UNIVERZITA KARLOVA V PRAZE
LÉKAŘSKÁ FAKULTA V PLZNI

DIZERTAČNÍ PRÁCE

MUDr. JITKA SEIDLEROVÁ

PLZEŇ 2009

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Interactive influences of environmental and genetic factors on the properties of large arteries in relation to sodium handling

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Table of Contents

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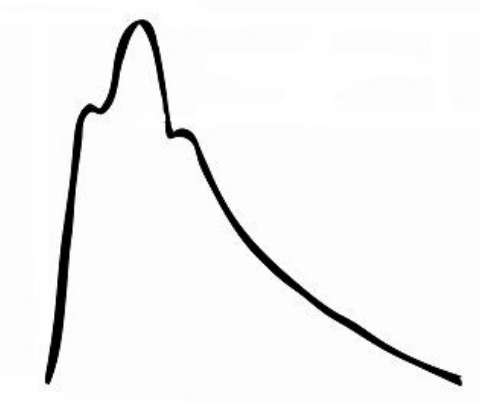
List of Abbreviations

95%CI	95% percent confidence interval
ACE	angiotensin converting enzyme
AGT	angiotensinogen
AIx	augmentation index
AT1R	angiotensin II receptor type 1 gene
AT1	angiotensin II type 1 receptor
CAIx	central augmentation index
CC	cross-sectional compliance
CPP	central pulse pressure
CYP11B2	aldosterone synthase gene
DC	distensibility coefficient
EPOGH	European Project on Genes in Hypertension
FLEMENGHO	Flemish Study on Environment, Genes and Health Outcomes
MAP	mean arterial pressure
PAIx	peripheral augmentation index
PPp	peripheral pulse pressure
PPc	central pulse pressure
aPWV	aortic pulse wave velocity
RAAS	renin-angiotensin-aldosterone system
SBP	systolic blood pressure
SD	standard deviation
SE	standard error
SNP	single nucleotide polymorphism



Chapter 1

Introduction

This doctoral dissertation focuses on environmental and genetic factors, which interactively influence the properties of large arteries. From the inception of our research plans, in line with Guyton's work, we hypothesized that arterial properties might depend on renal sodium handling, which itself changes with salt intake, an environmental factor, and with variation in a large number of genes.

Before presenting the results of our research proper, in this Introduction, we will give a short overview of the arterial system, explain the techniques to measure arterial properties and renal sodium handling, summarize the main determinants of arterial stiffness, clarify why we selected specific candidate genes in family-based population studies.

1.1. Arterial system

1.1.1. Structure of the arterial tree

Starting from the heart, the blood flows first through elastic arteries, such as the aorta and the carotid artery, which gradually give way to muscular or distributing arteries, such as the femoral, brachial and radial arteries. In all these vessels, the endothelium is reinforced by a network of elastic fibres, smooth muscle cells and collagen. These components of the vessel wall vary in proportion and arrangement in different segments of the system, but basically the organization of the arterial wall is similar in that three concentric layers or tunics can be distinguished. The *tunica intima* is an endothelial tube with the long axis of the endothelial cells oriented longitudinally. The *tunica media* is a well organized structure consisting of vascular smooth muscle cells and extracellular matrix proteins. The vascular smooth muscle cells produce elastin and collagen. Vascular smooth muscle cells represent the active component of arterial tone by varying the diameter of the arterial lumen under control of circulating hormones, paracrine substances and neurotransmitters. Vascular smooth muscle cells are circumferentially arranged. Elastin fibres contain the vascular smooth muscle cells in concentric layers. The *tunica adventitia* is made up of fibroblastic and fibrous elements, that are predominantly longitudinally oriented.

The large elastic arteries have walls containing many layers of elastin. They have a buffering function [1]. During systole elastic arteries are distended. The subsequent elastic recoil during diastole attenuates the pulsatile character of the flow and assures that the intermittent contraction of the left ventricle results in a continuous blood flow

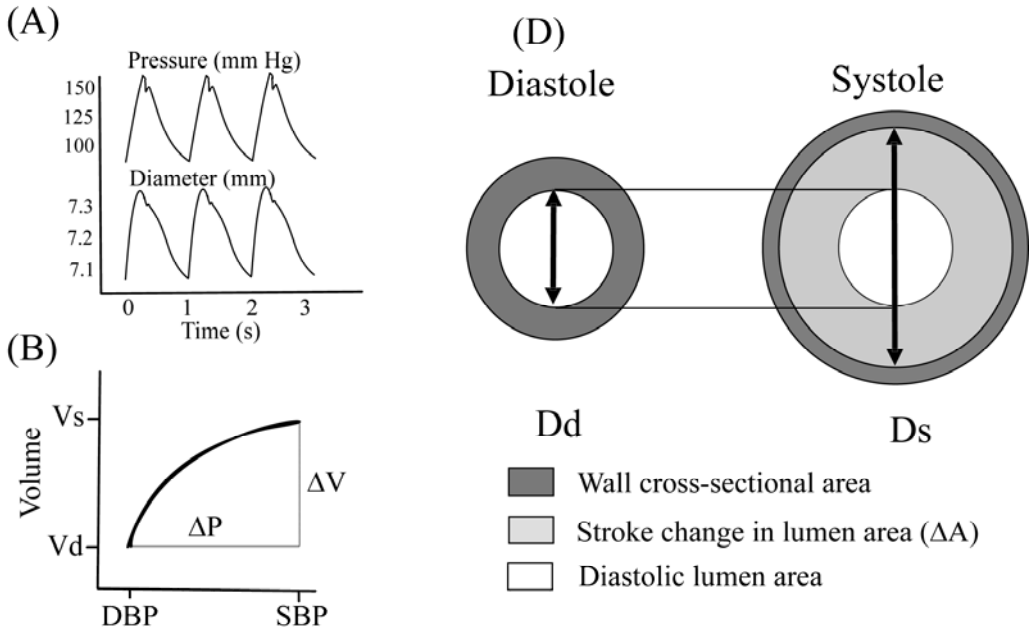
to the periphery. Non-elastic collagen reinforces the arterial wall, limiting distension at elevated transmural pressure. The muscular arteries have a conduit function to deliver blood from the central to the peripheral arteries with a minimal fall in mean arterial pressure (MAP). The vascular smooth muscle cells can actively alter diameter of conduit arteries to adjust blood flow in function of the needs of the irrigated region.

1.1.2. Compliance and distensibility

Compliance, defined as a change of volume per unit of pressure, reflects the capacity to store volume. Distensibility is the relative change in volume per unit of pressure and reflects the strain on the arterial wall exerted by the pressure wave of each heart beat. Arterial compliance (CC) is related to arterial distensibility (DC) and arterial volume (V) by the formula CC = DC × V (Fig. 1.1).

Figure 1.1 Local arterial distensibility

(A) Simultaneous recording of stroke changes in BP and diameter. (B) Pressure–diameter curve. (C) Calculation of distensibility. (D) Schematic representation of the stroke change (ΔA) in lumen cross-sectional area. With permission from *Eur Heart J* 2006; 27:2588-2605.



(C) Distensibility = ΔV/V∗ΔP = ΔA/A∗PP Compliance = ΔV/ΔP = ΔA/PP

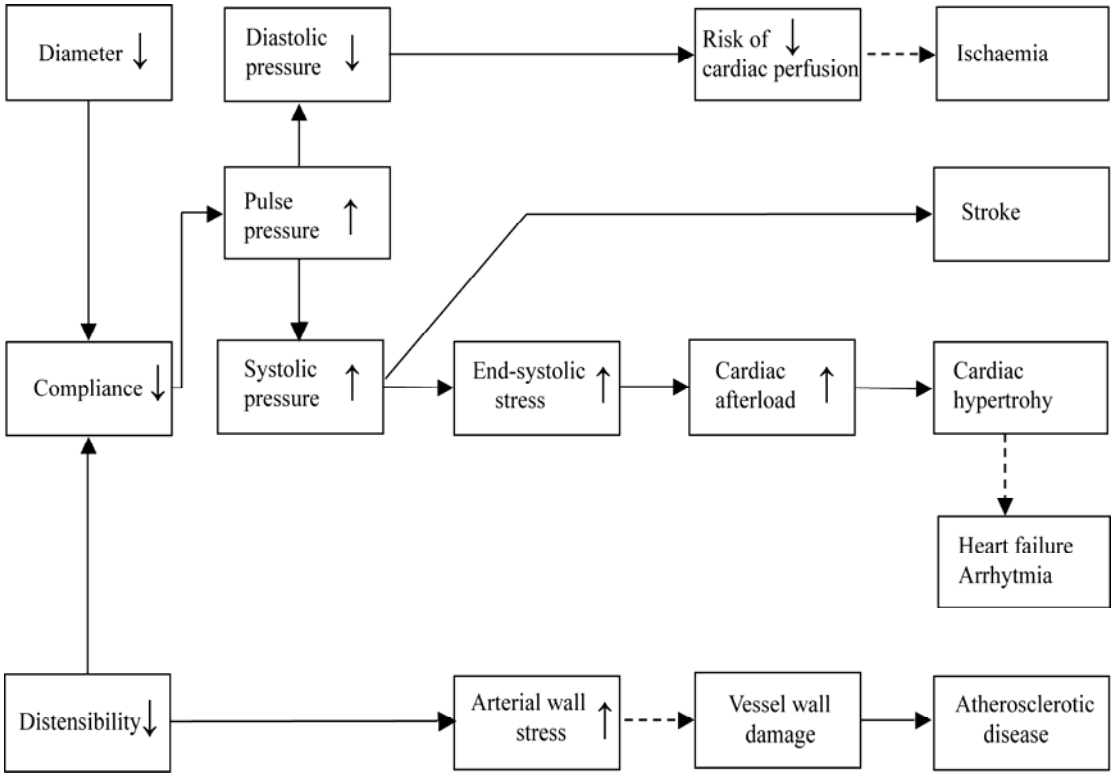
Arterial compliance and total peripheral resistance are major determinants of cardiac afterload. A decreasing distensibility leads to an increasing pulse pressure and

contributes to a higher "mechanical" afterload of the heart. Distensibility is a determinant of the pulsatile stress on the vessel wall, which induces degeneration of the tunica media [2]. The cyclic stress is also a significant risk factor for endothelial lesions and plaque rupture [3], which are important feature of the atherosclerotic disease process.

1.1.3. Arterial stiffness and cardiovascular complications

Decrease in arterial distensibility means stiffer arteries [4]. Carotid-femoral pulse wave velocity (PWV), also called aortic pulse wave velocity (aPWV), an index of arterial stiffness, doubles between 15 and 70 years of age [5]. The arterial pressure wave consists of a forward component generated by the heart and reflected waves returning to the heart from peripheral sites [5,6]. In healthy young adults, the reflected waves coincide with diastole, raise diastolic pressure, and boost coronary perfusion. With arterial stiffening, which arises with aging or in advanced hypertension, the reflected waves move faster, reach the proximal aorta during systole, and cause an augmentation of late systolic pressure, whereas diastolic pressure decreases [5,6]. Lowered diastolic pressure can impair coronary blood flow and predispose to myocardial ischaemia (Fig 1.2). Increased systolic pressure augments cardiac work and can lead to heart failure and arythmia (Fig 1.2). The age-related changes in the elastic properties of the large arteries and in the wave reflections also account for the greater brachial artery pressure than central aortic pressure in young patients, whereas in the elderly or in patients with end-stage renal disease [6] both values tend to be similar.

Figure 1.2 Relation between arterial stiffness and cardiovascular complications



1.2. Measurement of arterial stiffness

1.2.1. Pulse wave velocity

Arterial stiffness is the reciprocal of arterial distensibility. The "gold standard" to measure arterial stiffness is pulse wave velocity [7]. aPWV is a direct measure of arterial stiffness. PWV corresponds to the widely accepted propagation model of arterial system [8]. PWV can be expressed by the Bramwell-Hill equation:

$$PWV = \sqrt{\frac{1}{\rho_b} \cdot \frac{\Delta P}{\Delta A} \cdot A},$$

where ρ_b is the density of blood and A is the cross-sectional area of the arterial lumen in diastole. This relation enables the study of compliance by measuring PWV. Measured along the aortic and aorto-iliac pathway, it has great clinical relevance, because this segment of the arterial system is directly coupled to the left ventricle. aPWV is a strong predictor of cardiovascular outcome, over and beyond other risk factors, not only in patients with hypertension [9-11], diabetes mellitus [12] and end-stage renal disease [13], but in the general population as well [14]. In contrast, PWV

measured outside the aortic tract, at the arm (carotid-brachial PWV) or at the leg (femoro-tibial PWV), has no predictive value in patient with end-stage renal disease [15] (Table 1.1).

In our studies, we measured aPWV using the foot-to-foot velocity method. The foot of the wave is defined at the end of diastole, when the steep rise of the wavefront begins. The foot-to-foot method involves measurement of the arterial waveform at the right common carotid artery and the right femoral artery, and the time delay (Δt or transit time) between the feet of the two waveforms (Fig. 1.3).

Figure 1.3 Measurement of aortic PWV with the foot to foot method

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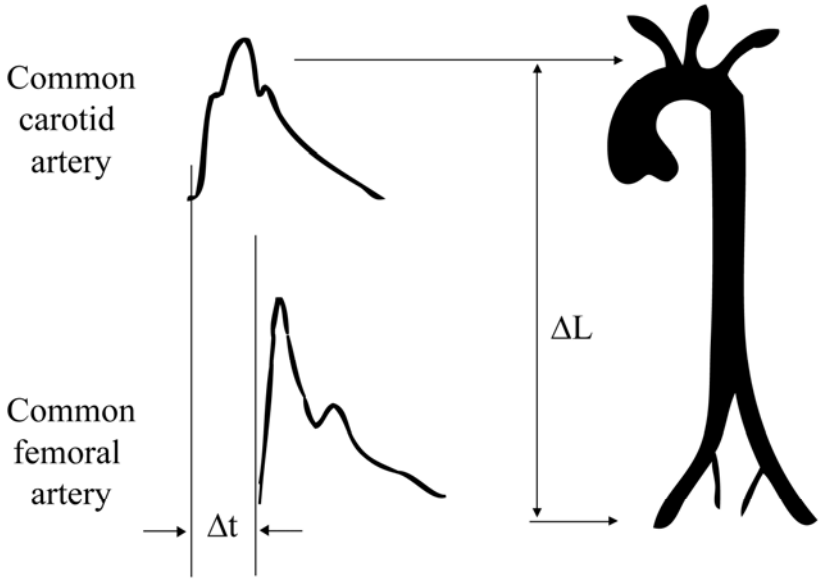


We measured waveforms, using either applanation tonometry [16] or a distension curve registered by ultrasound [17]. The distance (D) covered by the wave was the distance on the body surface between the two recording sites. aPWV was calculated as the ratio of the distance in meter to the transit time in seconds. Some investigators recommend either (i) using the total distance between the carotid and femoral sites of measurement, or (ii) subtracting the distance from the carotid location to the sternal notch from the total distance, or (iii) subtracting the distance from the carotid location to the sternal notch from the distance between the sternal notch and the femoral site of measurement [17,18]. We used latter approach (iii).

The measurement of aPWV also has some potential limitations. The femoral pressure waveform may be difficult to record accurately in patients with metabolic syndrome, obesity, diabetes, and peripheral arterial disease [18]. In the presence of aortic, iliac, or proximal femoral stenosis, the femoral pressure wave may be attenuated and delayed. Abdominal obesity, particularly in men, and large breast size in women can make the surface distance measurements inaccurate [18].

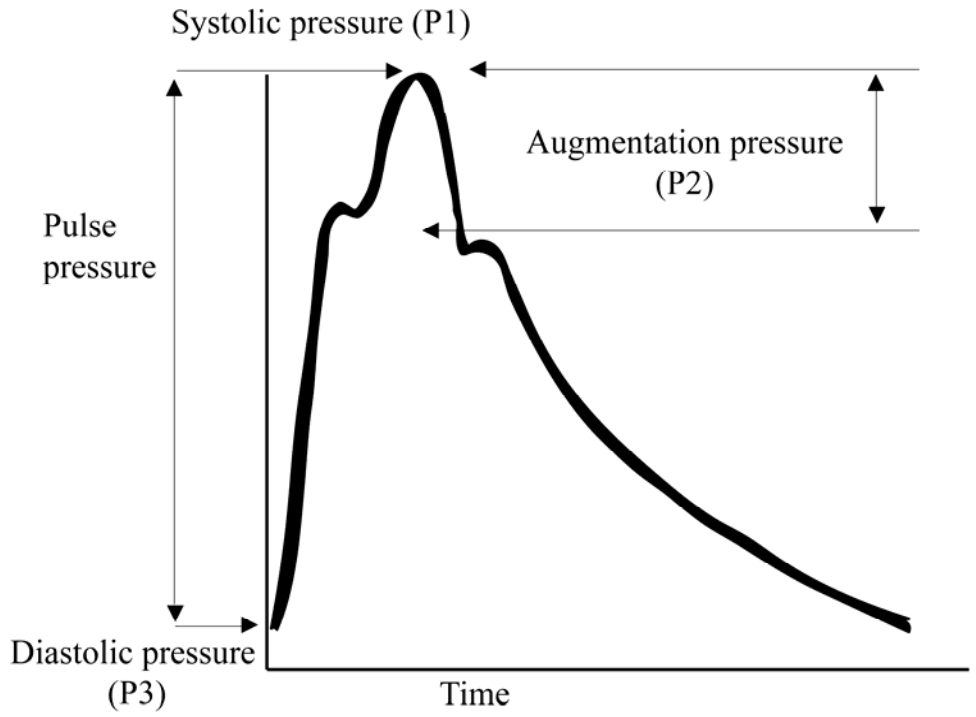
1.2.2. Pulse wave analysis

As described in section 1.1.3 [4], the arterial pressure waveform is a composite of the forward pressure wave created by the ventricular contraction and a reflected wave. Waves are reflected from the periphery, mainly at branching points or sites of impedance mismatch. In elastic vessels, because PWV is low, reflected waves tend to arrive back at the aortic root during diastole. In the case of stiff arteries, PWV rises and the reflected wave arrives back at the central arteries earlier, adding to the forward wave and augmenting the systolic pressure. This phenomenon can be quantified through the augmentation index (AIx) – defined as the difference between the second and first systolic peaks (P2 – P1) expressed as a percentage of the pulse pressure (Figure 1.4) [7]. Apart from a high PWV, also changes in reflection sites can influence the AIx.

The arterial pressure waveform should be preferentially analyzed at the central level, i.e. the ascending aorta, since the central AIx represents the true load imposed on the left ventricle and the walls of the central arteries. The aortic pressure waveform can be estimated from the radial waveform by means of the transfer function [19,20]or it can be approximated from the common carotid waveform. At both arteries, the pressure waveform can be recorded noninvasively with a pencil-type probe, incorporating a highfidelity Millar strain gauge transducer (SPT-301, Millar Instruments). The most widely used approach is to perform tonometry at the radial artery and then to apply a transfer function (Sphygmocor, AtCor, Sydney, Australia) to calculate from the radial waveform the aortic pressure waveform [16,19,20]. Indeed, in contrast to the carotid artery, the radial artery is well supported by bony tissue, making optimal applanation easier to achieve.

Figure 1.4 Pressure waveform recorded by applanation tonometry

The height of the late systolic peak (P1) above the inflection (P2) defines the augmentation pressure, and the ratio of augmentation pressure to PP defines the AIx (in percent). With permission from *Eur Heart J* 2006; 27:2588-2605.



1.2.3. Local determination of arterial stiffness

In our studies, we measured local arterial stiffness at the level of the carotid, femoral and brachial arteries by means of a wall-tracking ultrasound device (Wall Track System; Pie Medical, Maastricht, the Netherlands). This apparatus uses the radiofrequency signal and improves the precision of the measurement 6 to 10 times as compared with video-imaging systems, which are limited by the spatial resolution of pixel analysis. The measurement of local arterial compliance and distensibility requires computation of the local pulse pressure (see section 1.2.4).

1.2.4. Peripheral and central pulse pressure

Peripheral (brachial) pulse pressure (PP) is an age-related index reflecting arterial stiffness, the amplitude and velocity of reflected waves, and cardiac stroke volume [5]. Although an indirect measure of increased arterial stiffness, a high brachial PP is a harbinger of a poor prognosis in treated and untreated hypertensive patients [21] and

in older subjects randomly selected of European [22] or North American [23] populations.

The central PP in the ascending aorta may have high prognostic value [24]. Furthermore, measurement of the arterial compliance and distensibility at the carotid and femoral arteries requires computation of the local PP, which can be achieved by applying a transfer function or by the method suggested by Van Bortel et al. [25]. Applanation tonometry provides pressure waves which are almost identical to those obtained intra-arterially. At the reference artery (i.e. brachial artery), peak and nadir of the pressure wave are assigned systolic and diastolic pressures determined by a conventional method (i.e. sphygmomanometry). The pulse pressure at the target artery (PP_{tar}) is calculated from the pulse pressure at the reference artery (PP_{ref}) and the K factor at target and reference arteries (K_{tar} and K_{ref}, respectively) by the formula:

$$PP_{tar} = PP_{ref} \times \frac{K_{ref}}{K_{tar}}.$$

The K factor is calculated as: $K = \frac{A}{P}$;

where A = MAP-DAP (mean arterial pressure – diastolic blood pressure) and P = pulse pressure. MAP is calculated from the numeric integral of the calibrated pressure wave. With assignment of the same mean and diastolic pressures to the target artery (for instance carotid artery), the pressure wave at the target artery is calibrated throughout the cardiac cycle. This calibration procedure is based on the observation that MAP is constant throughout the large artery tree and that diastolic pressure does not change substantially [26].

1.3. Renal sodium handling

The kidneys play a central role in the pathogenesis of essential hypertension. Blood pressure starts to rise when the kidney requires a higher than usual blood pressure to maintain extracellular fluid volume within normal limits. Measuring the clearance of endogenous lithium provides a way to estimate sodium handling in the nephron. Indeed, lithium ions are freely filtered at the glomerulus and reabsorbed in the proximal tubule in parallel with sodium and water. Although lithium may be partially reabsorbed in the loop of Henle, distal tubular handling of lithium is minimal. Expressing the renal clearance of endogenous lithium as a fractional excretion provides a measure that is factored for the glomerular filtration rate. This limits

possible sources of bias, such as differences in flow rate and incomplete urine collection. The fractional excretion of lithium (FE_{Li}) and fractional distal reabsorption of sodium (RNa_{dist}) are non-invasive markers of proximal tubular sodium handling and the proportion of sodium escaping reabsorption in the proximal tubule, that is not eliminated in the urine, respectively [27].

We computed parameters of renal sodium handling as follow. Clearances (C) were calculated as $C_x = U_x \times V/P_x$, where U_x and P_x were the urinary and plasma concentrations of the solute x, and V was the volume of the urine sample. We computed the fractional excretion of sodium (FE_{Na}) and lithium (FE_{Li}) by dividing the sodium (C_{Na}) and lithium (C_{Li}) clearances by the creatinine clearance. We expressed these ratios as a percentage. Fractional distal reabsorption of sodium (RNa_{dist}) was estimated as $[(FE_{Li} - FE_{Na})/FE_{Li}] \times 100$ [27]. We defined the fractional proximal sodium reabsorption (RNa_{prox}) as $100 - FE_{Li}$.

1.4. Determinants of arterial stiffness

Arterial stiffness results from complex interactions between genetic, environmental, and lifestyle factors [28]. Known and postulated determinants of arterial stiffness include sex, age, blood pressure, antihypertensive treatment, physical activity, smoking, salt intake and various neurohormonal factors. Moreover, central PP and AIx are dependent on the speed of both the forward and reflected wave, the reflectance point, and the duration of ventricular ejection, especially with respect to changes in heart rate and ventricular contractility. These covariables of arterial stiffness are shortly discussed and have to be taken into account in any genetic study of arterial stiffness.

1.4.1. Demographic determinants

Women compared with men have narrower arteries, shorter body height, and higher heart rate [29], which impact on various arterial properties. Moreover, after menopause, systolic blood pressure increases more steeply in women than in age-matched men, implying a protective effect of circulating estrogen [29,30]. After menopause, peripheral PP, but not PWV [29] increased more than in age-matched men.

Aging is strongly associated with the development of isolated systolic hypertension, probably the most common clinical manifestation of arterial stiffening [31]. Enlargement of mean diameter with reduction of pulsatile diameter, expansion of the extracellular matrix, and progressive disappearance and degeneration of elastic elements of the arterial wall are hallmarks of this process. Collagen in the human aorta is much stiffer (at least 500 times) than elastin, and it more than doubles in content from age 20 to 70 years [32], which corresponds to a 2-fold increase in aPWV [17]. Moreover, the effect of age on different indexes of arterial stiffness is not linear. Indeed, in the Anglo-Cardiff Collaborative Study [33], AIx increased more than aPWV before the age of 50 years, and aPWV increased more than AIx in subjects older 50 years. In the Asklepios study, the distensibility of the elastic carotid artery decreased with age, but no effect of age was observed on femoral distensibility [34].

Body weight and height are other important determinants of arterial stiffness. Smaller body height, as seen in women, favors reflection sites closer to the heart and therefore higher systolic peak values [35]. Increased body weight associated with metabolic abnormalities promotes arterial stiffening [36,37].

1.4.2. Blood pressure

Apart from structure, arterial stiffness also depends on the distending pressure, because the pressure/diameter relationship is non-linear, through progressive recruitment of collagen fibres as an artery is distended. Moreover, there is a different pattern observed in elastic compared with muscular arteries. Indeed, in elastic arteries, during vasodilation, transmural pressure decreases, causing a passive decrease in wall stiffness and a decrease in PWV. A change in stiffness of muscular arteries is primarily due to acute changes in smooth muscle tone. During vasodilation, diameter increases whereas pressure decreases, causing a decrease in arterial stiffness [32]. Up to date, many studies have provided evidence that both peripheral (muscular) and central (elastic) arteries are stiffer in subjects with mixed (systolic/diastolic) hypertension compared with normotensive subjects [38].

1.4.3. Heart rate

Heart rate shows an inverase correlation with the AIx [39]. With slower heart rate, left ventricle contraction lasts longer, so that the reflected wave meets the forward wave in systole and thus increases central systolic pressure. On the other hand, the relation

between aPWV and heart rate might be more complex. Indeed, a significantly stronger correlation of AIx with heart rate was observed in subjects with higher levels of aortic stiffness as compared with those with lower level. Namely, the same increase in the heart rate between subjects, induced a greater decrease in the AIx at higher compared with lower aPWV levels [40].

1.4.4. Lifestyle factors

Active and also passive [41] smoking increased arterial stiffness both in acute [42,43] and chronic terms [44]. Moderate alcohol consumption was associated with lower arterial stiffness both in cross-sectional [45-47] and longitudinal [48] studies. This effect was more pronounced in women than in men [47].

1.5. Candidate genes and arterial stiffness

1.5.1. Heritability

Before engaging in genetic association studies, one should be sure that traits under study are heritable. Many studies to date confirmed a significant heritable component for the majority of the properties of large arteries, as shortly summarized below.

In analyses adjusted for the linear and squared terms of age, body weight, and height, the heritability of the aPWV was 0.40 among 817 pedigrees in the Framingham Heart Study [49]. With adjustments applied for sex and age, the heritability of aPWV was 0.36 in a single extended pedigree recruited in the framework of Erasmus Rucphen Family Study [50]. With additional adjustments for mean arterial pressure, HR, low-density lipoprotein cholesterol and blood glucose, the estimate was 0.26.

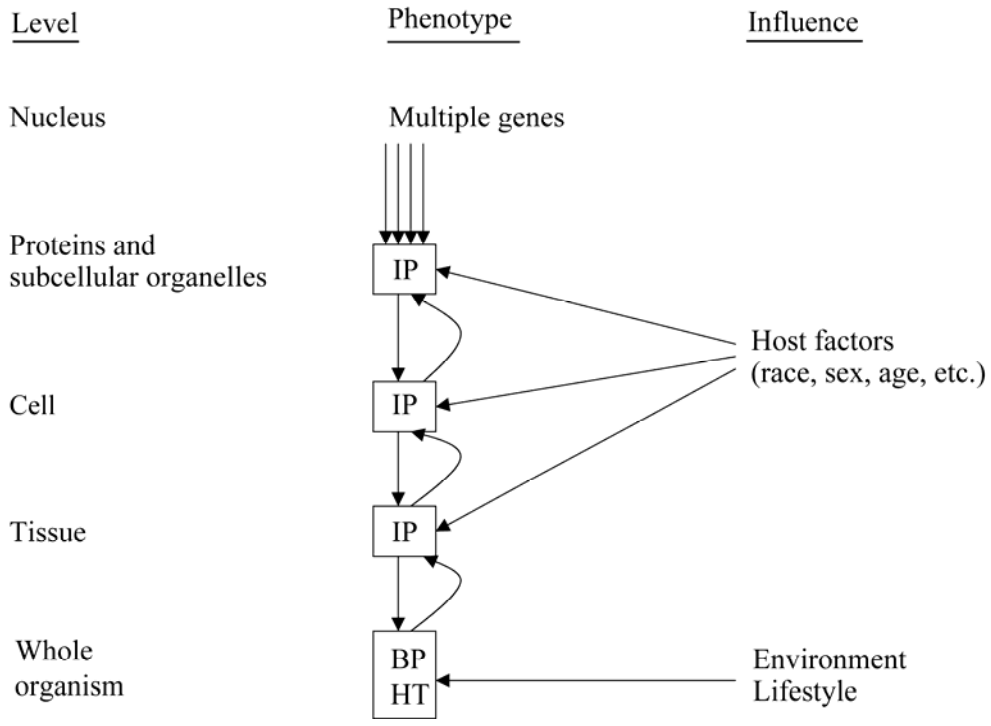
Two studies reported on the heritability of the central AIx. Among 225 monozygotic and 594 dizygotic female twin pairs, the heritability of the AIx was 0.37 in analyses adjusted for age, height, mean arterial pressure and HR [51]. In 32 extended families, the heritability of the augmentation index was 0.18 with cumulative adjustments for anthropometric characteristics, hypertension, diabetes and cholesterol [52].

Several studies investigated the heritability of the peripheral PP. The adjusted heritability estimate was 0.13 in a study of white female twin pairs with wide age range (18–73 years) [51]. In young twins (10–26 years), the heritability of peripheral

PP was similar among European and African Americans and averaged 0.53 with adjustments applied for ethnicity, sex, age, body mass index, and their interactions [53]. In family-based studies, in Blacks [54-56], Whites [54,57-59], and other ethnicities [54,60,61], the multivariate-adjusted heritability of peripheral PP ranged from 0.13 to 0.51. Heritability of carotid distensibility was 0.17 in high-risk Caribbean Hispanic families [62].

Figure 1.5 Schematic representation of arterial phenotype as a complex multigenic trait, affected by host and environmental factors and feedback control.

