11B regio, tonen dat twee RNAs tot uiting komen in menselijke hart- en vaatweefsel cellijnen, met onder andere aorta ring, gladde spier cellen van de aorta, hart fibroblasten en HUVECs. We tonen aan dat het niet coderende deel van VRK1-BCL11B gen een regulatie locus bevat dat stijfheid van de aorta beïnvloed.

Deel twee van dit proefschrift beschrijft de cardiovasculaire gevolgen van leeftijdsafhankelijke veranderingen van het vasculaire stelsel.

De relatie tussen arteriële vaatwandstijfheid en hypertensie gaat twee richtingen op en kan het best omschreven worden als een voortgaande vicieuze cirkel. In **hoofdstuk 4.1** bevestigen we het verband tussen arteriële vaatwandstijfheid en geïsoleerde systolische hypertensie en laten we zien dat deelnemers met geïsoleerde systolische hypertensie een stijvere aorta hebben ten opzichte van deelnemers met een normale bloeddruk en deelnemers met gecombineerde systolische en diastolische hypertensie. Dit verschil is het grootst op oudere leeftijd.

Orthostatische hypotensie komt veel voor bij ouderen en in patiënten met hypertensie. In **hoofdstuk 4.2** beschrijven we de relatie tussen orthostatische hypotensie, hart en vaatziekten en algehele mortaliteit. Orthostatische hypotensie verhoogt het risico op coronaire hartziekten en algemene mortaliteit.

Hartfalen is een groeiend gezondheidsprobleem in de vergrijzende populatie. Om behandeling van hartfalen te verbeteren, zullen mechanismen tot het ontwikkelen van hartfalen moeten worden onderzocht. In **hoofdstuk 5.1** bevestigen we de relatie tussen systolische bloeddruk en hartfalen, daarnaast laten we zien dat er een verband is tussen zowel polsdruk als arteriële vaatwandstijfheid met het ontwikkelen van hartfalen.

Zowel populatie brede primaire preventie en individuele gezondheidsinterventies voor hart- en vaatziekten hebben bijgedragen aan het verlagen van de mortaliteit aan hart- en vaatziekten. Als je gezonde mensen die het risico lopen voor het ontwikkelen van hart- en vaatziekten kan identificeren en behandeling kan geven om het risico te verlagen op hart- en vaatziekten, kan een groot gedeelte van dodelijke en niet dodelijke hart- en vaatziekte events worden voorkomen. In **hoofdstuk 5.2** onderzoeken we de toegevoegde waarde van de metingen voor aorta vaatwandstijfheid aan de bekende Framingham risico factoren op de risicovoorspelling van coronaire hartziekten, door middel van reclassificatie van risicoclassificatie modellen. Het toevoegen van aorta vaatwandstijfheid leidt tot minimale reclassificatie van 10-jaars risico op coronaire hartziekten. Aorta vaatwandstijfheid metingen hebben hiermee een lage toegevoegde waarde in het klinische beleid van coronaire vaatziekten bij ouderen.

In **hoofdstuk 6** bespreken we de belangrijkste bevindingen van de studies in dit proefschrift tezamen met de recente literatuur, belichten we de klinische mogelijkheden, en geven we ideeën voor verder onderzoek.

Chapter 8.

Dankwoord

PhD Portfolio

List of Publications

About the author

Dankwoord

`Waar je aan begint, moet je ook afmaken`, deze woorden zijn me vaker door het hoofd geschoten dan ik vooraf had kunnen bedenken. Het waren de woorden van mijn vader als ik vroeger ergens vol enthousiasme mee begon en het heeft me het nodige doorzettingsvermogen gegeven. Ondanks alle hobbels op de weg naar de afronding van mijn promotie, heb ik dankzij deze woorden nooit de gedachte gehad om te stoppen met mijn promotie. Gelukkig had ik dan ook veel hulp, steun of sportieve afleiding van collega´s, vrienden en dierbaren. Op deze plaats wil ik graag iedereen bedanken, die betrokken is geweest bij het tot stand komen van mijn proefschrift. Waarschijnlijk lukt het me niet om in dit dankwoord iedereen de eer toe te kennen die ze toekomen, mocht ik je vergeten zijn, bij deze hartelijke dank!

In de eerste plaats wil ik mijn co-promoter, Dr. F.U.S. Mattace-Raso bedanken. Lieve Francesco, vanaf dag één heb je mij met jouw enthousiasme over onderzoek aangestoken. We hebben de afgelopen jaren nogal wat meegemaakt met hoogte- en dieptepunten, maar altijd zijn we blijven werken voor hetzelfde doel. Naast je intensieve begeleiding, dat je altijd te bereiken was voor alle vragen over onderzoek en je snelle respons, kon ik ook altijd terecht voor een warm woord, steun of gewoon gezelligheid. Ik heb bewondering voor je hoe je het afgelopen jaar na het verlies van Annet verder bent gegaan. Ik ben dan ook blij dat we de komende jaren collega´s blijven en verder werken aan het onderzoek, maar bovenal ook vrienden zijn.

Mijn promotoren, Prof.dr. E.J.G. Sijbrands en Prof.dr. C.M. van Duijn. Beste Eric, je enthousiasme voor onderzoek straalt eraf. Meestal was je wat meer op de achtergrond betrokken omdat Francesco mijn eerste aanspreekpunt was, maar je hield wel altijd mijn traject in de gaten en waren onze meetings een mooie intellectuele aanvulling. Dank ook voor je geduld op het moment dat het allemaal niet zo liep en je optimisme dat het goed zou komen, gelukkig ging het de laatste maanden in een stroomversnelling. Best Cock, ik was een geadopteerde onderzoeker op de genetische epidemiologie, maar dankzij jouw steun en kennis heb ik me hier altijd thuis gevoeld. Dankzij jouw begeleiding hebben we met de GWA projecten van bloeddruk een aantal mooie publicaties kunnen realiseren. Dank ook dat je wilde invallen als promotor en mijn niet-genetische stukken wilde beoordelen na het wegvallen van Jacqueline.

Prof.dr. J.C.M. Witteman, beste Jacqueline, jij was diegene die vertrouwen in me had en me na mijn Master onderzoek de mogelijkheid gaf om te starten met mijn promotieonderzoek. Daarnaast wist je ook altijd je vinger op de zwakke methodologische punten te leggen en daarmee mijn onderzoek te verbeteren. Ik vind het jammer dat je weg bent bij de Rotterdam Studie. Prof.dr. A. Hofman, beste Bert als hoofd van de research opleiding NIHES en hoofd van de Rotterdam Studie, heb jij mij de kans gegeven de eerste stappen van onderzoek te doen op je eigen afdeling Epidemiologie. Met je memorabele presentaties en enthousiasme kon ik me geen betere start wensen.

