

CARDIOVASCULAR AND ANTHROPOMETRIC TWIN STUDIES

PhD thesis

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Cardiovascular and anthropometric twin studies

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The list of Abbreviations

A = additive genetic influence
AC = arterial compliance
AIC = Akaike's Information Criterion
Aix = augmentation index
BIA = bioelectrical impedance analysis
BMI = body mass index
BP = blood pressure
C = common familial environmental factors
CC = correlation coefficient
CCA = common carotid artery
CI = confidence interval
CT = computed tomography
CVD = cardiovascular disease
D = non-additive (dominant) genetic factors
DBP = diastolic blood pressure
DD = diastolic intraluminal diameter
Di = distensibility
DNA = deoxyribonucleic acid
DZ = dizygotic
E = unique environmental factors
GWAS = genome wide association study
ICA = internal carotid artery
IMT = intima-media thickness
MRI = magnetic Resonance Imaging
MZ = monozygotic
P = probability
PP = pulse pressure
PWV = pulse wave velocity

SBP = systolic blood pressure

SID = systolic intraluminal diameter

SD = standard deviation

SNP = Single Nucleotide Polymorphism

USA = United States of America

V = arterial volume

1. Introduction

1.1. Background of twin studies

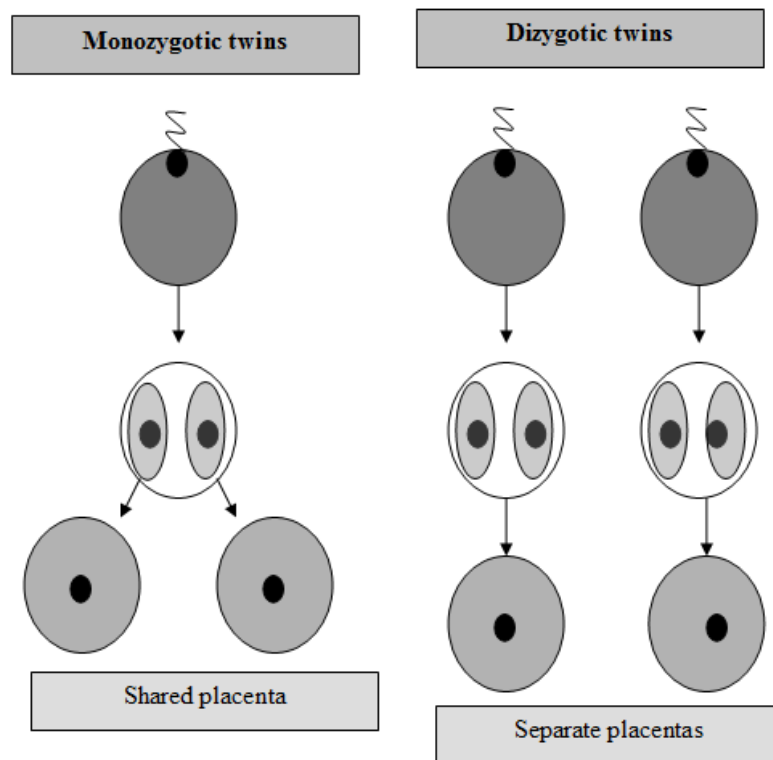
The subject of „nurture or nature” has always been a question in medicine, namely, whether genetic or environmental effects determinate a certain trait. In the 19th century, determinism and indeterminism represented the two inordinated viewpoints (Métneki 2005). While the deterministics believed that genetic traits are already defined at birth and definitely specify the individual’s walk of life (without any environmental effects), indeterministics showed evidences that the environment and the education is essential only (Métneki 2005).

Nowadays we know that the source of different traits derives from environmental and/or genetic effects as well. There are diseases which are only genetically defined without any role of environmental factors (eg., chromosome or monogenous defects), or determined by the environment only (eg., infections, nutritional disorders, injuries). However, most of the traits (diabetes, hypertension, epilepsy, etc.) have multifactorial background, namely, both genetic and environmental factors have importance, but in different portions. Francis Galton, grandnephew of Darwin realised in the 19th century that twin studies are of help to calculate the portion of genetic and environmental influence in percentage in case of any traits (Métneki 2005).

Monozygotic (MZ) twins share nearly 100% of their genes, since the two embryos originate from one fertilized egg (**Figure 1**). Therefore, the observed differences between the two members of a monozygotic twin pair are attributed to environmental factors (Métneki 2005). The timing of the division of the fertilized egg determinates whether the monozygotic twin embryos will develop in the same or different caul, and if the nutrition will be provided from identical or different placenta. Accordingly, monozygotic twins may be dichorionic diamnionic (in case of early detachment: on the 2nd - 3rd day after fertilization), monochorionic diamnionic (if the detachment happens on the 4-8th days after fertilization), and finally, monochorionic monoamnionic (in case of late detachment after the 9th day

after fertilization). Accordingly, monozygotic twins can be only same-sex, and their all genetically determined traits (eg., blood group) are the same (Métneki 2005). On the other hand, fraternal or dizygotic (DZ) twins share 50% of their genes, as the not at the same time born siblings, therefore, the observed differences between the members of a dizygotic twin pair are due to either genetic or environmental effects. Dizygotic twinning happens if two offsprings born of the same pregnancy and develop from two ova that were released from the ovary simultaneously and fertilized at the same time. Dizygotic twins may be of either same or opposite sex, and have two separate and distinct placentas and membranes, therefore, they are always dichorionic diamnionic. Nearly two-third of the twin pregnancies are dizygotic (Métneki 2005).

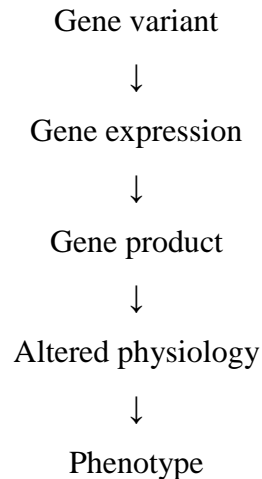
Figure 1. Development of identical and fraternal twins



Twin studies contribute to the discovery of the relationship of genes, environment and diseases, and help to explore the source and genetic determinacy of disorders. Nowadays, twin studies are combined with new gene technologies and have an

outstanding importance in gene localisation and exploring metabolic pathways which might open new ways in prevention and therapy (Métneki 2005).

Genetic etiology of a disease (Métneki 2005):

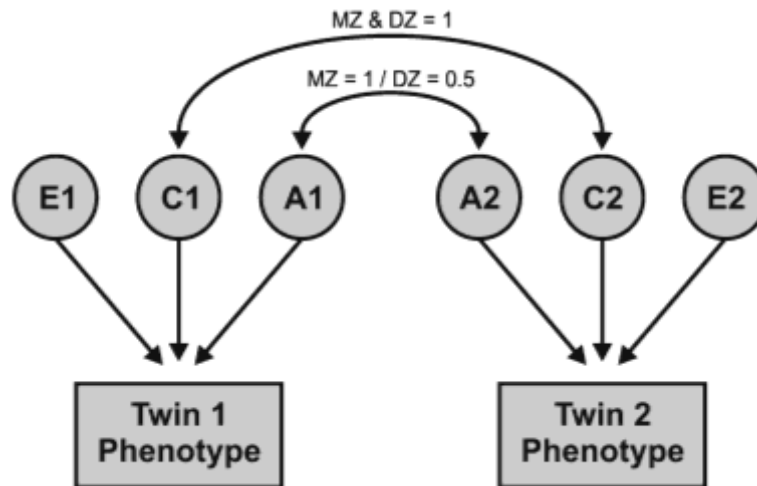


Nowadays, three types of twin studies include classical twin studies (1), the investigation of only monozygotic twins (2), and monozygotic twins reared apart (3) (Métneki 2005).

1. *Classical twin study design* involves both monozygotic and dizygotic twins. Higher correlation in monozygotic than in dizygotic twin pairs provides evidence for additive genetic influence (A) on a phenotype (effects due to genes at multiple loci or multiple alleles at one locus). Similarity of correlations suggests a contribution of the common familial environmental factors (C) shared by the twins (e.g., familiar socialization, diet, exposure to high levels of air pollution, shared womb, etc.). Finally, unique environmental factors (E), or unshared environmental factors that affect one twin but not the other, are estimated using the deviation from perfect MZ co-twin correlation (Neale et al 2006). Univariate quantitative genetic A-C-E models are fitted to decompose phenotypic variance of the considered parameters into additive genetic effects, or heritability (Neale et al 2006). The A-C-E model is able to estimate these components by capitalizing on several reasonable assumptions (**Figure 2**). Thus, identical twins share their genome ($r=1.0$) while this correlates $r=0.5$ for fraternal twins. Moreover, on average both monozygotic and dizygotic twins equally share their common environment ($r=1$ for both

monozygotic and dizygotic twins). Accordingly, environmental confounders are minimized because twin children are usually exposed to similar environments. The unique environment of co-twins remains uncorrelated for both zygositys. In the structural equation model, A-C-E components are latent variables but these variables for both co-twins are related to each other based on the described structure giving us the possibility to estimate the proportions of interest. In addition, longitudinal classical twin studies are useful for investigating the longitudinal contribution of genes on a phenotype (Métneki 2005, Neale et al 2006).

Figure 2. *Univariate A-C-E model. Rectangles denote the observed variables (i.e., cardiovascular or anthropometric parameters) and circles denote the latent variables. Curved arrows denote correlations (fixed at the highlighted values). Straight arrows signify the estimated impact of the latent factor on variance of the observed phenotype. Letters A, C, and E stand for additive genetic, common environmental, and unique environmental influences, respectively. (Courtesy of Dr. Tamás Horváth, Institute of Human Physiology and Clinical Experimental Research, Semmelweis University, Budapest, Hungary)*



2. *Investigation of only monozygotic twins* is of the increasing interest of current twin research (Métneki 2005). Disturbance of DNA methylation leading to aberrant gene expression has been implicated in the etiology of many diseases. Since monozygotic twins share a common DNA sequence, their study represents an ideal design for investigating the contribution of epigenetic factors to a disease etiology (Métneki 2005). In the recent years, therefore, twin

researchers have been started to focus on monozygotic twin pairs discordant for a phenotype (eg., obesity) in order to assess the influence of epigenetic factors which are responsible for discordance between the members of monozygotic twin pairs. These epigenetic factors mean cellular modifications (usually comprise DNA methylation and histone modifications) that can be heritable to the next generation, but appear unrelated to DNA sequence changes, and can be modified by environmental stimuli (Bell and Spector 2011, Holliday 1994). Recent studies have focused on the investigation of DNA methylation patterns in twins, and reported increasing differences in epigenetic profile by aging due to common or unique environmental exposures (eg., X-chromosome inactivation patterns) (Kaslow et al 1987, Vickers 2001, Fraga 2005). The most interesting first study assessed the epigenetic profile (DNA methylation and histone acetylation) at multiple genomic regions in 3-year-old and 50-year-old monozygotic twins and reported highly similar intrapair epigenetic profiles, whose epigenetic variability increased with age across multiple tissues especially in twins with greater difference in lifestyle (Fraga 2005). Furthermore, genome-wide analysis of DNA methylation on peripheral blood DNA samples are obtained on these samples of monozygotic twin pairs discordant for a certain disease (Fraga 2005).

3. The most challenging twin study profile is the examination of *monozygotic twins reared apart* which is appropriate for studying genetic effects in different environments (Métneki 2005). Mainly psychologic studies are carried on these kind of twin pairs, however, it is getting even more difficult to collect enough sample due to less frequent switch-baby incidents in the hospital (Jonason 2012, Segal and Stohs 2007, Segal 2009).

Twin studies have advantages in comparison with family and genome wide association studies (GWAS) or whole genome association studies which will be discussed later.