IP=intermediate phenotypes. BP=blood pressure. HT=hypertension. Curved arrows represent feedback loops.



As a consequence of heritability of the shared environment, risk factors and cardiovascular disorders cluster within families. Cardiovascular disease has a complex multifactorial etiology [4]. Until now, geneticists have failed to identify single common genes with large effects on cardiovascular disease. It is conceivable that such genes do not exist and that cardiovascular diseases are dependent on a mosaic of many loci, each with a small influence or with a contribution differing according to host factors or lifestyle. Throughout life, genetically determined host factors (i.e. sex and age) continuously interact with environmental influences including lifestyle (Fig

1.5). Any resulting change in cardiovascular phenotypes is initially counteracted by self-organizing homeostatic mechanisms, which encompass intracellular signaling, metabolic and hormonal regulation at cell and tissue levels, and systemic feedback loops involving the whole body. Disease finally develops as a consequence of interactions between the innate condition, as coded in the genotype, and exposures to environmental agents indexed by time and space that are integrated by dynamic, regulatory networks at levels above the genome [4].

1.5.2. The candidate gene approach

There are two different strategies to identify trait/disease susceptibility genes: the candidate gene approach and the genome wide scan. In the former approach, based on available knowledge on the pathogenesis of a disease, genes are selected and tested for association with arterial stiffness. A genome wide scan does not require any a priori hypothesis about where in the genome the relevant candidate genes are located. Usually, this technique makes use of genetic markers covering the whole genome, which are subsequently investigated for linkage with a trait/disease. Because a genome scan involves testing of multiple markers, false positive results are likely to occur and conventional significance levels are not applicable.

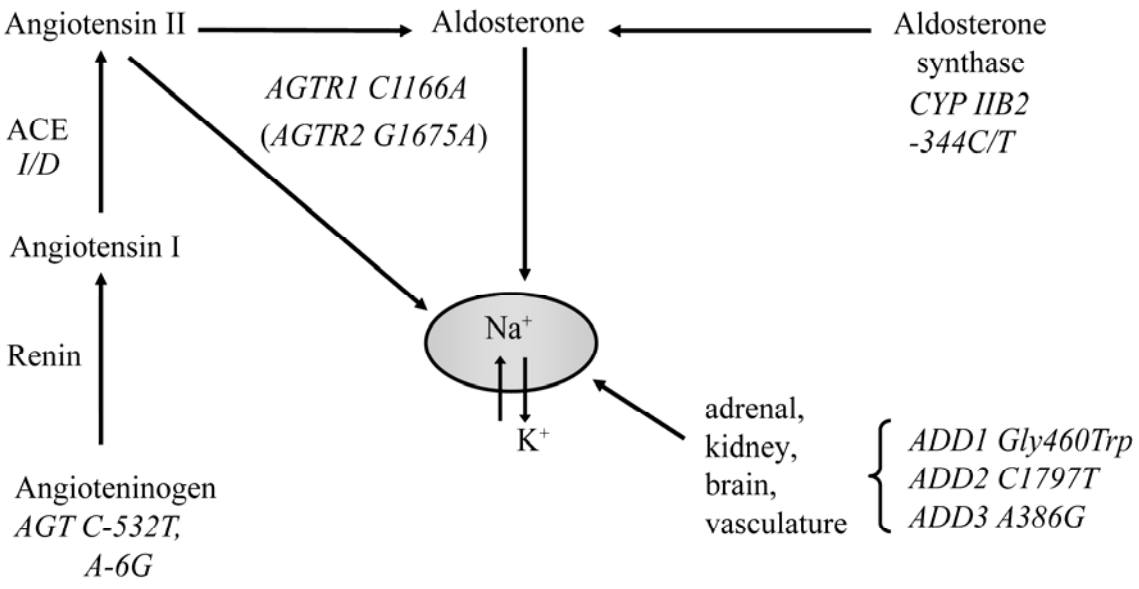
Recently two genome-wide scans focusing on tonometrically measured arterial stiffness were reported [63,64]. Both studies originated from Framingham Heart Study. The first report included 204 families [49]. Microsatellite markers covered the genome at 10-cM intervals. Variance components linkage analysis identified two regions of linkage for reflected wave amplitude: chromosome 4 at 181 cM (logarithm of odds [LOD]=4.93, permuted P=0.002) and chromosome 8 at 33 cM (LOD=3.27, permuted P=0.058). There was one region of linkage for forward wave amplitude on chromosome 7 at 174 cM (LOD=2.88, permuted P=0.017). There were several regions of suggestive linkage for aPWV: chromosome 2 at 94 cM (LOD=2.46), chromosome 7 at 29 cM (LOD=2.50), chromosome 13 at 108 cm (LOD=2.10), and chromosome 15 at 108 cM (LOD=2.48). There was one region of suggestive linkage for MAP on chromosome 1 at 192 cM (LOD=2.18). In the second report [64], the Framingham investigators investigated genome-wide SNPs using the Affymetrix 100K GeneChip. They analyzed a total of 70 987 autosomal SNPs with minor allele frequency ≥ 0.10, genotype call rate ≥ 0.80, and Hardy-Weinberg equilibrium $P \geq$ 0.001. The phenotypes of interest available in 644 subjects, were aPWV, carotid-

brachial PWV, the forward and reflected pressure wave amplitude, and MAP. For these five phenotypes, 5 SNPs had *P* value <10-5. The lowest *P* value were for reflected were for reflected wave (rs6063312, P = 2.1 × 10-6) and carotid-brachial PWV (rs770189, P = 2.5 × 10-6).

Several studies reported on genome-wide scans of PP, but produced inconsistent results. Zintzaras et al. [65] did a meta-analysis of 7 genome-wide scans of which three involved subjects of European descent. These investigators divided the whole genome in 120 bins and identified bins that ranked high on average in terms of linkage statistics across genome scans unweighted or weighted by study size. Of the 120 bins, 5 bins had significant average rank ($P_{rank} \leq 0.05$) by either unweighted or weighted analyses, 4 of which (bins 21.2: 21q22.11 to 21q22.3, 18.3: 18q12.2 to 18q21.33, 18.4: 18q21.33 to 18q23, and 6.2: 6p22.3 to 6p21.1) were significant by both. In subjects of European descent, 3 bins (22.1: 22q11.1 to 22q12.3, 22.2: 22q12.3 to 22q13.3, 10.4: 10q22.1 to 10q23.32) had $P_{rank} \leq 0.05$ with both unweighted and weighted analyses [65]. Further investigation of these regions might help to direct the identification of candidate genes for PP variation.

Because genome-wide scans produced inconsistent results, we opted for the candidate gene approach. We selected genes with known functionality or pathophysiological significance (Table 1.2 and Fig 1.6).

Figure 1.6 Schematic relation between renin-angiotensin-aldosterone system and adducin



1.5.3. Adducin

Adducin is a ubiquitously expressed membrane skeleton protein, which consists either of α- and ß- or α- and γ-subunits [66]. Adducin modulates actin-spectrin assembly and influences cell surface exposure of integrins and adhesion molecules [67]. Mutation of the α-adducin gene (*ADD1*) is linked with increased Na^+,K^+-ATPase activity [67,68] and increased renal tubular sodium reabsorption[66]. Variation in the Na^+,K^+-ATPase activity and in the intracellular Na^+ concentration might influence the sodium-dependent transmembranous Ca^{2+} transport in vascular smooth muscle cells and via this mechanism might affect arterial tone [4].

In the participants of the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), interaction between the genes encoding *ADD1* (*Gly460Trp* polymorphism), the angiotensin-converting enzyme and the angiotensin II type-1 receptor influenced the distensibility, cross-sectional compliance, and intima-media thickness of the femoral artery[69, 70]. In the same Flemish population [71] and in Polish and Russian subjects [72], my colleagues noticed that blood pressure and the prevalence of hypertension were associated with the *C1797T* polymorphism in the β-adducin subunit (*ADD2*), particularly in postmenopausal women [71]. *T* allele carriers had significantly higher 24-hour systolic blood pressure than *CC* homozygotes [72]. Moreover, Cwynar et al reported interaction between the *ADD1 Gly460Trp* polymorphism and the *A386G* polymorphism in the γ-adducin subunit (*ADD3*) [73]. Peripheral and central pulse pressures were higher in carriers of both the *ADD1 Trp* allele and the *ADD3 G* allele [73].

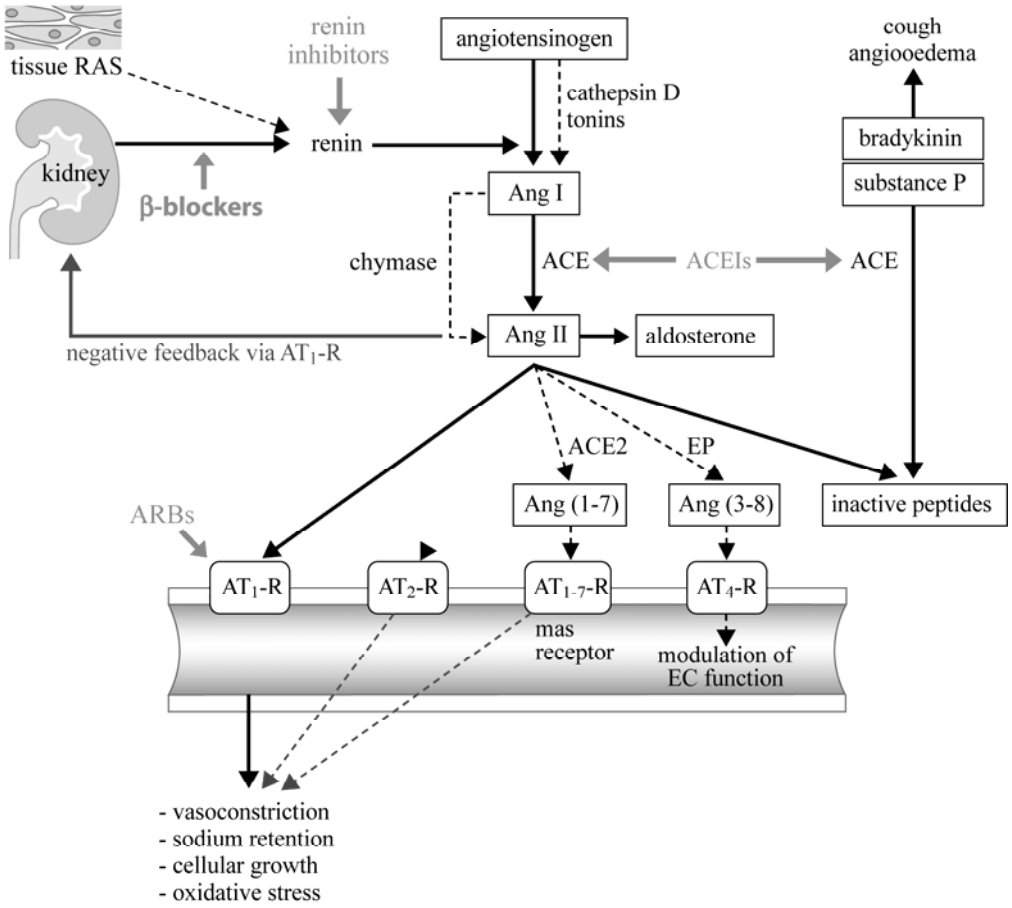
1.5.4. Angiotensinogen promoter area gene variations

The cleavage of AGT by renin is the rate-limiting step in cascade of enzymatic events leading to Ang II, which is promoter of vasoconstriction and vascular hypertrophy (Fig 1.7). As a consequence, there is a positive relation between plasma levels of AGT and Ang II. The *AGT G–6A* variant represents a guanine-to-adenine substitution 6 base pairs upstream from the initiation site of transcription. This nucleotide substitution is associated with a slightly higher basal rate of *AGT* gene transcription, which could account for the increase in plasma AGT in carriers of the *–6A* allele [74]. Studies involving French families or healthy subjects also showed higher plasma AGT levels in carriers of the *AGT –532T* allele [75,76]. As the *–532* site is located

within a consensus sequence of the *AGT* gene-binding transcription factor AP-2, the *C–532T* polymorphism might also modulate *AGT* gene transcription [75].

Figure 1.7 Bioenzymic cascade of the renin-angiotensin sytem

Black arrows show stimulation and grey arrows show inhibition. Dotted lines show alternative pathways mainly documented in experimental studies.



Few researches explored whether functional polymorphisms in the promoter area of the *AGT* gene might be associated with carotid intima-media thickness [77,78]. Indeed, in single-gene analysis, carotid intima-media thickness was not associated with the *AGT G–6A* polymorphism in 422 white Caucasian students (mean age 23.5 years) [77]. Chapman et al [78] investigated in 1111 subjects from the Perth Carotid Ultrasound Disease Assessment Study, whether polymorphisms in the *AGT* (*G-6A* and *A-20C*) and angiotensin II receptor type 1 (*AGTR1 A1166C*) genes might be associated with increased intima-media thickness or the presence of plaques in carotid arteries. After adjustment for conventional risk factors, the *AGT-6A* allele ($P<0.001$) and the *AGT-20C* allele ($P<0.03$) were significantly associated with increased mean

carotid intima-media thickness in females but not in males. The *AGTR1 A1166C* polymorphism did not show any significant relationship to mean IMT. None of the polymorphisms investigated were significantly associated with the presence of carotid plaques. To our knowledge no previous study investigated the effect of genetic variation in *AGT* promoter area on arterial stiffness.

1.5.5. Angiotensin II receptor type 1

The human *AT1R* gene consists of at least five exons and leads to four distinct alternatively spliced transcripts in which relative abundance varies form one tissue to another. More than 20 SNPs have been identified, none of them being a coding SNP. One of the most studied is a nucleotide change in the 3'untranslated region (*A1166C*). Sethupathy et al. [79] showed that this SNP occurs in a *cis*-regulatory site, which is recognized by a specific microRNA, miR-155. When *+1166C*-allele is present, base pairing complementarity is interrupted, and the ability of miR-155 to interact with the *cis*-regulatory site is decreased. As a consequence, miR-155 no longer attenuates translation, resulting in increased angiotensin II type receptor 1 densities [79]. To our knowledge, only one study investigated effect of this polymorphism on arterial stiffness [80]. Indeed, in cross-sectional study, 201 hypertensive patients without evidence of cardiovascular complications and 201 age and sex-matched normotensive Malay subjects were studied. aPWV was significantly higher among *1166C* allele carriers as compared with *AGTR1 AA* homozygotes (10.52±1.82 *vs*. 10.15±1.80, P=0.040) in the overall population, but not in the hypertensive and normotensive populations separately [79].

Table 1.1 Methods for measuring of arterial stiffness in clinical investigation

Method	Main features and definition	Limitation	Predictive value for cardiovascular events	Degree of technical expertise
Carotid-femoral PWV	Gold standard for arterial stiffness Speed of travel of the pulse along an arterial segment (L/Δt in m/s)	Pressure-dependent No data on arterial geometry	+++	+
Central pulse wave analysis (carotid and aortic pressure waves)	Central pulse pressure Central SBP Central augmentation pressure (AP) Central AIx with Aix = AP/PP	Inaccuracy of distance measurement Indirect information on arterial stiffness	++	+
Local arterial stiffness	Distensibility Compliance Young's modulus Takes into account blood pressure level	Required echotracking system Requires local PP	+	+++

With permission from *Eur Heart J* 2006; 27:2588-2605.

Table 1.2 Genes, polymorphism and methods of genotyping

Gene	Location	Polymorphism	Oligonucleotide		Method of genotyping
ADD1	Chr4p16.3	*Gly460Trp* (rs4961)	Forward 5'-CGTCCACACCTTAGTCTTCGACTT-3'	primer	Genotyping was performed by hybridization with an allele-specific oligonucleotide. The PCR fluid contained 50 ng DNA, 300 nmol primers, 100 nmol FAM-probe and 50 nmol TET-probe per 25 µl. The amplification conditions were: 50ºC for 2 min, 95ºC for 10 min, 95ºC for 15 min and 62ºC for 1 min for 40 cycles.
			Reverse 5'-GGAGAAGACAAGATGGCTGAACTC -3'	primer	
			460Gly 5'-FAM-TTCCATTCTGCCCTTCCTCGGA-TAMRA -3'	probe	
			460Trp 5'-TET-TTCCATTCTGCCATTCCTCGGAA-TAMRA -3'	probe	
ADD2	Chr2p14-13	*C1797T7* (rs4984)	Forward 5'-AGGAACGAGAGCCAGGCTCT-3'	primer	Genotyping was performed by hybridization with an allele-specific oligonucleotide. The PCR fluid contained 50 ng DNA, 200 nmol primers, 50 nmol FAM-probe and 100 nmol VIC-probe. The amplification conditions were 50ºC for 2 minutes and 95ºC for 10 minutes, followed by 40 cycles at 95º for 15 seconds and 62ºC for 1 minute.
			Reverse 5'-TTCATCA AAACACACACCTACCAAT-3'	primer	
			1797C 5'-VIC-TTCTTCAGCGTTGCCCTCCACAT-TAMRA-3'	probe	
			1797T 5'-FAM-TTCTTCAGTGTTGCCCTCCACATCTG-TAMRA-3'	probe	
ADD3	Chr10q24.2-24.3	IVS11 + *386A>G* (rs3731566) intron 11	Forward 5'-TGGAGGTGGGAATTGAAGAGA-3'	primer	The PCR fluid contained: 50 ng genomic DNA, 1200 nmol/l primers, 400 nmol/l FAM-probe and 120 nmol/l VIC-probe. The amplification conditions were: 50ºC for 2 min; 95ºC for 10 min; followed by 42 cycles at 92ºC for 15 s and at 60ºC for 1 min.
			Reverse 5'-CCCGAATCTGAATTGAAAAACAA-3'	primer	
			386A 5'-FAM-TGTCAAATAGTAAGCTTTT-MGB-3'	probe	
			386G 5'-VIC-TGTCAAGTAGTAAGCTTT-MGB-3'	probe	

Table 1.2 Genes, polymorphism and methods of genotyping (continues)

Gene	Location	Polymorphism	Oligonucleotide			Method of genotyping
AGT	Chr1q42-43	*G-6A* promoter area (rs5051)	Forward 5'-TTCCAGAAGGCACTTTTCAC-3'		primer	Genotyping was performed by hybridization with an allele-specific oligonucleotide. The PCR products were denaturated and blotted onto nylon membranes. Membranes were then neutralized in 2xSSC and cross-linked with UV light. Each membrane was hybridized in 7% polyethylene glycol-10% sodium dodecyl sulphate at specific tº for 4 hours with 100 pmol of either of the 2 oligonucleotides end labeled with (γ-^{32}P)dATP. The membranes were washed twice in 1xSSC at 52ºC, followed by autoradiography.
			Reverse 5'-TAGTACCCAGAACAACGGCA-3'		primer	
			Allele specific 5'-ACCCGGCCAGGGGAAGA-3'	*G*	*–6*	
			Allele specific 5'-TCTTCCCCCGGCCGGGT-3'	*A*	*–6*	
AGT	Chr1q42-43	*C-532A* promoter area	Forward 5'-TTCCAGAAGGCACTTTTCAC-3'		primer	Genotyping was performed by hybridization with an allele-specific oligonucleotide (see above, AGT *G–6A)*
			Reverse 5'-TAGTACCCAGAACAACGGCA-3'		primer	
			Allele specific 5'-TGTGTTTTCCCCAGTGT-3'	*C*	*–532*	
			Allele specific 5'-ACACTGGGAAAAACACA-3'	*T*	*–532*	
AGTR1	Chr3q21-25	*A+1166C,* 3' UT area (rs5186)	Forward 5'-AGAAGCCTGCACCATGTTTTGAG-3'		primer	The PCR products were digested overnight by the addition of 5 U of *Ddel* restriction enzyme. In the presence of the *1166C* allele, the PCR product (404 bp) was cut into 2 fragments of 118 bp and 286 bp in length and visualized on ethidium bromide-stained 1.5% agarose gels.
			Reverse 5'-CCTGTTGCTCCTCTAACGATTTA-3'		primer	

1.6. Main objectives

Building on the evidence produced by the experimental and clinical studies reviewed before, this doctoral dissertation addressed the question to what extent properties of large arteries are associated with renal sodium handling and with variations in candidate genes encoding RAAS and adducin. Phenotypes and genotypes were collected in three populations in the framework of the European Project on Genes in Hypertension (EPOGH) and the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO).

In ***Chapter 2,*** we first investigated differences in arterial stiffness indexes in offspring with normotensive or hypertensive parents.

In ***Chapter 3*****,** we evaluated the heritability, familial aggregation and genetic and environmental correlations of the arterial stiffness indexes.

Chapter 4, based on the FLEMENGHO study, focuses on the relation between renal sodium handling and arterial properties.

Chapter 5 concentrates on the relation between local arterial stiffness indexes and genetic variation, using population-based as well as family-based approaches. Chapter 5 consists of two parts:

- ***Part 1*** deals with the association between arterial properties and *ADD1* (*Gly460Trp*), *ADD2* (*C1797T7)* and *ADD3 (A386G)*.
- ***Part 2*** involves *ADD1* (*Gly460Trp*) and several candidate genes from RAAS, such as *AGT* (*G-6A, C-532A*) and *AT1R* (*A1166C*).

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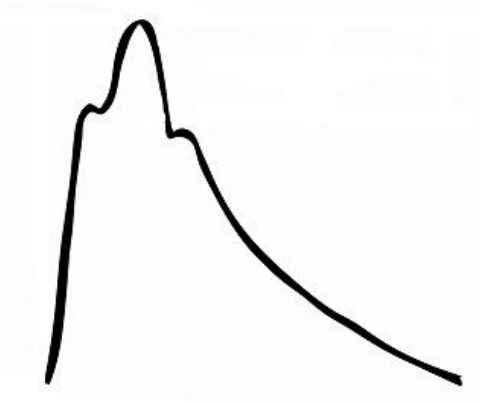

Chapter 2

Arterial characteristics in normotensive offspring of parents with or without a history of hypertension

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Abstract—In this study we compared the arterial characteristics and blood pressure (BP) of normotensive offspring of two normotensive parents (OFF/NT) and normotensive offspring who had at least one hypertensive parent (OFF/HT). A total of 174 OFF/HT (17 to 40 years of age) and 59 OFF/NT (16 to 34 years) were recruited in Cracow, Poland (n=138) and Pilsen, Czech Republic (n=95). Peripheral pulse pressure (PPp) was determined from conventional and 24-h ambulatory BP. A SphygmoCor device was used to measure the central (CAIx) and peripheral (PAIx) augmentation indexes, central pulse pressure (PPc), and the aortic pulse wave velocity (aPWV). In multivariate analyses family clusters and significant covariates were accounted for. The OFF/HT had higher ($0.14<P<0.0007$) conventional BP and PPp on conventional BP measurement (121/75 *vs.* 114/71 mm Hg and 46 *vs.* 42 mm Hg) as well as on 24-h ambulatory monitoring (118/70 *vs.* 114/67 mm Hg and 48 *vs.* 47 mm Hg). OFF/HT, compared with OFF/NT, also had higher ($0.05<P<0.0008$) PPc (28 *vs.* 26 mm Hg), PAIx (54.7% *vs.* 44.9%), CAIx (108.8% *vs.* 99.8%), and aPWV (7.4 *vs.* 6.6 m/sec). However, complex adjustment including mean arterial pressure and age removed the differences between the offspring in the PAIx, CAIx, and aPWV. Large-artery properties are altered in OFF/HT compared with OFF/NT. The findings from this cross-sectional study suggest that the alterations in arterial function in subjects with a family history of hypertension are determined mainly by an increased BP and age-related hemodynamic changes.

Introduction

Offspring of hypertensive parents have higher blood pressure and increased stiffness of the carotid artery compared with offspring of normotensive parents [1]. Pulse wave analysis, as implemented by O'Rourke and Gallagher in the SphygmoCor device [2] provides a simple and reproducible method to assess various indexes of arterial stiffness, including the peripheral and central augmentation indexes and aortic pulse wave velocity. To our knowledge only one study based on the SphygmoCor technique [3] reported an increased augmentation index, but not higher brachial pulse wave velocity, in offspring of hypertensive compared with normotensive parents. The goal of the present study was to compare the aforementioned indexes of arterial stiffness as well as the conventional and ambulatory blood pressure among offspring with a different family history of hypertension.

Methods

Study population

The European Project on Genes in Hypertension (EPOGH) was ethically approved and conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects [4]. Participants gave informed written consent.

Two EPOGH centers (Cracow and Pilsen) opted to take part in vascular phenotyping, using the SphygmoCor device. They randomly recruited nuclear families of Caucasian extraction, including offspring with minimum age of 16 years and parents with a maximum age of 68 years. Overall, the response rate was 82%. Of the 482 participants recruited in Cracow (Poland, n=299) or Pilsen (the Czech Republic, n=183), we discarded 80 subjects from analysis, because the recorded pulse wave was of insufficient quality (n=15), because offspring were hypertensive (n=12), or because of missing information concerning the hypertensive or normotensive status of parents (n=53). Out of offspring of hypertensive parents, 25 had both parents hypertensive. In those families considered as normotensive, both parents had to have a normal blood pressure, according to the 2003 criteria of the European Society of Hypertension [5] Thus, the overall number of participants statistically analyzed totaled 402 of whom 233 were offspring. All measurements of blood pressure and arterial parameters were completed in all participants.

Measurement of blood pressure

The conventional blood pressure phenotype in parents and offspring was the average of five consecutive readings obtained at one home visit. Hypertension was defined as a conventional blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic or as the use of antihypertensive medication [5]. We programmed oscillometric and properly validated 90202 or 90207 SpaceLabs monitors (Redmond, Washington, USA) to obtain blood pressure readings at intervals of 20 min from 8 AM until 10 PM and at 45 min from 10 PM to 8 AM. We monitored the ambulatory blood pressure within one week of the home visit. From unedited recordings, we calculated the average over 24-h blood pressure with weights according to the time intervals between successive readings. From the conventional and 24-h ambulatory blood pressure, we derived peripheral pulse pressure as the difference between systolic and diastolic blood pressure.

Measurements of arterial properties

All arterial measurements were performed in the clinic. After the subjects had rested for 15 min, we recorded, during an 8-s period, the radial arterial waveform at the dominant arm by applanation tonometry. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, Texas, USA) interfaced with a laptop computer running the SphygmoCor software, version 6.31 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms exceeded 10% or when the amplitude of the pulse wave signal was less than 80 mV. We calibrated the pulse wave by measuring blood pressure at the contralateral arm immediately before the recordings. From this blood pressure reading, we calculated mean arterial pressure as diastolic pressure plus one third of pulse pressure.

From the radial signal, the SphygmoCor software derives the aortic pulse wave by means of a validated generalized transfer function [6;7]. The radial augmentation index was defined as the ratio of the second to the first peak of the pressure wave expressed in percent. The aortic augmentation index was the difference between the second and first systolic peak given as a percentage of the aortic pulse pressure. From the aortic pulse wave we derived central pulse pressure. For statistical analysis, we

used the average of the peripheral and central waveforms over the 8-s measurement period.

We measured aortic pulse wave velocity by sequential recordings of the arterial pressure wave at the carotid and femoral arteries and by measurement of the distance between two recording sites. The distance was defined as: (distance from the suprasternal notch to femoral artery) – (distance from carotid artery to the suprasternal notch). Aortic pulse wave velocity was calculated as the ratio of the distance in meters to the transit time in seconds.

Other measurements

We administered a standardized questionnaire to obtain information on each subject's medical history, smoking and drinking habits and use of medications. After the subject had fasted overnight, we collected venous blood sample for measurement of blood glucose and serum lipids by means of standardized automated methods.

Statistical methods

For database management and statistical analysis, we used SAS software version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). We compared means and proportions by Student's t-test and the χ^2-statistic, respectively. We searched for possible covariates of the phenotypes using stepwise multiple regressions. Those covariates with *P* value less than 0.15 were used for further analysis. We used the PROC MIXED procedure of the SAS package [8] to account for the non-independence of observation within families while adjusting for covariates, including center, sex, age, body height, pulse rate and mean arterial pressure (taken immediately before arterial measurements), current smoking, regular alcohol intake (>5g/day), and serum total cholesterol, as appropriate.

Results

Characteristics of participants

Overall, the study population included 233 offspring from 123 nuclear families. The number of sibs per family amounted to one in 22 families, two in 94 families, three in 6 families and five in one family. Table 2.1 gives the general characteristics of the offspring according to presence or absence of parental hypertension. Offspring of hypertensive parents compared with those of normotensive parents were on average

2.5 years older, 4.4 kg heavier, and had a body mass index, which was 1.5 kg/m2 higher. Otherwise, there were no differences in lifestyle and biochemical measurements between the two groups of offspring.

Hypertensive parents (n=110; 60% women) compared with normotensive parents (n=59; 52.5% women) were slightly older (51.9 *vs.* 48.7 years; P<0.0001) and had higher mean values of body mass index (29.6 *vs.* 26.5 kg/m^2; P<0.0001), systolic and diastolic blood pressures (143.6 *vs.* 121.2 mm Hg and 89.5 *vs.* 77.8 mm Hg; P<0.0001), peripheral and central augmentation indexes (87.3 *vs.* 76.2% and 144.2 *vs.* 132.7%; P=0.0009) and aortic pulse wave velocity (9.6 *vs.* 8.0 m/s; P<0.0001). Otherwise the characteristics of the hypertensive and normotensive parents were similar.

Hemodynamic measurements

In unadjusted analyses (Table 2.2), most hemodynamic measurements, including blood pressure and peripheral pulse pressure at the subjects' homes, the 24-h ambulatory blood pressure, the central pulse pressure, the central and peripheral augmentation indexes and aortic pulse wave velocity were significantly higher in offspring of hypertensive compared with normotensive parents. In contrast, the 24-h peripheral pulse pressure (Table 2.2) and pulse rate (71.6 *vs.* 72.4; P=0.62) measured during the vascular examination were similar in the two groups.

Based on the results of stepwise regression, we adjusted all hemodynamic measurements for center, sex, age, pulse rate, current smoking, alcohol intake and serum total cholesterol. In addition, we adjusted blood pressure for body mass index and all vascular measurements for mean arterial pressure. The augmentation indexes were also adjusted for body height.

In fully adjusted analyses (Table 2.3), systolic blood pressure and peripheral pulse pressures on conventional measurement at home and the 24-h systolic and diastolic blood pressure remained significantly higher in offspring of hypertensive parents compared with offspring of normotensive parents. In contrast, in fully adjusted analyses, the differences in the arterial characteristics between the two groups of offspring disappeared. As shown in Fig. 2.1, this was mainly due to the introduction in the regression model of age, mean arterial pressure or both covariates. These results were consistent, when we ran separate analyses in offspring with one (n=149) or two (n=25) hypertensive parents (data not shown).