Prof.dr. F. Zijlstra, Prof.dr. G.A. Rongen en Prof.dr. O.H. Franco wil ik bedanken voor hun bereidheid om plaats te nemen in de kleine commissie en voor de inhoudelijke beoordeling van mijn proefschrift. Beste professor Zijlstra ik kijk ernaar uit om mijn opleiding tot cardioloog op uw afdeling te volbrengen. Dear professor Franco, thank you for reading my manuscripts as the successor of Jacqueline. Prof.dr. A.H.J. Danser en dr. Kofflard wil ik bedanken voor hun bereidheid plaats te nemen in de grote commissie. Beste Jan, de afgelopen jaren zijn we bij een aantal projecten samen betrokken geweest. Ik wil je bedanken voor je altijd snelle beoordeling en reacties en het in de gaten houden van de tijdlijn. Beste dr. M.J.M. Kofflard dank u wel voor de mogelijkheid om ervaring op te doen op de afdeling cardiologie in het Albert Schweitzer Ziekenhuis en uw steun voor de opleiding tot cardioloog. Prof.dr. A. Avolio, thank you for being part of the committee.

Alle co-auteurs wil ik bedanken voor hun bijdrage en waardevolle commentaar op de verschillende manuscripten. Speciaal wil ik Hans Hofland bedanken voor het samenwerken aan het HSD3B1 project. Dank ook voor je geduld, omdat het precies op een moment kwam waarop ik persoonlijk niet mijn sterkste tijd had. Prof.dr. A. Hoeks en Prof.dr. R. Reneman, hartelijk dank voor al jullie constructieve adviezen en snelle respons voor mijn papers. In addition, I would like to thank all the members of the CHARGE blood pressure , AortaGen and ICBP consortium for the collaboration on the GWA projects. Especially, Georg Ehret, Louise Wain, Daniel Levy and Gary Mitchell, we spent many hours on the phone. I think I would recognize you more by voice than by appearance. Graag wil ik ook gebruik maken van de gelegenheid om duizenden deelnemers aan het ERGO-onderzoek te bedanken en de huisartsen en apothekers die de data beschikbaar hebben gesteld. Hun bijdrage is onmisbaar voor het ERGO-onderzoek. Daarnaast wil ik de dames van het ERGO-centrum, onder leiding van Anneke Korving en de ERGO fup-sters bedanken voor hun inzet en voor de gezellige dagen op het ERGO-centrum. Anneke, het was altijd fijn om weer op het ERGO-centrum te komen, met je hartelijke ontvangst was het altijd een feestje. Hannie, dank voor je altijd lieve woorden. Natuurlijk een speciaal woord voor de dames van het cardiovasculaire blok. Lieve Inge, Toos en Saskia dank voor alle gezellige dagen, maar zeker ook leerzame tijd. Ik heb me altijd als onderdeel van het team gezien en ik maak nu grote indruk met mijn echo ervaring die ik bij jullie heb kunnen opdoen. Toos wat fijn dat je de foto´s wilt maken, na alles wat ik de afgelopen jaren van je werk heb gezien, kan ik alleen maar uitkijken naar deze foto´s.

Natuurlijk wil ik ook de dames van het secretariaat bedanken voor hun ondersteuning en de heren van het datamanagement en de automatisering voor de technische ondersteuning. Frank, bedankt voor de aanlevering van data. Nano, dank voor al je technische hulp als ik weer eens computer problemen had. Jolande Verkroost dank voor het aanleveren en verwerken van alle events, maar daarnaast ook altijd een woord van steun en uitlaatklep als ik het even niet meer zag zitten.

Gedurende de jaren van mijn onderzoek heb ik het geluk gehad met veel verschillende collega´s samen te werken en maakte dat ik veel zin had om naar mijn werk te gaan. Ik ben alle (ex)-collega´s veel dank verschuldigd. In eerste plaats mijn kamergenoten van het eerste uur, Quirijn en Charlotte, dank voor alle gezellige uurtjes. Sorry dat ik jullie wel eens van het werk heb gehouden met alweer een bizar verhaal van mij en dan vooral jullie nuchter commentaar. Ik hoor en zie de collega´s nog lachen over onze discussies. Bouwe, jij kwam na Charlotte. Dank voor de echte mannenkamer vanaf toen met discussies over sporten en vooral het kijken van de tour de France, Olympische Spelen en het WK voetbal. Ik kan met recht zeggen dat kamer Ee21.28 de gezelligste , leukste, tofste kamer was van het Erasmus MC, maar wel met een luguber fracturen tintje. Verder wil ik ook graag andere collega´s van de hart- en vaatziekten groep Abbas, Isabella, Jan, Janine, Frank, Maarten, Maryam en Rachel bedanken. Dank voor alle constructieve kritiek gedurende hart- en vaatziekte besprekingen. Abbas, dank voor alle hulp bij de genetische analyses. Maryam, you were a very nice and pleasant colleague. Janine samen zijn we bezig geweest om het echoblokje uit te breiden, ik hoop dat er mooie data uitkomen. Maarten, dank voor alle laatste updates. Ik hoop je de komende jaren als collega cardioloog te zien. Natuurlijk wil ik ook mijn collega´s Albert-Jan, Arfan, Ben, Daan, Eline, Elisabeth, Gabriella, Jory, Lintje, Marielle, Mark, Martina, Matthijs, Meike, Michiel, Monika, Monique, Renske, Rikje, Sandra, Seppe, Sjoerd, Toke, Vincent, Virginie en Wishal van de afdeling Epidemiologie bedanken. Naast alle beslommeringen herinner ik me vooral de gezellige borrels. Eline, dank je wel voor alle gesprekken, je was echt het zonnetje van de afdeling en gaf me altijd weer de broodnodige energie. Ik vind het super dat we bij dezelfde sportvereniging zitten . Ik hoop dat het een keer beter gaat met je blessure en we weer samen kunnen sporten. Elisabeth we zijn tegelijkertijd begonnen op de afdeling, dank voor alle gezellige uurtjes. Aaron and Najaf, thanks for all the help with the genetic analyses. I owe you some cake.

Naast mijn collega´s van de afdeling Epidemiologie, wil ik ook mijn collega´s van de afdeling Interne Geneeskunde bedanken. Ondanks dat ik geen kamer bij jullie had, voelde ik me altijd welkom en hadden we leuke discussies tijdens de research meeting, maar misschien nog meer buiten werktijd tijdens de borrels en fietstochten. Ilse en Jorie, dank voor alle gezellige uurtjes op de werkvloer, maar ook daarnaast. Met jullie kan ik mijn twee grootste hobby´s combineren, eten en sporten. Ik mag graag terugdenken aan alle fietstochten die we gemaakt hebben en jullie inspirerende woorden maar ook geduld als ik weer een berg aan het opzwoegen ben en jullie al lang boven staan te wachten. Ik kijk uit naar alle mooie tochten die we nog gaan maken, van Bourgondisch tot op de fiets. Luit, Thijs, Joep, Els, Koen mijn fiets staat klaar om nadat dit proefschrift is afgerond weer wat intensiever te gaan trainen. Mandy en Jeroen ik heb niet lang met jullie samen gewerkt, maar memorabel zijn wel de presentaties die we met zijn allen gemaakt hebben. Pieter, Lieke, Mariëlle, Ton, Joep, Wendy en Joost, ik vergeet het EK voetbal in Berlijn nooit meer. Edith en Carolien, dank voor al jullie hulp met de administratie. Zonder jullie was ik nu nog bezig.