Family-based association studies assess the appearance of an investigated phenotype in a multi-generation model and thus provide important information on the genetic etiology of certain disease traits via considering transmission of genotype within a family (Cupples 2008). These kind of studies not only identify and quantify the family risk and heritability of certain phenotypes but also permit segregation, linkage and association analyses, and have importance even in the modern GWAS era (Cupples 2008). The favorable features of family studies are the following (Borecki and Province 2008): 1. Studies of extended pedigrees or nuclear families, are likely to represent a more homogeneous and limited set of causative genes and pathways which enhance statistical power for gene discovery. In addition, this advantage leads to discovery of novel loci and pathways (Borecki and Province 2008). 2. Because family members share a predictable extent of their genetic background and environmental exposures identical-by-descent, the background genetic variation is controlled to some extent as a function of the degree of relationship, which can be modeled as a polygenic component (Borecki and Province 2008).

We must note that familial aggregation of a phenotype does not necessarily indicate that the disease is primarily determined by genetic components. Non-genetic factors may have similar effect on observed phenotypic variance since families share not only gene polymorphisms, but also many cultural, behavioral, and dietary habits that define critical environmental exposures (Susser and Susser 1987). An important drawback of family studies is that even if they are useful to determine inter-generation resemblance or difference, but these kind of studies cannot reliably distinguish heritability and common environmental effects (Susser and Susser 1987). Thus, genetic variance can be confounded by shared environmental factors that exist among related individuals and are usually environmentally context-specific (Susser and Susser 1987). Heritability estimates for individuals exclusively from one environment might differ from those calculated for individuals from disparate environments (Vitzthum 2003). Therefore, better understanding may be derived from studies based on the twin design, which is particularly suitable to distinguish heritability from effects of shared and unshared environmental influence. Accordingly, twin studies can take the shared family environment into

account, including potentially deleterious and confounding effects (eg., salt intake, alcohol use, and lack of physical exercise).

Two general ways for gene discovery include *linkage and association studies* (Borecki and Province 2008, Borecki and Province 2008). Linkage studies investigate the alleles at loci that are close together to be transmitted together as an intact unit (haplotype) within families („where is the gene?") (Borecki and Province 2008). For linkage studies, DZ twin pairs can be also studied as sib-pairs since they perfectly match their ages and thus reduce the environmental variation affecting the phenotype (Borecki and Province 2008). On the other hand, MZ twins are used to estimate allele frequencies for the markers tested (Luft 2001). By contrast, association studies can be performed either in families or in unrelated individuals. GWAS provides an opportunity to comprehensively examine associations of genetic variation with disease phenotypes.

On the course of GWAS, DNA is extracted from collected blood specimens of two groups (case-control method: a person with disease and another without), and the investigated gene variants are characterized using Single Nucleotide Polymorphisms (SNPs) represented on chips (these are usually high-density custom arrays that capture DNA variation at regions identified by well-powered GWAS meta-analyses for diseases and traits) and finally compared. Accordingly, if one allele is more frequent in the individuals with the investigated disease, the SNP is said to be "associated" with that certain disease. Although recent genome-wide studies have yielded to valuable genetic results on various traits by investigating the entire genome, only a relatively little of the heritability of most complex traits have been explained (Eichler et al 2010). This debate has led to the search of the 'missing heritability' of complex diseases. Accordingly, genome-wide linkage analysis usually underreport heritability (Vineis and Pearce 2010, Eichler et al 2010).

Limitation of twin studies must be noted (Pearson 2008). Environmental exposures may not be identical even in MZ twins. MZ twins can have different gene expressions. The risk of the genotype may be heterogenous between twin

pairs. Ascertainment bias: Co-twin with disease is more likely to participate in twin studies as compared to unaffected co-twin (Pearson 2008).

Statistical twin study methods

1. The basic *univariate quantitative genetic A-C-(D)-E model*, which was described briefly above, is able to estimate the contribution of additive genetic (A), non-additive genetic (D), common or shared environmental (C) and unique or unshared (E) environmental variance components (Neale and Cardon 1992, Littvay 2012) (**Figure 2**). A descriptive estimate of the genetic influence on a single trait can be calculated using the within-pair (co-twin) correlation in MZ and DZ pairs separately. A higher within-pair correlation in MZ than in DZ pairs provides evidence for existing genetic influences on the phenotype (Neale and Cardon 1992, Littvay 2012). Structural equation modeling is used to estimate the genetic and environmental influences on the investigated variables as well as the degree to which genetic and environmental factors overlap with another trait. According to the classical twin study methodology, genetic variation can be divided into additive and dominant genetic effects, which have an expected correlation of 1 within MZ pairs and correlations of 0.5 and 0.25, respectively, within DZ pairs. The A component refers to the sum of the allelic effects on the phenotype over all susceptible loci, whereas D refers to interaction effects between alleles at the same locus. The environmental variation can be either common to both twins within a pair or unique to each twin individual, and has (by definition) a correlation of 1 and 0, respectively, within both MZ and DZ twin pairs. The common environment includes all environmental factors that make the twin pair similar for the trait, such as shared childhood experiences or parental socioeconomic status. The unique environment includes all environmental factors and experiences that make the twin pair dissimilar, such as diseases or accidents that have affected only one sibling within a pair. The E component also includes measurement error. In case of a relatively small sample size the degrees of freedom is not strong enough to analyze the four components (A, D, C, E) together. Accordingly, three components are usually measured (if no additive genetic effect is present, it is not worth to examine the dominant genetic influence) (Neale and Cardon 1992, Littvay 2012).

In case of a large sample size, different combinations of these components (e.g. ACE, ADE, AE, CE) can be hypothesized to account for the pattern of variation in twin data, but effects due to dominance and common environmental effects cannot be estimated simultaneously with data limited to that from twins reared together (Hansen et al 2007).

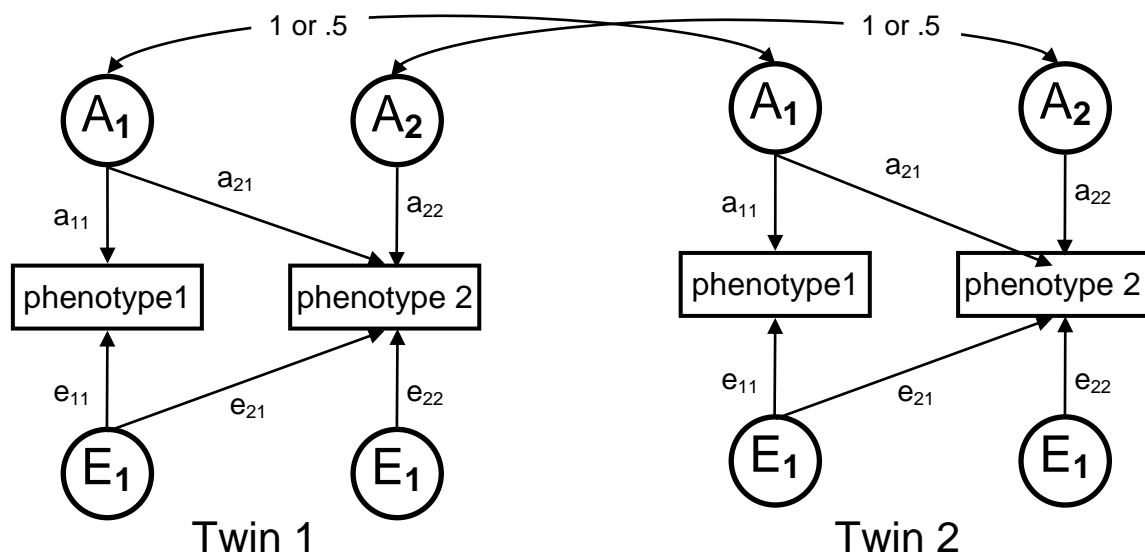
2. Beyond the classical A-C-E model, a *bivariate Cholesky decomposition model* can be also carried out to derive the magnitude of covariation between two investigated phenotypes of interest and to estimate what proportion of this correlation is attributable to common underlying genetic and environmental factors (**Figure 3**) (Neale and Cardon 1992, Littvay 2012). In order to estimate the amount of overlap between genes or environment that influences the two parameters, genetic (r_g) and environmental correlations (r_c , r_e) between a pair of measures are calculated. For example, the genetic correlation indicates the extent to which genetic effects on one trait correlate with genetic effects on another trait, independently of the heritability of the two traits. A genetic correlation of 1.0 would indicate that genetic influences on the two traits completely overlap, whereas a genetic correlation of 0 would indicate that entirely different genes influence the two traits. By including the heritability of the measures, it is also possible to estimate the extent to which genetic and environmental factors contribute to the observed phenotypic correlation between two traits (bivariate heritability) and the extent to which the two traits share common genetic variance (r_g^2) (Neale and Cardon 1992, Littvay 2012).

3. For both *univariate and bivariate modeling*, the significance of each parameter in the model is tested by dropping the parameter and evaluating the change in $-2 \log$ likelihood between the full model and the nested submodel. Model comparisons are made with likelihood ratio χ^2 tests, where a significant change in χ^2 indicates that dropping the parameter significantly decreases model fit, suggesting that the parameter should be retained in the model. Akaike's Information Criterion (AIC) is always used to compare non-nested models. Within-pair intraclass correlations and heritabilities are usually always adjusted for age and

sex, and further adjustments can be also taken into account after rank-transformation to normality of the outcome variables (Neale and Cardon 1992, Littvay 2012).

Figure 3. Path diagram of the bivariate Cholesky AE model

Squares represent the observed variables. Circles represent latent variables, including additive genetic (A) and unique environment (E) components. a_{11} , Additive genetic influence acting on phenotype 1. a_{22} , Additive genetic influence acting on phenotype 2. e_{11} , unique environmental influence acting on phenotype 1, e_{22} , unique environmental influence acting on phenotype 2. a_{21} , genetic covariance between phenotype 1 and phenotype 2. e_{21} , unique environmental covariance between phenotype 1 and phenotype 2.



1.2 Cardiovascular twin studies outside Hungary

Twins have always been important 'tools' in the cardiovascular genetic research. Blood pressure was the first investigated cardiovascular trait which was shown to be heritable in a German twin study (Weitz 1925, Frohlich 1937, Luft 2001). Several years later, an English twin study certified the heritability of blood pressure

in school children (Stocks 1930). In the past decades, numerous twin studies assessed the heritability of blood pressure in order to search for ethnic and age group differences in genetic predisposition of these phenotypes (Borhani et al 1976, Hong et al 1994, Rao et al 1993, Somes et al 1995, Grim et al 1990). In addition, intermediary blood pressure phenotypes, such as components of the renin-angiotensin and sympathetic nervous system (plasma aldosterone, catecholamines), renal function, and the facility in excreting electrolytes, turned to be also heritable beyond blood pressure traits (Luft 2001).

Beyond the blood pressure traits, further cardiovascular phenotypes have been also studied in twins. In the early 1990's, cardiovascular reactivity was studied in an adult male American twin cohort (Carmelli et al 1991). Genetic influence was found in blood pressure reactivity to the mental arithmetic task after adjustments for baseline and performance, but no genetic variance for blood pressure reactivity to the cold pressor test was observed (Carmelli et al 1991). In addition, numerous twin studies reported heritability values of traditional cardiovascular risk factors, eg. lipoproteins, alcohol consumption or obesity (Lamon-Fava et al 1991, McGue et al 1992, Carmichael et al 1995). Additional studies were also carried out concerning blood pressure regulation which yielded to identification of blood pressure-related SNPs, such as on β -2 AR gene which underscored the importance of the β -2 AR gene to blood pressure regulation, heart size, and probably to the development of hypertension (Luft 2001, Busjahn et al 2000). In the recent decades, studies aimed to explore the genetic contribution to the newly „detected” blood pressure-related phenotypes, such as central blood pressure, arterial aging and arterial stiffness which will be described below in more detail.