Table 2.1 General characteristics of offspring

Characteristics	Normotensive parents	Hypertensive parents*	*P*
Number of offspring	59	174	
Anthropometrics			
Age, y	23.0±3.9	25.5±5.4	0.001
Female gender, *n* (%)	32 (54.2)	87 (50)	0.58
Body height, cm	172.0±7.7	172.0±9.3	0.93
Body weight, kg	66.1±11.0	70.5±14.8	0.04
Body mass index, kg/m^2	22.2±2.9	23.7±4.0	0.01
Lifestyle			
Current smokers, *n* (%)	15 (25.4)	41 (23.6)	0.77
Alcohol intake > 5g/day, *n* (%)	32 (54.2)	79 (45.4)	0.24
Biochemistry			
Blood glucose, mmol/l	4.6±0.8	4.7±0.8	0.84
Serum total cholesterol, mmol/l	4.6±1.0	4.7±1.0	0.61
Serum HDL cholesterol, mmol/l	1.6±0.3	1.6±0.4	0.41

Values are arithmetic mean ± SD or percentage of subjects. HDL indicates high density lipoprotein. *P* values refer to the comparison of offspring of normotensive and hypertensive parents.

*Hypertension was a blood pressure measured at home (average of 5 readings) of at least 140 mm Hg systolic or 90 mm Hg diastolic or the use of antihypertensive drugs.

Table 2.2 Unadjusted hemodynamic measurements in offspring

Characteristics	Normotensive parents	Hypertensive parents*	*P*
Number of offspring	59	174	
Measurements at home*			
Systolic pressure, mm Hg	113.5±1.7	120.5±1.0	0.0007
Diastolic pressure, mm Hg	71.3±1.3	74.7±0.7	0.02
Peripheral pulse pressure, mm Hg	42.2±1.3	45.8±0.8	0.02
24-h ambulatory measurements			
Systolic pressure, mm Hg	113.7±1.2	118.2±0.7	0.01
Diastolic pressure, mm Hg	66.9±0.9	69.9±0.5	0.05
Peripheral pulse pressure, mm Hg	46.8±0.9	48.4±0.5	0.14
Arterial measurements			
Central pulse pressure, mm Hg	26.0±0.9	28.2±0.5	0.05
Peripheral augmentation index, %	44.9±2.5	54.7±1.4	0.0008
Central augmentation index, %	99.8±2.6	108.8±1.5	0.003
Aortic pulse wave velocity, m/s	6.6±0.3	7.4±0.2	0.03

Values are arithmetic mean ± SE adjusted for family clusters. *P* values refer to the comparison of offspring of normotensive and hypertensive parents. For further explanation, see Table 2.1.

*Average of 5 measurements obtained at one home visit.

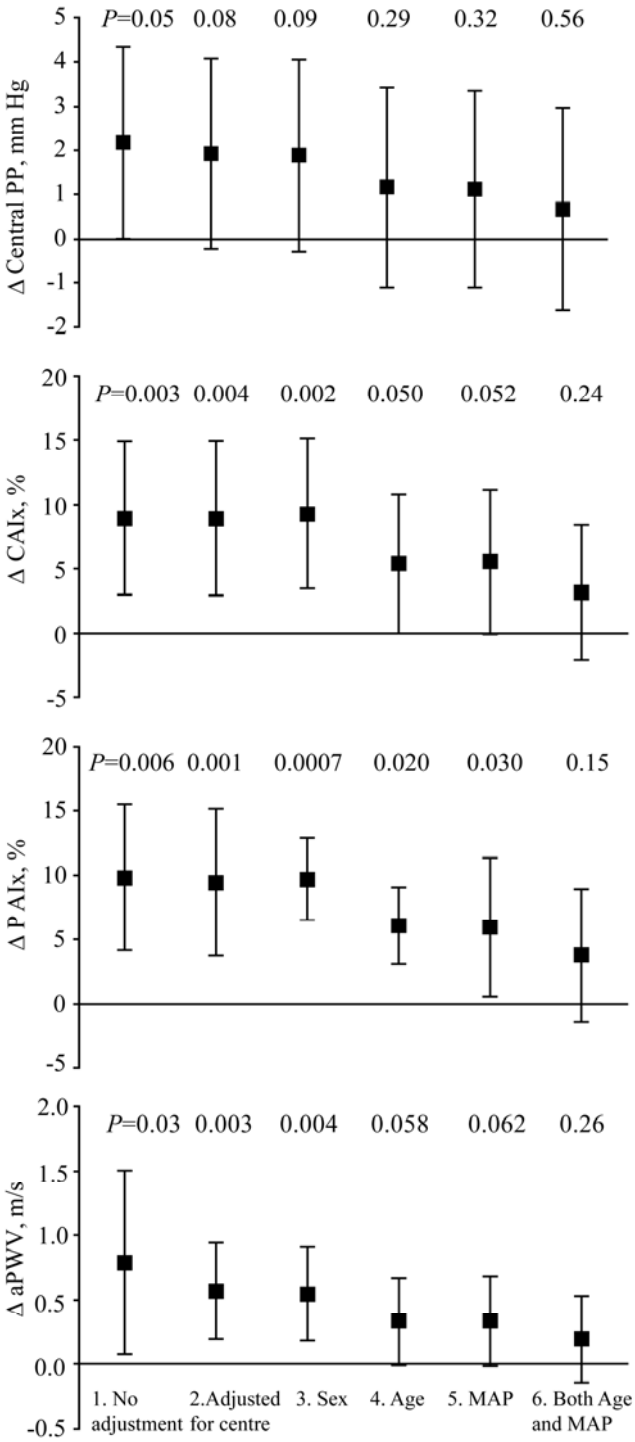
Table 2.3 Adjusted hemodynamic measurements in offspring

Characteristics	Normotensive parents	Hypertensive parents*	*P*
Number of offspring	59	174	
Measurements at home*			
Systolic pressure, mm Hg	115.2±1.5	120.0±0.9	0.009
Diastolic pressure, mm Hg	72.5±1.2	74.2±0.7	0.24
Peripheral pulse pressure, mm Hg	42.5±1.2	45.8±0.7	0.02
24-h ambulatory measurements			
Systolic pressure, mm Hg	114.6±1.1	117.9±0.6	0.01
Diastolic pressure, mm Hg	67.3±0.8	69.7±0.5	0.02
Peripheral pulse pressure, mm Hg	47.3±0.7	48.3±0.4	0.24
Arterial measurements			
Central pulse pressure, mm Hg	27.4±0.9	27.6±0.5	0.87
Peripheral augmentation index, %	50.9±1.9	52.5±1.1	0.50
Central augmentation index, %	105.8±1.9	106.6±1.1	0.72
Aortic pulse wave velocity, m/s	6.9±0.1	7.1±0.1	0.23

Values are arithmetic mean ± SE, adjusted for center, sex, age, pulse rate, current smoking, alcohol intake and serum total cholesterol. Additional adjustments included: (1) for blood pressure: body mass index; (2) for the augmentation indexes: body height and mean arterial pressure; (3) for aortic pulse wave velocity and central pulse pressure: mean arterial pressure. *P* values for the comparison of offspring of normotensive and hypertensive parents also account for family clusters.

*Average of 5 measurements obtained at one home visit.

Figure 2.1 Effect of cumulative adjustments on the differences in arterial characteristics between offspring of normotensive and hypertensive parents.



Mean differences with 95% confidence intervals and corresponding *P* values are given (e.g. ΔPPc = 2.2 mm Hg indicates that PPc in offspring of hypertensive parents was higher on average by 2.2 mm Hg than in offspring of normotensive parents). PAIx, CAIx, and aPWV indicate peripheral augmentation index, central augmentation index, and aortic pulse wave velocity, respectively.

Adjustments: unadjusted (1), adjusted for center (2), center and sex (3), center, sex and age (4), center, sex and mean arterial blood pressure (5), center, sex and both age and mean arterial blood pressure (6).

Discussion

The key finding of the present study was that in unadjusted analyses systolic and diastolic blood pressures on conventional as well as 24-h ambulatory measurement, peripheral pulse pressure on conventional blood pressure measurement, the central and peripheral augmentation indexes and aortic pulse wave velocity were significantly higher in normotensive offspring with at least one hypertensive parent compared with normotensive offspring of two normotensive parents. Fully adjusted analyses confirmed these findings for systolic pressure irrespective of the type of blood pressure measurement, for peripheral pulse pressure on conventional measurement, and for the 24-h diastolic blood pressure. However, adjustment for center, sex, age, mean arterial pressure, pulse rate, current smoking, alcohol intake and serum total cholesterol removed the differences between the two groups of offspring in the augmentation indexes and aortic pulse wave velocity.

To our knowledge only three studies dealt with large artery properties in young subjects at risk of hypertension. Meaney and coworkers [1] studied 100 nonobese offspring, aged 10–20 years, who were descendants of hypertensive or normotensive parents. By means of an ultrasound technique, they studied the characteristics of the ascending aorta and the common carotid artery. Carotid, but not aortic, stiffness and maximum velocity flow in the aorta were significantly higher in the offspring of the hypertensive parents; the comparisons were, however, not adjusted for blood pressure which was already higher in this group.

Yasmin and colleagues [3] recruited offspring of families with essential hypertension (mean age 39 years) and normotensive controls (mean age 43 years). They measured blood pressure at the brachial artery and applied the same SphygmoCor technique as we employed. They observed that offspring of hypertensive compared with normotensive parents had higher systolic/diastolic blood pressure (123/75 *vs.* 118/71 mm Hg). They also reported that offspring of hypertensive parents had higher peripheral pulse pressure (49 *vs.* 47 mm Hg; $P<0.01$), higher central pulse pressure (35 *vs.* 35; $P<0.01$) and higher central augmentation index (19.1 *vs.* 17.8%; $P<0.01$), but similar brachial pulse wave velocity (8.40 *vs.* 8.24 m/s); aortic pulse wave velocity was not studied. However, when we tried to replicate the calculations reported in Table 1 of Yasmin's paper, we found nonsignificant P values for peripheral pulse pressure ($t=1.82$; $P=0.08$), central pulse

pressure (t=0.23; P=0.78), and the augmentation index (t=0.85; P=0.30). The sample studied by Yasmin et al. differed from ours: the mean age was higher in the Yasmin's study and the offspring of hypertensive parents had higher HDL cholesterol, creatinine and blood glucose levels and smoking prevalence, whereas in our study these parameters were similar between the two groups.

Rajzer and colleagues [9] studied the effect of selected clinical and biochemical measurements on the pulse wave velocity in 70 young normotensives. The subjects were subdivided into two groups: those with and without a family history of arterial hypertension. They observed that pulse wave velocity did not differ between these two groups (9.7 *vs.* 9.3 m/s; P=0.52).

Other authors used the techniques which focus on the functional abnormalities separately in small and large arteries [10;11]. The results showed that especially small artery elasticity (the C2 component of the modified Windkessel model of circulation) may correlate closely with blood pressure [10] and predict future cardiovascular events [11].

The recently published study by Dernellis and Panaretou provided evidence that increased aortic stiffness precedes hypertension [12]. The authors examined 2512 subjects (aged 35 to 94 years) who were not hypertensive at baseline and followed them for four years. Aortic stiffness measured at baseline by means of echocardiographic technique predicted progression to hypertension in both sexes and in younger as well as older subjects. The results were consistent for all the three aortic stiffness indexes used and for different BP parameters (increase in systolic, in diastolic and in pulse pressure and hypertension incidence) and remained significant after adjustment for baseline BP, age and all other classic cardiovascular risk factors. Thus, aortic wall properties are likely to play a role in the pathogenesis of hypertension already in its early stages. On the other hand, the relationship of BP and arterial properties is reciprocal: it has been shown in the Bogalusa Heart Study [13] that childhood BP predicted arterial stiffness assessed on the mean 26.5 years later by brachial-ankle pulse wave velocity.

Our study was cross-sectional and therefore, the question of cause and consequence cannot be answered on the basis of our data. However, it adds to the longitudinal studies [12;13], because it suggests that an increased blood pressure and increased arterial stiffness run in parallel in hypertensive families. This might be due to genetic factors, shared environmental influences and/or their interaction. In a study of 225

monozygotic and 594 dizygotic female twin pairs, aged 18 to 73 years Snieder and colleagues [14] noticed that the heritability of the central augmentation index was 37% and was largely independent of age, blood pressure, heart rate and height. In 950 North-American Indians from 32 extended families, North and coworkers [15] reported that the heritability of carotid stiffness and the central augmentation index were 23% and 18%, respectively. These two studies underscore the importance of genetic factors in pathogenesis of arterial stiffness.

Our results have to be interpreted within the context of its limitations. Our sample size was relatively small, although still larger than in the three previously published studies with similar design [1;3;9]. This may be one of the reasons why the differences in arterial parameters were no more significant after the complex adjustment. On the other hand, we collected six measurements reflecting arterial stiffness and found great consistency among these measurements in the comparison between the two groups of offspring.

In conclusion, compared with normotensive offspring of normotensive parents, normotensive offspring of hypertensive parents have increased blood pressure and impaired arterial properties, namely aortic stiffness and pulse wave reflection as measured noninvasively by assessing aortic pulse wave velocity and radial augmentation index. The present cross-sectional findings, in keeping with the large prospective study by Dernellis and Panaretou [12] suggest that the alteration in arterial function is present already in nonhypertensive subjects at risk of hypertension and it may contribute to the progression to hypertension in later life.

Acknowledgement

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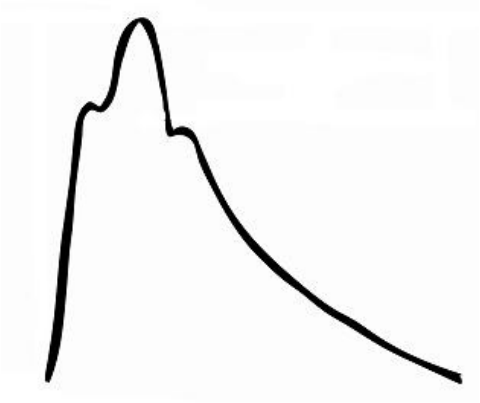

Chapter 3

Heritability and intrafamilial aggregation of arterial characteristics

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Abstract—We investigated in the same subjects, heritability and familial aggregation of various indexes of arterial stiffness and we partitioned the phenotypic correlation between these traits into shared genetic and environmental components. Using a family-based random sampling frame, we recruited 204 parents (mean age, 51.7 years) and 290 offspring (29.4 years) from the population in Cracow, Poland (62 families), Hechtel-Eksel, Belgium (36), and Pilsen, the Czech Republic (50). We measured peripheral pulse pressure (PPp) sphygmomanometrically at the brachial artery; central pulse pressure (PPc), the peripheral (PAIx) and central (CAIx) augmentation indexes by tonometry at the radial artery; and aortic pulse wave velocity (aPWV) by tonometry or ultrasound. In multivariate-adjusted analyses, we used the ASSOC and PROC GENMOD procedures as implemented in S.A.G.E. and SAS, respectively. All traits, with the exception of PPc (P=0.79) and aPWV (P=0.08), showed significant heritability ($P \leq 0.0001$), ranging from 0.37 for PPp to 0.41 for CAIx. The genetic correlation between aPWV and the other arterial indexes were significant ($\rho_G \geq 0.29$; $P<0.0001$). The corresponding environmental correlations were only significantly positive for PPp (ρ_E=0.10, P=0.03). Intrafamilial concordance was significant for all arterial indexes ($r \geq 0.12$; $P \leq 0.02$), with the exception of PPc (r=-0.007; P=0.90) in parent-offspring pairs. The sib-sib correlations were also significant for CAIx (r=0.22; P=0.001). The observation in the same group of subjects of significant intrafamilial concordance and heritability of various indexes of arterial stiffness as well as the genetic correlations among arterial phenotypes strongly support the search for shared genetic determinants underlying these traits.

Introduction

Aortic pulse wave velocity, augmentation and pulse pressure as measured in central or peripheral arteries are indexes of arterial stiffness. Aortic pulse wave velocity is a powerful predictor of cardiovascular outcome in the general population [1] as well as in patients with hypertension [2], diabetes, or end-stage renal disease [3]. Similarly, several other indexes of arterial stiffness also predict cardiovascular outcome [4;5], although especially for pulse pressure in relation to stroke, the evidence is more equivocal [6].

Understanding to what extent genetic and environmental factors contribute to arterial stiffening is an important issue in view of the relation of arterial properties with outcome. Previous studies included heritability estimates for aortic pulse wave velocity [7;8], augmentation index[9;10] and/or pulse pressure [9;11-19]. However, to our knowledge, no previous publication reported estimates of familial aggregation and the contribution of shared genetic and environmental factors along with the heritability of indexes of arterial stiffness. We addressed this issue in nuclear families recruited from the general population in three European countries in the framework of the European Project on Genes in Hypertension.

Methods

Study population

EPOGH was conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects [20]. The Ethics Committee of each institution approved the protocol. Participants gave informed written consent. Three EPOGH centers opted to take part in arterial phenotyping. They randomly recruited nuclear families of Caucasian extraction, including offspring with a minimum age of 10 years in Belgium and 18 years in the two other countries. Overall, the response rate was 82%.

We administered a standardized questionnaire to obtain information on each subject's medical history, smoking and drinking habits, and use of medications. From the type and quality of the alcoholic beverages used, we computed alcohol consumption in grams per day. We defined regular drinking as an alcohol consumption of at least five grams per day. To exclude consanguinity among families, parents provided a 3□generation pedigree. We checked for Mendelian

inconsistencies, using the ABO and rhesus blood groups. Our study population consisted of 525 subjects, who underwent arterial measurements. Because the recorded pulse wave was of insufficient quality, we discarded 31 participants from analysis. The 494 participants statistically analyzed were recruited from the population of Cracow, Poland (*n*=201), Hechtel-Eksel, Belgium (*n*=115), and Pilsen, the Czech Republic (*n*=178).

Measurements of blood pressure

The conventional blood pressure phenotype was the average of five consecutive readings obtained at a single home visit. We defined pulse pressure (PP) as the difference between systolic and diastolic BP. Mean arterial pressure was diastolic pressure and one-third of PP.

Measurements of arterial characteristics

The number of observers involved in the arterial measurements amounted to two in Cracow, and one in Hechtel-Eksel and in Pilsen. To ensure steady state, the arterial measurements were obtained in a quiet examination room, after the subjects had rested for 15 minutes in the supine position and had refrained from smoking, heavy exercise, and drinking alcohol or caffeinated beverages for at least 2 hours prior to the examination.

We recorded the radial arterial waveform during 8 seconds in the dominant arm by applanation tonometry. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, Texas, USA) interfaced with a laptop computer running the SphygmoCor software, version 6.31 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms exceeded 10% or when the amplitude of the pulse wave signal was less than 80 mV. We calibrated the pulse wave by measuring blood pressure at the contralateral arm immediately before the recordings. From the radial signal, the SphygmoCor software calculates the central pulse wave by means of a validated [21;22] generalized transfer function. The radial augmentation index was defined as the ratio of the second to the first peak of the pressure wave expressed as a percentage. The central augmentation index was the difference between the second and first systolic peak given as a percentage of the aortic pulse pressure.

We computed the aortic pulse wave velocity from recordings of the arterial pressure wave at the carotid and femoral arteries. We measured the distance between the site of the carotid recordings and the suprasternal notch and between the suprasternal notch and the site of the femoral recordings. Aortic pulse wave velocity was calculated as the ratio of the travel distance in meters to the transit time in seconds. We measured pulse wave velocity, using the Complior device (Complior, Colson, Les Lilas, France) [23] in Cracow, a pulsed ultrasound wall-tracking system (Wall Track System, Pie Medical, Maastricht, the Netherlands) [24;25] in Hechtel-Eksel, and the SphygmoCor device [26] in Pilsen.

Statistical methods

For database management and statistical analysis, we used SAS software version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). We compared population means and proportions by Tukey's t-test for multiple comparisons and the χ^2-statistic, respectively. For analyses of heritability and intrafamilial aggregation, we used center-specific standardized distributions. We winsorized the phenotypes under study at the 3% level (1.5% at each end of the distribution) to minimize the influence of outlying data.

Heritability

To estimate heritability, we used a maximum likelihood approach as implemented in the ASSOC procedure of the Statistical Analysis in Genetic Epidemiology (S.A.G.E.) package, version 5.1 [27] We estimated heritability (h^2) by assuming multivariate normality after a simultaneously estimated power transformation. ASSOC uses a linear regression model, in which the residual variance is partitioned into the sum of an additive polygenic component and a subject-specific random component. Heritability is the polygenic component divided by the total residual variance [28].

Genetic and environmental correlation

We calculated genetic and environmental correlations between traits after adjusting for covariates as follows. Assuming no dominance variance and no interaction between the genetic and environmental variance components, the variance of a trait is given by: $V = G + E$, where G is the additive polygenic component and E is the environmental component. The (phenotypic) correlation between two traits (ρ_P) has

both a genetic (ρ_G) and an environmental (ρ_E) contribution given by the equation: $\rho_P = \rho_G \sqrt{h_2^2}\, h_2^2 + \rho_E \sqrt{(1-h_2^2)}\, (1-h_2^2)$, where ρ_G and ρ_E are the genetic and environmental correlations, respectively. Significance of ρ_G and ρ_E suggest that the traits are influenced by shared genes and/or by shared environmental factors [28].

Intrafamilial correlation

We calculated the correlation coefficients between members of the same family as a measure of concordance (positive correlations) or discorcondance (negative correlation). Hence, in the context of this article, the terms correlation and concordance are used interchangeably. To estimate the intrafamilial correlations, we used generalized estimating equations as implemented in the PROC GENMOD procedure of the SAS package. In these analyses, we adjusted for confounders, we treated pairs of relatives as clusters, and we defined the working correlation matrix as unstructured. Adjustments were cumulative and performed in three steps to check consistency of the parameter estimates, while controlling for an increasing number of variables known to influence arterial stiffness. First, in model 1, we adjusted for center, sex, age and age squared. Model 2 also included body height and weight, mean arterial pressure and pulse rate (as appropriate), and antihypertensive treatment. Finally, we considered various lifestyle factors, such as smoking and regular alcohol intake.

Results

Characteristics of participants

Our study population (*n*=494) included 204 parents (88 fathers and 116 mothers) and 290 offspring (137 sons and 153 daughters). The number of offspring per family amounted to one in 30 families, two in 102 families, three in 10 families, and more than three in 6 families. We did not detect any case of consanguinity or Mendelian inconsistency.

Tables 3.1 and 3.2 list the characteristic of the participants by generation, center and sex. The mean age of parents and offspring (±SD) was 51.7±6.5 years and 29.4±10.8 years, respectively. In comparison with offspring, parents had higher body mass index and blood pressure, more elevated indexes of arterial stiffness and more frequently used antihypertensive drugs (39.2 *vs.* 4.1%; $P<0.0001$). Among parents and offspring,

the proportion of smokers was slightly higher in men than in women (31.1 *vs.* 23.0%; P=0.05). Men also more frequently reported alcohol intake (64.9 *vs.* 30.1%; P<0.0001). Among smokers, the median daily tobacco consumption was 15 cigarettes (interquantile range [IQR], 10–20) in men and 10 (IQR, 5–20) in women. Among drinkers, the median daily alcohol intake was 20.0 grams (IQR, 10.0–31.6) in men and 10.0 grams (IQR, 6.2–18.0) in women.

Heritability

We adjusted heritability estimates for center, sex, the linear and squared terms of age, body height and weight, mean arterial pressure, pulse rate, antihypertensive treatment, smoking and alcohol intake. We excluded covariates if they were the trait under study. We also did not consider pulse rate as a covariate for peripheral pulse pressure. For all traits, there were no among-center differences in the polygenic and total variances ($F \leq 3.03$; $P \geq 0.05$).

Table 3.3 lists the multivariate-adjusted heritability estimates for the peripheral and central hemodynamic measurements as well as for the anthropometric characteristics. The heritability for pulse wave velocity was 0.19 (P=0.08). For all other traits, with the exception of central pulse pressure (P=0.79), heritability was statistically significant ($P \leq 0.0001$).

In further analyses, we included a sibship component of variance, which represents dominance variance and shared environmental factors within sibships. For all phenotypes listed in Table 3.3, the sibship component did not significantly differ from zero, suggesting no significant departure from an additive genetic model.

Genetic and environmental correlation

In analyses adjusted as before, we studied the genetic and environmental correlations between the hemodynamic phenotypes (Table 3.4). We excluded central pulse pressure because of its low heritability. We observed significant genetic correlations between pulse wave velocity and the other hemodynamic measurements ($0.29 < \rho_G < 0.49$; P<0.0001), whereas the corresponding environmental correlations were either significantly positive (peripheral pulse pressure) or significantly negative (central augmentation index) or not significant (peripheral augmentation index).

Intrafamilial correlation coefficients

In parent-offspring pairs, the multivariate-adjusted intrafamilial correlation coefficients were significant for all traits with the exception of central pulse pressure, irrespective of the level of adjustment. This was also the case for the correlation coefficients for mean arterial pressure and the central augmentation index in sib-sib pairs. In spouse-spouse pairs, the only significant intrafamilial correlation was for mean arterial pressure (Fig. 3.1).

With adjustments applied for center, sex and age, the intrafamilial correlations for body height were 0.50 in parent-offspring pairs, 0.46 in sib-sib pairs, and 0.37 in spouse-spouse pairs. The corresponding correlation coefficients for body weight were 0.24, 0.46 and 0.38 and those for body mass index were 0.19, 0.30, and 0.30, respectively ($P \leq 0.006$ for all intrafamilial correlations of anthropometric measurements).

Table 3.1 Characteristics of parents

Characteristics	Cracow		Hechtel-Eksel		Pilsen	
	Fathers	Mothers	Fathers	Mothers	Fathers	Mothers
Number	41	55	10	16	37	45
Age, y	50.7±4.5	49.9±5.4	58.3±11.7^{C}	57.9±10.6^{C}	52.2±4.7^{H}	50.6±5.1^{H}
Height, cm	173.6±6.9	161.8±5.3‡	171.3±6.6	159.4±8.8†	178.0±6.2^{CH}	163.3±5.1‡
Weight, kg	85.2±12.9	75.9±14.6†	83.3±10.2	68.6±10.4†	89.8±10.9	74.6±13.9‡
Peripheral hemodynamics						
Systolic pressure, mm Hg	136.7±18.2	136.1±18.0	131.3±13.3	127.2±15.7	129.7±19.1	126.8±18.2^{C}
Diastolic pressure, mm Hg	86.6±11.4	86.4±9.6	81.4±11.8	77.3±8.3^{C}	83.7±11.5	78.7±10.2*^{C}
Mean arterial pressure, mm Hg	103.3±13.1	103.0±11.8	98.1±10.9	93.9±9.5^{C}	99.1±13.4	94.8±12.1^{C}
Pulse pressure, mm Hg	50.1±11.0	49.7±11.9	49.9±12.3	49.9±13.0	46.0±11.8	48.1±12.2
Augmentation index, %	79.6±16.6	90.6±15.0†	69.9±18.2	85.0±19.2	70.6±19.0	85.5±17.4†
Central hemodynamics						
Pulse pressure, mm Hg	37.9±10.6	42.5±11.4*	31.3±5.2	38.3±9.1*	30.8±10.1^{C}	34.9±11.7^{C}
Augmentation index, %	133.2±20.3	148.8±21.7†	126.8±21.2	148.6±23.2*	128.3±18.4	146.6±20.1‡
Pulse wave velocity, m/s	10.4±1.4	10.6±1.7	7.4±1.7^{C}	7.3±2.7^{C}	7.0±1.1^{C}	7.1±1.4^{C}

Values are arithmetic means±SD. Peripheral blood pressure was the average of 5 readings at a single home visit. Significance of the within-center differences between men and women: *P<0.05, †P<0.01 and ‡P<0.001. Significance of the between-centers differences, by sex, were adjusted for multiple comparisons by Tukey's test: $^{C}P\leq 0.05$ versus Cracow, $^{H}P\leq 0.05$ versus Hechtel-Eksel.

Table 3.2 Characteristics of offspring

Characteristics	Cracow		Hechtel-Eksel		Pilsen	
	Sons	Daughters	Sons	Daughters	Sons	Daughters
Number	54	51	38	51	45	51
Age, y	22.8±3.8	24.6±5.5	38.2±11.2^{C}	40.5±14.3^{C}	27.3±5.6^{CH}	25.5±5.5^{H}
Height, cm	178.5±7.2	166.2±5.3‡	175.2±7.3	164.6±6.9‡	179.7±6.1^{H}	166.3±6.0‡
Weight, kg	72.4±11.4	63.9±11.8†	81.9±14.3^{C}	65.5±13.0‡	83.3±14.7^{C}	63.3±13.3‡
Peripheral hemodynamics						
Systolic pressure, mm Hg	123.9±12.3	113.0±11.8‡	123.4±8.9	118.5±13.5^{C}	124.8±10.8	112.1±9.0‡^{H}
Diastolic pressure, mm Hg	76.1±9.0	71.6±7.1†	78.1±9.6	74.3±10.3	77.5±8.6	71.3±8.3†
Mean arterial pressure, mm Hg	92.0±9.0	85.4±8.0‡	93.2±8.7	90.0±10.7*	93.3±8.5	84.9±8.0‡
Pulse pressure, mm Hg	47.8±10.3	41.4±8.4†	45.3±7.7	44.2±8.9	47.3±8.8	40.9±6.4‡
Augmentation index, %	47.7±15.9	59.7±18.8†	58.3±17.3^{C}	72.1±21.9†^{C}	46.6±17.6^{H}	53.7±18.9^{H}
Central hemodynamics						
Pulse pressure, mm Hg	28.7±6.6	28.8±7.3	32.1±5.5^{C}	31.8±8.7	27.1±6.9^{H}	25.2±5.4^{CH}
Augmentation index, %	100.3±11.9	113.7±19.5‡	116.2±20.2^{C}	130.0±27.3*^{C}	103.3±19.0^{H}	109.5±20.7^{H}
Pulse wave velocity, m/s	8.7±1.1	8.1±1.3*	6.0±1.3^{C}	6.0±1.6 ^{C}	5.8±0.9^{C}	5.5±0.9^{C}

For further explanation, see Table 3.1.