Ook wil ik mijn collega´s van de intensive care uit het Albert Schweitzer ziekenhuis in Dordrecht bedanken. Ik heb een super jaar achter de rug en ontzettend veel geleerd.

Mijn nieuwe collega´s van de Cardiologie uit het Erasmus MC wil ik bedanken voor het warme welkom. Ik ben blij dat ik per 1 oktober mag beginnen met de opleiding.

Ferry Drop, super bedankt voor het ontwerpen van de voorkant en de lay-out. Het is precies geworden zoals ik het wilde!

Graag wil ik mijn vrienden bedanken voor alle steun en interesse. Annemieke, Els, Jacqueline en Marijn (en natuurlijk hierbij ook Pieter en Martin) dank voor alle gezellige etentjes, weekendjes weg en afleiding. Ik vind het super dat we na onze opleiding nog steeds goede vrienden zijn, ondanks dat we allemaal onze eigen wegen gaan. We hebben wat afgelachen, maar ook wel wat tranen gelaten. Dank jullie wel voor alle steun op het moment dat het even wat minder gaat. Els, ik kijk nog graag terug op onze huisgenoten tijd waarbij we beide onderzoek deden. Jacq, ik kijk ernaar uit dat we collega´s worden. Ilona, onze lekkere meidenavonden zijn zo fijn en heerlijk om even onze beslommeringen in de medische wereld te delen. Sandra, het is allemaal begonnen met badminton, maar gaat zo veel verder. Memorabel zijn onze stapavonden, maar tegenwoordig is het vooral lekker fietsen, wandelen en high-tea. Dank voor alle broodnuchtere relativering en gewoon even geen medische verhalen. Dieuwke vanaf de middelbare school zijn we al bevriend. Heerlijk dat jij altijd met je recht door zee benadering en nuchtere inslag, me een andere kijk op de zaak weet te geven

Dat sporten een belangrijk onderdeel van mijn leven is mag duidelijk zijn. Ik wil dan ook mijn sportvrienden bedanken voor alle afleiding van de medische en onderzoek beslommeringen. Mijn waterpolovereniging heeft naast deze afleiding ook een inhoudelijke bijdrage geleverd door zeer enthousiast proefpersoon te zijn toen ik mijn echodiploma wilde behalen. Het waterpolodames team, sorry dat ik zo veel wedstrijden mis, maar dank dat jullie me bij de overige wedstrijden laten spelen. Agnes, Josephine en Lineke, dank voor alle gezellige etentjes en jullie regelmatige realiteit check, dat er ook een wereld is buiten het ziekenhuis. De triatlon vereniging, het is echt super hoe iedereen in zijn waarde wordt gelaten. Van beginner tot professionele atleet, we zijn allemaal welkom en krijgen de ruimte om te sporten en veel tips om ons te verbeteren. Wieb, dank voor alle gezellige avonden. Jean-Michel, Koos en Tom, dank voor alle sportieve tips en jullie geduld om met mij te sporten.

Mijn paranimfen, Charlotte en Maxine. Lieve Charlotte, naast ex-kamergenoten en collega´s zijn we vooral ook vrienden. Ik vind het super dat je naast me wilt staan. Je staat altijd voor me klaar, met je kennis over alles en altijd snelle respons heb je me erg vaak geholpen. Ik hoop dat we in de toekomst nog veel vaker zullen samenwerken. Lieve Max, mijn kleine grote zus, dat je mijn paranimf zou worden stond vast op dag één dat ik aan mijn promotieonderzoek begon. Ik moest je alleen wel even overtuigen dat je toch echt niet veel inhoudelijks hoeft bij te dragen. Naast zus ben je ook mijn beste vriendin. Je bent altijd een voorbeeld voor mij geweest over wat je met doorzettingsvermogen kan bereiken. Ondanks alle problemen in onderwijswereld, sla je, je er goed doorheen. Een andere carrière is altijd nog een optie. Ik weet zeker dat je met jouw kwaliteiten alles kunt bereiken, heb vooral vertrouwen in jezelf.

Lieve Mam, waar moet ik beginnen met bedanken. Dank voor je onvoorwaardelijke steun en vertrouwen waarmee je me alle mogelijkheden hebt gegeven om me te ontwikkelen tot de persoon die ik nu ben. Nooit is iets te veel of te gek en je staat altijd achter mijn keuzes. Ik vind het super knap hoe je de afgelopen jaren staande hebt gehouden. Lieve pap, ik had zo graag gewild dat je bij mijn promotie aanwezig kon zijn. Rust zacht. Ik weet dat je trots op me was.

Germaine

PhD Portfolio

Name	Germaine Claudette Verwoert
Erasmus MC Department	Epidemiology and Internal Medicine
Research School	Netherlands Institute for Health Sciences (NIHES) COEUR
PhD period	2008-2010
Advisors	Prof.dr. E.J.G. Sijbrands Prof.dr. C.M. van Duijn Dr. F.U.S. Mattace-Raso

1.PhD training

Research skills

2009	English writing course
2005-2008	MSc in Clinical Epidemiology, Netherlands Institute for Health Sciences, Erasmus University, Rotterdam, The Netherlands.

In-dept courses

2008	R-Course, Department of Biostatistics, Erasmus MC, Rotterdam, The Netherlands.
2008	Echocardiography course, In Holland, Haarlem, The Netherlands.
2007	Summer Institute, John Hopkins University, Baltimore, USA.

Presentations

Oral presentations

2011	Arterial stiffness and cardiovascular prediction, Wetenschapsdagen Internal Medicine, Antwerp, Belgium.
2011	Insulin, fatty-acid synthase, homocysteine levels and measure of arterial properties. The Rotterdam Study, Hypertension 2011, Milano, Italy.
2009	Is aortic stiffness ready for clinical practice? Results from the Rotterdam Study, Artery 9, Cambridge, United Kingdom.
2009	Arterial stiffness causes isolated systolic hypertension: an age-dependent effect, Hypertension 2009, Milano, Italy.
2009	The value of arterial stiffness in the prediction of cardiovascular disease, Coeur, Rotterdam, the Netherlands.
2008	The risk of heart failure is increased in subjects with raised arterial stiffness, Hypertension 2008, Berlin, Germany.
2008	Orthostatic hypotension and the risk of cardiovascular disease in the elderly, Geriatriedagen, Rotterdam, The Netherlands.

Poster presentations

2009 The risk of heart failure is increased in subjects with raised arterial stiffness, Wetenschapsdagen, Internal Medicine, Antwerp, Belgium.

2008 The risk of heart failure is increased in subjects with raised arterial stiffness, Artery 8, Gent, Belgium.

2008 Orthostatic hypotension and the risk of cardiovascular disease in the elderly, Hypertension 2008, Berlin, Germany.

International conferences

2011 Hypertension 2011, Milano, Italy.

2009 4th CHARGE Meeting in Washington, United States of America.