Thanks to twin studies, it was also shown that genetic effects have an important influence in the development of certain heart and vascular diseases as well (Berg 1987, Lindpaintner 1994, Busjahn et al 1998, Busjahn et al 1999). For example, cardiac hypertrophy, arrhythmia, long QT associated sudden cardiac death and coronary heart disease was also turned to be heritable (Berg 1987, Lindpaintner 1994, Busjahn et al 1998, Busjahn et al 1999).

Due to the increasing number of investigable cardiovascular phenotypes, twin studies will continue to gain in importance and utility, particularly in elucidating normal human genetic diversity in the future (Luft 2001).

1.3 Arterial stiffness and hemodynamic variables

Blood pressure regulation involves complex interactions among genetic and nongenetic factors, providing major challenges to dissection of the genetic components that influence blood pressure and hypertension. Twin studies (Vinck et al 2001, Snieder et al 2003) and nuclear family studies (Knuiman et al 1996, Mitchell et al 1996, An et al 1999, Rotimi et al 1999, Livshits and Gerber 2001, North et al 2003) have shown that a sizeable proportion of systolic blood pressure and diastolic blood pressure variance is due to the effect of genes. More limited data are available on the heritability of pulse pressure (Mitchell et al 2005, Bochud et al 2005, Fava et al 2004, van Rijn et al 2007) and of central pressure (Cecelja et al 2009). Arterial stiffness is regulated by the amount, density and spatial organization of stiff wall material (Laurent et al 2005). Changes in the expression of genes associated with cell signaling and mechanical regulation of vascular structure may play an equally important role in the regulation of arterial stiffness (Durier et al 2003). In recent years, several genes associated with the renin-angiotensin-aldosterone system, beta-adrenergic and endothelin receptors, inflammatory molecules, and the transcriptional pathways controlling gene expression, differentiation of vascular smooth muscle cells, apoptosis of endothelial cells, and the immune response within the vascular wall have all been associated with arterial stiffness (Lacolley et al 2009). Although there is a consensus that genetic factors play a role in atherogenesis (Franklin et al 2009), the precise magnitude of the genetic influence and association of arterial stiffness, central systolic blood pressure and pulse pressure is poorly described.

1.3.1 Biomechanic background of arterial stiffness and hemodynamic variables

In recent decade, great emphasis has been placed on the role of arterial stiffness in the development and management of cardiovascular diseases. Arterial stiffness is a dynamic property, determined both by vascular function like vascular smooth muscle tone and by the structure of the vessel wall like elastin/collagen content (Van Bortel et al 2002). Arterial stiffness is inversely related to arterial distensibility (D_i), which is considered a determinant of stress on the vessel wall and defined as the relative change in volume per unit of pressure ($[\Delta V/V]/\Delta P$) (Safar and London 1994). In addition, arterial compliance (C) is also an important vessel wall property which is defined as the change in volume per unit of pressure ($\Delta V/\Delta P$) and reflects the afterload on the heart and the buffering function of the vessel (Lévy and Safar 1990, Guyton 1986, Van Bortel et al 1995). Arterial compliance is related to arterial distensibility (D_i) and arterial volume (V) by the formula $C=D_i \times V$ (Guyton 1986).

Pulse wave velocity is inversely related to arterial wall distensibility and its calculation is a widely used method. Pulse wave velocity (as an arterial distensibility index) can be calculated from measurements of pulse transit time and the distance traveled by the pulse between two recording sites (eg., carotid-femoral) (Avolio 1991). Determination of pulse wave velocity enables one to evaluate indirectly arterial distensibility and stiffness.

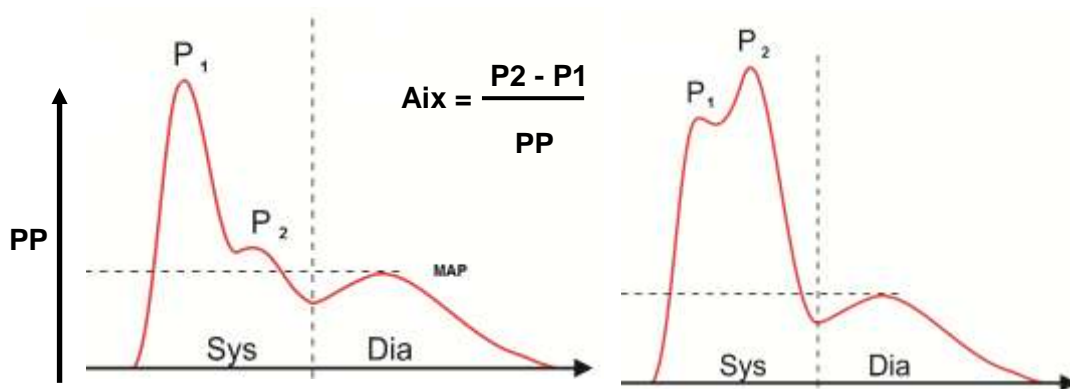
New risk factors have been determined in 2007 Guidelines for the Management of Arterial Hypertension including increased arterial stiffness (Mancia et al 2007). According to this latest guideline, an increase in arterial stiffness, due to pathological changes in large artery walls, is a direct measure of target organ damage (Mancia et al 2007). Endothelial dysfunction, associated with altered arterial stiffness, is a marker of increased cardiovascular risk (Kuvin et al 2001). Pulse wave velocity (PWV) can be estimated non-invasively and has an independent predictive value for cardiovascular events (Laurent et al 2006). Augmentation index (AIx) is given by the ratio of the augmentation pressure and the pulse pressure (PP), being used ever more often in studies as parameters of

wave reflection (Snieder et al 2000) (**Figure 4**). Augmentation indices can be calculated both at brachial and aortic sites.

Figure 4. Measurement of augmentation index.

Augmentation index informs us about wave reflection. Two characteristic pulse waveforms can be distinguished. The first reflected wave (P_1) is a forward wave following the heart ejection, and the second one (P_2) is the reflection from the periphery, coming still in systole (reflected wave). Normally, the second peak is located the amplitude of the first peak (on the left). In case of impaired vascular function (on the right), as the peripheral vascular function is getting worse, the wave reflection increases (pulse pressure, PP). The augmentation index shows the difference between the two peaks' heights and gives an evaluable and understandable value of this increase.

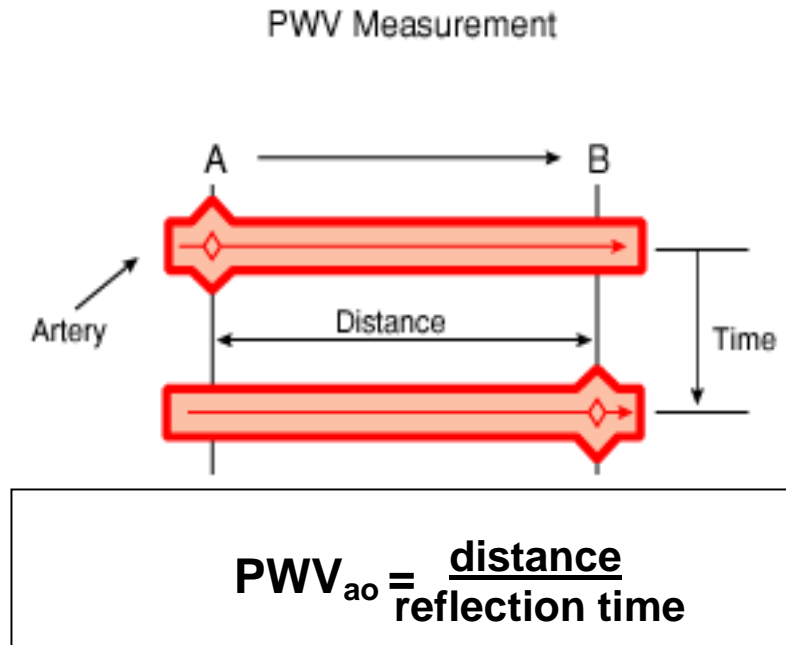
Courtesy of Ágnes Lannert and Balázs Varga (Medexpert Ltd.)



PWV, the most important measure of arterial stiffness, is characterized by the distance traveled (s) by the wave divided by the time (t) for the wave to travel that distance (**Figure 5**):

$$PWV = \Delta s / \Delta t.$$

Figure 5. Measurement of aortic pulse wave velocity based on forward traveling wave between points A and B. Courtesy of Ágnes Lannert and Balázs Varga (Medexpert Ltd.)



It has also been shown that measurement of arterial vascular stiffness and wave reflection can stratify patients with a high risk of cardiac and cerebral events who might profit from more aggressive cardiovascular treatment (Baulmann et al 2004, Nilsson et al 2009). It is well known that arterial stiffness increases with age mainly due to the development of isolated systolic hypertension in elderly (Zeki et al 2013). In addition, certain diseases are also related to increased arterial stiffening, such as diabetes mellitus, hypercholesterolaemia, hypertension and end-stage renal disease (Glasser et al 1997). In general, these conditions increase the central stiffness due to the unfavorable effect of risk factors resulting less compliant central arteries (**Figure 6.**). On the course of pulse wave analysis, the properties of the pressure pulse wave, which travels from the heart toward the peripheral arteries, are analysed (eg., amplitude, frequency). Frequency domain analysis allows the analysis of the pulse pressure wave at the aorta in time. Augmentation pressure is the increase of the pulse pressure wave due to the reflected wave. This pulse pressure amplification occurs in the large elastic and smaller conduit arteries in association with changes in the magnitude (amplitude) of each harmonic component of the wave (Avolio et al 2009).

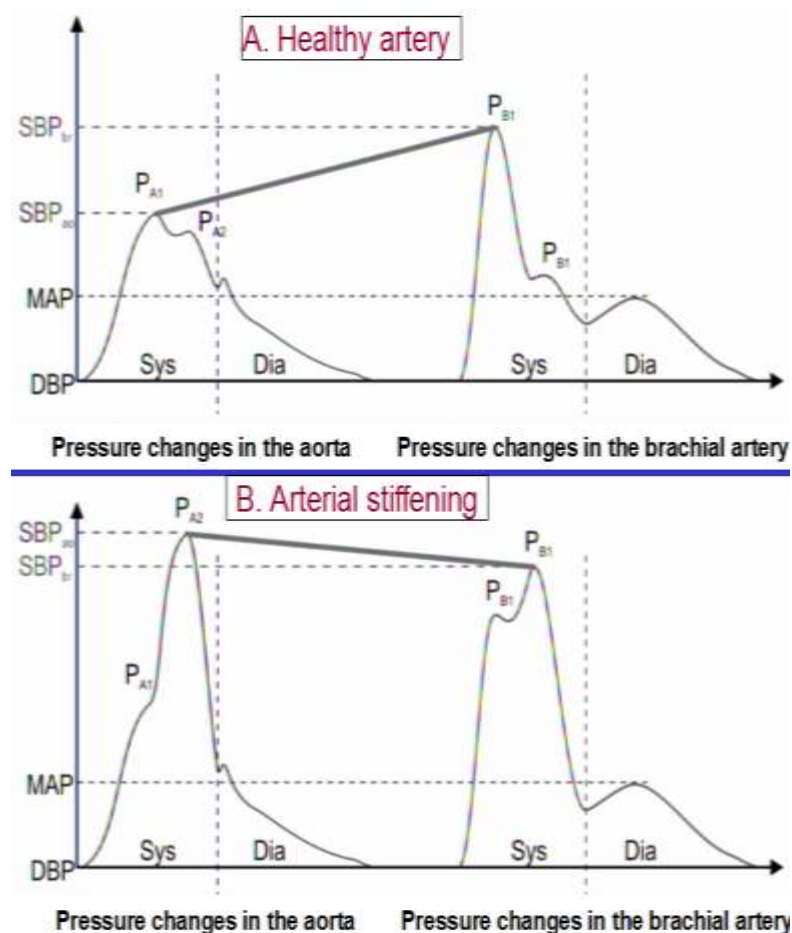
Figure 6. Development of arterial stiffening.