Table 3.3 Heritabilities of anthropometric and hemodynamic measurements

Trait	h2±SE	*P*	Proportion of variance attributable to covariates	λ_1
Anthropometrics				
Height	0.85±0.07	<00.001	0.54	0.82
Weight	0.54±0.08	<0.0001	0.30	0.00
Body mass index	0.43±0.09	<0.0001	0.25	0.00
Peripheral hemodynamics				
Mean arterial pressure	0.39±0.09	<0.0001	0.32	0.00
Pulse pressure	0.37±0.10	0.0001	0.17	0.00
Augmentation index	0.39±0.10	0.0001	0.65	0.44
Central hemodynamics				
Pulse pressure	0.02±0.08	0.79	0.31	0.15
Augmentation index	0.41±0.09	<0.0001	0.62	0.40
Pulse wave velocity	0.19±0.11	0.08	0.42	0.00

Peripheral blood pressure was the averages of 5 readings at a single home visit. h^2 indicates heritability. λ_1 is the power transformation (0 corresponds to log) to normalize the residuals. Anthropometric characteristics were adjusted for center, sex and age. Hemodynamic measurements were adjusted for center, sex, age, age squared, height and weight, antihypertensive treatment, smoking and alcohol intake. In addition, pulse pressure, pulse wave velocity and the augmentation indexes were adjusted for mean arterial pressure and pulse rate.

Table 3.4 Genetic and environmental correlations between hemodynamic phenotypes

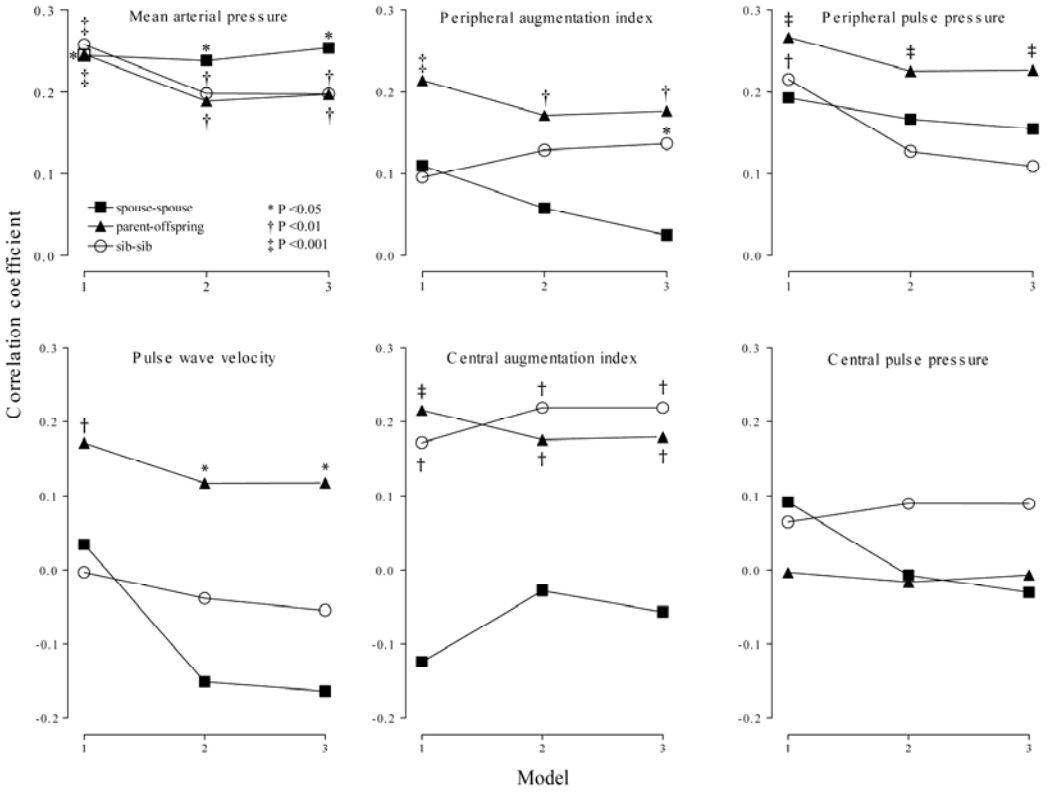
	Pulse wave velocity		Central augmentation index		Peripheral augmentation index		Peripheral pulse pressure	
Genetic correlation	ρ_G	*P*	ρ_G	*P*	ρ_G	*P*	ρ_G	*P*
Central augmentation index	0.443	<0.0001						
Peripheral augmentation index	0.433	<0.0001	0.952	<0.0001				
Peripheral pulse pressure	0.298	<0.0001	0.097	0.031	0.075	0.096		
Mean arterial pressure	0.489	<0.0001	0.105	0.020	0.084	0.063	–0.080	0.076
Environmental correlation	ρ_E	*P*	ρ_E	*P*	ρ_E	*P*	ρ_E	*P*
Central augmentation index	–0.105	0.020						
Peripheral augmentation index	–0.042	0.353	0.786	<0.0001				
Peripheral pulse pressure	0.098	0.030	0.030	0.507	0.042	0.353		
Mean arterial pressure	–0.039	0.389	0.010	0.825	–0.020	0.658	0.036	0.426

Peripheral blood pressure was the averages of 5 readings at a single home visit.

Figure 3.1 Intrafamilial correlation coefficients

Model 1 is adjusted for center, sex, and age (linear and squared terms). The two other models reflect further cumulative adjustments for height and weight, mean arterial pressure, pulse rate (if applicable) and antihypertensive treatment (model 2), and, in addition, for lifestyle factors such as smoking and alcohol intake (model 3).

Significance of the intraclass correlation coefficients: **P*<0.05, †*P*<0.01 and ‡*P*<0.001.



Discussion

We investigated several indexes of arterial stiffness within families. We estimated in the same subjects familial aggregation, heritability and the contribution of shared genetic and environmental factors. We found significant intrafamilial concordance in parent-offspring pairs for aortic pulse wave velocity, peripheral and central augmentation index and peripheral pulse pressure. In addition, the estimates of heritability for the aforementioned traits ranged from 0.19 for pulse wave velocity to 0.41 for the central augmentation index. The genetic correlations of pulse wave velocity with the augmentation indexes and peripheral pulse pressure were significant, whereas the corresponding environmental correlations were only significantly positive

for peripheral pulse pressure. Our findings suggest that common genes influence arterial stiffness. A shared environment, over and beyond the lifestyle factors for which we accounted, apparently plays only a minor role in the familial aggregation of these traits. In our hands, covariates explained 42% of the variance of pulse wave velocity, which experts consider as the gold standard to quantify arterial stiffness [29].

In analyses adjusted for the linear and squared terms of age, body weight and height, the heritability of the carotid-femoral pulse wave velocity was 0.40 among 817 pedigrees in the Framingham Heart Study [7]. With adjustments applied for sex and age, the heritability of carotid-femoral pulse wave velocity was 0.36 in a single extended pedigree recruited in the framework of Erasmus Rucphen Family Study [8]. With additional adjustments for mean arterial pressure, heart rate, low-density lipoprotein cholesterol and blood glucose, the estimate was 0.26. Heritability estimates of pulse wave velocity in previous studies [7;8] tended to be larger than our current estimate, probably because we adjusted for more covariates. Indeed, when we only accounted for center, sex and age, the heritability of pulse wave velocity was 0.30 (P=0.0021).

Two studies reported on the heritability of the central augmentation index. Among 225 monozygotic and 594 dizygotic female twin pairs, the heritability of the augmentation index was 0.37 in analyses adjusted for age, height, mean arterial pressure and heart rate [9]. In 32 extended families, the heritability of the augmentation index was 0.18 with cumulative adjustments for anthropometric characteristics, hypertension, diabetes and cholesterol [10]. In the current study, the multivariate-adjusted heritability for the central and peripheral augmentation indexes were 0.41 and 0.37, respectively. In general, heritability estimates from family-based studies tend to be lower than those from twin studies. It remains unclear to what extent differences in recording techniques, the number of observers involved in the measurement, reproducibility, or adjustment for confounders explain the variability across studies in the heritability estimates for augmentation indexes. In our hands, the intra-observer and inter-observer coefficients of variation across the three centers were less than 2.76% and 5.30%, respectively [30].

Several studies investigated the heritability of the peripheral pulse pressure. The adjusted heritability estimates were 0.13 in a study of White female twin pairs with wide age range (18–73 years) [9]. In young twins (10–26 years), the heritability of peripheral pulse pressure was similar among European and African Americans and

averaged 0.53 with adjustments applied for ethnicity, sex, age, body mass index, and their interactions [11]. In family-based studies, in Blacks [12-14], Caucasians [12;15-17], and other ethnicities [12;18;19] the multivariate-adjusted heritability of peripheral pulse pressure ranged from 0.13 to 0.51.

To our knowledge, only one previous study reported on the heritability of central pulse pressure as extrapolated from the tonometrically registered pulse pressure calibrated on the basis of the oscillometrically measured brachial blood pressure [7]. The heritability of the central pulse pressure, adjusted for age, age squared, height and weight was 0.35 [7], whereas in our current analysis it was zero both with minimal adjustment for center, sex and age (h^2=0.02) and after additional cumulative adjustment for anthropometric characteristics, mean arterial pressure, heart rate, smoking, alcohol intake, and use of anti-hypertensive medication (h^2=0.02). We analyzed the pulse wave at the radial artery to assess central pulse pressure. Such an approach may have led to a small degree of error in central pressure estimation, although the transfer function involved has been previously validated [21;22]. The strong consistency in the relations of peripheral and central hemodynamic measurements with age, as observed in previous studies [30;31] also excludes any distortion by the transfer function. Our heritability estimate of central pulse pressure was consistent with the absence of intrafamilial aggregation of this trait.

Numerous previous studies, spanning a time interval from 1965 until today addressed the familial aggregation of systolic and diastolic pressure, but to our knowledge few, if any, reported on the intrafamilial correlation of peripheral pulse pressure. We are also not aware of any previous investigation showing intrafamilial aggregation of pulse wave velocity, the central and peripheral augmentation indexes or the central pulse pressure. Our current study, in line with our heritability results, showed concordance of these traits with the exception of central pulse pressure in parent-offspring pairs, but not in spouse-spouse pairs. The high spouse-spouse correlation of mean arterial pressure is surprising. However, significant concordance among spouses in systolic and diastolic pressure were reported previously and have been attributed to assortative mating [32], the contribution of a common environment within the same generation of relatives or the home environment shared by members of the same household. To what extent these factors contributed to the currently observed parent-offspring correlations remains to be elucidated.

The present study must be interpreted within the context of its limitations and strengths. One potential limitation is that we used different devices to measure aortic pulse wave velocity. However, each of these devices allows recordings with acceptable and well documented intra- and inter-observer variability [23;24;26]. Because the measurement technique of pulse wave velocity was standardized within center and because we expressed the values in units of the within-center standard deviation, we believe that differences in the measurement technique can only have a minor influence, if any, on our estimates of heritability or intrafamilial aggregation. Second, our total sample size of 494 analyzable family members was smaller than in some other family-based studies [7;8;10;12;13;16-19]. On the other hand, we implemented a quality control programme to minimize error in the conventional blood pressure readings [33] from which we calculated peripheral pulse pressure and mean arterial pressure. As previously reported [30], we found high intra- and inter-observer reproducibility across our three centers for the central and peripheral augmentation indexes. We adjusted for a large number of potentially important covariates. Finally, heritability estimates are population-specific, because they are influenced by family structure [34], population-specific genetic and environmental factors. In our analyses, we pooled subjects drawn from three European populations, but only after we had checked that the additive polygenic and total phenotypic variances did not differ significantly across populations.

Our study demonstrated moderate heritability of various indexes of arterial stiffness, including peripheral pulse pressure, the central and peripheral augmentation indexes, and aortic pulse wave velocity. We confirmed the contribution of genetic factors to these traits by significant intrafamilial concordance in parent-offspring pairs and by the absence of a significantly positive environmental component in the phenotypic associations among most of these arterial phenotypes. We showed for the first time weak, but significant, genetic correlations between several indexes of arterial stiffness, which strongly suggest that shared genes must contribute to these traits. Several genome-wide scans showed linkage of peripheral pulse pressure with loci on chromosomes 7 [12;17;19] and 8 [17-19]. Some gene polymorphisms were also shown to be associated with age related increase in peripheral pulse pressure [35]. The Framingham Study reported linkage of pulse wave velocity with loci on chromosomes 1, 7, 13, 15 [7]. Our study therefore highlights the necessity of further research into the genes that affect arterial stiffness. From a practical point of view, we

would plea for a more standardized approach across studies in the adjustment of arterial phenotypes for host and lifestyle factors and potential confounders. Such uniform approach might increase the comparability and external validity of future studies on the heritability and intrafamilial concordance of arterial properties.

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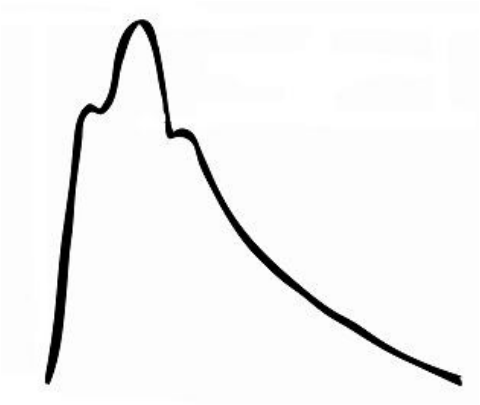

Chapter 4

Association between arterial properties and renal sodium handling in a general population

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Abstract—Mean arterial pressure (MAP) drives pressure-natriuresis and determines arterial structure and function. In a population sample, we investigated the relation between arterial characteristics and renal sodium handling as assessed by the clearance of endogenous lithium. We ultrasonographically measured diameter, cross-sectional compliance (CC) and distensibility (DC) of the carotid, brachial and femoral arteries in 1069 untreated subjects (mean age, 41.6 years; 50.1% women; 18.8% hypertensives). While accounting for covariates and standardizing for the sodium excretion rate, in both sexes, CC and DC of the femoral artery increased with higher fractional distal sodium reabsorption (RNa_{dist}). Differences associated with a 1-SD change in RNa_{dist} were 51.7 $mm^2/kPa \times 10^{-3}$ (95% CI, 23.9–79.5; *P*=0.0002) and 0.56 $10^{-3}/kPa$ (95% CI, 0.17–0.94; *P*=0.004) for femoral CC and DC, respectively. In women as well as in men, a 1-SD increment in fractional proximal sodium reabsorption (RNa_{prox}) was associated with decreases in femoral and brachial diameter, amounting to 111.6 μm (95% CI, 38.2–185.1; *P*=0.003) and 52.5 μm (CI, 10.0–94.9; *P*=0.016), respectively. There was no consistent association between the properties of the elastic carotid artery and renal sodium handling. In conclusion, higher fractional sodium reabsorption in the distal nephron is associated with higher femoral CC and DC and higher proximal sodium reabsorption is associated with decreased brachial and femoral diameters. These findings demonstrate that there might be an influence of renal sodium handling on arterial properties or vice versa, or that common mechanisms might influence both arterial and renal function.

Introduction

The kidneys play a central role in the pathogenesis of essential hypertension. Blood pressure starts to rise when the kidney requires a higher than usual blood pressure to maintain extracellular fluid volume within normal limits. Measuring the clearance of endogenous lithium provides a way to estimate sodium handling in the nephron. Indeed, lithium ions are freely filtered at the glomerulus and reabsorbed in the proximal tubule in parallel with sodium and water. Although lithium may be partially reabsorbed in the loop of Henle, distal tubular handling of lithium is minimal. Expressing the renal clearance of endogenous lithium as a fractional excretion provides a measure that is factored for the glomerular filtration rate. This limits possible sources of bias, such as differences in flow rate and incomplete urine collection. The fractional excretion of lithium (FE_{Li}) and fractional distal reabsorption of sodium (RNa_{dist}) are non-invasive markers of proximal tubular sodium handling and the proportion of sodium escaping reabsorption in the proximal tubule, that is not eliminated in the urine, respectively [1].

Mean arterial pressure drives pressure-natriuresis and influences arterial structure and function. The renin-angiotensin-aldosterone system is an important determinant of renal sodium handling and the properties of the arterial wall. To our knowledge, no prior study addressed the relation between renal sodium handling and arterial properties, while accounting for the activity of the renin-angiotensin-aldosterone system. Therefore, in the framework of the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), we investigated the functional and structural properties of three large arteries in relation to renal sodium handling as assessed by the clearance of endogenous lithium. We also measured plasma renin activity and the urinary aldosterone excretion rate.

Methods

Study population

The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) [2] is part of the European Project on Genes in Hypertension (EPOGH) [3]. From August 1985 until July 2003, we recruited a random sample of families from a geographically defined area in Northern Belgium. The Ethics Committee of the

University of Leuven approved the study. All participants or their parents gave informed written consent. The participation rate averaged 64.3%.

Of 1306 participants who underwent a vascular ultrasound examination [2], 1291 (98.8%) had high-quality measurements available at the three target arteries, and 1278 (97.9%) also underwent an assessment of renal sodium handling by measurement of the clearance of endogenous lithium. Of these 1278 subjects, we excluded 36 because of a very high lithium concentration in serum (≥1.0 μmol/l) or urine (≥20 μmol/l), suggestive of external contamination, 14 because of missing questionnaire data, and 159 because of current intake of antihypertensive drugs. Thus, the number of subjects analyzed totalled 1069.

Clinical measurements

For at least 3 hours before the examination, the participants refrained from heavy exercise, smoking, and alcohol or caffeine-containing beverages. Trained nurses measured the subjects' anthropometric characteristics and blood pressure. They administered a questionnaire to collect information about each participant's recent medical history, smoking and drinking habits, and intake of medications. Each subject's blood pressure was the average of five consecutive readings measured at the clinic after at least five minutes of sitting rest. Mean arterial pressure was diastolic pressure plus one third of pulse pressure. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic. Body mass index was weight in kilograms divided by the square of height in meters.

Vascular measurements

By means of a pulsed ultrasound wall-tracking system (Wall Track System; Pie Medical, Maastricht, the Netherlands), three trained researchers obtained vascular measurements at the common carotid artery 2 cm proximal of the carotid bulb, at the femoral artery 1 cm proximal of the bifurcation into the profound and superficial branches, and at the right brachial artery 2 cm proximal of the antecubital fossa [2].

During the ultrasound examination, an automated oscillometric device (Dinamap 845; Critikon Inc., Tampa, Florida, USA) recorded blood pressure at the upper arm at 5-minute intervals. As for the conventional auscultatory measurements, cuff size was adjusted to the circumference of the upper arm [3]. Standard cuffs had an inflatable bladder of 12 x 24 cm. As described elsewhere [4], the observers used applanation

tonometry with a pencil-shaped probe (Millar Instruments Inc., Houston, Texas, USA) and calibration to mean arterial pressure and diastolic blood pressure at the brachial artery to derive the local pulse pressure at the other arteries. We computed cross-sectional compliance (CC) and the distensibility coefficient (DC) from the diastolic cross-sectional area (A), the systolic increase in cross-sectional area (ΔA) and the local pulse pressure (PP) [5]: $CC = \Delta A / PP$ and $DC = (\Delta A / A)/PP$. A and ΔA were calculated from (D) diameter and the change in diameter (ΔD) as $A = \pi \times (D/2)^2$ and $\Delta A = \pi \times [(D + \Delta D)/2)^2 - \pi \times (D/2)^2$, respectively. The intra-observer intrasession variability was below 10% for the carotid measurements, below 5% for the brachial and femoral diameter, and amounted to 10% to 15% for brachial and femoral cross-sectional compliance and distensibility [6]. The intraobserver intersession and interobserver intrasession variability were of the same order of magnitude [6].

Renal sodium handling

On the day of the vascular measurements, venous blood samples were drawn to measure the serum concentrations of sodium, lithium, and creatinine and plasma renin activity. The participants also collected an exactly timed urine sample over 4 to 6 hours to measure the excretion of sodium, lithium, creatinine and aldosterone.

We measured the sodium concentration in serum and urine by flame photometry, and serum and urinary creatinine by an automated enzymatic method. We determined plasma renin activity (RIA-0180, DRG Instruments GmbH, Marburg/Lahn, Germany) and the urinary aldosterone concentration (DSL-8600 Active®, Diagnostic Systems Laboratories, Inc., Webster, Texas, USA) by radioimmunoassay, according to the instructions provided by the manufacturers of the analytic kits. Endogenous trace lithium was measured with an electrothermal atomic absorption spectrophotometer (model AAS 300) with a HGA-700 graphite furnace (Perkin-Elmer Inc., Boston, Massachusetts, USA) [1].

Clearances (C) were calculated as $C_X = U_X \times V/P_X$, where U_X and P_X are the urinary and plasma concentrations of the solute x, and V is the volume of the urine sample. We computed the fractional excretion of sodium (FE_{Na}) and lithium (FE_{Li}) by dividing the sodium (C_{Na}) and lithium (C_{Li}) clearances by the creatinine clearance. We expressed these ratios as a percentage. Fractional distal reabsorption of sodium (RNa_{dist}) was estimated as $[(FE_{Li} - FE_{Na})/FE_{Li}] \times 100$ [1]. RNa_{dist} is a measure of the

amount sodium that escapes reabsorption in the proximal tubules and is reabsorbed in the postproximal tubules. We defined the fractional proximal sodium reabsorption (RNa_{prox}) as $100 - FE_{Li}$.

Statistical methods

For database management and statistical analysis, we used SAS software (SAS Institute, Cary, North Carolina, USA), version 9.1. We normalized the distributions of plasma renin activity and the aldosterone excretion rate by a logarithmic transformation. We report the central tendency and spread of these measurements as the geometric mean and the interquartile range, respectively. We compared means and proportions using the large sample z-test and Fisher's exact test, respectively. Our statistical methods also included single and multiple linear regression. We searched for possible covariates of the arterial and renal phenotypes, using stepwise multiple regression with the *P*-value for independent variables to enter and stay in the model set at 0.15. Covariates considered for entry into the model were observer, age, body mass index, pulse rate, mean arterial pressure, current smoking, and alcohol intake. We initially analyzed women and men separately, but we combined both sexes when the interaction terms between explanatory variables and sex were nonsignificant ($P>0.15$). We standardized RNa_{prox} and RNa_{dist} to the mean sodium excretion rate for the whole study population (8.3 mmol per hour).

Results

Characteristics of participants

Women and men had similar age (mean, 41.6 years; range, 10.9–81.5). Table 4.1 summarizes their demographic characteristics. The study sample included 201 (18.8%) hypertensive patients not taking any antihypertensive medication.

Fewer women than men reported smoking or alcohol intake. Of the 535 women and 534 men, 146 women (27.3%) and 176 men (33.0%) were smokers and 166 women (31.0%) and 326 men (61.0%) reported intake of alcohol. In smokers, median tobacco use was 15 cigarettes per day (interquantile range, 8–23). In drinkers, the median alcohol consumption was 16 grams per day (interquantile range, 8–30). Among women, 171 (32.0%) reported natural or surgical menopause, while 123 (23.0%) used oral contraceptives and 7 (1.3%) took hormonal replacement therapy.

Arterial properties

Table 4.2 gives the vessel wall properties by sex and vascular territory. Across the three vascular territories, arterial diameter and cross-sectional compliance and local pulse pressure were consistently smaller (P<0.0002) in women than men, whereas the opposite was the true for arterial distensibility (P<0.0007).

Renal measurements

The excretion rate of sodium, lithium and creatinine and creatinine clearance were significantly lower in women than in men (Table 4.1). Compared to women, men had higher FE_{Na}, whereas opposite was true for RNa_{dist} (Table 4.1).

RNa_{prox} changed curvilinearly with age, while RNa_{dist} decreased after middle age (Fig. 4.1). The sodium excretion rate was a strong and independent determinant of FE_{Na}, RNa_{prox} and RNa_{dist} (Table 4.3). In all further analyses, we therefore standardized RNa_{prox} and RNa_{dist} to the average excretion rate of sodium in the whole study population (8.3 mmol/h).

Both before and after adjustment for age, body mass index, pulse rate as index of sympathetic modulation, and mean arterial pressure, plasma renin activity was positively and independently associated with RNa_{dist} in men. A 1-SD increase in RNa_{dist} (3%) was associated with an increase in plasma renin activity by 0.132 ng/ml/h (95% confidence interval [CI], 0.065–0.198; P<0.0001). In women, but not men, the aldosterone excretion rate was significantly associated with RNa_{prox} (Table 4.4) with a 1 SD increase in RNa_{prox} (10%) resulting in a decrease in urinary aldosterone by 0.097 nmol/h (CI, 0.031–0.162; P=0.004).

Arterial wall properties in relation to renal sodium handling

Stepwise multiple regression analysis showed significant and independent associations between one or more arterial properties and age, body mass index, mean arterial pressure, pulse rate, smoking, and alcohol intake. We therefore adjusted our further analyses for these covariates and additionally also for observer. In analyses combining women and men, we also adjusted for sex.

In both sexes, the cross-sectional compliance and the distensibility of the femoral artery significantly increased with higher RNa_{dist}. The RNa_{dist}-by-sex interaction terms for these associations were nonsignificant (P>0.63), indicating consistent

results in women and men. In all 1069 subjects combined, a 1-SD increment in RNa_{dist} (3%) was associated with an increase in femoral cross-sectional compliance by 51.7 $mm^2/kPa \times 10^{-3}$ (CI, 23.9–79.5; *P*=0.0002) and with an increase in femoral distensibility by $0.56 \times 10^{-3}/kPa$ (CI, 0.17–0.94; *P*=0.004). In women as well as in men, the diameter of the brachial and femoral arteries decreased with higher RNa_{prox}, whereas the diameter of the carotid artery increased with higher RNa_{dist} in men, but not women (Table 4.5).

Both before and after adjustment for age, body mass index, pulse rate, smoking, and alcohol intake, there was in women as well as in men an inverse association of mean arterial pressure and diastolic blood pressure with RNa_{prox} (Table 4.4). An ≈1-SD increase in RNa_{prox} (10%) was associated with a decrease in mean arterial pressure by 0.95 mm Hg (CI, 0.42–1.49; *P*=0.0005) and a decrease in diastolic blood pressure by 1.13 mm Hg (CI, 0.59–1.66; *P*=0.0009).

Sensitivity analyses produced consistent results. After exclusion of 130 women taking oral contraceptives or on hormonal replacement therapy, adjusted effect sizes associated with a 1 SD increase in RNa_{dist} were 41.3 $mm^2/kPa \times 10^{-3}$ (CI, 16.0–66.6; *P*=0.001) and $0.42 \times 10^{-3}/kPa$ (CI, 0.09–0.76; *P*=0.01) for the increases in femoral cross-sectional compliance and distensibility. Those associated with a 1 SD increase in RNa_{prox} were 49.2 μm (CI, 6.7–91.6; *P*=0.025) and 107.5 μm (CI, 32.4–182.6; *P*=0.005) for the decreases in the brachial and femoral diameters, respectively. After we had excluded 301 women with an active menstrual cycle, the corresponding effect sizes were 51.7 $mm^2/kPa \times 10^{-3}$ (CI, 24.0–79.5; *P*=0.0002) and $0.55 \times 10^{-3}/kPa$ (CI, 0.17–0.94; *P*=0.004) for the increases in femoral cross-sectional compliance and distensibility and 52.5 μm (CI, 10.0–94.9; *P*=0.016) and 111.6 μm (CI, 38.2–185.1; *P*=0.0027) for the decreases in brachial and femoral diameters, respectively. Finally, our results also remained consistent after exclusion of subjects younger than 20 years. The aforementioned estimates then were 52.4 $mm^2/kPa \times 10^{-3}$ (CI, 24.6 –80.2; *P*=0.0003) and $0.65 \times 10^{-3}/kPa$ (CI, 0.26–1.03; *P*=0.0009) for femoral compliance and distensibility, and 63.3 μm (CI, 19.2–107.3; *P*=0.005) and 132.4 μm (CI, 55.7–209.0; *P*=0.0007) for the brachial and femoral diameters.

Table 4.1 Characteristics of participants by sex

Characteristics	Women (*n*=535)	Men (*n*=534)	*P*
Anthropometry			
Age, y	41.2±14.7	42.1±14.5	0.32
Body mass index, kg/m^2	24.3±4.2	25.4±3.7	<0.0001
Systolic pressure, mm Hg	122.1±14.9	128.0±13.1	<0.0001
Diastolic pressure, mm Hg	76.6±9.9	81.0±10.4	<0.0001
Mean arterial pressure, mm Hg	91.8±10.7	96.7±10.1	<0.0001
Pulse pressure, mm Hg	44.8±11.1	48.3±11.9	<0.0001
Hypertensive, *n* (%)	72 (13.5)	129 (24.2)	<0.0001
Serum biochemistry			
Sodium, mmol/l	141.0±3.3	142.6±2.4	<0.0001
Lithium, µmol/l	0.17±0.08	0.19±0.10	0.009
Creatinine, µmol/l	73.5±12.7	90.4±14.0	<0.0001
Plasma renin activity, ng/ml/h	0.43 (0.20-0.86)	0.43 (0.22-0.87)	0.79
Urinary excretion rate			
Sodium, mmol/h	7.1±5.4	9.4±6.6	<0.0001
Lithium, µmol/h	0.17±0.13	0.22±0.17	<0.0001
Creatinine, mmol/h	0.41±0.31	0.61±0.37	<0.0001
Aldosterone, nmol/h	1.01 (0.51-2.02)	0.98 (0.57-1.77)	0.43
Renal function			
Creatinine clearance, ml/min	87.3±24.8	105.9±32.0	<0.0001
FE_{Na}, %	0.92±0.44	1.00±0.45	0.006
RNa_{prox}, %	79.3±8.4	79.5±8.3	0.64
RNa_{dist}, %	95.0±3.1	94.6±3.2	0.02

Values are mean±SD, geometric mean (interquantile range) or number (%) of subjects. FE_{Na} indicates the fractional excretion of sodium. RNa_{prox} represents the fractional sodium reabsorption along the proximal tubules. RNa_{dist} is the calculated reabsorption of sodium in the postproximal tubules.

Table 4.2 Arterial characteristics by sex

Characteristics	Women (*n*=535)	Men (*n*=534)
Common carotid artery		
Diameter, μm	6732±735	7498±842
Pulse pressure, mm Hg	44.8±11.1	48.3±11.9
Cross-sectional compliance, mm^2/kPa	1.01±0.45	1.12±0.47
Distensibility coefficient, 10^{-3}/kPa	29.0±14.1	25.6±11.0
Brachial artery		
Diameter, μm	3892±705	4807±697
Pulse pressure, mm Hg	45.3±8.9	50.0±9.9
Cross-sectional compliance, mm^2/kPa	0.15±0.11	0.19±0.15
Distensibility coefficient, 10^{-3}/kPa	13.2±11.3	11.4±10.0
Femoral artery		
Diameter, μm	8485±1126	10003±1346
Pulse pressure, mm Hg	49.4±12.0	53.6±12.1
Cross-sectional compliance, mm^2/kPa	0.67±0.40	0.77±0.48
Distensibility coefficient, 10^{-3}/kPa	12.0±7.0	9.9±5.7

Values are mean±SD. All sex differences were statistically significant (P<0.0007).

Figure 4.1 Fractional sodium excretion (FE_{Na}), fractional proximal sodium reabsorption (RNa_{prox}) and fractional distal sodium reabsorption (RNa_{dist}) by age

Values are mean±SE. The number of subjects contributing to the means is given for each age group.