2009 Artery 9, Cambridge, United Kingdom.

2009 Hypertension 2009, Milano, Italy.

2009 3rd CHARGE Meeting, Rotterdam, The Netherlands.

2008 Artery 8, Gent, Belgium.

2008 Hypertension 2008, Berlin, Germany.

National conferences

2009 Netherlands Hypertension Congress, Utrecht, The Netherlands.

2008 Special KNAW conference on: The role of DNA polymorphisms in complex traits and diseases, Amsterdam, The Netherlands.

2008 Geriatriedagen, Rotterdam, The Netherlands.

Seminars and workshops

2008-2010 COEUR research seminars, Erasmus MC, Rotterdam, The Netherlands.

2007-2010 Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands.

2010 Joint Valorisation Workshop NCHA/CGC, Erasmus MC, Rotterdam, The Netherlands

2009 Pathophysiology of ischemic heart disease, COEUR PhD training, Erasmus MC Rotterdam, The Netherlands.

2009 Peripheral and intracranial obstructive vascular disease, COEUR PhD training, Erasmus MC Rotterdam, The Netherlands.

2009 Arrhythmia research methodology, COEUR PhD training, Erasmus MC, Rotterdam, The Netherlands.

2008 Basic data analysis on gene expression arrays, MolMed, Erasmus MC, Rotterdam, The Netherlands.

Other

2008-2009 Organization and program coordination of the Research seminars, Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands.

2. Teaching activities

Lecturing

2008-2010 Junior Med School, Erasmus MC, Rotterdam, The Netherlands.

Supervising practical

2008-2009 Principles of Research in Medicine, Erasmus Summer Program, Rotterdam, The Netherlands.

2007-2008 Statistics, 4th year medical student, Department of Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

2007 Clinical epidemiology, 4th year medical students, Erasmus MC, Rotterdam, The Netherlands.

Supervising Master theses

2008 "Measures of body composition and risk of heart failure in the elderly", Marno van Lieshout, Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands.

3. Grants, prizes

2011 Travelgrant 22th Meeting on Hypertension, ESH

2009 Travelgrant Artery 9

2009 Travelgrant 20th Meeting on Hypertension, ESH

2008 Travelgrant 19th Meeting on Hypertension, ESH

List of Publications

Manuscripts based on the studies described in this thesis

Chapter 2.1

Levy D*, Ehret GB*, Rice K*, **Verwoert GC***, Launer LJ*, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V#, Larson MG#, Chakravarti A#, Psaty BM#, van Duijn CM#. Genome-wide association study of blood pressure and hypertension. Nat Gen 2009 May; 41:677-687.
(*# equal contribution)

Chapter 2.2

International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, **Verwoert GC**, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sõber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND; CARDIoGRAM consortium; CKDGen Consortium; KidneyGen Consortium; EchoGen consortium; CHARGE-HF consortium, Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Köttgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Artigas MS, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stanáková A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT Jr, Mosley TH, Seshadri S, Shrine NR, Wain LV,

Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen LP, Soininen P, Tukiainen T, Würtz P, Ong RT, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Järvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T, Tang H, Knowles J, Hlatky M, Fortmann S, Assimes TL, Quertermous T, Go A, Iribarren C, Absher D, Risch N, Myers R, Sidney S, Ziegler A, Schillert A, Bickel C, Sinning C, Rupprecht HJ, Lackner K, Wild P, Schnabel R, Blankenberg S, Zeller T, Münzel T, Perret C, Cambien F, Tiret L, Nicaud V, Proust C, Dehghan A, Hofman A, Uitterlinden A, van Duijn C, Levy D, Whitteman J, Cupples LA, Demissie-Banjaw S, Ramachandran V, Smith A, Gudnason V, Boerwinkle E, Folsom A, Morrison A, Psaty BM, Chen IY, Rotter JI, Bis J, Volcik K, Rice K, Taylor KD, Marciante K, Smith N, Glazer N, Heckbert S, Harris T, Lumley T, Kong A, Thorleifsson G, Thorgeirsson G, Holm H, Gulcher JR, Stefansson K, Andersen K, Gretarsdottir S, Thorsteinsdottir U, Preuss M, Schreiber S, Meitinger T, König IR, Lieb W, Hengstenberg C, Schunkert H, Erdmann J, Fischer M, Grosshennig A, Medack A, Stark K, Linsel-Nitschke P, Bruse P, Aherrahrou Z, Peters A, Loley C, Willenborg C, Nahrstedt J, Freyer J, Gulde S, Doering A, Meisinger C, Wichmann HE, Klopp N, Illig T, Meinitzer A, Tomaschitz A, Halperin E, Dobnig H, Scharnagl H, Kleber M, Laaksonen R, Pilz S, Grammer TB, Stojakovic T, Renner W, März W, Böhm BO, Winkelmann BR, Winkler K, Hoffmann MM, O'Donnell CJ, Voight BF, Altshuler D, Siscovick DS, Musunuru K, Peltonen L, Barbalic M, Melander O, Elosua R, Kathiresan S, Schwartz SM, Salomaa V, Guiducci C, Burtt N, Gabriel SB, Stewart AF, Wells GA, Chen L, Jarinova O, Roberts R, McPherson R, Dandona S, Pichard AD, Rader DJ, Devaney J, Lindsay JM, Kent KM, Qu L, Satler L, Burnett MS, Li M, Reilly MP, Wilensky R, Waksman R, Epstein S, Matthai W, Knouff CW, Waterworth DM, Hakonarson HH, Walker MC, Mooser V, Hall AS, Balmforth AJ, Wright BJ, Nelson C, Thompson JR, Samani NJ, Braund PS, Ball SG, Smith NL, Felix JF, Morrison AC, Demissie S, Glazer NL, Loehr LR, Cupples LA, Dehghan A, Lumley T, Rosamond WD, Lieb W, Rivadeneira F, Bis JC, Folsom AR, Benjamin E, Aulchenko YS, Haritunians T, Couper D, Murabito J, Wang YA, Stricker BH, Gottdiener JS, Chang PP, Wang TJ, Rice KM, Hofman A, Heckbert SR, Fox ER, O'Donnell CJ, Uitterlinden AG, Rotter JI, Willerson JT, Levy D, van Duijn CM, Psaty BM, Witteman JC, Boerwinkle E, Vasan RS, Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, Parsa A, Gao X, Yang Q, Smith AV, O'Connell JR, Li M, Schmidt H, Tanaka T, Isaacs A,