A. In healthy arteries the situation is the following: because of the tissue differences (elastic aorta, having the windkessel function and less elastic muscular type peripheral arteries) the blood pressure differs in the central arteries (aortic root) from the peripheral measured, and is lower in the elastic part.

B. If the total peripheral resistance and/or the cardiac output increases (among other factors), and the arteries have less compliance due to the unfavorable effect of risk factors, higher blood pressure can be measured in the aorta, while such a big difference cannot be observed in the periphery (brachial blood pressure).

Accordingly, worsening arterial function has unfavorable hemodynamic effects for the central arteries and the heart. Lower pulse pressure amplification increases the left ventricular afterload due to the increased stiffness of central arteries.

Courtesy of Ágnes Lannert and Balázs Varga (Medexpert Ltd.)



The definition of hypertension and the assessment of its prognostic value have long been based upon brachial systolic and diastolic blood pressure (BP) measurement (Lewington et al 2002). More recently, pulse pressure (PP), a measure of the pulsatile component of BP, has also been recognized as an important predictor of future cardiovascular events (Gasowski et al 2002). In elderly subjects, it has been more closely associated with the future development of cardiovascular disease than either systolic or diastolic BP (Franklin et al 2001, Glynn et al 2000, Vaccarino et al 2000, Franklin et al 2009). High brachial pulse pressure was shown as an independent predictor of cardiovascular mortality in both hypertensive and normotensive individuals, and in middle-aged and elderly subjects (Benetos et al 1998, Franklin et al 1999). In addition, long-term spontaneous increase in PP portends a high cardiovascular risk independently of absolute values of BP and other risk factors (Benetos et al 2000).

Although mean BP varies little from the aorta to the brachial artery, the oscillating pulse wave generated in the arterial tree by left ventricular contraction undergoes a progressive distortion with distance, due to changes in stiffness of the different arterial segments and the presence of wave reflections from the peripheral arteries (**Figure 6**). Thus, brachial systolic BP and pulse pressure provide an inaccurate measure of the corresponding values measured at the level of the ascending aorta (Nichols and O'Rourke 2005).

Central BP, a more direct measure than peripheral BP of the hemodynamic stress imposed on the myocardium and the coronary and cerebral circulation, has a closer relation to target organ damage (Roman et al 2010, Wang et al 2009) than peripheral BP and may be a more robust predictor of future cardiovascular complications (Roman et al 2007, Chirinos et al 2005, Pini et al 2008). An increase in central BP is influenced by arterial stiffness, pressure wave reflections from the geometry and vasomotor tone of small arteries, stroke volume, and heart rate (Mancia et al 2007). In a meta-analysis of 5 prospective studies, central PP was associated with a higher risk of future cardiovascular events, although its predictive superiority over brachial PP only bordered statistical significance (Vlachopoulos et al 2010). Increased central PP independently predicts cardiovascular mortality in

end-stage renal disease (Safar et al 2002). High PP involves an excessive arterial pressure pulsatility on the aortic wall which is associated with microvascular damage in the heart, brain, kidneys and contributes to an increased risk for major adverse clinical events involving these organ systems (Mitchell et al 2008). Notably, the different effects of BP-lowering drugs on brachial and central BP may partly explain their different vascular protective properties (Williams et al 2006). Aortic stiffness has also recently emerged as a strong, independent predictor of cardiovascular mortality and morbidity in patients with essential hypertension (Laurent et al 2001, Boutouyrie et al 2002). In hypertensive patients, increased carotid incremental elastic modulus can be observed independently of age, end-stage renal disease and mean blood pressure (Blacher et al 1999).

1.3.2 Noninvasive measurement of arterial stiffness

In order to detect changes in the pulse, volume or distension arterial waveforms before the appearance of clinically apparent vascular disease, several methods were developed for the assessment of arterial stiffness. Since evidences have shown that arterial stiffness is influenced, especially using pulse wave analysis, by body position, blood pressure, cardiac function, vasomotor tone, smoking, diurnal variation, recent nutrition, drinking and alcohol consumption, subject conditions must be standardized (van Bortel et al 2002). Accordingly, subjects must be in rest in a quiet room at least 10 minutes before the measurement, cannot sleep during the measurements, and subjects cannot drink coffee containing beverages, have meal and must refrain from smoking 3 hours prior to the assessment (Benowitz 1988, Kool et al 1991, Waaler et al 1991, Failla et al 1997, Wilkinson et al 1998, van Bortel et al 2002).

The assessment methods can be structured as follows (Cseprekál 2011, Laurent et al 2006):

A. Local arterial stiffness

Local arterial stiffness can be assessed either by echo tracking (ultrasound), applanation tonometry or video magnetic resonance imaging. Local arterial stiffness can be determined from (preferentially simultaneous) measurements of stroke changes in diameter and local pulse pressure (Laurent et al 2006). Most important assessed parameters include (Hayashi et al 1974, Kawasaki et al 1987, Hoeks et al 1990):

1. local PWV (eg., carotid);
2. strain, in brief, a percentage change of the lumen diameter during the cardiac cycle, or the amount of deformation relative to the unstressed state and expressed as percent change in the arterial diameter: $\text{strain} = (\text{SID} - \text{DD}) / \text{DD}$, where SID is the systolic and DD the diastolic intraluminal CCA diameter (mm);
3. stiffness (β) representing the stress (SBP-DBP)-to-strain ratio, as $\ln(\text{SBP}/\text{DBP}) / \text{strain}$, where SBP and DBP are brachial blood pressures measured in the systolic and diastolic cardiac cycle, respectively;
4. distensibility as the absolute and relative change in cross-sectional area (ΔA) of the vessel per unit of pressure change;
5. a) measurement of intima-media thickness allows calculation of Young's incremental elastic modulus characterizes the elastic properties of the arterial wall material by taking into account the thickness of the arterial wall. Young's modulus can be calculated as $[3 \times (1 + \text{Lcsa}/\text{IMcsa})] / \text{DC}$ where Lcsa means lumen cross sectional area IMcsa means intima-media cross sectional area, and DC characterize distensibility coefficients.
- b) pressure-strain elastic modulus (Peterson elastic modulus; inversely related to cross-sectional distensibility, and elastic properties of large arteries): $\text{EM} = K(\text{SBP} - \text{DBP}) / \text{strain}$, where $K = 133.3$ is the conversion factor for mmHg to Nm^{-2} .

B. Regional arterial stiffness

Regional arterial stiffness which measures the change in waveform between two sites. Oscillometry, tonometry (Sphygmocor, PulsePen), mechanotransducer method (Complior) and Doppler technique are the most common methods of the

measurement of regional arterial stiffness characterized by aortic, carotid-femoral, carotid-radial and femoro-tibial PWV (Cseprekál 2011, Laurent et al 2006).

C. Systemic arterial stiffness (waveform shape analysis)

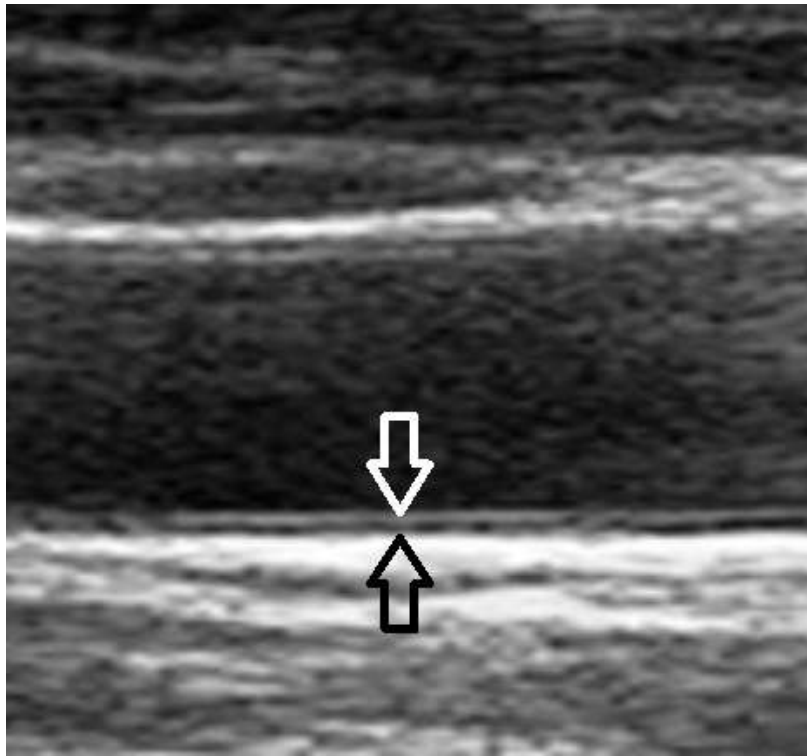
Systemic arterial stiffness can be estimated from models of the circulation based on analogies with electrical models combining capacitance and resistance in series (Laurent et al 2006). The measurement possibilities of systemic arterial stiffness include tonometry, oscillometry, mechanotransducer method, stroke volume and pulse pressure assessment, and finally, decrease of diastole and windkessel function using pulse wave analysis (Mahomed 1877). Accordingly, most important variables include proximal capacitive compliance, distal oscillatory compliance and systemic arterial compliance (Laurent et al 2006).

1.4 Carotid intima-media thickness

Carotid intima-media thickness (IMT), or the thickness of the intima and media layers of the carotid artery (*Figure 7.*) is a surrogate marker for atherosclerosis, and is associated with prevalent and incident cardiovascular disease (Bots et al 1997, Hollander et al 2002, Simon et al 2002, O’Leary and Polak 2002).

The carotid IMT can be easily, safely, reliably and inexpensively measured with B-mode ultrasound, and the predictive value increases when carotid IMT is measured at multiple extracranial carotid sites (Simon et al 2002, Paul et al 2012). Even if carotid IMT can be assessed at the near-wall and the far-wall of the carotid artery as well, studies suggest that the assessment of the far wall is more accurate due to the easier detection of media-adventitia interface at this location (Wikstrand and Wendelhag 1994, Wong et al 1993, Paul et al 2012). Color Duplex imaging mode can help in the surface detection as well by clear imaging of the circulating red blood cells in the arterial lumen.

Figure 7. Intima-media thickness measurement by ultrasound
(white arrow: intima, black arrow: media layer)



The normal carotid IMT measures 0.74 ± 0.14 mm in general (Mohan et al 2000). A recent 2012 study showed similar findings in a healthy Indian and Bangladesian population: 0.75 ± 0.12 mm was the mean carotid IMT value (Paul et al 2012). According to recent studies, normal carotid IMT is considered if it is <0.8 mm, and a value of carotid IMT ≥ 1 mm is associated with atherosclerosis and a significantly increased cardiovascular disease (CVD) risk in any age group (Simon et al 2002, Wikstrand et al 1994, Paul et al 2012). In addition, carotid IMT values increase with age and it is especially higher in the age group of 60-88 years than lower age groups (Stein et al 2004, Paul et al 2012). Moreover, carotid IMT is always higher in men than women (Kablak-Ziembicka et al 2005, Paul et al 2012).

Recent studies have shown that carotid IMT is mostly determined by genetic factors (Zannad et al 1998, Zannad and Benetos 2003, Zhao et al 2008). Heritability estimates of carotid IMT ranged from 24% to 59% based on three twin studies (Zhao et al 2008, Swan et al 2003, Jartti et al 2002).