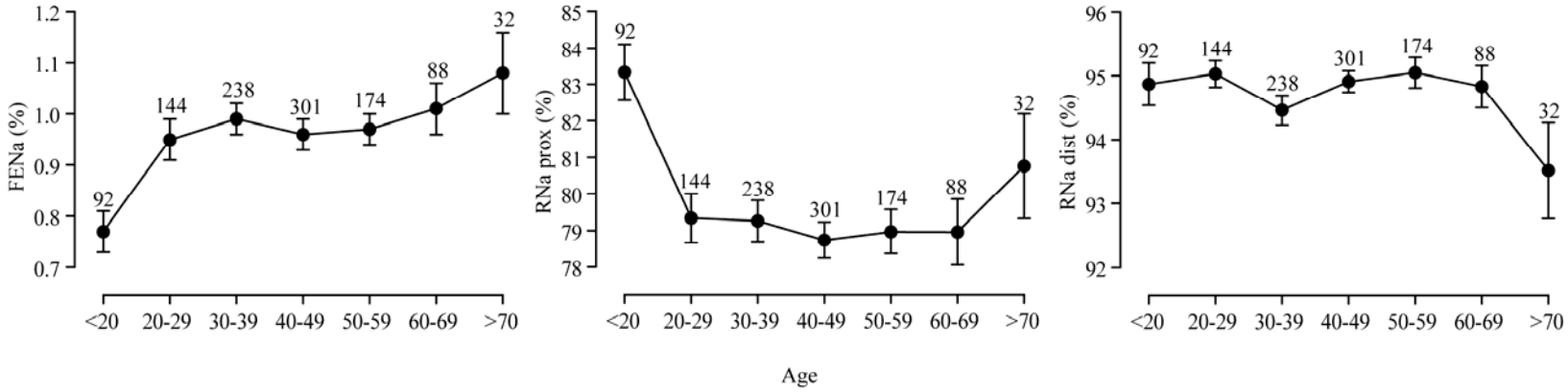

Table 4.3 Determinants of renal sodium handling

Determinants	FE_{Na}	RNa_{prox}	RNa_{dist}
R^2	0.306	0.046	0.383
Intercept	0.34†	90.9†	97.0†
Partial regression coefficient ±SE			
Age (year x 10^{-3})	3.46±0.89†	–387.3±86.7†	–11.8±5.9*
Age squared (year^2 x 10^{-3})	NC	4.10±0.97†	NC
Body mass index (kg/m^2)	–0.010±0.003†	0.149±0.071*	0.041±0.020*
Mean arterial pressure (mm Hg)	0.0043±0.0013†	–0.089±0.028†	NS
Female gender (0, 1)	NS	NS	–0.35±0.16*
Sodium excretion rate (mmol/h)	0.039±0.002†	0.117±0.042†	–0.374±0.015†
Current smoker (0, 1)	–0.068±0.025†	1.388±0.560*	NS

FE_{Na} indicates the fractional excretion of sodium. RNa_{prox} represents the fractional sodium reabsorption along the proximal tubules. RNa_{dist} is the calculated reabsorption of sodium in the postproximal tubules. Significance of the partial regression coefficients: NS $P>0.15$; * $P\leq0.05$ and † $P\leq0.01$. NC indicates that age squared was not considered.

Table 4.4 Blood pressure, plasma renin activity and aldosterone excretion in relation to 1-SD increments in proximal or distal sodium reabsorption

	RNa_{prox}			RNa_{dist}		
	Group	Estimate (95% CI)	*P*	Group	Estimate (95% CI)	*P*
Blood pressures						
Systolic pressure, mm Hg	All	–0.61 (-1.37 to 0.15)	0.11	All	–0.71 (-1.48 to 0.05)	0.068
Diastolic pressure, mm Hg	All	–1.13 (-1.66 to -0.59)	0.0009	All	0.32 (-0.21 to 0.86)	0.24
Mean arterial pressure, mm Hg	All	–0.95 (-1.49 to -0.42)	0.0005	All	–0.03 (-0.57 to 0.52)	0.93
Pulse pressure, mm Hg	All	0.08 (-0.60 to 0.76)	0.82	All	0.05 (-0.64 to 0.74)	0.90
Renin system components						
Plasma renin activity, ng/ml/h	All	–0.024 (-0.073 to 0.025)	0.34	Women	0.018 (-0.051 to 0.086)	0.62
				Men	0.132 (0.065 to 0.198)	<0.0001
Aldosterone excretion rate, nmol/h	Women	–0.097 (-0.162 to -0.031)	0.004	All	0.032 (-0.014 to 0.078)	0.17
	Men	–0.001 (-0.064 to 0.062)	0.98			

RNa_{prox} and RNa_{dist} represent the fractional sodium reabsorption along the proximal and postproximal tubules, respectively. RNa_{prox} and RNa_{dist} were standardized to a sodium excretion rate of 8.3 mmol/h. Regression coefficients for blood pressures were adjusted for sex, age, body mass index, pulse rate, smoking and alcohol. Regression coefficients for renin system components were adjusted for sex (if appropriate), age, body mass index, pulse rate and mean arterial pressure. *P* refers to the significance of the partial regression coefficients. We reported results for women and men separately, if the *P*-value for the interaction term between the indexes of renal sodium handling and sex was less than 0.15.

Table 4.5 Arterial properties in relation to 1-SD increments in proximal or distal sodium reabsorption

		RNa_{prox}			RNa_{dist}		
		Group	Estimate (95% CI)	*P*	Group	Estimate (95%CI)	*P*
Carotid artery	Diameter, µm	All	10.0 (–32.5 to 52.5)	0.63	Women	–5.6 (-61.6 to 50.3)	0.84
					Men	72.9 (11.0 to 134.8)	0.021
	Pulse pressure, mm Hg	All	0.04 (–0.684 to 0.77)	0.91	All	0.07 (-0.67 to 0.81)	0.85
	Compliance, $mm^2/kPa \times 10^{-3}$	All	–12.56 (–40.0 to 12.0)	0.32	Women	–2.5 (-33.9 to 28.8)	0.87
					Men	39.8 (2.6 to 76.9)	0.037
	Distensibility coefficient, $10^{-3}/kPa$	Women	0.29 (–0.50 to 1.08)	0.47	All	0.15 (-0.38 to 0.68)	0.57
		Men	–0.33 (–0.99 to 0.33)	0.33			
Brachial artery	Diameter, µm	All	–52.5 (–94.9 to -10.0)	0.016	All	32.8 (-9.8 to 75.5)	0.13
	Pulse pressure, mm Hg	All	–0.07 (–0.67 to 0.52)	0.81	All	–0.47 (-1.07 to 0.13)	0.13
	Compliance, $mm^2/kPa \times 10^{-3}$	All	5.8 (–0.7 to 12.4)	0.085	Women	–9.7 (-17.1 to -2.4)	0.010
					Men	–0.3 (-10.2 to 9.6)	0.95
	Distensibility coefficient, $10^{-3}/kPa$	All	0.46 (–0.05 to 0.96)	0.076	All	–0.56 (-1.08 to -0.05)	0.031
Femoral artery	Diameter, µm	All	–111.6 (–185.1 to –38.2)	0.003	All	67.5 (-6.1 to 141.0)	0.071
	Pulse pressure, mm Hg	All	0.35 (–0.44 to 1.13)	0.38	All	–0.11 (-0.89 to 0.67)	0.78
	Compliance, $mm^2/kPa \times 10^{-3}$	Women	–27.7 (–62.3 to 6.9)	0.11	All	51.7 (23.9 to 79.5)	0.0002
		Men	–68.6 (–112.3 to –24.8)	0.002			
	Distensibility coefficient, $10^{-3}/kPa$	Women	–0.06 (–0.62 to 0.51)	0.84	All	0.56 (0.17 to 0.94)	0.004
		Men	–0.55 (–1.07 to –0.04)	0.034			

RNa_{prox} and RNa_{dist} represent the fractional sodium reabsorption along the proximal and postproximal tubules, respectively. RNa_{prox} and RNa_{dist} were standardized to a sodium excretion rate of 8.3 mmol/h. Regression coefficients were adjusted for observer, age, mean arterial pressure (not for pulse pressure as dependent variable), pulse rate, body mass index, smoking and alcohol intake. *P* refers to the significance of the partial regression coefficients. We reported results for women and men separately, if the *P*-value for the interaction term between the indexes of renal sodium handling and sex was less than 0.15.

Discussion

The key finding of our study was that while accounting for covariates and standardizing for the sodium excretion rate the cross-sectional compliance and distensibility of the femoral artery increased with higher distal sodium reabsorption, while the brachial and femoral diameters lessened with higher proximal sodium reabsorption. To our knowledge, no prior study addressed the possible influence of renal sodium handling on arterial properties or conversely the possible effects of arterial characteristics on renal sodium handling.

According to Guyton's hypothesis, the pathogenesis of hypertension, irrespective of the primary causal factor, always involves the pressure-natriuresis relation in the kidney, with higher pressures being required to excrete a given sodium load. In keeping with this concept, we found an inverse and independent relation of RNa_{prox} with mean arterial pressure and diastolic blood pressure. Higher mean arterial pressure is associated with elevated renal interstitial hydrostatic pressure and therefore with less sodium reabsorption in the proximal tubules, which are responsible for reuptake of 80% of the filtered sodium load [7].

Our cross-sectional epidemiological study cannot directly address the mechanisms underlying the positive relation of femoral compliance and distensibility with RNa_{dist}. A possible explanation might be that a common mechanism, operating in both the arterial wall and the renal tubules, might influence arterial properties and renal sodium handling. For instance, genetic variability in ion transport across cell membranes might be involved. In the present population, we previously demonstrated that mutations of the cytoskeleton protein α adducin (*460 Gly→Trp*) and the angiotensin-converting enzyme (*ACE I→D*) jointly predicted the incidence of hypertension [8]. In cross-sectional analyses of the same population, we also noticed that the combination of these two functional mutations [9;10] was associated with raised serum creatinine concentration [11], increased femoral intima-media thickness [12] and 24-hour proteinuria [11]. In this context, it is conceivable that the constitutive activation of the sodium pump in α-adducin *Trp* allele carriers not only occurs in renal tubular cells, but that it might also be present in vascular smooth muscle cells. In vascular myocytes enhanced Na^+,K^+-ATPase activity might reduce the sarcolemmal Na^+/Ca^{2+} exchange and through calcium-dependent pathways modulate excitation-contraction coupling [13].

Oxidative stress might represent another common pathway, which potentially affects both arterial and renal function. Indeed, studies in patients with end-stage renal disease demonstrated that asymmetrical dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase, behaves as an independent risk factor, associated with endothelial dysfunction, arterial stiffening, carotid atherosclerosis and the risk of cardiovascular events [14]. In patients with essential hypertension [15] and in salt-sensitive Dahl rats [16], sodium loading increases [15;16], whereas salt depletion decreases [15], the plasma levels of ADMA. In healthy men, infusion of ADMA increased renovascular resistance and lowered effective renal plasma flow as well as sodium excretion and FE_{Na} [17].

In women and men, we noticed a negative relation between the brachial and femoral diameters and RNa_{prox}. Vasoconstriction is the hallmark of hypertension. Animal models of hypertension [18], hypertensive patients [19], normotensive subjects with one first-degree relative with hypertension [20] and patients with white-coat hypertension [19] have higher RNa_{prox}. Moreover, Draaijer and coworkers reported a significantly lower arterial compliance in sodium-sensitive than in sodium-resistant men with borderline hypertension, independent of cardiac output, blood pressure and hormonal factors [21]. Along similar lines, salt sensitivity rather than salt intake seems to be a more important determinant of the mechanical properties of the carotid arteries in Dahl rats [22].

In women, the aldosterone excretion rate was negatively associated with RNa_{prox}, whereas in men there was a positive association between plasma renin activity and RNa_{dist}. These relations are physiologically plausible. Indeed, enhanced proximal sodium reabsorption leads to inhibition of the renin-angiotensin-aldosterone system. Conversely, under conditions requiring sodium conservation, the distal tubule is the ultimate target of the activated renin system. Heterogeneity in the study population or residual confounding, might explain why these relations were not consistent in women and men. For instance, our analysis did not take salt sensitivity into account, the prevalence of which is ≈26% in normotensive subjects and ≈51% in hypertensive patients [23]. Furthermore, there might be physiological differences between women and men in renal sodium handling. Progesterone binds to the human mineralocorticoid receptor with nearly the same affinity as aldosterone [24]. Progesterone and its metabolites antagonize aldosterone [25]. The renal tubular response to a sustained

increase in sodium intake was similar in women during the follicular phase of their cycle compared with men. In contrast, in women examined during the luteal phase of their menstrual cycle, increasing sodium intake led to a marked sodium escape from the distal nephron, with no change in proximal sodium reabsorption [26]. We did not record the phase of the menstrual cycle, but our results remained consistent after we excluded from analysis women taking oral contraceptives or hormonal replacement therapy or those with an active menstrual cycle.

The central elastic arteries, such as the carotid artery, and the more peripheral muscular conduit vessels, including the brachial and femoral arteries have different properties [27]. Going from the central to the peripheral arteries, the collagen-to-elastin ratio reverses, vascular smooth muscle cells become the predominant component of the arterial wall, and the phenotype of the vascular smooth muscle cells changes [27]. In line with this diversity along the arterial tree, we earlier noticed that genetic influences on the cross-sectional compliance and distensibility of large arteries depended on vascular territory [28]. In clinical trials [29; 30], diuretics decreased the carotid, but not the brachial and femoral diameters, and they increased brachial and femoral, but not carotid distensibility. Finally, the relation between arterial stiffness and body mass index is more complex in the carotid than muscular arteries, because carotid properties in relation to body mass index vary according to sex and age [2]. These observations [2;27-30] help to understand the heterogeneity in the current associations of arterial properties with renal sodium handling.

The present study must be interpreted within the context of its limitations. The endogenous lithium clearance provides only an indirect measurement of renal tubular sodium transport in vivo. The fractional excretion of sodium and the creatinine clearance, which we used to standardize data, showed large inter-individual variability. Although the measurement of trace lithium in biological fluids at concentrations down to 0.03 μmol/l shows intra- and inter-day variation of less then 10% [31], the intra-individual variability in RNa_{prox} and RNa_{dist} on repeated measurement must be large, mirroring the day-to-day variation in the 24-hour urinary sodium excretion [32]. However, large variability would rather tend to weaken than strengthen the observed relations of the indexes of renal sodium handling with femoral compliance and distensibility and the brachial and femoral diameters.

The novel finding in our study is the significant association between arterial properties and renal sodium handling. If confirmed, our findings might be relevant for unraveling the pathogenesis of essential hypertension and its renal and vascular complications. We will further explore to what extent genetic variability in the regulation of sodium homeostasis, in particular the adducin polymorphisms [33] contributed to the present findings.

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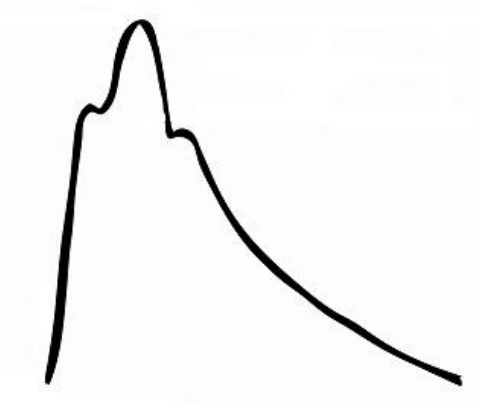

Chapter 5

Relation between local arterial stiffness and genetic variation in adducin subunit and renin-angiotensin system

Part 1

Arterial properties in relation to genetic variation in the adducin subunits in a White population

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Abstract—Adducin is a membrane skeleton protein, which consists either of α- and ß- or α- and γ-subunits. We investigated whether arterial characteristics might be related to the genes encoding *ADD1* (*Gly460Trp-rs4961*), *ADD2* (*C1797T-rs4984*) and *ADD3* (*IVS11+386A>G-rs3731566*). We randomly recruited 1126 Flemish subjects (mean age, 43.8 years; 50.3% women). Using a wall-tracking ultrasound system, we measured the properties of the carotid, femoral and brachial arteries. We studied multivariate-adjusted phenotype-genotype associations, using a population- and family-based approach. In single-gene analyses, brachial diameter was 0.15 mm (P=0.0022) larger, and brachial distensibility and cross-sectional compliance were 1.55 10^{-3}/kPa (P=0.013) and 0.017 mm^2/kPa (P=0.0029) lower in *ADD3 AA* than *ADD3 GG* homozygotes with an additive effect of the *G* allele. In multiple-gene analyses, the association of brachial diameter and distensibility with the *ADD3 G* allele only occurred in *ADD1 GlyGly* homozygotes. Otherwise, the associations between the arterial phenotypes in the 3 vascular beds and the *ADD1* or *ADD2* polymorphisms were not significant. In family-based analyses, the multivariate-adjusted heritability was 0.52, 0.38 and 0.30 for brachial diameter, distensibility, and cross-sectional compliance, respectively (P<0.001). There was no evidence for population stratification ($0.07 \leq P \leq 0.96$). Transmission of the mutated *ADD3 G* allele was associated with smaller brachial diameter in 342 informative offspring (–0.12±0.04 mm; P=0.0085) and in 209 offspring, who were *ADD1 GlyGly* homozygotes (–0.14±0.06 mm; P=0.018). In *ADD1 GlyGly* homozygotes, the properties of the brachial artery are related to the *ADD3* (*A386G*) polymorphism, but the underlying mechanism needs further clarification.

Introduction

Adducin is a ubiquitously expressed membrane skeleton protein, which consists either of α- and ß- or α- and γ-subunits. Mutation of the α-adducin gene (*ADD1*) is linked with increased Na^+,K^+-ATPase activity [1,2] and increased renal tubular sodium reabsorption [3]. Variation in the Na^+,K^+-ATPase activity and in the intracellular Na^+ concentration might influence the sodium-dependent transmembranous Ca^{2+} transport in vascular smooth muscle cells and via this mechanism might affect arterial tone [4].

In the participants of the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), interaction between the genes encoding *ADD1* (*Gly460Trp* polymorphism), the angiotensin-converting enzyme and the angiotensin II type-1 receptor influenced the distensibility, cross-sectional compliance, and intima-media thickness of the femoral artery [5,6]. In the same Flemish population [7] and in Polish and Russian subjects [8], we noticed that blood pressure and the prevalence of hypertension were associated with the *C1797T* polymorphism in the β-adducin subunit (*ADD2*), particularly in postmenopausal women [7]. *T* allele carriers had significantly higher 24-hour systolic blood pressure than *CC* homozygotes [8]. Moreover, Cwynar et colleagues reported interaction between the *ADD1 Gly460Trp* polymorphism and the *A386G* polymorphism in the γ-adducin subunit (*ADD3*) [9]. Peripheral and central pulse pressures were higher in carriers of both the *ADD1 Trp* allele and the *ADD3 G* allele [9].

To our knowledge, no previous study investigated whether arterial properties are related to genetic interactions between the three adducin subunits. We addressed this question in the FLEMENGHO participants with available ultrasonographically measured arterial phenotypes.

Methods

Study population

The FLEMENGHO study is part of the European Project on Genes in Hypertension (EPOGH) [10] and is embedded in the InGenious HyperCare Network of Excellence. From August 1985 until July 2003, we recruited a random sample of families from a geographically defined area in Northern Belgium. The Ethics Committee of the University of Leuven approved the study. All participants or their parents gave informed written consent. The participation rate averaged 64.3%.

Of 1306 participants, who underwent a vascular ultrasound examination [11], 1180 (90.3%) had high-quality images obtained at the common-carotid, femoral and brachial arteries. We excluded 39 participants, because of missing genotypes, and 7 because of incomplete information on important covariates. In addition, we detected 8 cases of inconsistency in Mendelian segregation. Thus, the number of subjects analyzed totaled 1126.

Clinical and biochemical measurements

For at least 3 hours before the examination, the participants refrained from heavy exercise, smoking, and alcohol or caffeine-containing beverages. Trained nurses measured the subjects' anthropometric characteristics, heart rate and blood pressure. They administered a questionnaire to collect information about each participant's recent medical history, smoking and drinking habits, and intake of medications. Each subject's blood pressure was the average of 5 consecutive readings measured before the ultrasound examination after the subjects had rested in the sitting position for at least 5 minutes. Mean arterial pressure was diastolic pressure plus one third of pulse pressure. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic, and/or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters.

Arterial measurements

By means of a pulsed ultrasound wall-tracking system (Wall Track System; Pie Medical, Maastricht, the Netherlands), 3 trained researchers obtained vascular measurements at the common carotid artery 2 cm proximal of the carotid bulb, at the femoral artery 1 cm proximal of the bifurcation into the profound and superficial branches, and at the right brachial artery 2 cm proximal of the antecubital fossa.

During the ultrasound examination, an automated oscillometric device (Dinamap 845; Critikon Inc., Tampa, Florida, USA) recorded blood pressure at the upper arm at 5-minute intervals. As for the conventional auscultatory measurements, cuff size was adjusted to the circumference of the upper arm [10]. Standard cuffs had an inflatable bladder of 12 x 24 cm. Larger cuffs had a 15 x 35 cm bladder. As described elsewhere [12], the observers used applanation tonometry with a pencil-shaped probe (Millar Instruments Inc., Houston, Texas, USA) and calibration to mean arterial pressure and diastolic blood pressure at the brachial artery to derive the local pulse pressure at the

other arteries. We computed the distensibility (DC) and cross-sectional compliance (CC) from the diastolic cross-sectional area (A), the systolic increase in cross-sectional area (ΔA) and the local pulse pressure (PP) [13]: DC = (ΔA/ A)/PP and CC = ΔA/ PP. A and ΔA were calculated from (D) diameter and the change in diameter (ΔD) as $A = \pi \times (D/2)^2$ and $\Delta A = \pi \times [(D + \Delta D)/2)^2 - \pi \times (D/2)^2$, respectively. The intra-observer intra-session variability was below 10% for the carotid measurements, below 5% for the femoral and brachial diameter, and amounted from 10% to 15% for the femoral and brachial cross-sectional compliance and distensibility [14]. The intra-observer inter-session and inter-observer intra-session variability were of the same order of magnitude [14].

Genotypes

We extracted DNA from white blood cells. For genotyping, we used a 5' nuclease detection assay implemented on an ABI Prism 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, California, USA). Primers, probes and PCR conditions for *ADD1 Gly460Trp* (rs4961) [15], *ADD2 C1797T* (rs4984) [7] and *ADD3 IVS11+386A>G* (rs3731566) [9] genotyping have already been described in detail elsewhere.

Statistical methods

For database management and statistical analyses, we used SAS software (SAS Institute, Cary, North Carolina, USA), version 9.1. We compared means and proportions, using the large sample z-test and Fisher's exact test, respectively. We assessed Hardy-Weinberg proportions in unrelated founders, using an exact test based on Monte Carlo permutations. Our statistical methods also included single and multiple linear regressions. We searched for possible covariates of the arterial phenotypes, using a stepwise regression procedure with the *P*-values for independent variables to enter and to stay in the model set at 0.15. As covariates, we considered observer, sex, age, body mass index, heart rate, mean arterial pressure, and binary variables (0, 1), coding for current smoking, alcohol intake, and use of antihypertensive drugs.

We performed both population- and family-based analyses. In the former approach, we applied a generalization of the standard linear model as implemented in the PROC MIXED procedure of the SAS package to test the associations between phenotypes

and SNPs, while accounting for the nonindependence of phenotypes within families and adjusting for covariates. In the family-based analyses, we evaluated the within- and between-family components of phenotypic variability, using the orthogonal model proposed by Abecasis and colleagues [16]. We implemented the quantitative transmission disequilibrium test (QTDT), using a mixed model with similar adjustments as in population-based analyses. The within-family component of phenotypic variance is robust to population stratification.

Results

We divided study participants into founders (n = 187) and unrelated subjects (n=273) as compared with offspring (n=696). Subjects in the founders group (mean age 51.5, range 12.6–81.5 years) were older compared with offspring (39.0; range 10.9-81.0 years). Table 5.1.1 summarizes their demographic characteristics. In contrast to founders more offspring reported alcohol intake. In drinkers, the median alcohol consumption was 10 grams per day (interquantile range, 4–20). In smokers, median tobacco use was 14 cigarettes per day (interquantile range, 8–20). Table 5.1.2 lists the arterial properties by generation and vascular territory. Brachial distensibility and cross-sectional compliance were higher in founders than in offspring.

Table 5.1.3 summarizes the genotype and allele frequencies for the adducin genes in the whole study population. In the founder generation ($0.41<P<0.67$), the genotype frequencies complied with Hardy-Weinberg proportions.

Population-based analyses

In stepwise multiple regression, in line with our previous publications [11], we identified the following covariates as significant determinants of one or more of the arterial phenotypes in the 3 vascular beds under study: sex, age, body mass index, mean arterial pressure, heart rate, smoking, daily alcohol intake in excess of 5 grams, and use of antihypertensive drugs. We adjusted all phenotype-genotype associations for these covariates, and in addition for observer.

In single-gene analyses (Fig. 5.1.1), brachial diameter was 0.15 mm (95% confidence interval [CI], 0.05–0.24; P=0.0022) larger, and brachial distensibility and cross-sectional compliance were 1.55 10^{-3}/kPa (CI, 0.33–2.77; P=0.013) and 0.017 mm^2/kPa (CI, 0.002–0.032; P=0.0029) lower in *ADD3 AA* homozygotes than in their *GG* counterparts. As shown by the P-values for linear trend, the *G* allele had an

additive effect in relation to these phenotypes. In single-gene analyses, we did not find any other significant association between the arterial phenotypes under study in the 3 vascular beds and the *ADD1* ($P \geq 0.26$) or *ADD2* ($P \geq 0.27$) polymorphisms.

In multiple-gene analyses, there was a significant gene-gene interaction between *ADD1* and *ADD3* in relation to the diameter of the brachial artery ($P=0.042$; Fig. 5.1.2). In carriers of the wild type *ADD1*, brachial diameter was 0.23 mm (CI, 0.11–0.34; $P=0.0001$) larger in *ADD3 AA* homozygotes than in *GG* homozygotes with a significant *P*-value for linear trend ($P=0.0014$). This was not the case in *ADD1 Trp* allele carriers (*P* for linear trend, 0.55). Furthermore, in carriers of the wild type *ADD1*, brachial distensibility was 2.54 10^{-3}/kPa (CI, 0.95–4.13; $P=0.0018$) lower in *ADD3 AA* homozygotes than in their *GG* counterparts with a significant *P*-value for trend ($P=0.011$). Although in *ADD1 Trp* allele carriers brachial distensibility did not change with the *ADD3* genotype ($P=0.92$), the *P*-value for the *ADD1*-by-*ADD3* gene-gene interaction was only 0.26. We did not find any significant gene-gene interaction between carotid and femoral arterial properties and the adducin subunits polymorphisms. In a sensitivity analysis, from which we excluded 147 patients on antihypertensive drugs, we reproduced our results for the properties of the brachial artery (Fig. 5.1.3).

Family-based analyses

Our study sample consisted of 58 pedigrees, of which 27 spanned more than 2 generations, and additionally of 273 unrelated subjects. The number of offspring per pedigree was less than 3 in 24 pedigrees, ranged from 3 to 8 in 30 families, and amounted to more than 8 in 4 pedigrees.

The multivariate-adjusted heritability estimates, as reported by Abecasis' software [16], were 0.52 for the brachial diameter, 0.38 distensibility, and 0.30 for cross-sectional compliance ($P<0.001$ for all). Abecasis' orthogonal model did not reveal population stratification ($0.07 \leq P \leq 0.96$). In 342 informative offspring, transmission of the mutated *ADD3* G allele was associated with a significant decrease in brachial diameter (–0.12±0.04 mm; $\chi^2=6.99$; $P=0.0085$). We observed a similar trend in 209 informative offspring, who were homozygous for the *ADD1 Gly* allele (–0.14±0.06 mm; $\chi^2=5.72$; $P=0.018$). For brachial distensibility or cross-sectional compliance, these estimates were not significant ($0.28 \leq P \leq 67$).

Table 5.1.1 Characteristics of participants

	Founders and unrelated participants (*n*=430)	Offspring (*n*=696)	*P*
Anthropometry			
Women, *n* (%)	219 (50.9)	347(49.9%)	0.76
Age, y	51.5±12.4	39.0±15.1	<0.0001
Height, cm	167.3±9.1	169.4±9.3	0.0002
Weight, kg	71.5±12.6	71.1±14.2	0.60
Body mass index, kg/m^2	25.5±3.6	24.7±4.2	0.0011
Systolic pressure, mmHg	130.5±15.8	124.3±14.4	<0.0001
Diastolic pressure, mmHg	81.8±9.8	77.3±11.0	<0.0001
Mean arterial pressure, mmHg	98.1±10.5	93.0±11.1	<0.0001
Heart rate, bpm	62.5±9.9	91.9±9.4	0.34
Questionnaire data			
Hypertensives, *n* (%)	173 (40.2)	164 (23.6)	<0.0001
Antihypertensive treatment, *n* (%)	71 (16.5)	76 (10.9)	0.0082
Current smokers, *n* (%)	128 (29.8)	205 (29.4)	0.95
Alcohol intake ≥5g/day, *n* (%)	132 (30.7)	365 (52.4)	<0.0001

Values are mean±SD or number (%) of subjects. *P* values are for the differences between founders and unrelated participants compared with offspring.

Table 5.1.2 Arterial properties

	Founders and unrelated participants (*n*=430)	Offspring (*n*=696)	*P*
Common carotid artery			
Diameter, mm	7.35±0.94	7.04±0.89	<0.0001
Pulse pressure, mmHg	48.4±13.5	47.2±12.1	0.13
Distensibility, 10^{-3}/kPa	21.4±10.2	28.9±14.1	<0.0001
Cross-sectional compliance, mm^2/kPa	0.89±0.39	1.09±0.45	<0.0001
Femoral artery			
Diameter, mm	9.24±1.39	9.16±1.57	0.36
Pulse pressure, mmHg	53.0±13.0	52.2±12.9	0.33
Distensibility, 10^{-3}/kPa	9.0±5.6	11.5±7.3	<0.0001
Cross-sectional compliance, mm^2/kPa	0.58±0.35	0.73±0.45	<0.0001
Brachial artery			
Diameter, mm	4.28±0.83	4.36±0.85	0.10
Pulse pressure, mmHg	48.8±10.8	48.3±9.3	0.45
Distensibility, 10^{-3}/kPa	16.3±11.4	10.8±10.3	<0.0001
Cross-sectional compliance, mm^2/kPa	0.21±0.14	0.15±0.13	<0.0001

Values are mean±SD. *P* values are for the differences between founders and unrelated participants compared with offspring.