Ketkar S, Hwang SJ, Johnson AD, Dehghan A, Teumer A, Paré G, Atkinson EJ, Zeller T, Lohman K, Cornelis MC, Probst-Hensch NM, Kronenberg F, Tönjes A, Hayward C, Aspelund T, Eiriksdottir G, Launer LJ, Harris TB, Rampersaud E, Mitchell BD, Arking DE, Boerwinkle E, Struchalin M, Cavalieri M, Singleton A, Giallauria F, Metter J, de Boer J, Haritunians T, Lumley T, Siscovick D, Psaty BM, Zillikens MC, Oostra BA, Feitosa M, Province M, de Andrade M, Turner ST, Schillert A, Ziegler A, Wild PS, Schnabel RB, Wilde S, Munzel TF, Leak TS, Illig T, Klopp N, Meisinger C, Wichmann HE, Koenig W, Zgaga L, Zemunik T, Kolcic I, Minelli C, Hu FB, Johansson A, Igl W, Zaboli G, Wild SH, Wright AF, Campbell H, Ellinghaus D, Schreiber S, Aulchenko YS, Felix JF, Rivadeneira F, Uitterlinden AG, Hofman A, Imboden M, Nitsch D, Brandstätter A, Kollerits B, Kedenko L, Mägi R, Stumvoll M, Kovacs P, Boban M, Campbell S, Endlich K, Völzke H, Kroemer HK, Nauck M, Völker U, Polasek O, Vitart V, Badola S, Parker AN, Ridker PM, Kardia SL, Blankenberg S, Liu Y, Curhan GC, Franke A, Rochat T, Paulweber B, Prokopenko I, Wang W, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Shlipak MG, van Duijn CM, Borecki I, Krämer BK, Rudan I, Gyllensten U, Wilson JF, Witteman JC, Pramstaller PP, Rettig R, Hastie N, Chasman DI, Kao WH, Heid IM, Fox CS, Vasan RS, Glazer NL, Felix JF, Lieb W, Wild PS, Felix SB, Watzinger N, Larson MG, Smith NL, Dehghan A, Grosshennig A, Schillert A, Teumer A, Schmidt R, Kathiresan S, Lumley T, Aulchenko YS, König IR, Zeller T, Homuth G, Struchalin M, Aragam J, Bis JC, Rivadeneira F, Erdmann J, Schnabel RB, Dörr M, Zweiker R, Lind L, Rodeheffer RJ, Greiser KH, Levy D, Haritunians T, Deckers JW, Stritzke J, Lackner KJ, Völker U, Ingelsson E, Kullo I, Haerting J, O'Donnell CJ, Heckbert SR, Stricker BH, Ziegler A, Reffelmann T, Redfield MM, Werdan K, Mitchell GF, Rice K, Arnett DK, Hofman A, Gottdiener JS, Uitterlinden AG, Meitinger T, Blettner M, Friedrich N, Wang TJ, Psaty BM, van Duijn CM, Wichmann HE, Munzel TF, Kroemer HK, Benjamin EJ, Rotter JI, Witteman JC, Schunkert H, Schmidt H, Völzke H, Blankenberg S, Chambers JC, Zhang W, Lord GM, van der Harst P, Lawlor DA, Sehmi JS, Gale DP, Wass MN, Ahmadi KR, Bakker SJ, Beckmann J, Bilo HJ, Bochud M, Brown MJ, Caulfield MJ, Connell JM, Cook HT, Cotlarciuc I, Davey Smith G, de Silva R, Deng G, Devuyst O, Dikkeschei LD, Dimkovic N, Dockrell M, Dominiczak A, Ebrahim S, Eggermann T, Farrall M, Ferrucci L, Floege J, Forouhi NG, Gansevoort RT, Han X, Hedblad B, Homan van der Heide JJ, Hepkema BG, Hernandez-Fuentes M, Hypponen E, Johnson T, de Jong PE, Kleefstra N, Lagou V, Lapsley M, Li Y, Loos RJ, Luan J, Luttropp K, Maréchal C, Melander O, Munroe PB, Nordfors L, Parsa A, Peltonen L, Penninx BW, Perucha E, Pouta A, Prokopenko I, Roderick PJ, Ruokonen A, Samani NJ, Sanna S, Schalling M, Schlessinger D, Schlieper G, Seelen MA, Shuldiner AR, Sjögren M, Smit JH, Snieder H, Soranzo N, Spector TD, Stenvinkel P, Sternberg MJ, Swaminathan R, Tanaka T, Ubink-Veltmaat LJ, Uda M, Vollenweider P, Wallace C, Waterworth D, Zerres K, Waeber G, Wareham NJ, Maxwell PH, McCarthy MI, Jarvelin MR, Mooser V, Abecasis GR, Lightstone L, Scott J, Navis G, Elliott P, Kooner JS. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011 Sep 11;478(7367):103-9.

Chapter 2.3

Verwoert GC*, Hofland J*, Amin N, Mattace-Raso FUS, Sijbrands EJG, Hofman A, Uitterlinden AG, van Duijn CM, de Jong FH, Danser AHJ. Expression and gene variation studies deny association of human 3ß-hydroxysteroid dehydrogenase type 1 gene (HSD3B1) with aldosterone production or blood pressure. Submitted American Journal of hypertension (* equal contribution).

Chapter 3.1

Wain LV*, **Verwoert GC***, O'Reilly PF*, Shi G*, Johnson T*, Johnson AD, Bochud M, Rice KM, Henneman P, Smith AV, Ehret GB, Amin N, Larson MG, Mooser V, Hadley D, Dörr M, Bis JC, Aspelund T, Esko T, Janssens AC, Zhao JH, Heath S, Laan M, Fu J, Pistis G, Luan J, Arora P, Lucas G, Pirastu N, Pichler I, Jackson AU, Webster RJ, Zhang F, Peden JF, Schmidt H, Tanaka T, Campbell H, Igl W, Milaneschi Y, Hottenga JJ, Vitart V, Chasman DI, Trompet S, Bragg-Gresham JL, Alizadeh BZ, Chambers JC, Guo X, Lehtimäki T, Kühnel B, Lopez LM, Polašek O, Boban M, Nelson CP, Morrison AC, Pihur V, Ganesh SK, Hofman A, Kundu S, Mattace-Raso FU, Rivadeneira F, Sijbrands EJ, Uitterlinden AG, Hwang SJ, Vasan RS, Wang TJ, Bergmann S, Vollenweider P, Waeber G, Laitinen J, Pouta A, Zitting P, McArdle WL, Kroemer HK, Völker U, Völzke H, Glazer NL, Taylor KD, Harris TB, Alavere H, Haller T, Keis A, Tammesoo ML, Aulchenko Y, Barroso I, Khaw KT, Galan P, Hercberg S, Lathrop M, Eyheramendy S, Org E, Sõber S, Lu X, Nolte IM, Penninx BW, Corre T, Masciullo C, Sala C, Groop L, Voight BF, Melander O, O'Donnell CJ, Salomaa V, d'Adamo AP, Fabretto A, Faletra F, Ulivi S, Del Greco M F, Facheris M, Collins FS, Bergman RN, Beilby JP, Hung J, Musk AW, Mangino M, Shin SY, Soranzo N, Watkins H, Goel A, Hamsten A, Gider P, Loitfelder M, Zeginigg M, Hernandez D, Najjar SS, Navarro P, Wild SH, Corsi AM, Singleton A, de Geus EJ, Willemsen G, Parker AN, Rose LM, Buckley B, Stott D, Orru M, Uda M; LifeLines Cohort Study, van der Klauw MM, Zhang W, Li X, Scott J, Chen YD, Burke GL, Kähönen M, Viikari J, Döring A, Meitinger T, Davies G, Starr JM, Emilsson V, Plump A, Lindeman JH, Hoen PA, König IR; EchoGen consortium, Felix JF, Clarke R, Hopewell JC, Ongen H, Breteler M, Debette S, Destefano AL, Fornage M; AortaGen Consortium, Mitchell GF; CHARGE Consortium Heart Failure Working Group, Smith NL; KidneyGen consortium, Holm H, Stefansson K, Thorleifsson G, Thorsteinsdottir U; CKDGen consortium; Cardiogenics consortium; CardioGram, Samani NJ, Preuss M, Rudan I, Hayward C, Deary IJ, Wichmann HE, Raitakari OT, Palmas W, Kooner JS, Stolk RP, Jukema JW, Wright AF, Boomsma DI, Bandinelli S, Gyllensten UB, Wilson JF, Ferrucci L, Schmidt R, Farrall M, Spector TD, Palmer LJ, Tuomilehto J, Pfeufer A, Gasparini P, Siscovick D, Altshuler D, Loos RJ, Toniolo D, Snieder H, Gieger C, Meneton P, Wareham NJ, Oostra BA, Metspalu A, Launer L, Rettig R, Strachan DP, Beckmann JS, Witteman JC, Erdmann J, van Dijk KW, Boerwinkle E, Boehnke M, Ridker PM, Jarvelin MR, Chakravarti A, Abecasis GR, Gudnason V, Newton-Cheh C, Levy D, Munroe PB#, Psaty BM#, Caulfield MJ#, Rao DC#, Tobin MD#, Elliott P#, van Duijn CM#. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet. 2011 Sep 11;43(10):1005-11. (*equal contribution)