1.5 Anthropometric cardiovascular risk factors and their assessment modalities

Obesity is a complex condition of excessive fat accumulation linked to major adverse health effects including the development of type 2 diabetes, cardiovascular disease, and certain forms of cancer (Field et al 2001, Bianchini et al 2002). Obesity has reached epidemic proportions worldwide with more than one billion overweight adults of which at least 300 million are clinically obese (Nguyen and El-Serag 2010). On the basis of body fat percentage, prevalence of obesity was 17.9% for boys and 12.8% for girls in a Hungarian study conducted among schoolchildren (Antal et al 2009). In an American study performed among schoolchildren, 30.5% of girls and 26.8% of boys were above the 95th percentiles for BMI-for-age (Caballero et al 2003). Underlying mechanisms of the rising obesity epidemic are still unclear. Environmental effects undeniably contribute to the body's energy balance through modifying caloric intake and physical activity (Herrera and Lindgren 2010, Qi and Cho 2008). However, not everyone becomes obese and there is considerable variation in individual responsiveness to obesogenic environments (Wardle et al 2008). Large epidemiological studies based on family, adoption, and twin relations indicate that genetic influences contribute substantially to variation in obesity (Allison et al 1996, Haworth et al 2008). Collectively, these findings suggest that gene-environment interactions may particularly increase the risk of obesity among those who are genetically predisposed to weight gain.

Body mass index (BMI) is a simple but quantitative anthropometric estimate of obesity based on height and weight that applies to adult men and women. However, the relationship between BMI and body fat may significantly vary by age, gender, and ethnicity (Gallagher et al 2000). For instance, athletes can present with high values of BMI but normal or low fat percentage (Seagle et al 2009). Moreover, the definition of obesity based on BMI alone may highly vary by geographical areas (Shiwaku et al 2004). Accordingly, body fat percentage that exceeds normal levels may indicate obesity more reliably than BMI alone and there is a recommendation

for the normal range of body fat content in both genders (Position of the American Dietetic Association, 2009) (*Table 1*).

*Table 1. Recommended amount of body fat according to gender
(Position of the American Dietetic Association, 2009)*

For women:	<ul style="list-style-type: none"> • The recommended amount of body fat is 20-21% (at least 10%). • The average American woman has approximately 22-25% body fat. • A woman with more than 30% body fat is considered obese.
For men:	<ul style="list-style-type: none"> • The recommended amount of body fat is 13-17% (at least 8%). • The average American man has approximately 17-19% body fat. • A man with 25% body fat or higher is considered obese.

Body fat percentage refers to the amount of body fat mass in regards to the total body weight expressed as a percentage as follows:

Body fat percentage (%) = (Body fat mass / Body weight) × 100 where body fat mass and body weight are expressed in kg.

Interpretation of body fat percentage results based on NIH/WHO guidelines for age are shown in *Table 2* (Gallagher et al 2000).

*Table 2. Interpreting Body Fat Percentage values according to age
(Gallagher et al 2000)*

Gender	Age	Low	Normal	High	Very High
Female	20 - 39	< 21.0%	21.0 - 32.9%	33.0 - 38.9%	> 39.0%
	40 - 59	< 23.0%	23.0 - 33.9%	34.0 - 39.9%	> 40.0%
	60 - 79	< 24.0%	24.0 - 35.9%	36.0 - 41.9%	> 42.0%
Male	20 - 39	< 8.0%	8.0 - 19.9%	20.0 - 24.9%	> 25.0%
	40 - 59	< 11.0%	11.0 - 21.9%	22.0 - 27.9%	> 28.0%
	60 - 79	< 13.0%	13.0 - 24.9%	25.0 - 29.9%	> 30.0%

Levels significantly above these amounts may indicate excess body fat. Athletes, leaner individuals, and more muscular individuals will have a body fat percentage lower than these levels. Furthermore, body composition is a sensitive indicator of health and nutritional status. There are different methods to estimate body composition parameters (*Table 3*).

Table 3. Different methods of body composition measurements (Kiebzak et al 2000)

Method	Description
Calipers	It measures the thickness of subcutaneous fat in multiple places on the body (abdominal area, subscapular region, arms, buttocks and thighs).
Bioelectrical impedance analysis (BIA)	It uses the resistance of electrical flow through the body to estimate body fat
Air displacement plethysmography	Subjects enter a sealed chamber that measures their body volume through the displacement of air in the chamber. Body volume is combined with body weight (mass) in order to determine body density.
Dual energy X-ray absorptiometry	Measures body composition, bone mineral content and density, lean tissue mass, fat tissue mass, and % fat values
Hydrostatic weighing	Following the dry weight of the subject is determined the subject sits on a specialized seat and is lowered into the tank until all body parts are emerged. The person must remain motionless underwater while the underwater weight is recorded.
Measurement of total body water by deuterium oxide dilution	Deuterium oxide isotope is given orally to subjects after saliva sample has been obtained to determine background deuterium in body water. After a 3.5 h period, another saliva sample is collected to measure deuterium content by mass spectrometry and calculate total body water from the extent of dilution.
Magnetic Resonance Imaging (MRI)	The most precise body composition measures to date.
Computed Tomography (CT)	Comparable to MRI, but involves radiation exposure.

Bioelectrical impedance analysis determines electrical impedance, or the opposition to current flow of an electric current through body tissues, in order to estimate total body water (Kyle et al 2004). In early studies, bioelectrical impedance analysis

highly varied and was not considered an accurate measure of body composition parameters, except for individuals with very low or very high BMI (Biaggi et al 1999). More recently, bioelectrical impedance analysis has become a commonly used, low-cost approach to assess body composition in a variety of health care settings (Jaffrin 2009). For instance, a Danish study found that BMI correlates with body fat assessed by bioelectrical impedance ($r=0.9$), indicating that BMI reflects body fat very well (Schousboe et al 2004).

2. Objectives

To decide whether genetic or environmental variances determinate the cardiovascular and anthropometric traits of interest, twin studies are necessary. Twin studies by comparing identical with non-identical twins produce information on the relative contribution of genes and environment, and how the two interact. The development or the progression of a heritable phenotype predisposing to a disease can be avoided or postponed, if proper screening methods are available. On the other hand, if the unique environmental factors determinate the certain trait, prevention (eg., by the modification of lifestyle) must be highlighted. Accordingly, our aims can be summarized in the following points:

1. Although the heritability of few hemodynamic variables has been shown (Vinck et al 2001, Snieder et al 2003, Mitchell et al 2005, Bochud et al 2005, Fava et al 2004, van Rijn et al 2007, Cecelja et al 2009), the precise magnitude of the genetic influence on novel hemodynamic variables (eg., central SBP, PP, arterial stiffness) and the association of arterial stiffness with central SBP and PP is poorly described. The first goal of our investigation was to assess the heritability of arterial stiffness, central SBP and PP and of brachial PP and the phenotypic/genotypic correlations between central pressure and arterial stiffness measures using a twin sample.
2. Previous studies have shown that carotid IMT is determined by genetic factors but largely influenced by environmental variance on certain segments (Zannad et al 1998, Zannad and Benetos 2003, Zhao et al 2008, Swan et al 2003, Jartti et al 2002, Lee et al 2012). However, narrow age-range cohorts or samples involving only one gender (eg., females) were applied and the precise extent to which genetic predisposition explains the variance of carotid IMT on multiple segments in a wide age-range population (including both males and females) is unclear. In addition, it is poorly investigated what connection is between carotid IMT and arterial stiffness measures. The second goal of this investigation, was to assess the heritability of carotid IMT, and to estimate phenotypic correlations between carotid IMT and arterial stiffness and augmentation index measures using the same twin sample.
3. Several studies have investigated the interplay of genetic and environmental influences on anthropometric parameters related to obesity by studying twin cohorts

(Fabsitz et al 1992, Korkeila et al 1995). In this study, we aimed to assess body composition components by using bioelectrical impedance analysis to determine the heritability of key anthropometric attributes in twins. Our goal was to demonstrate the ease with which this relatively simple method may confirm the role of genetics in body composition attributes and prove practical in identifying individuals who would primarily benefit from lifestyle changes to prevent obesity-related adverse health events.

3. Methods

3.1 Subjects

In this classical twin study, we examined in total 391 twin pairs, including 166 Hungarian (59 DZ and 107 MZ, mean age 42 ± 17 years \pm standard deviation /SD/, range 18-82 years), 50 American (3 DZ and 47 MZ, age 46 ± 17 years, range 18-76 years) and 175 Italian (97 DZ and 78 MZ, age 55 ± 12 years, range 22-74 years) pairs. In the three substudies, different sample sizes were included in the analysis as follows (*Table 4*):

3.1.1 Sample of the hemodynamic study

In this analysis, 146 Hungarian and 50 American twin pairs were analyzed.

3.1.2 Sample of the carotid intima-media thickness study

205 twin pairs (44 Hungarian, 124 Italian, 37 American twin pairs) were included in the second substudy analysis.

3.1.3 Sample of the anthropometric study

380 twin pairs (157 Hungarian, 174 Italian and 49 American; 230 monozygotic and 150 dizygotic pairs) were included in this cross-sectional twin substudy.

Table 4. Basic sample characteristics of the three studies

	Hemodynamic study	Carotid intima-media thickness study	Anthropometric study
Subjects, n	392	410	760
Monozygotic:dizygotic, n	308:84	270:140	460:300
Male:female	90:302	127:283	241:519
Age, years (mean \pm SD)	43.4 \pm 17.0	49.0 \pm 16.4	49.1 \pm 15.4

3.2 Study design

Subjects were recruited as part of the International Twin Study 2009 project. Twins above the age of 18 years were invited to participate. Exclusion criteria were race other than white (to exclude the influence of ethnicity), pregnancy, medical conditions possibly interfering with compliance during test procedures, arrhythmia, morbid obesity, and anorexia.

Hungarian subjects were enrolled from our Hungarian Twin Registry (Littvay et al 2012) and examined during local twin festivals (at locations in Ágfalva and Szigethalom) and in two large hospitals in Budapest (Semmelweis University Department of Radiology and Oncotherapy; Military Hospital Department of Cardiology) between July, 2009 and June, 2010.

Italian twins were enrolled by the Italian Twin Registry (Fagnani et al 2006) and tested in Roman, Paduan, and Perugian hospitals in September, 2009 and in March, 2010.

American twins were tested at the Twins Day Festival in Twinsburg, OH, USA in August, 2009.

Hemodynamic and anthropometric measurements were facilitated by the author and the author's twin brother (ADT and DLT) at all research sites together in order to avoid inter-observer variation. The carotid ultrasounds were performed by multiple sonographers beyond ourselves due to technical reasons.

In the absence of genotypic data, zygosity was assessed in Hungary and in the USA through a multiple widely used and accepted self-reported questionnaire of a >99% accuracy (Heath et al 2003). In Italy, zygosity was determined by using a validated self-report questionnaire (Kyvik et al 1995), which included questions on the similarities of twins during their childhood. To assess past medical history and personal habits (diet, smoking, alcohol consumption, and history of physical activity), all study subjects were requested to complete an on-site questionnaire.

The research was conducted in accordance with the Declaration of Helsinki. The local responsible university IRB committees have approved the study (in Hungary: Semmelweis University Regional and Institutional Committee of Science and

Research Ethics, TUKEB, 29/2009). Written informed consent was obtained from all subjects/patients.