Table 5.1.3 Allele and genotype frequencies

Gene	Allele		Genotype		
ADD1	*Gly*	*Trp*	*GlyGly*	*GlyTrp*	*TrpTrp*
	1722 (76.53)	530 (23.5)	666 (59.2)	390 (34.6)	70 (6.2)
ADD2	*C*	*T*	*CC*	*CT*	*TT*
	2000 (88.8)	252 (11.2)	888 (78.9)	224 (19.9)	14 (1.2)
ADD3	*A*	*G*	*AA*	*AG*	*GG*
	1297 (57.6)	955 (42.4)	388 (34.4)	521 (46.3)	217 (19.3)

Values indicate number of alleles or genotypes (%).

Figure 5.1.1 Diameter, distensibility and cross-sectional compliance of the brachial artery in relation to the *ADD3 A386G* polymorphism

The analyses were adjusted for observer, sex, age, body mass index, mean arterial pressure, heart rate, smoking, alcohol intake and the use of antihypertensive drugs and account for family clusters. Values are least square means ± SEs. The number of subjects contributing to each plotted point are given. *P*-values are for linear trend across the *ADD3* genotypes.

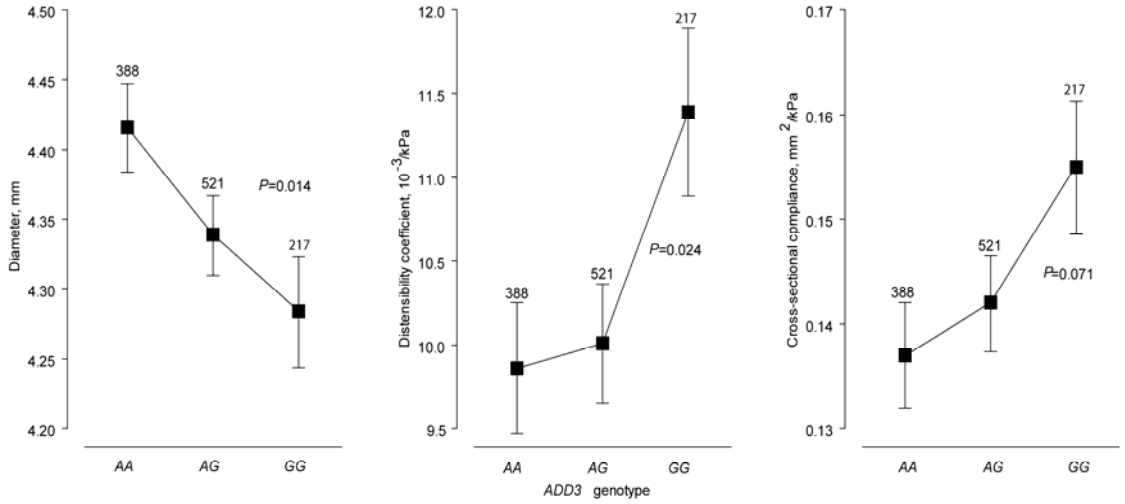


Figure 5.1.2 Diameter, distensibility and cross-sectional compliance of brachial artery in relation to the *ADD1 Gly460Trp* and *ADD3 A386G* polymorphisms.

P_{int} indicates the significance of the 2-way interaction between *ADD1* and *ADD3*. For further explanation, see Figure 5.1.1.

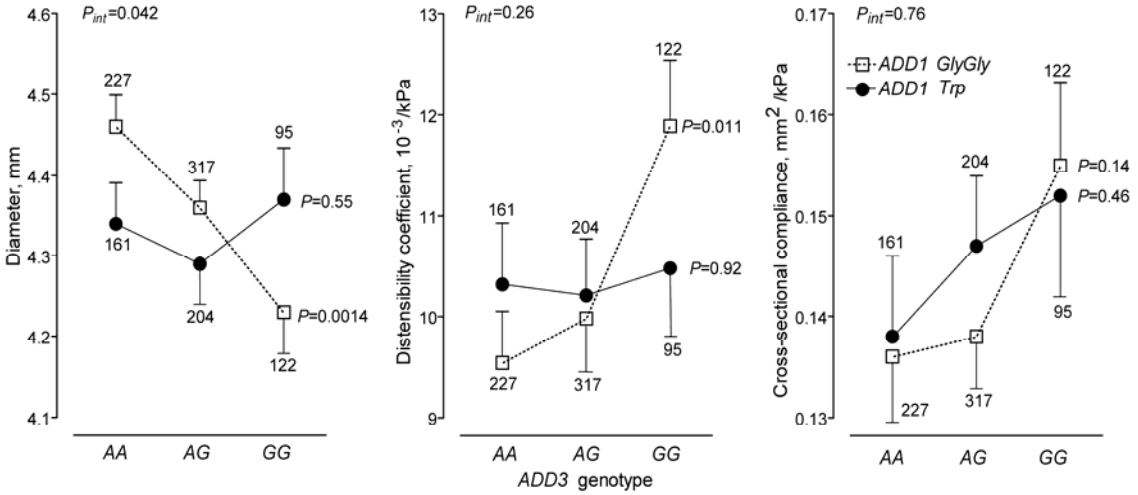
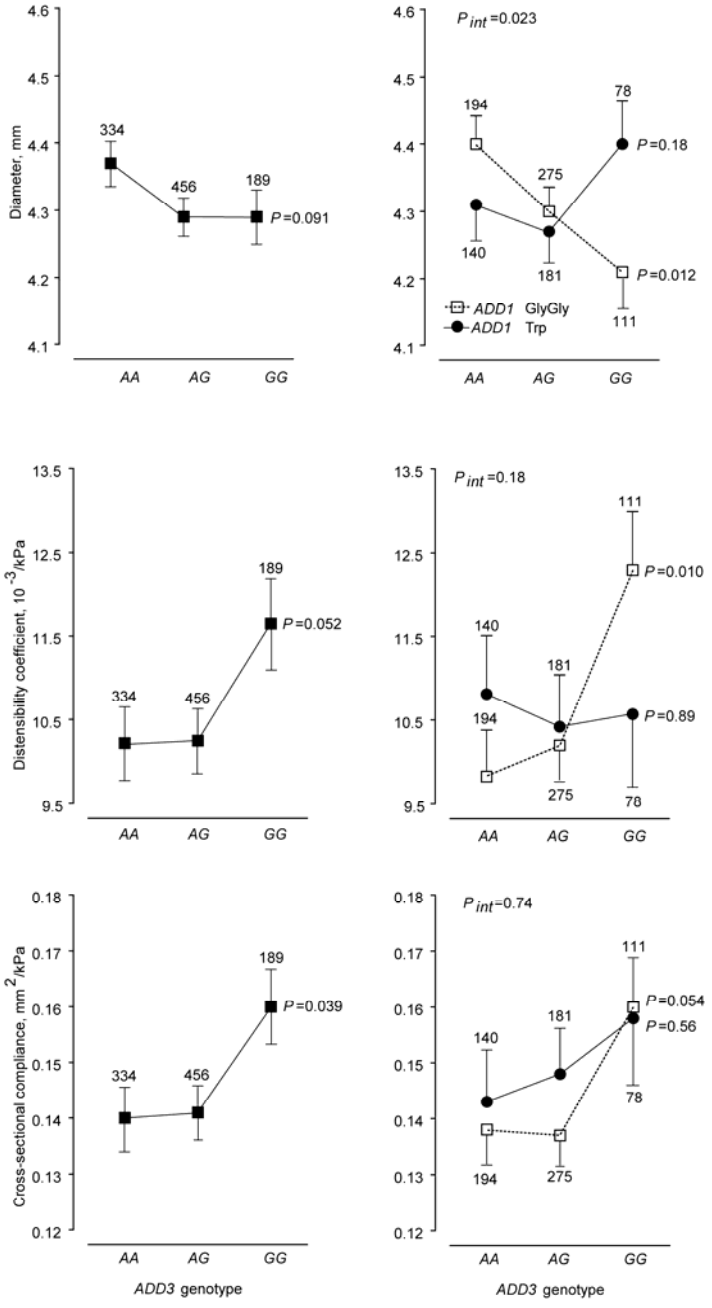


Figure 5.1.3 Diameter, distensibility and cross-sectional compliance of brachial artery in relation to the *ADD1 Gly460Trp* and *ADD3 A386G* polymorphisms in 979 untreated subjects

For further explanation, see Figure 5.1.1.



Discussion

The key finding of our study was that brachial diameter decreased, while brachial distensibility increased with the *ADD3 G* allele, and that these associations were confined to *ADD1 GlyGly* homozygotes. In the family-based analyses, we did not find

any evidence for population stratification. Transmission of the *ADD3 G* allele was associated with smaller brachial diameter in all informative offspring as well as in offspring homozygous for *ADD1 Gly* allele. *ADD1* and *ADD2,* alone or in combination with each other, were not associated with the arterial properties in the three arterial beds. This was also true for *ADD1* and *ADD3* in relation to the carotid and femoral phenotypes.

Adducin functions within the cell as a tissue-specific heterodimer, which consists either of α- and ß- or α- and γ-subunits. This provides the physiological and biochemical basis for studying the interaction among the three adducin genes, which map to different chromosomes [3]. To our knowledge, no prior study investigated the genetic interactions between the three adducin subunits in relation to arterial properties. In the present population, we previously demonstrated that interaction between the *ADD1*, the angiotensin-converting enzyme (*ACE I/D)* and the angiotensin II type-1 receptor (*AT1R C1166A*) polymorphisms modulated the properties of the femoral artery. Indeed, in the presence of the *ADD1 460Trp* allele, femoral intima-media thickness was higher in *ACE D* carriers than *II* homozygotes [5]. Moreover, in *ACE DD* homozygotes, carriers of mutated *ADD1* had higher femoral distensibility and cross-sectional compliance than those with the wild type *ADD1* [6].

Our epidemiological study only allows speculation about the reasons why there might be an association between the properties of the brachial artery, a small muscular artery, and the *ADD3 A386G* polymorphism. One possible mechanism is that the polymorphism might affect the neurogenic tone of vascular smooth muscle cells. Indeed, in rat models, increased blood pressure was associated with a decrease in the hypothalamic levels of γ-adducin mRNA and protein [17.18]. Furthermore, inhibition of γ-adducin by intracellular delivery of γ-adducin-specific antibodies increased the neuronal firing rate possibly via regulation of Na^+,-K^+-ATPase [17]. On the other hand, given that the phenotypes representative of two other arterial segments did not shown any association with the adducin SNPs, suggests that local mechanisms in the brachial artery, rather than the central nervous system, might underlie the demonstrated findings.

We did not find any association between the polymorphisms under study and the properties of the elastic carotid artery. The central elastic arteries, such as the carotid artery, and the more peripheral muscular conduit vessels, including the brachial and femoral arteries, have different properties [19]. Going from the central to the

peripheral arteries, the collagen/elastin ratio reverses, vascular smooth muscle cells become the predominant component of the arterial wall, and the phenotype of the vascular smooth muscle cells changes [19]. Thus, the effects of genetic variation in adducin subunits on different arteries must be complex and may depend on the vessel wall component that is involved.

Our study should be interpreted within the context of its limitations. First, our epidemiological study demonstrated association of arterial properties with variation in *ADD3*, but did not provide direct information on the mechanisms underlying these phenotype-genotype associations. Second, we did not use the tagging SNP strategy to investigate genotype-phenotype associations. However, we have extensively investigated nucleotide and haplotype variation in both *ADD1* and *ADD3*, but in experimental studies the additional common SNPs did not enhance the phenotype-genotype association, over and beyond the single SNPs *ADD1 rs4961* and *ADD3 rs3731566*. We will publish these results elsewhere. Therefore, we focused on *Gly460Trp* polymorphism of the *ADD1* gene, because it is functional, increases Na^+,K^+-ATPase activity [1,2], and is associated with various cardiovascular [5,6,9] and renal phenotypes [3]. The *A* to *G* substitution in *ADD3* is located in intron 11 (*IVS11 +386A>G* –rs3731566) [3]. Neither previous publications nor genome browser databases provided any suggestion about the functional role of the *ADD3 A386G* polymorphism. A nucleotide variation analysis is needed to elucidate the pattern of linkage disequilibrium of the entire *ADD3* locus and to establish whether this intronic common polymorphism is linked with the ‘’causal’’ SNP or is the regulatory variant ‘’per se’’. According to the latter hypothesis, preliminary data showed that *ADD3* mRNA level was significantly enhanced in *GG* homozygotes compared with the other genotypes in 39 kidney cortex samples from human donors (Lorena Citterio, personal communication, 2008). Third, arterial measurements are quantitative traits prone to measurement error. However, the repeatability and reproducibility of the arterial phenotypes collected in the present study is high [14]. We adjusted for observer bias. Moreover, for the brachial diameter in relation to the *ADD3* and *ADD1* polymorphisms, there was consistency between the population-based and the family-based analyses. We did not find any evidence for population stratification. Fourth, we did not adjust significance levels for multiple testing. However, arterial diameter, distensibility and cross-sectional compliance are

correlated phenotypes. In such case, multiple testing is not indicated, because each test does not provide a completely independent opportunity for type I error [20].

In conclusion, the properties of the brachial artery are related to the *ADD3* (*A386G*) polymorphism, particularly in *ADD1 GlyGly* homozygotes. Further clinical observations and experimental studies should confirm the present observation and eventually clarify the underlying mechanism.

Acknowledgements

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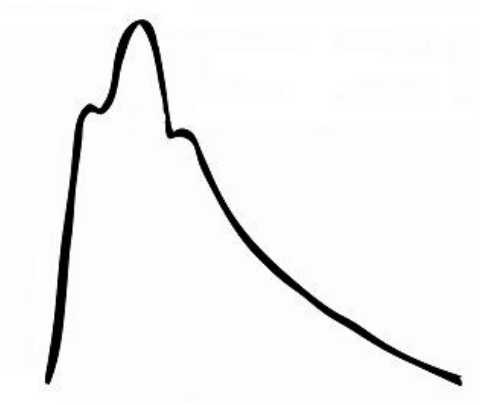

Chapter 5

Relation between local arterial stiffness and genetic variation in adducin subunit and renin-angiotensin system

Part 2

Arterial properties in relation to genetic variation in α-adducin and the renin-angiotensin-system in a White population

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Abstract—Previous studies demonstrated interaction between *ADD1* with *ACE* in relation to arterial properties. We investigated whether arterial characteristics might also be related to interactions of *ADD1* with other renin-angiotensin-system genes. Using a family-based sampling frame, we randomly recruited 1064 Flemish subjects (mean age, 43.6 years; 50.4% women). By means of a wall-tracking ultrasound system, we measured the properties of the carotid, femoral and brachial arteries. In multivariate-adjusted analyses, we assessed the multiple-gene effects of *ADD1* (*Gly460Trp*), *AGT* (*C–532T* and *G–6A*) and *AT1R* (*A1166C*). In *ADD1 Trp* allele carriers, but not in *ADD1 GlyGly* homozygotes (*P* value for interaction ≤0.014), femoral cross-sectional compliance was significantly higher (0.74 *vs.* 0.65 mm^2/kPa; *P*=0.020) in carriers of the *AT1R C* allele than in *AT1R AA* homozygotes, with a similar trend for femoral distensibility (11.3 *vs.* 10.2 10^{-3}/kPa; *P*=0.055). These associations were independent of potential confounding factors, including age. Family-based analyses confirmed these results. Brachial diameter (4.35 *vs.* 4.18 mm) and plasma renin activity (0.23 *vs.* 0.14 ng/ml/h) were increased (*P*≤0.0050) in *AGT CG* haplotype homozygotes compared with non-carriers, whereas the opposite was true for brachial distensibility (12.4 *vs.* 14.4 10^{-3}/kPa; *P*=0.011). There was no interaction between *AGT* and any other gene in relation to the measured phenotypes. *ADD1* and *AT1R* interactively determine the elastic properties of the femoral artery. There is a single-gene effect of the *AGT* promoter haplotypes on brachial properties and plasma renin activity.

Introduction

Adducin is a ubiquitously expressed membrane skeleton protein, which consists either of α- and ß- or α- and γ-subunits. Mutation of the α-adducin gene (*ADD1*) entails increased Na^+,K^+-ATPase activity [1,2], and increased renal tubular sodium reabsorption [3]. Variation in the Na^+,K^+-ATPase activity and in the intracellular Na^+ concentration might influence the sodium-dependent transmembranous Ca^{2+} transport in vascular smooth muscle cells [4].

We previously demonstrated that interaction between the *ADD1 Gly460Trp* and the angiotensin-converting enzyme *ACE I/D* polymorphisms modulates femoral artery properties. Indeed, in the presence of the *ADD1 460Trp* allele, femoral intima-media thickness was higher in *ACE D* carriers than *II* homozygotes [5]. Moreover, in *ACE DD* homozygotes, carriers of mutated *ADD1* had higher femoral distensibility and cross-sectional compliance than those with the wild type *ADD1* [6]. In the prospective Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), the combination of *ACE DD* homozygosity and mutated *ADD1* worsened cardiovascular prognosis to a similar extent as classical risk factors [7]. Experimental studies in fibroblasts from 51 subjects and in transfected cell models showed higher membrane-bound ACE activity in cells carrying the *ADD1 Trp* allele [7].

In view of the aforementioned evidence [1-7], we hypothesized that arterial properties might be related not only to interactions of *ADD1* with *ACE,* but also to interactions of *ADD1* with other genes encoding various components of the renin-angiotensin system. We therefore genotyped FLEMENGHO participants for the angiotensinogen (*AGT*) *C–532T* and *G–6A* promoter polymorphisms as well as for the intronic *A1166C* polymorphism in the angiotensin II type-1 receptor gene (*AT1R*).

Methods

Study population

The FLEMENGHO study is part of the European Project on Genes in Hypertension (EPOGH) [8] and is embedded in the InGenious HyperCare Network of Excellence. From August 1985 until July 2003, we recruited a random sample of families from a geographically defined area in Northern Belgium. The Ethics Committee of the University of Leuven approved the study. All participants or their parents gave informed written consent. The participation rate averaged 64.3%.

Of 1306 participants, who underwent a vascular ultrasound examination [9], 1180 (90.3%) had high-quality images obtained at the common-carotid, femoral and brachial arteries. We excluded 80 participants, because of missing genotypes, and 26 because of incomplete information on important covariates. In addition, we detected 10 cases of inconsistency in Mendelian segregation. Thus, the number of subjects analyzed totaled 1064.

Clinical and biochemical measurements

For at least 3 hours before the examination, the participants refrained from heavy exercise, smoking, alcohol, and caffeine-containing beverages. Trained nurses measured the subjects' anthropometric characteristics, heart rate and blood pressure. They administered a questionnaire to collect information about each participant's recent medical history, smoking and drinking habits, and intake of medications. Each subject's blood pressure was the average of 5 consecutive readings measured before the ultrasound examination after the subjects had rested in the sitting position for at least 5 minutes. Pulse pressure was systolic minus diastolic blood pressure. Mean arterial pressure was diastolic pressure plus one third of pulse pressure. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic, and/or the use of antihypertensive drugs. Body mass index was weight in kilograms divided by the square of height in meters. On the day of the vascular measurements, venous blood samples were drawn to measure the blood glucose concentration and plasma renin activity. The participants also collected an exactly timed urine sample over 4 to 6 hours to measure the excretion of sodium and aldosterone [10]. Diabetes mellitus was a self-reported diagnosis, use of antidiabetic medication, or a fasting or casual blood glucose concentration ≥7.0 mmol/l (126 mg/dl) or ≥11.1 mmol/l (200 mg/dl), respectively [11]. Hypercholesterolemia was a serum level of total cholesterol ≥5.16 mmol/L (200 mg/dl) or treatment with lipid-lowering drugs [12]. We determined plasma renin activity (RIA-0180, DRG Instruments GmbH, Marburg an der Lahn, Germany) and the urinary aldosterone concentration (DSL-8600 Active, Diagnostic Systems Laboratories Inc., Webster, Texas, USA) by radioimmunoassay, according to the instructions provided by the manufacturers of the analytic kits.

Vascular measurements

By means of a pulsed ultrasound wall-tracking system (Wall Track System; Pie Medical, Maastricht, the Netherlands), 3 trained researchers obtained vascular measurements at the common carotid artery 2 cm proximal of the carotid bulb, at the femoral artery 1 cm proximal of the bifurcation into the profound and superficial branches, and at the right brachial artery 2 cm proximal of the antecubital fossa.

During the ultrasound examination, an automated oscillometric device (Dinamap 845; Critikon Inc., Tampa, Florida, USA) recorded blood pressure at the upper arm at 5-minute intervals. As for the conventional auscultatory measurements, cuff size was adjusted to the circumference of the upper arm [8]. Standard cuffs had an inflatable bladder of 12 x 24 cm. Larger cuffs had a 15 x 35 cm bladder. As described elsewhere [13], the observers used applanation tonometry with a pencil-shaped probe (Millar Instruments Inc., Houston, Texas, USA) and calibration to mean arterial pressure and diastolic blood pressure at the brachial artery to derive the local pulse pressure at the other arteries. We computed the distensibility (DC) and cross-sectional compliance (CC) from the diastolic cross-sectional area (A), the systolic increase in cross-sectional area (ΔA) and the local pulse pressure (PP) [14]: $DC = (\Delta A/ A)/PP$ and $CC = \Delta A/ PP$. A and ΔA were calculated from the diameter (D) and the change in diameter (ΔD) as $A = \pi \times (D/2)^2$ and $\Delta A = \pi \times [(D + \Delta D)/2)^2 - \pi \times (D/2)^2$, respectively. The intra-observer intra-session variability was below 10% for the carotid measurements, below 5% for the femoral and brachial diameter, and amounted from 10% to 15% for the femoral and brachial cross-sectional compliance and distensibility [15]. The intra-observer inter-session and inter-observer intra-session variability were of the same order of magnitude [15].

Genotypes

We extracted DNA from white blood cells. For genotyping, we used a 5' nuclease detection assay implemented on an ABI Prism 7700 Sequence Detection System (Applied Biosystems Inc., Foster City, California, USA). Primers, probes and PCR conditions for *ADD1 Gly460Trp* (rs4961 dbSNP) [16], *AT1R C1166A* [17] and *AGT A–532C* and *G–6A* [17] genotyping have already been described in detail elsewhere.

Statistical methods

For database management and statistical analyses, we used SAS software (SAS Institute, Cary, North Carolina, USA), version 9.1. We normalized the distributions of plasma renin activity (PRA) and of the urinary excretion rates of sodium and aldosterone by a logarithmic transformation. We compared means and proportions, using the large sample z-test and Fisher's exact test, respectively. Our statistical methods also included single and multiple linear regressions. We searched for possible covariates of the arterial phenotypes, using a stepwise regression procedure with the *P*-values for independent variables to enter and to stay in the model set at 0.15. As covariates, we considered observer, sex, age, body mass index, heart rate, mean arterial pressure, and design variables (0, 1), coding for current smoking, alcohol intake, and use of antihypertensive drugs.

We tested linkage disequilibrium and we reconstructed haplotypes, using the SAS procedures PROC ALLELE and PROC HAPLOTYPE, as implemented in the genetics module of the SAS software. In our analyses, we only included those *AGT* haplotypes that were unambiguously determined.

In a population-based approach, we applied a generalization of the standard linear model as implemented in the PROC MIXED procedure of the SAS package to test the associations between phenotypes and SNPs or haplotypes, while accounting for the nonindependence of phenotypes within families and adjusting for covariates. We tested the interactions between genotypes and between genotypes and anthropometric characteristics by introducing the appropriate interaction terms into the models.

Furthermore, in the family-based analyses, we performed a transmission disequilibrium test for quantitative traits (QTDT). We evaluated the within- and between-family components of phenotypic variance, using the orthogonal model as implemented by Abecasis et al [18] in the QTDT software, version 2.4, available at http://www.sph.umich.edu/csg/abecasis/QTDT. The within-family component of phenotypic variance is robust to population stratification.

Results

Characteristics of participants

We divided study participants into founders (*n*=152) and unrelated subjects (*n*=252) as compared with offspring (*n*=660). Subjects in the founders group (mean age 51.0

years, range 11.8–81.8 years) were older than offspring (39.1 years; range 10.9-81.0 years). Table 5.2.1 summarizes their demographic characteristics. The study sample included 315 hypertensive patients (29.6%). Of 134 (12.6% of total study population) treated hypertensive patients, 53 (39.6%) were on monotherapy and 81 (60.4%) were taking different classes of antihypertensive drugs. Antihypertensive therapy included β-blockers in 80 (59.7%) patients, diuretics in 44 (32.8%), calcium-channel blockers in 28 (20.9%), angiotensin-converting enzyme inhibitors in 14 (10.4%), centrally acting drugs in 4 (3.0%), and α-blockers in 2 (1.5%) patients. The study sample included 580 (54.5%) patients with hypercholesterolemia, of whom 15 (2.6%) were receiving lipid lowering treatment. Offspring reported alcohol intake more frequently than founders (52.6% *vs*. 30.7%). For smoking, the corresponding frequencies were 29.2% and 30.2%, respectively. In smokers, median tobacco use was 14 cigarettes per day (interquartile range, 8–20). In drinkers, the median alcohol consumption was 10 grams per day (interquartile range, 4–20).

Table 5.2.2 lists the arterial properties by generation and vascular territory. Local pulse pressure at the 3 arteries under study was similar ($0.31<P<0.97$) in founders and offspring. Carotid and femoral distensibility and cross-sectional compliance and brachial diameter were lower in founders than in offspring. The opposite was true for brachial distensibility and cross-sectional compliance and carotid diameter.

Genotypes and haplotype frequencies

Genotype frequencies (Table 5.2.3) complied with Hardy-Weinberg proportions in the founder generation ($0.08<P<0.62$). The two polymorphisms in the *AGT* promoter were in significant linkage disequilibrium (Lewontin's D'=0.93; $P<0.0001$). In 10 subjects, we could not reliably reconstruct the haplotypes. The haplotype frequencies were 56.3% for the combination of *–532C* and *–6G* (*H1-CG*), 33.3% for the combination of *–532C* and *–6A* (*H2-CA*), and 10.4% for the combination *–532T* and *–6A* (*H3-TA*).

Population-based study of genotypes

In stepwise multiple regression analyses, in line with our previous publications [2], we identified the following covariates as significant determinants of one or more of the arterial phenotypes in the 3 vascular beds under study: sex, age, body mass index, mean arterial pressure, heart rate, serum total cholesterol, smoking, daily alcohol

intake in excess of 5 grams, and use of antihypertensive drugs (Table 5.2.4). We adjusted all phenotype-genotype associations involving arterial properties for these covariates, and in addition for observer.

In single-gene analyses, involving all subjects, none of the multivariate-adjusted phenotype-genotype associations reached statistical significance with the exception of brachial artery diameter in relation to the *AT1R* polymorphism. *C* allele carriers had a slightly larger brachial diameter compared with *AA* homozygotes (4.39±0.03 mm *vs.* 4.29±0.03 mm; *P*=0.016).

In multivariate-adjusted multiple-gene analyses (Table 5.2.5), we found significant interaction between *ADD1* and *AT1R* in relation to femoral cross-sectional compliance (*P*=0.014) and femoral distensibility (*P*=0.023). In *ADD1 Trp* allele carriers, but not in *ADD1 GlyGly* homozygotes, femoral cross-sectional compliance was significantly higher (*P*=0.020) in carriers of the *AT1R C* allele than in *AT1R AA* homozygotes, with a similar trend for femoral distensibility (*P*=0.055).

In sensitivity analyses, we narrowed the age range first by excluding 85 participants younger than 20 years and next by only including in our analyses subjects with an age range corresponding to the 10th to 90th percentile interval (20 – 70 years; *n*=931) or to the interquartile range (34 – 55 years; *n*=533). As shown in Table 5.1.5, the results of these sensitivity analyses were confirmatory. Similarly, when we repeated the sensitivity analyses excluding 142 treated patients on antihypertensive (*n*=134) or lipid lowering (*n*=15) drugs, we also obtained consistent results (Table 5.2.6). The genotype-by-age interactions in the whole study population were also not significant (*P*≥0.24).

Population-based study of haplotypes

Brachial diameter was significantly larger (Fig. 5.1.1) in *H1-CG* homozygotes compared with non-carriers of this haplotype (4.35±0.04 mm *vs*. 4.18±0.05 mm; *P*=0.0040). Furthermore, brachial distensibility was lower in *H1-CG* homozygotes than in those subjects not carrying this haplotype (12.4±0.6 × 10^{-3}/kPa *vs.* 14.4±0.7 × 10^{-3}/kPa; *P*=0.011). The number of copies of the *AGT H1-CG* haplotype had an additive effect, as exemplified by the *P*-values for linear trend (Figure 5.2.1). The aforementioned associations with brachial diameter and distensibility were consistent irrespective of sex (*P*≥0.40), age (*P*≥0.75), and sodium excretion rate (*P*≥0.26). We did not find any association of the properties of the carotid or femoral arteries with the

AGT haplotypes ($0.22 \leq P \leq 0.74$). There were also no gene-gene interactions between the *AGT* haplotypes and the *ADD1* ($P \geq 0.11$) or *AT1R* ($P \geq 0.20$) polymorphisms in relation to any of the arterial phenotypes in the 3 arterial beds.

In analysis adjusted for sex, age, body mass index, mean arterial pressure, heart rate and use of antihypertensive treatment, PRA was significantly higher in *H1-CG* homozygotes than in subjects not carrying this haplotype (0.23 ng/ml/h [95% confidence interval, 0.18–0.30 ng/ml/h] *vs*. 0.14 ng/ml/h [95%CI, 0.12–0.16 ng/ml/h]; $P=0.0050$; Fig. 5.2.1). These findings remained consistent after exclusion of 134 subjects on antihypertensive treatment (data not shown).

Family-based study of genotypes

Our study sample consisted of 58 pedigrees, of which 25 spanned more than 2 generations, and additionally of 252 unrelated subjects. The number of offspring per pedigree was less than 3 in 24 pedigrees, ranged from 3 to 8 in 30 families, and amounted to more than 8 in 4 pedigrees.

The multivariate-adjusted heritability estimates, as reported by Abecasis' software, were 0.31 for the femoral distensibility and 0.43 for the femoral cross-sectional compliance ($P<0.001$ for both). Abecasis' orthogonal model did not reveal population stratification ($0.11 \leq P \leq 0.97$). In 115 informative offspring, carrying the *ADD1 Trp* allele, transmission of the mutated *ATR1 C* allele was associated with a significant increase in femoral distensibility ($+2.46 \pm 1.06 \times 10^{-3}$/kPa; $\chi^2=5.39$; $P=0.022$) with a similar trend in femoral cross-sectional compliance ($+0.12 \pm 0.07$ mm^2/kPa; $\chi^2=2.63$; $P=0.11$). In 184 informative *ADD1 GlyGly* homozygotes, these effect sizes were $-0.19 \pm 0.85 \times 10^{-3}$/kPa ($\chi^2=0.05$; $P=0.82$) and $+0.01 \pm 0.06$ mm^2/kPa ($\chi^2=0.05$; $P=0.82$), respectively.