Chapter 3.2

Mitchell GF*, **Verwoert GC***, Tarasov KV*, Isaacs A, Smith AV, Yasmin, Rietzschel ER, Tanaka T, Liu Y, Parsa A, Najjar SS, O'Shaughnessy KM, Sigurdsson S, De Buyzere ML, Larson MG, Sie MP, Andrews JS, Post WS, Mattace-Raso FU, McEniery CM, Eiriksdottir G, Segers P, Vasan RS, van Rijn MJ, Howard TD, McArdle PF, Dehghan A, Jewell ES, Newhouse SJ, Bekaert S, Hamburg NM, Newman AB, Hofman A, Scuteri A, De Bacquer D, Ikram MA, Psaty BM, Fuchsberger C, Olden M, Wain LV, Elliott P, Smith NL, Felix JF, Erdmann J, Vita JA, Sutton-Tyrrell K, Sijbrands EJ, Sanna S, Launer LJ, De Meyer T, Johnson AD, Schut AF, Herrington DM, Rivadeneira F, Uda M, Wilkinson IB, Aspelund T, Gillebert TC, Van Bortel L, Benjamin EJ, Oostra BA, Ding J, Gibson Q, Uitterlinden AG, Abecasis GR, Cockcroft JR, Gudnason V, De Backer GG, Ferrucci L, Harris TB, Shuldiner AR, van Duijn CM, Levy D*, Lakatta EG*, Witteman JC*.Common genetic variation in the remote 3'BCL11B enhancer is associated with carotid-femoral pulse-wave velocity in a meta-analysis of 11 community based cohorts: the AortaGen Consortium. Circ Cardiovasc Genet. 2012 Feb 1;5(1):81-90.
(* equal contribution)

Chapter 4.1

Verwoert GC, O Franco , Hoeks APG, Reneman RS, Hofman A, CM v Duijn, Sijbrands EJG, Mattace-Raso FUS. Arterial stiffness and hypertension. The Rotterdam Study. Accepted Journal of hypertension

Chapter 4.2

Verwoert GC, Mattace-Raso FUS, Hofman A, Heeringa J, Stricker BH, Breteler MM, Witteman JC. Orthostatic hypotension and risk of cardiovascular disease in elderly people: the Rotterdam study. Journal of the American Geriatric Society 2008 Oct; 56:1816-20.

Chapter 5.1

Verwoert GC, O Franco, Hofman A, van Noord C, Stricker BHC, Deckers JW, CM v Duijn, Sijbrands EJG, Mattace-Raso FUS. Elevated aortic stiffness predict heart failure in an aging population. The Rotterdam Study. Submitted

Chapter 5.2

Verwoert GC, Elias-Smale SE, Rizopoulos D, Koller MT, Steyerberg EW, Hofman A, Kavousi M, Sijbrands EJG, Hoeks APG, Reneman RS, Mattace-Raso FUS, Witteman JCM. Does aortic stiffness improve the prediction of coronary heart disease in elderly? The Rotterdam Study. J Hum Hypertens. 2012 Jan;26(1):28-34

Other Manuscripts

Selwaness M, van Den Bouwhuijsen Q, Mattace-Raso FU, **Verwoert GC**, Hofman A, Franco OH, Witteman JC, van der Lugt A, Vernooij MW, Wentzel JJ. Arterial stiffness is associated with carotid intraplaque hemorrhage in the general population: The Rotterdam Study. Arterioscler Thromb Vasc Biol. 2014 Jan 30.
Pistis G, Okonkwo SU, Traglia M, Sala C, Shin SY, Masciullo C, Buetti I, Massacane R, Mangino M, Thein SL, Spector TD, Ganesh S; CHARGE Consortium Hematology Working, Pirastu N, Gasparini P, Soranzo N, Camaschella C, Hart D, Green MR, Toniolo D. Genome wide association analysis of a founder population identified TAF3 as a gene for MCHC in humans. PLS One. 2013 Jul 31; 8(7)

Olden M, Teumer A, Bochud M, Pattaro C, Köttgen A, Turner ST, Rettig R, Chen MH, Dehghan A, Bastardot F, Schmidt R, Vollenweider P, Schunkert H, Reilly MP, Fornage M, Launer LJ, **Verwoert GC**, Mitchell GF, Bis JC, O'Donnell CJ, Cheng CY, Sim X, Siscovick DS, Coresh J, Kao WH, Fox CS, O'Seaghdha CM; AortaGen, CARDIoGRAM, CHARGE Eye, CHARGE IMT, ICBP, NeuroCHARGE, and CKDGen Consortia. Overlap between common genetic polymorphisms underpinning kidney traits and cardiovascular disease phenotypes: the CKDGen consortium. Am J Kidney Dis.2013 Jun;61(6):889-98