3.3 Hemodynamic measurement

Aortic PWV, brachial and aortic AIx along with central blood pressure components can be assessed using Arteriograph (Medexpert Ltd., Budapest), a clinically validated oscillometric device (Baulmann et al 2008) which has been favorably compared with intra-arterial invasive measurements (Horváth et al 2010) (*Figure 8*). The measurements were performed in accordance with the suggestions of the European Society of Cardiology (Laurent et al 2006). Arteriograph calculates arterial stiffness parameters from oscillometrically recorded pressure waves of the brachial artery. Using inflatable upper arm cuffs with high fidelity sensors, pulsatile volume changes (resulting from pulsatile fluctuations of the brachial artery) are transduced into pressure curves. Pulse waves are recorded when the brachial artery is completely occluded at a cuff pressure that is 35-40 mmHg above systolic BP. Computer programs are used to further analyze the recorded pulse waves. Pulse transit time is determined from the decomposition of the generated aortic pressure wave using the reflection method (Qasem and Avolio 2008), based on the fact that the forward traveling pulse wave (generated by the ejection of the left ventricle) is reflected in the periphery creating a second reflected wave. Pulse transit time is determined from the time delay between the forward and the beginning of the reflected pressure wave, and aortic PWV is automatically calculated from transit time and traveling distance between jugulum (sternal notch) and symphysis pubica (according to the manufacturers recommendations) (jugulum-symphysis distance). Central hemodynamics including AIx and central BP were calculated from brachial pressure curves in combination with automated transfer algorithms.

All subjects were asked not to smoke three hours, not to eat one hour, not to drink alcohol and coffee ten hours prior to their visit. Subjects were examined in supine position on the dominant arm after at least 10 min of rest, and were asked not to speak or move during the measurements, and to keep their eyes closed during the

test. The same device was used for all tests except for circa 50 twin pairs on the twin festivals where parallel measurements were necessary due to the lack of time. If the automatic quality control was appropriate at the first measurement, i.e. SD of aortic PWV was <1 m/s, only one measurement was performed. In case of SD >1 m/s, the mean value obtained from the average of at least 3 measurements was considered.

Figure 8. Hemodynamic measurements of the American twin pairs (Twinsburg, OH)



3.4 Carotid ultrasonography (IMT measurement)

The measurement of carotid IMT was performed by B-mode ultrasound with linear array high frequency (5-10 MHz) transducers (in Rome: Esaote Technos MPX, in Padua: Philips iU22, in Perugia: Esaote Technos MP, in Hungary: Toshiba Power Vision and Esaote Mylab70, in the USA: Sonosite Titan). Sonographers were professional internists, neurologists or radiologists. Bilateral carotid arteries were assessed from the origin of the common carotid artery until the proximal 3-4 cm of the internal and external carotid arteries (**Figure 9**).

IMT of proximal and distal common carotid artery, and of proximal internal carotid artery was measured bilaterally using high resolution B-mode ultrasonography with standard techniques (Simon et al 2002, Wang et al 2003). IMT was quantified on

the far wall of the CCA 3-5 cm after its origin from subclavian artery (proximal CCA) and 1 cm proximally to the bifurcation (distal CCA). In addition, IMT was measured on the far wall at the proximal left and right ICA 1 cm distal to the bifurcation. For each segment, the sonographer used multiple different scanning angles to identify the longitudinal image of IMT showing the maximum IMT. At least 10 pictures for each segment were stored digitally, and measurements were made off-line using semiautomated computerized analytical software (Carotid Analyzer, Medical Imaging Applications LLC). In Padua, a dedicated software (QLAB) measured the IMT thickness automatically (since it was available in the ultrasound machine). Average values of the IMT of each of the six measurement spots (both proximal CCA, distal CCA, proximal ICA) were used as the IMT values for each twin in the analysis. We did not use electrocardiogram gating. In case of a carotid plaque we measured the carotid IMT at the end of the plaque.

Figure 9. *Ultrasonographic measurements of the Hungarian twin pairs*



3.5 Assessment of body composition

Body composition was determined by a clinically validated, portable body consistency monitor (OMRON BF500, Omron Healthcare Ltd., Kyoto, Japan) using bioelectrical impedance analysis (Bosy-Westphal et al 2008). The monitor leads weak electrical current of 50 kHz and less than 500 μ A through the subject's body to determine the amount of fat tissue. Muscles, blood vessels and some soft tissues including high water content conduct electricity easily while body fat has little electric conductivity. During the course of day, body water tends to gradually shift to the lower limbs making the ratio of water between the upper and lower body different in the morning and evening. Accordingly, the body's electrical impedance varies during the day. Use of electrodes for both hands and feet may reduce the influence of fluctuations on measured values. To further improve consistency, measurements were taken in the late morning hours and in the afternoon. To avoid electromagnetic interferences, subjects with pacemaker were excluded.

Under guidance of a trained assistant, current height, age, and gender information was entered in order to generate results. The subjects were instructed to stand on the scale barefooted with straight knees and back, looking straight ahead with horizontally raised arms and extended elbows (*Figure 10.*). The extended arms had at a 90° angle to the subject's body until the BMI, total body fat, fat-free mass rates were calculated. Body fat percentage refers to the amount of body fat mass with regards to total body weight expressed as follows:

$$\text{Body fat percentage (\%)} = [\text{Body fat mass (kg)} / \text{Body weight (kg)}] \times 100$$

Fat-free mass was interpreted as [100% - body fat percentage (%)]. Waist and hip circumferences were measured by placing a measuring tape in a horizontal plane mid-way between the top of the iliac bone and the bottom of the rib cage, and at hip level, respectively.

Figure 10. Body composition measurement of a Hungarian twin



3.6 Statistical analysis

3.6.1. Risk factor assessment

Initially we conducted a descriptive analysis (mean, standard deviation and the percentage for categorical variables) in MZ and DZ twins. Between-sex, between-zygosity and between-country differences were calculated using independent-samples t-test. The heritability model corrected for country of sample by regressing out its effects as these differences were significant at $p < 0.05$.

3.6.2. Estimating genetic influence on hemodynamic, carotid intima-media thickness and body composition parameters

A descriptive estimate of the genetic influence was calculated using the bivariate co-twin correlation in MZ (r_{MZ}) and DZ (r_{DZ}) pairs for each trait of interest. The corresponding 95% confidence intervals for r_{MZ} and r_{DZ} were calculated (McGraw and Wong 1996). If the within pair similarity for a phenotype is greater

in MZ than DZ pairs this provides evidence for genetic influence. Saturated models estimate means, variances and covariances separately for each group of twins (twin 1 vs twin 2) and by zygosity (MZ vs DZ pairs). Accordingly, we can get a baseline likelihood estimate against which to compare a genetic model.

Structural equation modeling was used to estimate heritability. Univariate quantitative genetic model (ACE) was performed to decompose phenotypic variance of the considered parameters into additive (A), common environmental (C) and unique environmental (E) effects (Neale and Cardon 1992). The additive genetic component measures the effects due to genes at multiple loci or multiple alleles at one locus. In case of carotid IMT parameters, non-additive or dominant (D) genetic component was also calculated which component measures the interaction between alleles at the same locus or on different loci. The common environmental component estimates the contribution of the shared family environment by both twins, whereas the unique environmental component estimates the effects that apply only to each individual twin, and includes measurement error. More formally, the covariance matrix of the MZ co-twins is modeled to be equal to $a^2+c^2+e^2$ (or the total phenotypic variance) and the off diagonal as a^2+c^2 (the components that the co-twin covariance consists of). For DZ twins the diagonal restrictions are the same for MZ twins but the off-diagonals are restricted to $0.5a^2+c^2$ (since genetic co-twin covariance is, on average, 0.5 for non-identical twins while their shared environmental correlation is the same as for MZ twins). With these restrictions in place the likelihood is maximized to obtain the estimates. The p-values for the 2 difference test of the likelihood function was calculated (sometimes insignificant results show that the model estimated covariance is not significantly worse than what is observed). The fitting model was determined on intraclass correlation: if twin correlations do not suggest shared environmental influence the (A+D) E model was considered (broad heritability); if twin correlations show evidence of common environmental effects the full ACE model was considered. Nested models were compared using likelihood-ratio χ^2 tests and Akaike Information Criteria (AIC) model selection was performed according to the principle of parsimony. Model fitting was done with the statistical software Mplus Version 6 (Muthén and Muthén 2010) with a full information maximum likelihood

in case of the hemodynamic variables by the Hungarian Twin Registry (Levente Littvay). Mx and STATA softwares were used to estimate model fit, to perform descriptive analyses and to determine statistical significance in case of the carotid IMT and body composition substudies by the staff of Italian Twin Registry (Neale et al 2006, Fagnani et al 2006). Shows significant deterioration of fit as compared to a model perfectly fitting the data. (It is strong indication of the model not fitting the data casting doubts on the results). Chi-Square model fit p-values are presented where the desired results show insignificant model misfit. Instead of a covariance matrix, the estimation procedure used the raw data matrix. Given the relatively small sample size, no component was fixed to 0 in the model. Due to the low number of American DZ twins, country-specific heritability estimates were not calculated.

3.6.3. Estimating the correlation between arterial stiffness and carotid intima-media thickness parameters

Correlation coefficients (CC) between brachial AIx or aortic PWV and carotid IMT variables were calculated to measure the strength and the direction of the relationship between variables, furthermore regression was used to show graphically the relationships between parameters.

3.6.4. Genetic covariance between hemodynamic variables

A bivariate Cholesky decomposition was carried out to derive the magnitude of covariation between the investigated phenotypes of interest and to estimate what proportion of this correlation is attributable to common underlying genetic and environmental factors. In order to estimate the amount of overlap between genes or environment that influences the two parameters, genetic and environmental correlations between those phenotypes were calculated (Neale and Cardon 1992, Littvay 2012).

4. Results

4.1 Clinical characteristics

Tables 4-8 present clinical characteristics of the three subsamples. Roughly two-third of the twins were monozygotic and female. Male and females were comparable with respect to age, body mass index, smoking habits, race and ethnicity.

Italian twin pairs were older than the other countries' samples (the involved twins in Rome were older). The prevalence of never smokers was higher in Hungarian and American sample compared to the Italians. Exsmokers' prevalence were significant lower in the Hungarian sample compared to the others. Prevalence of American current smoker twins was very low. Never smokers' rate was similar in both genders.

No significant country-specific difference was observed in hemodynamic parameters (**Table 5**). In contrast, statistically significant differences across countries were observed for some IMT variables (**Tables 6 and 7**).

No significant country-specific difference was observed in weight and waist circumference (**Table 8**). All anthropometric parameters showed normal distribution. Hip circumference was significantly higher in the USA compared to Hungary and Italy ($p < 0.01$ for both). Body fat percentage was significantly higher and the fat-free mass was significantly lower in the USA compared to Hungary ($p < 0.01$ in both cases). Significant gender-specific differences were found in all investigated parameters of body composition except hip circumference and physical activity.

Table 5. Clinical characteristics and measures of the hemodynamic study according to zygosity, gender and country (n=196 twin pairs)

	Total	Zygosity		Sex		Country	
		Monozygotic	Dizygotic	Male	Female	Hungarian	American
Subjects, n	392	308	84	90	302	292	100
Monozygotic:dizygotic, n	308:84	N/A	N/A	72:18	236:66	214:78	94:6
Age, years	43.4±17	42±17 [%]	48±14 [%]	43±17	44±17	43±17	46±17
Brachial AIx, %	-29.6±32	-30.5±33	-26.0±29	-43.3±26 [†]	-25.6±33 [†]	-29.0±32	-31.3±30
Central AIx, %	22.5±16	22.0±16	24.2±14	15.6±13 [†]	24.5±16 [†]	22.7±16	21.8±15
Aortic PWV, m/s	8.6±2.5	8.4±2.4	9.3±2.4	8.1±2.0 [†]	8.8±2.6 [†]	8.7±2.5	8.6±2.4
Brachial SBP, mmHg	127.2±17	127.2±17	127.1±17	132.0±16	125.8±17	127.5±17	126.3±17
Brachial DBP, mmHg	74.2±11	74.0±11	74.7±11	76.6±12	73.4±11	75.0±11	71.5±10
Central SBP, mmHg	120.2±20	119.8±20	121.4±20	120.5±17 [†]	120.1±21 [†]	120.9±20	118.2±18
Brachial PP, mmHg	53.1±11	53.3±11	52.4±11	55.4±11	52.4±11	52.5±10	54.8±12
Central PP, mmHg	46.2±12	46.1±12	46.7±12	43.7±8 ^{\$}	46.9±13 ^{\$}	45.9±12	47.1±13
BMI, kg/m ²	25.8±5.5	25.6±5.5	26.6±5.4	27.0±4.5 [†]	25.6±5.8 [†]	25.4±5.2 [^]	27.4±6.2 [^]
Never smokers, %	70.3	71.4	66.3	67.4	71.1	70.5	69.5
Ex smokers, %	17.1	16.2	20.5	19.1	16.5	13.7 ^{\$}	27.4 ^{\$}
Current smokers, %	12.6	12.5	13.3	13.5	12.4	15.8 ^{\$}	3.2 ^{\$}

Mean ± standard deviation. PP, pulse pressure; AIx, augmentation index; PWV, pulse wave velocity; SBP, systolic blood pressure;

DBP, diastolic blood pressure, BMI: body mass index, N/A: non applicable.