Family-based association study of AGT haplotypes

The multivariate-adjusted heritability estimates, as reported by Abecasis' software, were 0.52 for the brachial diameter, 0.38 for brachial distensibility, and 0.45 for PRA ($P<0.001$ for all). Abecasis' orthogonal model did not reveal population stratification ($0.06 \leq P \leq 0.81$). In 300 informative offspring, the orthogonal model did not show significant association of transmission of *H1-CG* with brachial diameter ($+0.07 \pm 0.06$

mm; χ^2=1.33; *P*=0.25), brachial distensibility (–0.55±0.76 × 10^{-3}/kPa; χ^2=0.53; *P*=0.46), or PRA (0.91 ng/ml/h [95%CI, 0.63–1.31 ng/ml/h]; χ^2=0.26; *P*=0.61).

Table 5.2.1 Characteristics of participants

	Founder and unrelated subjects (*n*=404)	Offspring (*n*=660)	*P*
Anthropometry			
Women, *n* (%)	209 (51.7)	327 (49.5)	0.53
Age, y	51.0±12.4	39.1±15.2	<0.0001
Body mass index, kg/m2	25.5±3.6	24.7±4.2	0.0008
Systolic pressure, mmHg	130.2±16.2	124.6±14.3	<0.0001
Diastolic pressure, mmHg	81.8±10.0	77.5±10.9	<0.0001
Mean arterial pressure, mmHg	97.9±10.8	93.2±11.1	<0.0001
Pulse pressure, mmHg	48.1±12.2	47.1±10.8	0.16
Heart rate, bpm	62.5±10.0	61.6±9.1	0.15
Questionnaire data			
Hypertensives, *n* (%)	158 (39.1)	157 (23.8)	<0.0001
Antihypertensive treatment, *n* (%)	61 (38.6)	73 (46.5)	0.057
Current smokers, *n* (%)	122 (30.2)	193 (29.2)	0.78
Alcohol intake ≥5g/day, *n* (%)	124 (30.7)	347 (52.6)	<0.0001
Diabetics, *n* (%)	4 (1.0)	5 (0.8)	0.74
Hypercholesterolemia, *n* (%)	270 (66.8)	310 (47.0)	<0.0001
Biochemistry			
Serum total cholesterol (mmol/l)	5.65±0.98	5.15±1.07	<0.0001
Plasma renin activity (ng/ml/h)	0.46 (0.22–1.03)	0.41 (0.22–0.81)	0.51
Sodium excretion (mmol/h)	6.36 (3.86–10.13)	6.96 (4.33–11.39)	0.76
Aldosterone excretion (nmol/h)	1.15 (0.66–2.16)	0.95 (0.54–1.70)	0.15

Values are mean±SD, geometric mean (interquartile range), or number (%) of subjects. *P* values are for the differences between founders and unrelated participants compared with offspring.

Table 5.2.2 Arterial properties

Characteristics	Founder and unrelated subjects (*n*=404)	Offspring (*n*=660)	*P*
Common carotid artery			
Diameter, mm	7.33±0.91	7.04±0.89	<0.0001
Pulse pressure, mmHg	48.0±13.2	47.3±12.0	0.31
Cross-sectional compliance, mm^2/kPa	0.90±0.39	1.10±0.49	<0.0001
Distensibility, 10^{-3}/kPa	21.7±10.2	29.1±15.5	<0.0001
Femoral artery			
Diameter, mm	9.26±1.39	9.16±1.56	0.33
Pulse pressure, mmHg	52.9±12.8	52.4±12.8	0.55
Cross-sectional compliance, mm^2/kPa	0.58±0.35	0.74±0.46	<0.0001
Distensibility, 10^{-3}/kPa	8.9±5.7	11.6±7.3	<0.0001
Brachial artery			
Diameter, mm	4.27±0.84	4.38±0.84	0.047
Pulse pressure, mmHg	48.5±10.5	48.5±9.3	0.97
Cross-sectional compliance, mm^2/kPa	0.21±0.13	0.15±0.13	<0.0001
Distensibility, 10^{-3}/kPa	16.4±11.4	11.0±10.8	<0.0001

Values are mean±SD. *P* values are for the differences between founders and unrelated participants compared with offspring.

Table 5.2.3 Allele and genotype frequencies

Gene	Allele		Genotype		
ADD1	*Gly*	*Trp*	*GlyGly*	*GlyTrp*	*TrpTrp*
	1636 (76.9)	492 (23.1)	636 (59.8)	364 (34.2)	64 (6.0)
ATR1	*A*	*C*	*AA*	*AC*	*CC*
	1465 (68.8)	663 (31.2)	507 (47.6)	451 (42.4)	106 (10.0)
AGT C-532T	*C*	*T*	*CC*	*CT*	*TT*
	1904 (89.5)	224 (10.5)	855 (80.4)	194 (18.2)	15 (1.4)
AGT G-6A	*G*	*A*	*GG*	*AG*	*AA*
	1203 (56.5)	925 (43.5)	345 (32.4)	513 (48.2)	206 (19.4)

Values indicate number of alleles or genotypes (%).

Table 5.2.4 Determinants of characteristics of femoral and brachial arteries in multiple regression analysis

Determinants	Diameter (mm)		Cross-sectional compliance (mm^2/kPa)		Distensibility coefficient (10^{-3}/kPa)	
	Femoral	Brachial	Femoral	Brachial	Femoral	Brachial
R^2	0.322	0.358	0.144	0.470	0.251	0.438
Intercept	7.59‡	3.65‡	1.99‡	0.037	36.20‡	5.40
Partial regression coefficient ±SE						
Female gender (0, 1)	–1.225±0.083‡	–0.793±0.046‡	–0.115±0.027‡	–0.058±0.007‡	1.224±0.403†	NS
Age (+10 years)	0.153±0.033‡	0.062±0.018†	NS	0.012±0.003‡	–0.739±0.158‡	0.448±0.226*
Body mass index (kg/m^2)	0.062±0.011‡	0.037±0.006‡	–0.010±0.003†	NS	–0.275±0.053‡	NS
Mean arterial pressure (+10 mm Hg)	0.091±0.041*	0.050±0.022*	–0.052±0.013‡	–0.016±0.003‡	–1.122±0.197‡	–1.543±0.282‡
Heart rate (+10 bpm)	–0.130±0.042†	NS	NS	NS	NS	NS
Serum total cholesterol (mmol/L)	–0.109±0.041†	–0.072±0.022†	–0.056±0.013‡	0.008±0.003†	–0.625±0.197†	1.157±0.282‡
Current smoking (0, 1)	–0.194±0.085*	NS	NS	0.015±0.007*	NS	1.550±0.585†
Alcohol intake ≥5g/day (0, 1)	NS	0.163±0.046†	NS	–0.023±0.007†	NS	–2.478±0.588‡
Antihypertensive treatment (0, 1)	–0.401±0.124†	NS	–0.088±0.040*	–0.026±0.010†	NS	–1.996±0.858*
Observer	0.198±0.042‡	–0.051±0.023*	NS	0.082±0.003‡	–0.697±0.205†	6.880±0.293‡

Significance of the partial regression coefficients: NS $P>0.15$; * $P\leq0.05$, † $P\leq0.01$ and ‡ $P\leq0.0001$.

Table 5.2.5 Femoral cross-sectional compliance and distensibility in subjects of different age groups in relation to the *ADD1* and *AT1R* genotypes

	ADD1 GlyGly homozygotes					*ADD1 Trp allele carriers*					
AT1R	*AA* homozygotes		*C* allele carriers			*AA* homozygotes		*C* allele carriers			
	n	Estimate±SE	*n*	Estimate±SE	*P*	*n*	Estimate±SE	*n*	Estimate±SE	*P*	P_{int}
Cross-sectional compliance (mm^2/kPa)											
All subjects	304	0.687±0.022	332	0.654±0.021	0.27	203	0.648±0.030	225	0.743±0.028	0.020	0.014
Subjects >20years	280	0.652±0.022	304	0.633±0.021	0.53	189	0.614±0.029	206	0.728±0.028	0.0051	0.012
Age 20 – 70 years	266	0.661±0.023	293	0.641±0.022	0.52	176	0.625±0.031	196	0.744±0.029	0.0052	
Age 34 – 55 years	157	0.658±0.030	174	0.612±0.028	0.27	96	0.625±0.038	106	0.744±0.036	0.027	0.015
Distensibility coefficient (10^{-3}/kPa)											
All subjects	304	10.79±0.34	332	10.13±0.32	0.16	203	10.157±0.43	225	11.28±0.40	0.055	0.023
Subjects >20years	280	10.04±0.33	304	9.65±0.32	0.40	189	9.55±0.43	206	10.81±0.41	0.033	0.038
Age 20 – 70 years	266	10.18±0.34	293	9.82±0.33	0.45	176	9.75±0.45	196	11.08±0.42	0.033	0.040
Age 34 – 55 years	157	10.00±0.44	174	9.60±0.42	0.52	96	9.45±0.54	106	11.29±0.51	0.015	0.020

Values are least square means ± SEs. The properties of the femoral artery were adjusted for observer, sex, age, mean arterial pressure, heart rate, body mass index, serum total cholesterol, smoking, alcohol intake, and antihypertensive drug treatment. *P*-values denote the significance of the differences between the *AT1R* genotypes. *Pint* indicates the significance of the interaction between *ADD1* and *ATR1*.

Table 5.2.6 Femoral cross-sectional compliance and distensibility in untreated subjects* of different age groups in relation to the *ADD1* and *AT1R* genotypes

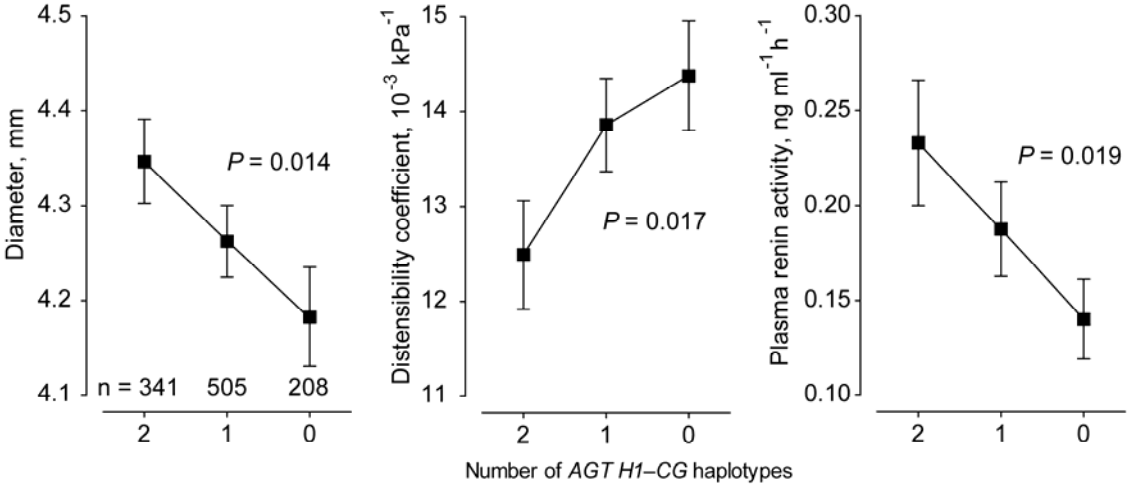
	ADD1 GlyGly homozygotes					*ADD1 Trp allele carriers*					
AT1R	*AA* homozygotes		*C* allele carriers			*AA* homozygotes		*C* allele carriers			
	n	Estimate±SE	*n*	Estimate±SE	*P*	*n*	Estimate±SE	*n*	Estimate±SE	*P*	P_{int}
Cross-sectional compliance (mm^2/kPa)											
All subjects	261	0.716±0.024	294	0.677±0.022	0.23	173	0.682±0.033	194	0.778±0.031	0.034	0.018
Subjects >20years	237	0.679±0.024	266	0.655±0.023	0.49	159	0.645±0.033	175	0.762±0.031	0.0093	0.017
Age 20 – 70 years	230	0.685±0.025	261	0.657±0.023	0.42	149	0.654±0.034	169	0.773±0.031	0.010	0.014
Age 34 – 55 years	139	0.677±0.031	164	0.616±0.029	0.15	85	0.651±0.041	96	0.759±0.038	0.057	0.017
Distensibility coefficient (10^{-3}/kPa)											
All subjects	261	11.24±0.37	294	10.56±0.35	0.18	173	10.73±0.48	194	11.87±0.45	0.086	0.039
Subjects >20years	237	10.41±0.36	266	10.06±0.34	0.48	159	10.09±0.49	175	11.35±0.46	0.062	0.074
Age 20 – 70 years	230	10.51±0.37	261	10.13±0.35	0.47	149	10.22±0.51	169	11.56±0.47	0.056	0.061
Age 34 – 55 years	139	10.16±0.46	164	9.57±0.43	0.35	85	9.88±0.58	96	11.48±0.54	0.045	0.031

Values are least square means ± SEs. The properties of the femoral artery were adjusted for observer, sex, age, mean arterial pressure, heart rate, body mass index, serum total cholesterol, smoking, and alcohol intake.

*Sensitivity analysis, excluding 142 patients on antihypertensive medication (*n*=134) or lipid lowering drugs (*n*=15). For further explanation, see Table 5.1.5.

Figure 5.2.1 Brachial diameter and distensibility and plasma renin activity according to the number of copies of *AGT H1-CG* haplotype

The analyses were adjusted for observer, sex, age, body mass index, mean arterial pressure, heart rate, serum total cholesterol, smoking, alcohol intake and use of antihypertensive drugs and account for family clusters. Values are least square means ± SEs. N indicates the number of subjects carrying 2, 1 or 0 copies of the *AGT H1-CG* haplotype. *P*-values are for linear trend.



Discussion

Our current study builds on previous findings showing interactions between the *ADD1, ACE,* and aldosterone synthase genes in relation to femoral intima-media thickness [5] and carotid and femoral distensibility [6]. We assessed whether the properties of the large arteries might also be related to interactions of *ADD1* with other genes of the renin-angiotensin system. The key finding of our study was that femoral cross-sectional compliance and distensibility were higher in *AT1R C* allele carriers than in *AT1R AA* homozygotes, but that this association was only observed in the presence of the mutated *ADD1 Trp* allele, and not in *ADD1 GlyGly* homozygotes. In the family-based analyses, we did not find any evidence for population stratification. Transmission of the *AT1R C* allele was associated with higher femoral distensibility in offspring carrying the mutated *ADD1 Trp* allele. Furthemore, single-gene analyses showed increased brachial diameter associated with the *AT1R C* allele and larger brachial diameter and lower brachial distensibility associated with the *AGT*

H1-CG haplotype. The latter association was not modified by interaction with the other genes under study.

Epidemiological findings only allow speculation about the plausibility of the observed associations and possible underlying mechanisms. In tissue extracted from 68 term placentas of European British ancestry, Abdollahi and colleagues demonstrated that allele and mRNA haplotypes carrying *AT1R 1166C* exhibited lower expression of angiotensin II type-1 receptor mRNA [19]. If these findings can be extrapolated to the brachial artery, they might explain the larger diameter, because of the lower sensitivity to the vasoconstrictive effects of angiotensin II.

Substitution of glycine to tryptophane in the α-subunit of the adducin cytoskeleton protein leads to higher activity of Na^+,-K^+-ATPase [1,2] and increased tubular sodium reabsorption in the kidney [3]. Higher body sodium suppresses the generation of angiotensin II. Combined with a lower expression of the AT1R in *AT1R C* allele carriers [19], this might explain the interaction between the 2 genes in relation to femoral cross-sectional compliance and distensibility. Alternatively, it is also conceivable that the constitutive activation of the sodium pump in *ADD1 Trp* allele carriers not only occurs in renal tubular cells, but that it might also be present in vascular smooth muscle cells [4]. Mutation of *ADD1* by decreasing intracellular sodium could enhance sarcolemmal Na^+/Ca^{2+} exchange and, through calcium-dependent pathways decrease excitation–contraction coupling [20], and hence increase the distensibility and cross-sectional compliance of muscular arteries, such as the femoral artery.

The *AGT G–6A* variant represents a guanine-to-adenine substitution 6 base pairs upstream from the initiation site of transcription. This nucleotide substitution is associated with a slightly higher basal rate of *AGT* gene transcription, which could account for the increase in plasma AGT in carriers of the *–6A* allele [21]. Studies involving healthy subjects or French families also showed higher plasma AGT levels in carriers of the *AGT –532T* allele [17,22]. As the *–532* site is located within a consensus sequence of the *AGT* gene-binding transcription factor AP-2, the *C–532T* polymorphism might also modulate *AGT* gene transcription [17]. In our study, the *C–532T* and *G–6A* polymorphisms of the *AGT* gene were in linkage disequilibrium. This finding is in keeping with other published data on the haplotype structure of the *AGT* gene [22, 23]. Thus, carriers of the *H2-CA* or *H3-TA* might have an elevated AGT concentration in both plasma and tissues. Our finding that *H2-CA* or *H3-TA* carriers

had lower plasma renin activity is in line with this hypothesis. In subjects with a suppressed renin-angiotensin system and lower circulating volume, the diameter of the brachial artery might be smaller, and hence brachial distensibility might be higher [24].

In keeping with our previous study [5], we did not find any association between the polymorphisms under study and the properties of the elastic carotid artery. The central elastic arteries, such as the carotid artery, and the more peripheral muscular conduit vessels, including the brachial and femoral arteries, have different properties [25]. Going from the central to the peripheral arteries, the collagen/elastin ratio reverses, vascular smooth muscle cells become the predominant component of the arterial wall, and the phenotype of the vascular smooth muscle cells changes [25]. Thus, the effects of genetic variations in *ADD1* and the renin-angiotensin system genes on arteries must be complex and may depend on the vessel wall component that is involved [6].

Limitations

Our study should be interpreted within the context of its limitations. First, our epidemiological study demonstrated association of arterial properties with variation in *ADD1* and various genes of the renin-angiotensin system, but did not provide direct information on the mechanisms underlying these phenotype-genotype associations. Second, arterial measurements are quantitative traits prone to measurement error. However, the repeatability and reproducibility of the arterial phenotypes collected in the present study was high [15]. Moreover, we adjusted for observer bias. For the interaction between the *ADD1* and *AT1R* genes, there was consistency between the population-based and the family-based analyses. We did not find any evidence for population stratification. Third, in contrast to the most recent guidelines from the European Societies of Hypertension and Cardiology (ESH/ESC) [26], we based mean arterial pressure and the diagnosis of hypertension only on office blood pressure measurement without confirmation by 24-hour ambulatory monitoring. Moreover, arterial phenotyping took place before the 2007 ESH/ESC guidelines [26] were issued.

In conclusion, *ADD1* and *AT1R* interactively determine the elastic properties of the femoral artery. The underlying mechanisms remain to be elucidated. There is a single-gene effect of the *AGT* promoter haplotypes on brachial properties, which might be

mediated by different circulating levels of angiotensinogen and angiotensin II as suggested by lower PRA in *H1-CG* haplotype homozygotes.

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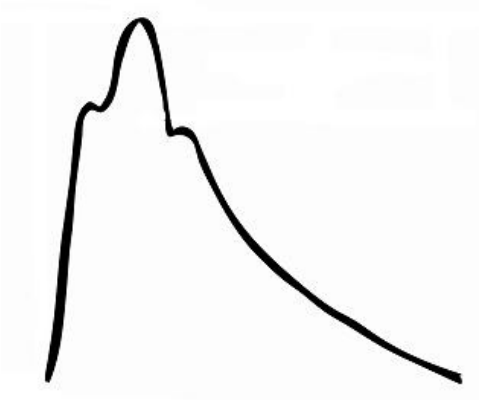

Chapter 6

General discussion

Salt intake has a potent effect on arterial stiffness. The kidneys play a central role in sodium handling. This process is influenced by the renin-angiotensin-aldosterone system as well as by cytoskeleton protein adducin. The question whether renal sodium handling is associated with arterial properties remains unanswered. In this doctoral dissertation we investigated the role of renal sodium handling and genetic variability in the regulation of sodium homeostasis on properties of large arteries.

Arterial phenotyping

In present thesis, we included several types of measurement of arterial properties. For genetic analyses as well as for association study between arterial properties and renal sodium handling we used local parameters measured at three arterial sites, reflecting properties of both elastic as well as muscular arteries. This method is indicated for mechanistic analyses in pathophysiology, pharmacology, and therapeutics [1]. We analyzed all arterial measurements as continuous traits. Indeed, in family-based association studies, quantitative traits are thought to provide higher statistical power than dichotomous traits.

One of the possible limitations of our study was that arterial phenotyping might be prone to measurement error, causing large interobserver variability. To minimize this potential source of bias, we standardized examination protocol and we adjusted for observer bias. Moreover, the repeatability and reproducibility of the arterial phenotypes used in our studies were high [2-5]. Second, we registered pulse wave at the radial but not at the carotid artery to assess CAIx. Tonometry at baseline took place from September 2000 until January 2002 and preceded the expert consensus document on arterial stiffness published in 2006 [1]. However, the radial-to-aortic transfer function for the measurement of central systolic blood pressure was already well established at the time of the study [6,7].

Statistical analyses

Association studies based on candidate genes are one of the major strategies used to identify genetic factors underlying complex traits, such as properties of large arteries. They are usually performed in samples of unrelated individuals, such as population-based case-control or cohort designs. In such study design, the effect of population stratification can not be overcome [8]. On the other hand, family-based designs such as analysis of general pedigrees have been proposed to counteract confounding due to

population stratification that can occur in case-control or other population-based designs [8]. The population-based analysis includes all available subjects. The family-based analysis, which only includes informative offspring, is complimentary to the information provided by the population-based analysis. Indeed, the family-based analysis provides important additional information. In case of a non-significant between-family component, we can exclude population stratification/admixture [9-11]. The within-family component provides an estimate of the genetic effect on the phenotype, which is robust to population stratification/admixture. Therefore, the family-based analysis serves as a confirmation of the population-based analysis.

In line of above mention evidence, in our analyses we used both population- and family-based approaches. Family-based design offers another advantage, including the potential to conduct linkage analysis and genotype-phase inference for haplotyping.

The FLEMENGHO and EPOGH study population consists of extended pedigrees and nuclear families, respectively. In this case, conventional statistics procedures are not valid and may lead to incorrect inferences. The mixed model offers the possibility for studying phenotype-genotype associations in samples of related subjects. This statistical method includes all available subjects and accounts for confounders as well as for the non-independence of observations within families. It also allows test for phenotype-genotype interaction and/or gene-gene interaction. Although dealing with family data, the mixed model does not overcome the risk of spurious associations due to uncontrolled stratification of the population. To minimize these limitations, we also investigated the possible association between quantitative traits and the transmission of alleles of interest across families by implementation of the transmission disequilibrium test (TDT) [9]. For continuous traits, Abecasis proposed a QTDT [10,11]. Using this method, it is possible to score allelic transmission that accommodates families of any size and uses all available genotypic information [10]. In general, in family design, alleles transmitted to affected individuals are compared with untransmitted alleles, providing a control sample that is inherently matched to the case sample with regard to population structure [12].

In view of the physiological consistency in the phenotype-genotype relations, it is unlikely that our finding just arose by chance. Adjustment for multiple comparisons is usually recommended to avoid rejecting null hypotheses too readily [13]. The theoretic basis for advocating routine adjustment for multiple comparisons is that

chance serves as the first order explanation for observed phenomena [13]. This hypothesis undermines one of the basic premises of epidemiological research, which holds that human biology follows regular laws that may be studied through observation of populations. Arterial diameter, distensibility and cross-sectional compliance, investigated in our genetic association studies, are correlated phenotypes. In such case, multiple testing is not indicated, because each test does not provide a completely independent opportunity for a type I error.

Familial aggregation

Properties of large arteries are complex multigenic traits and may be influenced by variation in many genes and their gene-gene and/or genotype-phenotype interactions. Classic heritability studies, involving twins or families, do provide some insights to what extent genetic factors impact on a trait or disease. Furthermore, knowledge that a trait of interest has high heritability can support a study that propose to investigate the genetic determinants of that trait. In our first study, we showed that aortic stiffness and pulse wave reflection are impaired in normotensive offspring of hypertensive parents compared with normotensive offspring of normotensive parents. These results suggest that the alteration in arterial function is present already in nonhypertensive subjects at risk of hypertension and it may contribute to the progression to hypertension in later life.

Furthermore, in our second study, we investigated familial aggregation. We found significant intrafamilial correlation in parent-offspring pairs for aPWV, peripheral and central augmentation indexes and peripheral pulse pressure (PPp). In addition, the estimates of heritability (h^2) for the aforementioned traits ranged from 0.19 for aPWV to 0.41 for central AIx. Our heritability estimates for arterial properties somewhat differed compared with other published studies [14-17]. Random variability is unlikely to explain our observations. Indeed, heritability estimates for anthropometric parameters in our study were similar to those published by other investigators [18]. In general, heritability estimates from family-based studies tend to be lower than those from twin studies. It remains unclear to what extent differences in recording techniques, the number of observers involved in measurement, reproducibility, or adjustment for confounders explain the variability across studies in heritability estimates for indexes of arterial stiffness.

Moreover, we also investigated contribution of shared genetic and environmental factors. The (phenotypic) correlation between 2 traits (ρ_p) in the same individual is related to the genetic correlation (ρ_G) and environmental correlation (ρ_E) by the following equation: $\rho_p = \rho_G \times \sqrt{h_1^2 \times h_2^2} + \rho_E \times \sqrt{(1-h_1^2) \times (1-h_2^2)}$, where h_1^2 and h_1^2 represent the heritability of each individual trait [19]. The genetic and environmental components between the traits represent the effects of shared genes and of shared (unmeasured) environmental factors. In our hands, the genetic correlations of aortic PWV with augmentation indexes and peripheral pulse pressure were significant, whereas the corresponding environmental correlations were only significantly positive for PPp. These findings suggest that common genes influences arterial stiffness. A shared environment, over and beyond the lifestyle factors for which we accounted, apparently plays only a minor role in the familial aggregation of these traits. These results advocates for further research into the genes that affect arterial stiffness.

Association of arterial properties with renal phenotypes

The kidneys play a central role in the pathophysiology of essential hypertension. Blood pressure starts to rise when the kidney requires a higher than usual blood pressure to maintain extracellular fluid volume within normal limits. Mean arterial pressure drives pressure-natriuresis and influences arterial structure and function. The renin-angiotensin-aldosterone system is an important determinant of renal sodium handling and the properties of the arterial wall. We therefore investigated the functional and structural properties of 3 large arteries in relation to renal sodium handling.

We demonstrated in our population-based study, that while accounting for covariates and standardizing for the sodium excretion rate, the compliance and distensibility of the femoral artery increased with higher distal sodium reabsorption, whereas the brachial and femoral diameters lessened with higher proximal sodium reabsorption. We did not find any consistent association between renal sodium handling and properties of elastic carotid artery.

Our cross-sectional epidemiological study cannot directly address the mechanisms underlying the positive relation of femoral compliance and distensibility with RNa_{dist}. A possible explanation might be that a common mechanism, operating in both the arterial wall and the renal tubules, might influence arterial properties and renal

sodium handling. For instance, genetic variability in ion transport across cell membranes might be involved. In the present population, we previously demonstrated that mutations of the cytoskeleton protein α-adducin (*460 Gly→Trp*) and the angiotensin-converting enzyme (*ACE I→D*) jointly predicted the incidence of hypertension [20]. In cross-sectional analyses of the same population, we also noticed that the combination of these two functional mutations [21,22] was associated with raised serum creatinine concentration [23], increased femoral intima-media thickness [24] and 24-hour proteinuria [23]. In this context, it is conceivable that the constitutive activation of the sodium pump in α-adducin *Trp* allele carriers not only occurs in renal tubular cells, but that it might also be present in vascular smooth muscle cells. In vascular myocytes enhanced Na^+,K^+-ATPase activity might reduce the sarcolemmal Na^+/Ca^{2+} exchange and through calcium-dependent pathways modulate excitation-contraction coupling [25].

Furthermore, we found a negative relation between the brachial and femoral diameters and RNA_{prox}. Vasoconstriction is the hallmark of hypertension. Animal model of hypertension [26], hypertensive patients [27], normotensive subjects with 1 first-degree relative with hypertension [28], and patients with white-coat hypertension [27] have higher RNA_{prox}.

The novel finding in our study is the significant association between arterial properties and renal sodium handling. If confirmed, our findings might be relevant for unraveling the pathogenesis of essential hypertension and its renal and vascular complications.

Genetic analyses

In recent genome-wide association study, the Framingham investigators measured indexes of arterial stiffness using arterial tonometry [29]. No association attained genome-wide significance. Whereas the genome-wide association approach is an unbiased search of the entire genome without any preconceptions about the role of a certain gene, the candidate gene approach allows researchers to investigate the validity of an “educated guess” about the genetic basis of a disorder. This approach involves assessing the association between a particular allele (or set of alleles) of a gene that may be involved in the disease (i.e., a candidate gene) and the disease itself. In other words, this type of association study tries to answer the question, “Is one

allele of a candidate gene more frequently seen in subjects with the disease than in subjects without the disease?" Furthermore, candidate gene studies are better suited for detecting genes underlying common and more complex diseases where the risk associated with any given candidate gene is relatively small [30,31].

The cytoskeleton protein adducin as well as the components of renin-angiotensin system are involved in sodium homeostasis. In line with our previous finding showing association between renal sodium handling and properties of large arteries, we further investigated whether genetic variation in above mentioned systems might be associated with arterial characteristics.

Properties of large arteries in relation to genetic variation in adducin subunits

Adducin functions within the cell as a tissue-specific heterodimer, which consists either of α- and ß- or α- and γ-subunits. This provides the physiological and biochemical basis for studying the interaction among the three adducin genes, which map to different chromosomes [32]. The key finding of our study was that brachial diameter decreased, while brachial distensibility increased with the *ADD3 G* allele, and that these associations were confined to *ADD1 GlyGly* homozygotes. In the family-based analyses, we did not find any evidence for population stratification. Transmission of the *ADD3 G* allele was associated with smaller brachial diameter in all informative offspring as well as in offspring homozygous for *ADD1 Gly* allele. *ADD1* and *ADD2,* alone or in combination with each other, were not associated with the arterial properties in the three arterial beds. This was also true for *ADD1* and *ADD3* in relation to the carotid and femoral phenotypes.