Singaraja RR, Sivapalaratnam S, Hovingh K, Dubé MP, Castro-Perez J, Collins HL, Adelman SJ, Riwanto M, Manz J, Hubbard B, Tietjen I, Wong K, Mitnaul LJ, van Heek M, Lin L, Roddy TA, McEwen J, Dallinge-Thie G, van Vark-van der Zee L, **Verwoert GC**, Winther M, van Duijn C, Hofman A, Trip MD, Marais AD, Asztalos B, Landmesser U, Sijbrands E, Kastelein JJ, Hayden MR. The impact of partial and complete loss-of-function mutations in endothelial lipase on high-density lipoprotein levels and functionality in humans. Circ Cardiovasc Genet.2013 Feb;6(1):54-62

Selwaness M, van den Bouwhuijsen QJ, **Verwoert GC**, Dehghan A, Mattace-Raso FU, Vernooij M, Franco OH, Hofman A, van der Lugt A, Wentzel JJ, Witteman JC. Blood pressure parameters and carotid intraplaque hemorrhage as measured by magnetic resonance imaging: the Rotterdam Study. Hypertension. 2013 Jan;61(1):76-81

Poels MM, Zaccai K, **Verwoert GC**, Vernooij MW, Hofman A, van der Lugt A, Witteman JC, Breteler MM, Mattace-Raso FU, Ikram MA. Arterial stiffness and cerebral small vessel disease: the Rotterdam Scan Study. Stroke. 2012Oct;43(10):2637-42

Durik M, Kavousi M, van der Pluijm I, Isaacs A, Cheng C, Verdonk K, Loot AE, Oeseburg H, Bhaggoe UM, Leijten F, van Veghel R, de Vries R, Rudez G, Brandt R, Ridwan YR, 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 JC, Duncker DJ, Danser AH, Hoeijmakers JH, Roks AJ.Nucleotide excision DNA repair is associated with age-related vascular dysfunction. Circulation. 2012 Jul 24;126(4):468-78

Fedorowski A, Franceschini N, Brody J, Liu C, **Verwoert GC**, Boerwinkle E, Couper D, Rice KM, Rotter JI, Mattace-Raso F, Uitterlinden A, Hofman A, Almgren P, Sjögren M, Hedblad B, Larson MG, Newton-Cheh C, Wang TJ, Rose KM, Psaty BM, Levy D, Witteman J, Melander O. Orthostatic hypotension and novel blood pressure-associated gene variants: Genetics of Postural Hemodynamics (GPH) Consortium. Eur Heart J. 2012 Sep;33(18):2331-41

Kavousi M, Elias-Smale S, Rutten JH, Leening MJ, Vliegenthart R, **Verwoert GC**, Krestin GP, Oudkerk M, de Maat MP, Leebeek FW, Mattace-Raso FU, Lindemans J, Hofman A, Steyerberg EW, van der Lugt A, van den Meiracker AH, Witteman JC. Evaluation of newer risk markers for coronary heart disease risk classification: a cohort study. Ann Intern Med. 2012 Mar 20;156(6):438-44.

Taal HR, **Verwoert GC**, Demirkan A, Janssens AC, Rice K, Ehret G, Smith AV, Verhaaren BF, Witteman JC, Hofman A, Vernooij MW, Uitterlinden AG, Rivadeneira F, Ikram MA, Levy D, van der Heijden AJ; Cohort for Heart and Aging Research in Genome Epidemiology and Early Genetics and Lifecourse Epidemiology consortia, Jaddoe VW, van Duijn CM. Genome-wide profiling of blood pressure in adults and children. Hypertension. 2012 Feb;59(2):241-7

Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, Holm H, Sanna S, Kavousi M, 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 PF, Peden JF, Rahmioglu N, Soininen P, Speliotes EK, Yuan X, Thorleifsson G, Alizadeh BZ, Atwood LD, Borecki IB, Brown MJ, Charoen P, Cucca F, Das D, de Geus EJ, 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 HL, Johnson T, Kangas AJ, Kema IP, Kühn JP, Lai S, Lathrop M, Lerch MM, Li Y, Liang TJ, Lin JP, Loos RJ, Martin NG, Moffatt MF, Montgomery GW, Munroe PB, Musunuru K, Nakamura Y, O'Donnell CJ, Olafsson I, Penninx BW, Pouta A, Prins BP, Prokopenko I, Puls R, Ruokonen A, Savolainen MJ, Schlessinger D, Schouten JN, 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; Alcohol Genome-wide Association (AlcGen) Consortium; Diabetes Genetics Replication and Meta-analyses (DIAGRAM+) Study; Genetic Investigation of Anthropometric Traits (GIANT) Consortium; Global Lipids Genetics Consortium; Genetics of Liver Disease (GOLD) Consortium; International Consortium for Blood Pressure (ICBP-GWAS); Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC), Abecasis GR, Ahmadi KR, 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 MJ, Stolk RP, Thomas HC, Thorsteinsdottir U, Uda M, Waeber G, Wareham NJ, Waterworth DM, Watkins H, Whitfield JB, Witteman JC, Wolffenbuttel BH, Fox CS, Ala-Korpela M, 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. Nat Genet. 2011 Oct 16;43(11):1131-8

Amin N, Byrne E, Johnson J, Chenevix-Trench G, Walter S, Nolte IM; kConFab Investigators, Vink JM, Rawal R, Mangino M, Teumer A, Keers JC, **Verwoert GC**, Baumeister S, Biffar R, Petersmann A, Dahmen N, Doering A, Isaacs A, Broer L, Wray NR, Montgomery GW, Levy D, Psaty BM, Gudnason V, Chakravarti A, Sulem P, Gudbjartsson DF, Kiemeney LA, Thorsteinsdottir U, Stefansson K, van Rooij FJ, Aulchenko YS, Hottenga JJ, Rivadeneira FR, Hofman A, Uitterlinden AG, Hammond CJ, Shin SY, Ikram A, Witteman JC, Janssens AC, Snieder H, Tiemeier H, Wolfenbuttel BH, Oostra BA, Heath AC, Wichmann E, Spector TD, Grabe HJ, Boomsma DI, Martin NG, van Duijn CM. Genome-wide association analysis of coffee drinking suggests association with CYP1A1/CYP1A2 and NRCAM. Mol Psychiatry. 2012 Nov;17(11):1116-29

Elias-Smale SE, Kavousi M, **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 people: the Rotterdam Study. Eur J Prev Cardiol. 2012 Aug;19(4):698-705

Van Lieshout MA, **Verwoert GC**, Mattace-Raso FU, Zillikens MC, Sijbrands EJ, Deckers JW, Hofman A, Witteman JC. Measures of Body Composition and Risk of Heart Failure in the Elderly. The Rotterdam Study. J Nutr Health Aging. 2011;15(5):393-7.