[%]Monozygotic vs dizygotic p<0.01; [†]Male vs female p<0.05; ^{\$}Male vs female p<0.001; [^]Hungarian vs American p=0.052; ^{\$} Hungarian vs American p<0.001

Table 6. Clinical characteristics and measures according to gender and country of the carotid intima-media thickness study (n=205 twin pairs)

	Sex		Country			Total
	Male	Female	Hungarian	Italian	American	
Subjects, n	136	274	88	248	74	410
Zygoty, MZ:DZ	97:39 [%]	186:88 [%]	34:10 [†]	66:58 [†]	35:2 [†]	135:70 [†]
Zygoty, %MZ:%DZ	29:71	68:32	77:23	53:47	94:6	66:34
Mean age, years (mean±SD),	49±18	49±16	44±16	58±9	47±17	49±16
Male, %	-	-	31	36	16	31
Female, %	-	-	69	64	84	69
BMI, kg/m ² (mean±SD)	26.6±3.2	26.8±3.8	24.6±4.5	26.6±4.4	27.4±6.3	26.3±4.8
Never smokers, %	59.6	60.7	73.9	41.4	70.2	60.5
Ex smokers, %	30.6	26.9	10.9	39.4	28.7	27.8
Current smokers, %	9.6	12.2	15.1	19.1	1.1	11.6
SBP, mmHg (mean±SD)	131.4±16.1	127.9±10.5	127.9±16.6	130.6±18.6	126.2±16.8	128.8±17.5
DBP, mmHg (mean±SD)	79.7±11.6	75.5±18.8	75.2±11.2	78.8±10.4	71.5±9.6	76.1±10.9

Mean ± standard deviation where applicable. MZ, monozygotic; DZ, dizygotic; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; [%] number of subjects, [†] number of twin pairs

Table 7. Mean, standard deviation values and confidence intervals of the investigated carotid intima media thickness parameters according to sample countries in the carotid intima-media thickness study (n=205 twin pairs)

	Hungarian	American	Italian	Total
Right CCA proximal IMT	0.62±0.16 [^]	0.60±0.14 [§]	0.67±0.14	0.65±0.14
Right CCA distal IMT	0.65±0.34	0.66±0.16 [*]	0.70±0.17	0.69±0.22
Right ICA proximal IMT	0.70±0.37	0.69±0.16	0.68±0.17	0.69±0.23
Left CCA proximal IMT	0.62±0.20 [†]	0.62±0.14 [§]	0.70±0.15	0.67±0.16
Left CCA distal IMT	0.65±0.27 [†]	0.62±0.15 [§]	0.73±0.20	0.69±0.21
Left ICA proximal IMT	0.66±0.42	0.71±0.20	0.69±0.19	0.69±0.25

Mean ± standard deviation where applicable. SD, standard deviation; IMT, intima media thickness; CCA, common carotid artery; ICA, internal carotid artery.

[^]Hungarian vs Italian p=0.02; [§]American vs Italian p<0.01; ^{*}American vs Italian p=0.04; [†]Hungarian vs Italian p<0.01

Table 8. Subject characteristics of study subjects by countries of origin in the anthropometric study

	Hungary			USA			Italy			Sex				Total				
	n	value	SD	n	value	SD	n	value	SD	Males		Females		n	value	SD		
										n	value (SD)	n	value (SD)					
Zygoty																		
MZ	204	65.0%	-	92	93.9%	-	164	47.1%	-	134	55.6%	326	62.8%	460	60.5%	-		
DZ	110	35.0%		6	6.1%		184	52.9%		107	44.4%	193	37.2%	300	39.5%			
Gender																		
Male	90	28.7%	-	16	16.3%	-	135	38.8%	-	-	-	-	-	241	31.7%	-		
Female	224	71.3%		82	83.7%		213	61.2%						519	68.3%			
Age, year ± SD	314	43.6	15.9*	98	46.9	16.8 †	348	54.6	12.4 *†	241	49.0	15.7	519	49.1	15.3	760	49.1	15.4
Weight, kg ± SD	312	71.4	15.4	98	73.7	17.1	347	70.8	12.7	240	81.6	11.5 ‡	517	66.8	13.3 ‡	757	71.5	14.5
Height, m ± SD	312	166.8	9.8§	98	163.9	9.4§	348	165.9	9.8	240	174.0	8.1‡	517	160.7	7.8‡	758	166.0	9.8
Waist circumference, cm ± SD	308	88.2	14.1	98	89.7	15.2	347	87.0	11.8	240	94.1	11.3 ‡	513	84.9	13.1 ‡	753	87.8	13.2

MZ, monozygotic; DZ, dizygotic; SD, standard deviation; §Hungarian vs American, p<0.05; ¶Hungarian vs American, p<0.01;

|Hungarian vs American, p<0.001; **Hungarian vs American, p<0.005; *Hungarian vs Italian p<0.001; |Hungarian vs Italian, p<0.005;

†American vs Italian p<0.001; ‡Males vs Females p<0.001; ***Males vs Females p<0.01; ****Males vs Females p<0.05

Table 8. Subject characteristics of study subjects by countries of origin in the anthropometric substudy (cont.)

	Hungary			USA			Italy			Sex						Total				
	n	value	SD	n	value	SD	n	value	SD	Males			Females			n	value	SD		
										n	value	SD	n	value	SD					
Hip circumference, cm ± SD	308	98.8	11.8	98	105.2	11.6 †	347	95.8	10.1 *	240	99.0	8.5	513	97.9	12.5	753	98.2	11.4		
Body fat, % ± SD	309	31.7	9.5¶	98	34.7	10.7¶	346	32.6	9.1	238	24.4	6.4‡	515	36.3	8.4‡	753	32.5	9.6		
Fat-free mass, % ± SD	309	68.3	9.5¶	98	65.3	10.7¶	346	67.4	9.1	238	75.6	6.4‡	515	63.7	8.4‡	753	67.5	9.6		
BMI, kg/m ² ± SD	312	25.7	5.1**	98	27.5	6.2 **†	347	25.7	3.8†	241	26.7	3.8 ***	519	26.0	5.3 ***	757	25.9	4.8		
Physical activity (Sport)																				
Yes	208	70.7%	-	63	69.2%	-	146	49.0%	-	152	67.6%****			254	57.5%****			417	61.1%	-
No	86	29.3%		28	30.8%		152	51.0%		73	32.4%			188	42.5%			266	38.9%	

MZ, monozygotic; DZ, dizygotic; SD, standard deviation; §Hungarian vs American, p<0.05; ¶Hungarian vs American, p<0.01;

|Hungarian vs American, p<0.001; **Hungarian vs American, p<0.005; *Hungarian vs Italian p<0.001; |Hungarian vs Italian, p<0.005;

†American vs Italian p<0.001; ‡Males vs Females p<0.001; ***Males vs Females p<0.01; ****Males vs Females p<0.05

4.2 Results of the hemodynamic twin study

4.2.1 Genetic and environmental effects on hemodynamic components

Age-, sex- and country-adjusted heritability was 60% for central SBP and 47% for central PP. Corresponding values for peripheral BP were 51% for brachial SBP and 30% for brachial PP. Heritability was 49% for aortic AIx, 47% for brachial AIx, and 50% for aortic PWV. Shared environmental factors had negligible influence. Unshared environmental effects accounted for the largest part of the environmental variance (**Table 2**). Models had a good fit except for aortic PWV (**Table 9**).

Table 9. Age-, sex- and country-adjusted parameter estimates and 95% confidence intervals of the Best-Fitting Univariate Models

Measure	A	C	E	Model fit (p)
Brachial SBP, mmHg	0.51 (0.30-0.64)	0.00 (0.00-0.48)	0.49 (0.37-0.60)	0.98
Brachial DBP, mmHg	0.64 (0.47-0.74)	0.00 (0.00-0.46)	0.36 (0.27-0.45)	0.81
Central SBP, mmHg	0.60 (0.45-0.69)	0.00 (0.00-0.00)	0.40 (0.31-0.50)	0.59
Brachial PP, mmHg	0.30 (0.00-0.53)	0.14 (0.00-0.46)	0.56 (0.43-0.69)	0.53
Central PP, mmHg	0.47 (0.12-0.61)	0.01 (0.00-0.46)	0.52 (0.40-0.66)	0.61
Brachial AIx, %	0.47 (0.01-0.74)	0.21 (0.00-0.62)	0.33 (0.24-0.42)	0.40
Aortic AIx, %	0.49 (0.02-0.74)	0.19 (0.00-0.64)	0.32 (0.24-0.41)	0.33
Aortic PWV, m/s	0.50 (0.26-0.67)	0.00 (0.00-0.00)	0.50 (0.33-0.72)	0.002

A indicates heritability; C, shared environmental variance component; E, unique environmental variance component; Model fit, Chi-square test of Model fit (p value); PP, pulse pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; AIx, augmentation index; PWV, pulse wave velocity.

4.2.2 Phenotypic correlation between brachial and central blood pressure, pulse pressure and arterial stiffness

Table 10 shows the results of the analysis aimed at estimating age, sex, country and family corrected phenotypic correlation between central blood pressure, pulse pressure and arterial stiffness. In a bivariate saturated model, both central SBP and PP had strong correlations with brachial and central AIx, as well as with aortic PWV (all $p < 0.001$). Brachial PP had a weak correlation with brachial AIx ($p < 0.05$) and central AIx ($p < 0.05$). No significant correlation was observed between brachial PP and aortic PWV.

Table 10. *Bivariate family, age, sex and population corrected phenotypic correlation from a bivariate structural equation saturated model of a genetic covariance decomposition model between brachial blood pressure, central blood pressure, pulse pressure, augmentation index and arterial stiffness parameters*

	Brachial AIx, %	Central AIx, %	Aortic PWV, m/s
Brachial SBP, mm Hg	0.135\$	0.132\$	0.248*
Central SBP, mm Hg	0.461*	0.457*	0.341*
Brachial PP, mm Hg	-0.118†	-0.122†	0.080#
Central PP, mm Hg	0.425*	0.419*	0.292*

AIx, augmentation index; PWV, pulse wave velocity; PP, pulse pressure; SBP, systolic blood pressure

* $p < 0.001$, \$ $p = 0.01$, † $p < 0.05$, # non significant

4.2.3 Genetic covariance of brachial and central blood pressure, pulse pressure and arterial stiffness

Since the heritability and bivariate correlation coefficients of brachial and central PP, AIx, central SBP and aortic PWV showed moderate and significant genetic influence, bivariate Cholesky decomposition model was run in order to investigate a common genetic background of these traits. **Table 11** shows standardized genetic, common and unique environmental components of the covariance in the investigated measures. Estimates of genetic and environmental proportion of variance mostly moderately overlapped with those obtained in the univariate analysis. Additive genetic component significantly ($p < 0.05$) accounted for 83-100% of covariance between central PP, brachial SBP, central SBP and aortic PWV. Furthermore, the unique environmental component accounted for 28-36% of the covariance between central PP, central SBP and AIx. No other significant genetic covariation was found. In addition, phenotypic correlations between central PP, brachial SBP, central SBP and aortic PWV, which represent the overlap between genetic influences, were estimated at 0.295, 0.252 and 0.344. Phenotypic correlations between central PP, brachial SBP, central SBP and aortic AIx accounted for 0.419, 0.132 and 0.455. Finally, phenotypic correlations between central PP, brachial SBP, central SBP and brachial AIx were calculated at 0.425, 0.135 and 0.460.