Our epidemiological study only allows speculation about the reasons why there might be an association between the properties of the brachial artery, a small muscular artery, and the *ADD3 A386G* polymorphism. One possible mechanism is that the polymorphism might affect the neurogenic tone of vascular smooth muscle cells. Indeed, in rat models, increased blood pressure was associated with a decrease in the hypothalamic levels of γ-adducin mRNA and protein [33,34]. Furthermore, inhibition of γ-adducin by intracellular delivery of γ-adducin-specific antibodies increased the neuronal firing rate possibly via regulation of Na^+,-K^+-ATPase [33]. On the other hand, given that the phenotypes representative of two other arterial segments did not shown any association with the adducin SNPs, suggests that local mechanisms in the

brachial artery, rather than the central nervous system, might underlie the demonstrated findings.

Properties of large arteries in relation to genetic variation in α-adducin subunit and renin-angiotensin system components

We built our study on previous findings showing interactions between the *ADD1, ACE,* and aldosterone synthase genes in relation to femoral intima-media thickness [24] and carotid and femoral distensibility [35]. We assessed whether the properties of the large arteries might also be related to interactions of *ADD1* with other genes of the renin-angiotensin system. The key finding of our study was that femoral cross-sectional compliance and distensibility were higher in *AT1R C* allele carriers than in *AT1R AA* homozygotes, but that this association was only observed in the presence of the mutated *ADD1 Trp* allele, and not in *ADD1 GlyGly* homozygotes. In the family-based analyses, we did not find any evidence for population stratification. Transmission of the *AT1R C* allele was associated with higher femoral distensibility in offspring carrying the mutated *ADD1 Trp* allele.

There is an apparent discrepancy between our analyses in which compliance of femoral artery was lower in the *AT1R AA* homozygotes carrying *ADD1 Trp* allele, whereas in other analyses of hypertensive subjects, arterial stiffness assessed as aPWV was increased with *AT1R C* allele [36-38]. However, these studies cannot be compared. Compliance of artery is parameter measured locally at one arterial site, while aPWW reflects characteristics of arterial segment between two sites of measurements, e.g. carotid and femoral artery. Elastic aorta and muscular femoral artery have different properties [39]. Moreover, observations collected in a general population should not be extrapolated to hypertensive patients. Indeed, the aforementioned association between aPWV and *AT1R C* allele was found only in hypertensive, but not in normotensive, individuals [37]. Finally, we did only demonstrate effect of *AT1R C* allele only in carriers of mutated *ADD1 Trp* allele, which implicates gene-gene interaction.

Furthemore, single-gene analyses showed larger brachial diameter and lower brachial distensibility associated with the *AGT H1-CG* haplotype. This association was not modified by interaction with the other genes under study. The *AGT G–6A* variant represents a guanine-to-adenine substitution 6 base pairs upstream from the initiation site of transcription. This nucleotide substitution is associated with a slightly

higher basal rate of *AGT* gene transcription, which could account for the increase in plasma AGT in carriers of the *–6A* allele [40]. Studies involving healthy subjects or French families also showed higher plasma AGT levels in carriers of the *AGT –532T* allele [41,42]. As the *–532* site is located within a consensus sequence of the *AGT* gene-binding transcription factor AP-2, the *C–532T* polymorphism might also modulate *AGT* gene transcription [41]. In our study, the *C–532T* and *G–6A* polymorphisms of the *AGT* gene were in linkage disequilibrium. This finding is in keeping with other published data on the haplotype structure of the *AGT* gene [42]. Thus, carriers of the *H2-CA* or *H3-TA* might have an elevated AGT concentration in both plasma and tissues. Our finding that *H2-CA* or *H3-TA* carriers had lower plasma renin activity is in line with this hypothesis. In subjects with a suppressed renin-angiotensin system and lower circulating volume, the diameter of the brachial artery might be smaller, and hence brachial distensibility might be higher [43].

In line with our previous studies [24], we did not find any association between the polymorphisms under study and the properties of the elastic carotid artery. The central elastic arteries, such as the carotid artery, and the more peripheral muscular conduit vessels, including the brachial and femoral arteries, have different properties [39]. Going from the central to the peripheral arteries, the collagen/elastin ratio reverses, vascular smooth muscle cells become the predominant component of the arterial wall, and the phenotype of the vascular smooth muscle cells changes [39]. One possible explanation for different gene expression along arterial tree might be level of applied wall shear stress [44]. It is well established that level of wall shear stress is an important determinant of arterial gene expression. Along arterial tree wall shear stress is not constant. Indeed, the highest mean wall shear stress on average 1.5 Pa was found in elastic carotid artery. In the muscular femoral and brachial arteries, the mean wall shear stress is substantially lower, varying on the average between 0.3 and 0.5 and between 0.4 and 0.5 Pa, respectively [44]. Thus, the effects of genetic variation in adducin subunits and components of renin-angiotensin-aldosterone system on different arteries must be complex and may depend on the vessel wall component that is involved.

Perspectives

Arterial stiffness, mainly aPWV is an independent predictor of cardiovascular outcome in the general population [45] as well as in patients with several pathological

condition [46,47]. Similarly, other indexes of arterial stiffness also predict cardiovascular outcome [48]. aPWW >12 m/s is also recognized in recent ESH/ESC Guidelines for the management of arterial hypertension as a factor influencing CV prognosis [49]. These findings might be explained by a phenomenon called "ventricular-arterial coupling". Arterial stiffening influence the phasic mechanical stresses imposed on the arteries that in turn is important for regulating smooth muscle tone, endothelial function, and arterial health. In addition, the heart typically adapts to confront higher and later systolic loads by both hypertrophy and ventricular stiffening. This creates altered coupling between heart and arteries that importantly affects cardiovascular reserve function [50] and leads to heart failure [51]. However, despite great effort of numerous investigators, the genetic research did not yet produce all information on genetic causes of accelerated arterial stiffening. The reason might be that arterial stiffness as a continuous polygenic trait is affected by multiple interacting genetic and environmental determinants. Genetic epidemiology provides powerful method to investigate possible genotype-phenotype associations.

For various indexes of arterial stiffness we confirmed a significant familial aggregation and heritability and demonstrated genetic correlations between these indexes. Building upon our previous studies we further explored an effect of genes encoding adducin subunits and different components of renin-angiotensin system on arterial characteristics. Properties of muscular brachial artery were related to the *ADD3 A386G* polymorphism and also to variation in the regulatory area of *AGT* gene. Moreover, we demonstrated an interactive effect of *ADD1 Gly460Trp* and *AT1R C1166A* on elastic properties of the femoral artery. We noted in a cross-sectional population study an association between arterial properties and renal sodium handling. These results have to be interpreted carefully. Epidemiological studies can only show associations but do not prove causation or lack thereof. From this point of view our results are only hypothesis generation.

Our findings may be relevant to the interpretation of published studies and the design of experimental and clinical research in the future. Population studies, which take into account gene-gene and/or gene-environment interactions, will increasingly be used to study complex phenotypes, such as arterial stiffness. Overall, standardized epidemiological observations such as presented in this work, reflect the experiment of nature and help to distinguish what is clinically relevant at the level of the general population. Recent studies showed that standardized phenotyping is the key for

recognizing important genetic variations. Together with other experimental and clinical investigators, we will continue our research in this field and we hope to witness in the not so distant future the translation of this collective scientific endeavor into clinical innovation.

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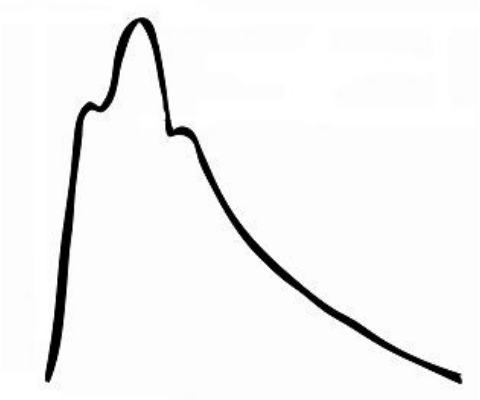

Chapter 7

Summary

In line with Guyton's work, the goal of our research was to explore in three European populations whether the properties of large arteries are associated with renal sodium handling, which itself changes with environmental factors and with variation in a large number of genes. Before engaging in the genetic analyses proper, we first studied the familial aggregation and the heritability of arterial properties. In all our analyses, we accounted for relatedness among participants and for covariables and confounders.

In a first study, we compared the arterial characteristics and blood pressure (BP) in normotensive offspring of two normotensive parents (OFF/NT) and normotensive offspring, who had at least one hypertensive parent (OFF/HT). We measured peripheral pulse pressure (PPp) by conventional and 24-h ambulatory BP. A SphygmoCor device was used to determine the central (CAIx) and peripheral (PAIx) augmentation indexes, central pulse pressure (PPc), and aortic pulse wave velocity (aPWV). Compared with OFF/NT (n=59; 16 to 34 years of age), the OFF/HT (n=174; 17 to 40 years) had higher ($0.14<P<0.0007$) BP and PPp on conventional measurement (121/75 *vs.* 114/71 mm Hg and 46 *vs.* 42 mm Hg) as well as on 24-h ambulatory monitoring (118/70 *vs.* 114/67 mm Hg and 48 *vs.* 47 mm Hg). OFF/HT, compared with OFF/NT, also had higher ($0.05<P<0.0008$) PPc (28 *vs.* 26 mm Hg), PAIx (54.7% *vs.* 44.9%), CAIx (108.8% *vs.* 99.8%), and aPWV (7.4 *vs.* 6.6 m/sec). However, complex adjustment including mean arterial pressure and age removed the differences between the offspring in CAIx, PAIx, and aPWV.

In a family-based population sample consisting of 204 parents (mean age, 51.7 years) and 290 offspring (29.4 years), we investigated the heritability and familial aggregation of PPp, CAIx, PAIx and aPWV. We partitioned the phenotypic correlation between these traits into shared genetic and environmental components. We found significant heritability for PPp, CAIx, PAIx, and mean arterial pressure ranging from 0.37 to 0.41; $P\leq0.0001$. The parent-offspring correlation coefficients were significant for all arterial indexes ($r\geq0.12$; $P\leq0.02$) with the exception of PPc (P=0.90). The sib-sib correlations were also significant for CAIx (r=0.22; P=0.001). The genetic correlation between aPWV and the other arterial indexes were significant ($\rho_G\geq0.29$, $P<0.0001$). The corresponding environmental correlations were only significantly positive for PPp (ρ_E=0.10. P=0.03).

In the Flemish population sample, we also ultrasonographically measured diameter, cross-sectional compliance (CC) and distensibility (DC) of the carotid, brachial, and femoral arteries. In multivariable-adjusted analyses of 1069 untreated subjects (mean age, 41.6 years), CC and DC of the femoral artery increased with higher fractional distal sodium reabsorption (RNa_{dist}), as assessed by the clearance of endogenous lithium. Differences associated with a 1-SD change in RNa_{dist} were 51.7 mm^2/kPa $\times 10^{-3}$ (P=0.0002) and 0.56×10^{-3}/kPa (P=0.004) for femoral CC and DC, respectively. In women as well as in men, a 1-SD increment in fractional proximal sodium reabsorption (RNa_{prox}) was associated with decreases in femoral and brachial diameter, amounting to 111.6 μm (P=0.003) and 52.5 μm (P=0.016), respectively. There was no consistent association between the properties of the elastic carotid artery and renal sodium handling.

In 1126 subjects from the same Flemish population (mean age, 43.8 years), we investigated whether arterial characteristics are related to the genes encoding *ADD1* (*Gly460Trp*), *ADD2* (*C1797T*) and *ADD3* (*A386G).* In single gene analyses, brachial diameter was 0.15 mm (P=0.0022) larger, and brachial CC and DC were 0.017 mm^2/kPa (P=0.0029) and 1.55 10^{-3}/kPa (P=0.013) lower in *ADD3 AA* than *ADD3 GG* homozygotes with an additive effect of the *G* allele. In multiple-gene analyses, the association of brachial diameter and DC with the *ADD3 G* allele only occurred in *ADD1 GlyGly* homozygotes. Otherwise, the associations between the arterial phenotypes in the three vascular beds and the *ADD1* or *ADD2* polymorphisms were not significant. There was no evidence for population stratification ($0.07\leq P\leq0.96$). Transmission of the mutated *ADD3 G* allele was associated with smaller brachial diameter in 342 informative offspring (–0.12±0.04 mm; P=0.0085) and in 209 offspring, who were *ADD1 GlyGly* homozygotes (–0.14±0.06 mm; P=0.018).

Finally, in 1064 Flemish subjects (mean age, 43.6 years), we assessed the multiple-gene effects of *ADD1* (*Gly460Trp*), *AGT* (*C–532T* and *G–6A*) and *AT1R* (*A1166C*). In *ADD1 Trp* allele carriers, but not in *ADD1 GlyGly* homozygotes (P-value for interaction ≤0.014), femoral CC was significantly higher (0.74 *vs.* 0.65 mm^2/kPa; P=0.020) in carriers of the *AT1R C* allele than in *AT1R AA* homozygotes, with a similar trend for femoral DC (11.3 *vs.* 10.2 10^{-3}/kPa; P=0.055). Family-based analyses confirmed these results. Brachial diameter (4.35 *vs.* 4.18 mm) and plasma renin activity (PRA, 0.23 *vs.* 0.14 ng/ml/h) were increased ($P\leq0.005$) in *AGT CG*

haplotype homozygotes compared with non-carriers, whereas the opposite was true for brachial DC (12.4 *vs.* 14.4 10^{-3}/kPa; P=0.011). There was no interaction between *AGT* and any other gene in relation to the measured phenotypes.

In conclusion, in this doctoral dissertation, we demonstrated significant familial aggregation and significant heritability of arterial properties. We also noticed that higher RNa_{dist} was associated with higher femoral CC and DC, and that higher RNa_{prox} was associated with decreased diameters of muscular arteries. The aforementioned observations justified our analyses of genes, which are involved in renal sodium handling. In *ADD1 GlyGly* homozygotes, the properties of the brachial artery were related to the *ADD3* (*A386G*) polymorphism. Furthermore, *ADD1* and *AT1R* interactively determined the elastic properties of the femoral artery. There was a single-gene effect of the *AGT* promoter haplotypes on brachial properties and PRA. Overall, our findings suggest, that there might be a genetically determined influence of renal sodium handling on arterial properties, or vice versa, or that common genetic pathways might influence both arterial and renal function.

Samenvatting

Ons onderzoek sluit aan bij het het werk van Guyton en had tot doel na te gaan of er, in 3 Europese populaties, een verband bestaat tussen de eigenschappen van de grote slagaders en de regulatie van de zouthuishouding in de nieren. Dit laatste wordt beïnvloed door zowel genetische als omgevingsfactoren. In een eerste fase bestudeerden we in welke mate de arteriële eigenschappen erfelijk bepaald zijn. Nadien onderzochten we de impact van verschillende genetische polymorfismen op the arteriële fenotypes. In onze analyses hielden we rekening met familieverbanden en met mogelijke verstrengelde factoren.

In een eerste studie vergeleken we de arteriële eigenschappen en de bloeddruk (BP) van normotensieve kinderen van 2 normotensieve ouders (OFF/NT) met deze van normotensieve kinderen die minstens 1 hypertensieve ouder hadden (OFF/HT). We maten de perifere polsdruk (PPp) zowel op conventionele wijze als door middel van 24-uur ambulante bloeddrukmeting. Een SphygmoCor toestel werd gebruikt om de centrale (CAIx) en perifere (PAIx) systolische augmentatie index, de centrale polsdruk (PPc) en de polsgolfsnelheid (aPWV) te bepalen. Vergeleken met OFF/NT (n=59; 16 tot 34 jaar oud) hadden de OFF/HT (n=174; 17 tot 40 jaar) hogere ($0.14<P<0.0007$) BP and PPp en dit zowel voor de conventionele (121/75 *vs.* 114/71 mm Hg and 46 *vs.* 42 mm Hg) als de 24-h ambulante meting (118/70 *vs.* 114/67 mm Hg and 48 *vs.* 47 mm Hg). De OFF/HT hadden ook hogere ($0.05<P<0.0008$) PPc (28 *vs.* 26 mm Hg), PAIx (54.7% *vs.* 44.9%), CAIx (108.8% *vs.* 99.8%) en aPWV (7.4 *vs.* 6.6 m/sec) dan de OFF/NT. Na correctie voor ondermeer gemiddelde arteriële bloeddruk en leeftijd verdwenen echter de verschillen in centrale en perifere augmentatie index en polsgolfsnelheid tussen de 2 groepen.

In een bevolkingsstaal bestaande uit 204 ouders (gemiddelde leeftijd, 51.7 jaar) en 290 kinderen (29.4 jaar) onderzochten we in welke mate PPp, CAIx, PAIx en aPWV overerfbaar zijn. De erfelijkheid van PPp, CAIx, PAIx en gemiddelde arteriële bloeddruk lag tussen 0.37 en 0.41 ($P\leq0.0001$), wat wil zeggen dat in deze populatie tussen de 37% en 41% van de varianties in de arteriële fenotypes veroorzaakt worden door genetische factoren. De ouder-kind correlaties waren significant voor alle arteriële fenotypes ($r\geq0.12$; $P\leq0.02$) uitgezonderd PPc (P=0.90). De correlaties tussen kinderen van dezelfde ouders waren ook significant voor CAIx (r=0.22; P=0.001). De genetische correlatie tussen aPWV en de andere arteriële fenotypes waren hoog

significant ($\rho_G \geq 0.29$, $P<0.0001$). Dit suggereert dat deze fenotypes waarschijnlijk gedeeltelijk beïnvloed worden door dezelfde genen. De overeenkomstige omgevingscorrelaties waren enkel significant positief voor PPp ($\rho_E=0.10$. $P=0.03$).

In een representatief bevolkingsstaal uit de Noorderkempen maten we ook de diameter, compliantie (CC) en distensibiliteit (DC) van de slagaders in hals, arm en lies. Bij 1069 onbehandelde personen (gemiddelde leeftijd, 41.6 jaar) waren CC en DC van de liesslagader positief geassocieerd met de fractionele distale sodium reabsorptie (RNa_{dist}), gemeten aan de hand van de endogene lithium klaring. Een toename van de RNa_{dist} met 1 standard deviatie (SD) kwam overeen met een stijging van de CC en DC van de arteria femoralis met respectievelijk 51.7 $mm^2/kPa \times 10^{-3}$ ($P=0.0002$) en 0.56×10^{-3}/kPa ($P=0.004$). Bij zowel vrouwen als mannen was een 1-SD hogere fractionele proximale sodium reabsorptie (RNa_{prox}) geassocieerd met een 111.6 μm ($P=0.003$) en 52.5 μm ($P=0.016$) kleinere diameter van respectievelijk de lies- en armslagader. Er was geen consistent verband tussen de eigenschappen van de halsslagader en de regulatie van de zouthuishouding in de nieren.

Bij 1126 subjecten van dezelfde Vlaamse populatie (gemiddelde leeftijd, 43.8 jaar) onderzochten we of er een verband bestaat tussen de arteriële eigenschappen en de genen die coderen voor *ADD1* (*Gly460Trp*), *ADD2* (*C1797T*) and *ADD3* (*A386G).* In genetische analyses waarbij slechts 1 gen tegelijkertijd bestudeerd werd, was de diameter van de armslagader 0.15 mm ($P=0.0022$) groter, en de CC en DC van de armslagader 0.017 mm^2/kPa ($P=0.0029$) en 1.55 10^{-3}/kPa ($P=0.013$) kleiner bij personen die homozygoot waren voor het *ADD3 A* allel vergeleken met personen die 2 copiën hadden van de *G* variant van het *ADD3* gen. Het effect van het *G* allel bleek bovendien additief te zijn. In modellen die het effect van meerdere genen tegelijkertijd bestuderen, was de associatie tussen de diameter en DC van de armslagader en het *ADD3 G* allel enkel significant bij *ADD1 GlyGly* homozygoten. De overige associaties tussen de verschillende arteriële fenotypes en de *ADD1* en *ADD2* polymorfismen waren niet significant. Er was geen evidentie voor populatie stratificatie ($0.07 \leq P \leq 0.96$). Bij 342 informatieve kinderen was de transmissie van het *ADD3 G* allel geassocieerd met een 0.12 ±0.04 mm ($P=0.0085$) kleinere diamater van de armslagader. We vonden dezelfde associatie bij 209 kinderen, die homozygoot waren voor het *ADD1 GlyGly* allel (0.14±0.06 mm; $P=0.018$).

Tenslotte onderzochten we, bij 1064 Vlaamse subjecten (gemiddelde leeftijd, 43.6 jaar), de gezamenlijke effecten van *ADD1* (*Gly460Trp*), *AGT* (*C–532T* en *G–6A*) en *AT1R* (*A1166C*). Bij dragers van het *ADD1 Trp* allel, doch niet bij *ADD1 GlyGly* homozygoten (*P*-waarde voor interactie ≤0.014), was de CC van de liesslagader significant groter (0.74 *vs.* 0.65 mm^2/kPa; *P*=0.020) bij dragers van het *AT1R C* allel vergeleken met *AT1R AA* homozygoten. Een gelijkaardige trend vonden we voor DC van dezelfde slagader (11.3 *vs.* 10.2 10^{-3}/kPa; *P*=0.055). Deze resultaten werden bevestigd door analyses die rekening hielden met familieverbanden. De diameter van de armslagader (4.35 *vs.* 4.18 mm) en plasma renine activiteit (PRA, 0.23 *v*s. 0.14 ng/ml/h) waren groter (*P*≤0.005) bij personen dit homozygoot waren voor het *AGT CG* haplotype vergeleken met de anderen, maar het tegenovergestelde vonden we voor DC van de armslagader (12.4 *vs.* 14.4 10^{-3}/kPa; *P*=0.011). Verder was er voor de gemeten fenotypes geen interactie tussen *AGT* en de andere genotypen.

Alles samenvattend toonden we in dit proefschrift aan dat de arteriële eigenschappen significant genetisch bepaald zijn. We noteerden ook dat grotere RNa_{dist} geassocieerd was met grotere CC en DC van de liesslagader, en dat grotere RNa_{prox} geassocieerd was met kleinere diameters van de musculaire arteries. Daarom kozen we voor onze genetische analyses die genen waarvan gekend zijn dat ze impact hebben op de regulatie van de sodium huishouding in de nieren. In *ADD1 GlyGly* homozygoten waren de eigenschappen van de armslagader gerelateerd aan het *ADD3* (*A386G*) polymorfisme. Bovendien bepaalden *ADD1* en *AT1R* interactief de elastische eigenschappen van de liesslagader. Er was een enkelvoudig genetisch effect van het *AGT* promoter haplotype op de eigenschappen van de arteria brachialis en PRA. Onze bevindingen suggereren dat de invloed van de renale sodium huishouding op de arteriële eigenschappen genetisch bepaald is, of omgekeerd, dat mutaties in dezelfde genen zowel de arteriële als de renale functie beïnvloeden.

Souhrn

V souladu s Guytonovou prací bylo cílem našeho výzkumu prozkoumat ve třech evropských populacích, zda jsou vlastnosti velkých tepen spojeny s ledvinným sodným hospodařením, jež samotné je ovlivněno zevními a genetickými vlivy. Před započetím genetických analýz jsme studovali rodinnou agregaci a děditelnost tepenných vlastností. Ve všech našich analýzách jsme zohlednili vliv příbuznosti mezi účastníky, stejně jako vliv významných proměnných.

V první práci jsem porovnávali vlastnosti tepen a krevní tlak (TK) mezi normotenzními potomky dvou normotenzních rodičů (OFF/NT) a normotenzními potomky, kteří měli alespoň jednoho hypertenzního rodiče (OFF/HT). Pulzní tlak (PPp) jsem měřili jak konvenčně, tak pomocí 24-h monitorace TK. Centrální (CAIx) a periferní (PAIx) augmentační index, centrální pulzní tlak (PPc) a rychlost pulzové vlny (aPWV) jsme měřili přístrojem SphygmoCor. V porovnání s OFF/NT (n=59; věk 16 až 34 let), měli OFF/HT (n=174; 17 až 40 let) vyšší (0,14<P<0,0007) konvenční TK, měřený jak konvenčně (121/75 *vs.* 114/71 mm Hg a 46 *vs.* 42 mm Hg) tak 24-h monitorací TK (118/70 *vs.* 114/67 mm Hg a 48 *vs.* 47 mm Hg). OFF/HT v porovnání s OFF/NT, měli také vyšší (0,05<P<0,0008) PPc (28 *vs.* 26 mm Hg), PAIx (54,7% *vs.* 44,9%), CAIx (108,8% *vs.* 99,8%), a aPWV (7,4 *vs.* 6,6 m/sec). Nicméně pro CAIx, PAIx a aPWV rozdíly mezi potomky vymizely po komplexní adjustaci zahrnující střední arteriální tlak a věk.

V populačním vzorku založeném na rodinách, skládající se z 204 rodičů (průměrný věk 51,7 let) a 290 potomků (29,4 let), jsme zkoumali děditelnost a rodinnou agregaci PPp, CAIx, PAIx a aPWV. Fenotypickou korelaci mezi těmito parametry jsme rozdělili na sdílenou genetickou a environmentální komponentu. Nalezli jsme významnou děditelnost pro PPp, CAIx, PAIx a střední arteriální tlak v rozmezí od 0.37 do 0.41 (P≤0,0001). Korelační koeficient mezi rodiči a potomky byl významný pro všechny tepenné indexy (r≥0,12; P≤0,02) s výjimkou PPc (P=0,90). Korelace mezi sourozenci byla významná pro CAIx (r=0,22; P=0,001). Genetické korelace mezi aPWV a ostatními tepennými indexy byly významné (ρ_G≥0,29, P<0,0001). Korespondující environmentální korelace byly významné pouze pro PPp (ρ_E=0,10. P=0,03).

Ve vlámské populaci jsme sonograficky měřili průměr, poddajnost (CC) a roztažnost (DC) karotické, brachiální a femorální tepny. V multivariátní analyze 1069

neléčených jedinců (průměrný věk 41,6 let), CC a DC femorální tepny vzrůstala s vyšší frakční reabsorpcí sodíku v distálním tubulu (RNa_{dist}) měřeného pomocí clearance endogenního lithia. Rozdíly spojené se změnou o 1-SD RNa_{dist} byly 51,7 $mm^2/kPa \times 10^{-3}$ (P=0,0002) a $0,56 \times 10^{-3}$/kPa (P=0,004) pro femorální CC a DC. U žen stejně jako u mužů, vzestup o 1-SD frakční proximální reabsorpce sodíku (RNa_{prox}) byl spojen s menším průměrem femorální a brachiální tepny čítajícím 111.6 μm (P=0,003) a 52,5 μm (P=0,016). Nenalezli jsme konzistentní spojitost mezi elastickou karotickou tepnou a ledvinným sodným hospodařením.

Ve stejné vlámské populaci čítající 1126 jedinců (průměrný věk 43,8 let) jsme zkoumali zda vlastnosti tepen mohou souviset s geny kódujícími *ADD1* (*Gly460Trp*), *ADD2* (*C1797T*) a *ADD3* (*A386G).* V single-gene analýze byl průměr brachiální tepny o 0,15 mm (P=0,0022) větší, a brachiální CC a DC byly o 0,017 mm^2/kPa (P=0,0029) a 1,55 10^{-3}/kPa (P=0,013) nižší u *ADD3 AA* než *ADD3 GG* homozygotů s aditivním účinkem alely *G*. V multiple-gene analýze, asociace mezi průměrem a DC brachiální tepny a *ADD3 G* alelou byla přítomna pouze u *ADD1 GlyGly* homozygotů. Jiné asociace mezi tepennými vlastnostmi sledovaných tří tepen a *ADD1* nebo *ADD2* polymorfismy nebyly významné. Nenašli jsem důkaz pro populační stratifikaci ($0.07 \leq P \leq 0.96$). Přenos mutované *ADD3 G* alely byl spojen s menším průměrem brachiální tepny u 342 informativních potomků (–0,12±0,04 mm; P=0,0085) a u 209 potomků, kteří byli homozygotní pro *ADD1 Gly* (–0,14±0,06 mm; P=0,018).

Nakonec jsme u 1064 vlámských jedinců (průměrný věk 43,6 let) hodnotili efekt genů pro *ADD1* (*Gly460Trp*), *AGT* (*C–532T* a *G–6A*) a *AT1R* (*A1166C*). U nositelů *ADD1 Trp* alely, ale ne u homozygotů pro *ADD1 Gly* (P hodnota pro interakci ≤0,014), CC femorální tepny byla významně vyšší (0,74 *vs.* 0,65 mm^2/kPa; P=0,020) u nositelů *AT1R C* alely než u *AT1R AA* homozygotů, s podobným trendem pro DC femorální tepny (11,3 *vs.* 10,2 10^{-3}/kPa; P=0,055). Family-based analýza potvrdila tyto výsledky. Průměr brachiální tepny (4,35 *vs.* 4,18 mm) a plasmatická reninová aktivita (PRA, 0,23 *vs.* 0,14 ng/ml/h) byly vyšší ($P \leq 0,005$) u homozygotů pro *AGT CG* haplotyp v porovnání s ostatními jedinci, zatímco opak byl pravdou pro DC brachiální tepny (12,4 *vs.* 14,4 10^{-3}/kPa; P=0,011). Nenašli jsme žádnou interakci mezi *AGT* a jinými geny v souvislosti se sledovanými fenotypy.

Závěrem lze říci, že v této doktorské práci jsme prokázali významnou rodinnou agregaci a významnou děditelnost tepenných vlastností. Dále jsme ukázali, že vyšší

RNa_{dist} byla spojena s vyšší femorální CC a DC, a že vyšší RNa_{prox} byla spojena s menším průměrem muskulárních tepen. Tato pozorování opravňovala naše další analýzy genů zapojených v ledvinném sodném hospodaření. U *ADD1 GlyGly* homozygotů, byly vlastnosti brachiální tepny spojeny s *ADD3* (*A386G*) polymorfismem. Dále, *ADD1* a *AT1R* společně ovlivňovali elastické vlastnosti femorální tepny. Mimo to jsme nalezli single-gene efekt haplotypů v promotoru *AGT* na vlastnosti brachiální tepny a PRA. Naše výsledky tedy naznačují, že geneticky determinované ledvinné sodné hospodaření ovlivňuje vlastnosti tepen, nebo naopak že společná genetická dráha může ovlivňovat jak tepenné tak ledvinné funkce.

Curriculum vitae

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Education:

1996-2003	Charles University in Prague, Faculty of Medicine in Pilsen Exams to obtain the degree of doctor in medicine (M.D.): June 2003
2003 to present	PhD student, Charles University in Prague
2004 to present	PhD student, University of Leuven, collaborative program with Charles University in Prague

Occupation

- Clinical Intership (Internal Medicine), II Internal Department, Faculty Hospital in Pilsen, the Czech Republic

Research experience

From 2006 to 2007, she was one of the key personnel in Pilsen of EPOGH Project (European Project on Genes in Hypertension). During this time, she was responsible for conducting the field work and technical examination. In 2008, she was responsible for conducting and organising of SAS (Study of Active Seniors) in Pilsen. In 2008, she was also participating in MONICA study.

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