Johnson AD, Newton-Cheh C, Chasman DI, Ehret GB, Johnson T, Rose L, Rice K, **Verwoert GC**, Launer LJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, Caulfield M, van Duijn CM, Ridker PM, Munroe PB, Levy D; on Behalf of the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium, Global BPgen Consortium, and Women's Genome Health Study. Association of Hypertension Drug Target Genes With Blood Pressure and Hypertension in 86 588 Individuals. Hypertension. 2011 May;57(5):903-10

Fox ER, Young JH, Li Y, Dreisbach AW, Keating BJ, Musani SK, Liu K, Morrison AC, Ganesh S, Kutlar A, Ramachandran VS, Polak JF, Fabsitz RR, Dries DL, Farlow DN, Redline S, Adeyemo A, Hirschorn JN, Sun YV, Wyatt SB, Penman AD, Palmas W, Rotter JI, Townsend RR, Doumatey AP, Tayo BO, Mosley TH Jr, Lyon HN, Kang SJ, Rotimi CN, Cooper RS, Franceschini N, Curb JD, Martin LW, Eaton CB, Kardia SL, Taylor HA, Caulfield MJ, Ehret GB, Johnson T; The International Consortium for Blood Pressure Genome-wide Association Studies (ICBP-GWAS); CARDIoGRAM consortium; CKDGen consortium; KidneyGen consortium; EchoGen consortium; CHARGE-HF consortium, Chakravarti A, Zhu X, Levy D. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Hum Mol Genet. 2011 Jun1;20(11):2273-84

Rutten JH, Mattace-Raso FU, **Verwoert GC**, Lindemans J, Hofman A, Witteman JC, van den Meiracker AH.. Arterial stiffness as determinant of increased levels of amino terminal pro-B-type natriuretic peptide levels in individuals with and without cardiovascular disease – The Rotterdam Study. J Hypertens. 2010 Oct;28(10):2061-7

Meyer TE#, **Verwoert GC**#, Hwang SJ#, Glazer NL#, Smith AV#, van Rooij FJ, Ehret GB, Boerwinkle E, Felix JF, Leak TS, Harris TB, Yang Q, Dehghan A, Aspelund T, Katz R, Homuth G, Kocher T, Rettig R, Ried JS, Gieger C, Prucha H, Pfeufer A, Meitinger T, Coresh J, Hofman A, Sarnak MJ, Chen YD, Uitterlinden AG, Chakravarti A, Psaty BM, van Duijn CM, Kao WH, Witteman JC, Gudnason V, Siscovick DS, Fox CS, Köttgen A; Genetic Factors for Osteoporosis Consortium; Meta Analysis of Glucose and Insulin Related Traits Consortium. Genome wide association studies of serum magnesium, potassium and sodium concentrations identify multiple novel loci influencing serum magnesium concentrations. PLoS Genet. 2012 Aug 5;6(8)
(# equal contribution)

Reference values for Arterial Stiffness Collaboration. Determinants of pulse-wave velocity in healthy people and in the presence of cardiovascular risk factors: ` establishing normal and reference values`. Eur Heart J. 2010 Oct;31(19):2338-50

Mattace-Raso FUS, **Verwoert GC**, HofmanA, Witteman JCM. Inflammation and incident isolated systolic hypertension in older adults. The Rotterdam Study. J Hypertens. 2010 May;28(5):892-5

Pfeufer A, van Noord C, Marciante KD, Arking DE, Larson MG, Smith AV, Tarasov KV, Müller M, Sotoodehnia N, Sinner MF, **Verwoert GC**, Li M, Kao WH, Köttgen A, Coresh J, Bis JC, Psaty BM, Rice K, Rotter JI, Rivadeneira F, Hofman A, Kors JA, Stricker BH, Uitterlinden AG, van Duijn CM, Beckmann BM, Sauter W, Gieger C, Lubitz SA, Newton-Cheh C, Wang TJ, Magnani JW, Schnabel RB, Chung MK, Barnard J, Smith JD, Van Wagoner DR, Vasan RS, Aspelund T, Eiriksdottir G, Harris TB, Launer LJ, Najjar SS, Lakatta E, Schlessinger D, Uda M, Abecasis GR, Müller-Myhsok B, Ehret GB, Boerwinkle E, Chakravarti A, Soliman EZ, Lunetta KL, Perz S, Wichmann HE, Meitinger T, Levy D, Gudnason V, Ellinor PT, Sanna S, Kääb S, Witteman JC, Alonso A, Benjamin EJ, Heckbert SR. Genome-wide association study of PR interval. Nat Genet. 2010 Feb;42(2):153-9.

Ganesh SK, Zakai NA, van Rooij FJ, Soranzo N, Smith AV, Nalls MA, Chen MH, Kottgen A, Glazer NL, Dehghan A, Kuhnel B, Aspelund T, Yang Q, Tanaka T, Jaffe A, Bis JC, **Verwoert GC**, Teumer A, Fox CS, Guralnik JM, Ehret GB, Rice K, Felix JF, Rendon A, Eiriksdottir G, Levy D, Patel KV, Boerwinkle E, Rotter JI, Hofman A, Sambrook JG, Hernandez DG, Zheng G, Bandinelli S, Singleton AB, Coresh J, Lumley T, Uitterlinden AG, Vangils JM, Launer LJ, Cupples LA, Oostra BA, Zwaginga JJ, Ouwehand WH, Thein SL, Meisinger C, Deloukas P, Nauck M, Spector TD, Gieger C, Gudnason V, van Duijn CM, Psaty BM, Ferrucci L, Chakravarti A, Greinacher A, O'Donnell CJ, Witteman JC, Furth S, Cushman M, Harris TB, Lin JP. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nature Genetics 2009 Nov; 41(11):1191-1198.

About the author

Germaine Claudette Verwoert was born on September 2nd, 1985 in Vlaardingen, The Netherlands. In 2003, she graduated from the Angelus Merula Athenaeum in Spijkenisse and started her medical school at the Erasmus University, Rotterdam. During the second year, she was invited to participate in the Master of Science in Clinical Epidemiology program by the Netherlands Institute of Health Sciences. During this program she received her initial training in epidemiology, part of which was spent at the John's Hopkins University, School of Public Health in Baltimore, USA during the 25th Annual summer Session. In 2007, she participated in research on orthostatic hypotension and the risk of cardiovascular disease and mortality at the department of Epidemiology of the Erasmus Medical Center in Rotterdam (supervisors: Dr. F.U.S. Mattace-Raso and Prof.dr. J.C.M. Witteman). In 2007 she obtained her doctoral medical degree and in 2008 she obtained her Master degree in Epidemiology.

At the beginning of 2008 she started the work described in this thesis at the cardiovascular epidemiology unit (head: Prof.dr.J.C.M. Witteman) and genetic epidemiology unit (head: Prof. dr.C.M. van Duijn) of the department Epidemiology (head: Prof.dr. A. Hofman) and at the vascular and metabolic unit (head: Dr. E.J.G. Sijbrands) of the department of Internal Medicine of the Erasmus Medical Center Rotterdam.

From January 2011 to December 2012, she finished her internship and completed her medical studies. From January 2013 to January 2014 she worked as a resident on the intensive care department of the Albert Schweitzer Hospital in Dordrecht. From January 2014 onwards she worked at the cardiac care unit at the Erasmus Medical Center to obtain a residency for Cardiology, which she will start from October 2014 onwards.