Table 11. Covariance of brachial and central systolic blood pressure, pulse pressure and arterial stiffness parameters as estimated from the best bivariate Cholesky model

	Brachial AIx	Central AIx	Aortic PWV
Brachial PP	A: 0.715 (-2.018; 4.273) C: 0.021 (-3.683; 2.502) E: 0.264 (-0.795; 1.331)	A: 0.536 (-2.283; 6.625) C: 0.218 (-5.948; 2.303) E: 0.246 (-0.687; 1.298)	A: 1.680 (-0.716; 13.96) C: -0.430 (-10.13; 1.315) E: -0.250 (-4.099; 1.308)
Central PP	A: 0.384 (-0.264; 0.944) C: 0.252 (-0.232; 0.818) E: 0.364 (0.164; 0.552) [†]	A: 0.403 (-0.248; 0.975) C: 0.231 (-0.288; 0.802) E: 0.367 (0.176; 0.562) [†]	A: 1.002 (0.215; 1.415) [†] C: -0.035 (-0.522; 0.341) E: 0.033 (-0.366; 0.282)
Brachial SBP	A: 0.419 (-2.271; 2.274) C: 0.420 (-0.901; 2.932) E: 0.161 (-0.770; 1.168)	A: 0.501 (-1.636; 2.350) C: 0.330 (-1.212; 2.499) E: 0.169 (-0.774; 1.254)	A: 0.841 (0.003; 1.321) [†] C: 0.000 (-0.018; 1.672) E: 0.159 (-0.288; 0.488)
Central SBP	A: 0.460 (-0.071; 0.856) C: 0.259 (-0.087; 0.713) E: 0.281 (0.119; 0.432) [†]	A: 0.486 (-0.115; 0.863) C: 0.232 (-0.102; 0.763) E: 0.282 (0.129; 0.434) [†]	A: 0.833 (0.157; 1.131) [†] C: 0.000 (0.000-1.116) E: 0.167 (-0.097; 0.375)

A, additive genetic factors; C, shared environmental factors; E, unique environmental factors; AIx, augmentation index; PWV, pulse wave velocity; PP, pulse pressure; SBP, systolic blood pressure. The numbers within the parentheses are the lower and upper limits of the 95% confidence interval. † p<0.05

4.3 Results of the carotid intima-media thickness twin study

4.3.1 Genetic and environmental effects on carotid IMT

Table 12 presents the age-adjusted intraclass correlation of IMT values by zygosity. Age-adjusted intraclass correlations were higher in MZ than in DZ pairs for proximal right CCA (MZ=0.19, DZ=0.06), proximal and distal left CCA (MZ=0.27, DZ=0.06; MZ=0.27, DZ=0.13, respectively), proximal left ICA (MZ=0.39, DZ=-0.54) suggesting a moderate genetic effect. Heritability was estimated to be 18% (95% CI: 3-33) for proximal right CCA, 26% and 27% for proximal and distal left CCA, respectively and 38% (95% CI: 26-49) for proximal left ICA. As regards distal right CCA and proximal right ICA, no genetic effects were detected.

Table 12. Twin intraclass correlation and standardized genetic and environmental variance components of carotid IMT values

Parameter	Zygo- sity	Intraclass correlation (age adjusted)	95% CI	A or A+D (95% CI)	C (95% CI)	E (95% CI)
Right CCA proximal IMT	MZ	0.19	0.02 - 0.34	0.18 (0.03 - 0.33)	0 (0.0- 0.0)	0.82 (0.67 - 0.97)
	DZ	0.06	-0.19 - 0.29			
Right CCA distal IMT	MZ	0.16	0.02 - 0.29	0 (0.0 - 0.29)	0.16 (0.0 - 0.29)	0.84 (0.71 - 0.97)
	DZ	0.27	-0.48 - 0.58			
Right ICA proximal IMT	MZ	0.27	0.14 - 0.40	0 (0.0 - 0.36)	0.30 (0.0 - 0.42)	0.70 (0.58 - 0.83)
	DZ	0.57	0.25 - 0.72			
Left CCA proximal IMT	MZ	0.27	0.12 - 0.41	0.26 (0.11 - 0.40)	0 (0.0 - 0.0)	0.74 (0.60 - 0.89)
	DZ	0.06	-0.19 - 0.31			
Left CCA distal IMT	MZ	0.27	0.12 - 0.40	0.27 (0.13 - 0.40)	0 (0.0 - 0.0)	0.73 (0.60 - 0.87)
	DZ	0.13	-0.20 - 0.40			
Left ICA proximal IMT	MZ	0.39	0.27 - 0.51	0.38 (0.26 - 0.49)	0 (0.0 - 0.0)	0.62 (0.51 - 0.74)
	DZ	-0.54	-0.71 - 0.35			

A, additive genetic variance; D, non-additive genetic variance; C, shared environmental variance; E, unique environmental variance; 95% CI, 95% confidence interval; IMT, intima media thickness; CCA, common carotid artery; ICA, internal carotid artery; MZ, monozygotic; DZ, dizygotic

4.3.2 Correlation between carotid IMT variables and arterial stiffness and augmentation index

Table 13 and **Figure 11** show the results of the analysis aimed to estimate phenotypic correlation coefficients between the brachial AIx, aortic PWV and carotid IMT parameters. Overall the estimated coefficients indicated a not significant linear correlation, and few slight differences in coefficient values between countries were

observed. After adjusting for age, correlations between carotid IMT, AIx and PWV appeared indicating only an age-related association.

Table 13. Correlation coefficients (CC) between brachial AIx or aortic PWV and carotid IMT parameters

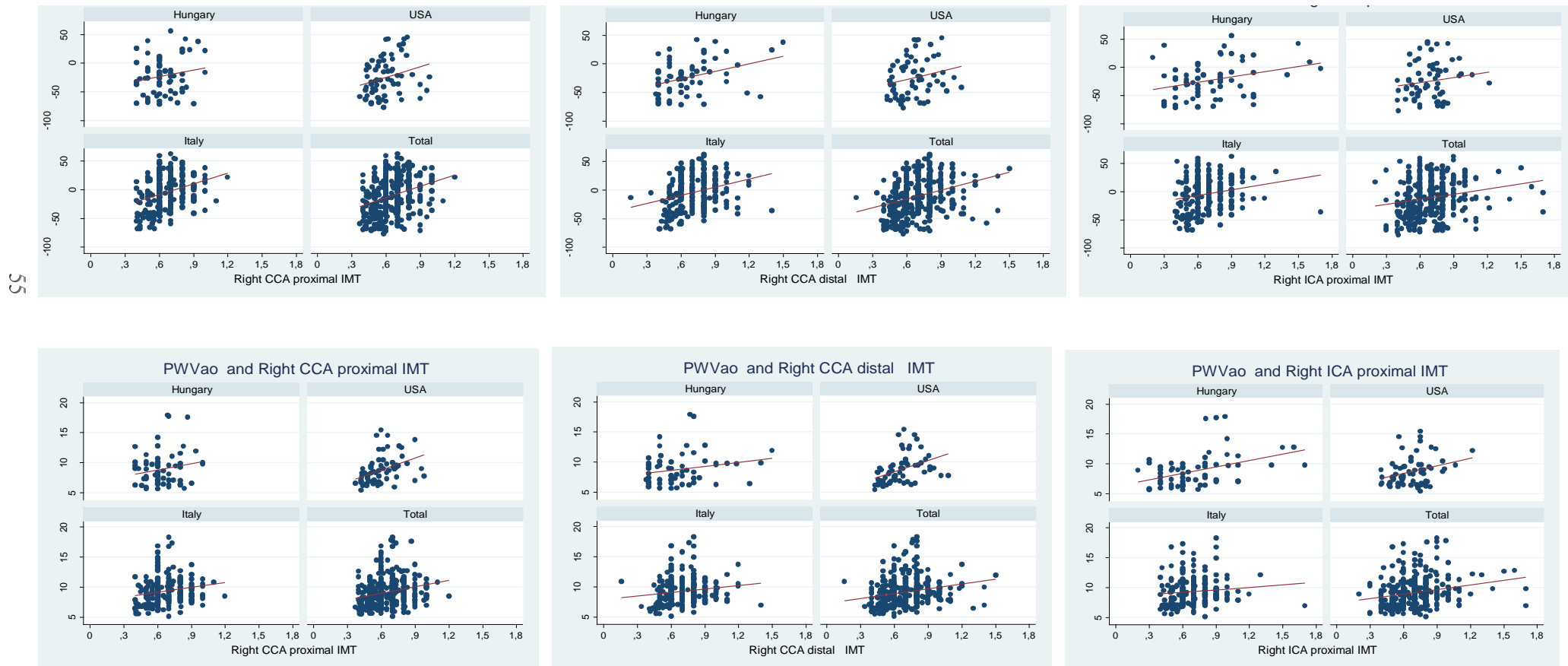
Parameter	Number of twin subjects	Brachial AIx		Aortic PWV	
		Partial CC (age adjusted)	Pearson CC (non age adjusted)	Partial CC (age adjusted)	Pearson CC (non age adjusted)
Right CCA proximal IMT	386	0.02	0.32*	-0.03	0.23*
Right CCA distal IMT	379	0.09	0.33*	0.07	0.27*
Right ICA proximal IMT	384	0.00	0.18*	0.10	0.23*
Left CCA proximal IMT	384	0.10*	0.38*	0.01	0.27*
Left CCA distal IMT	384	0.08	0.38*	0.03	0.30*
Left ICA proximal IMT	383	-0.02	0.17*	0.05	0.19*

CC, correlation coefficient; Brachial AIx, augmentation index on brachial artery; Aortic PWV, pulse wave velocity on aorta; MZ, monozygotic; DZ, dizygotic; IMT, intima media thickness; CCA, common carotid artery; ICA, internal carotid artery

*p<0.05

Figures 12-14 show the sample ultrasound images where the arterial stiffness was optimal, increased and abnormal.

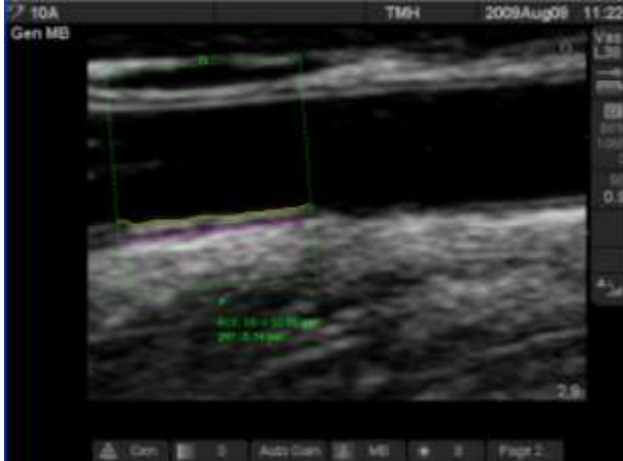
Figure 11. Regression for right carotid intima media thickness and arterial stiffness variables (brachial AIx and aortic PWV) showing some slight differences between countries



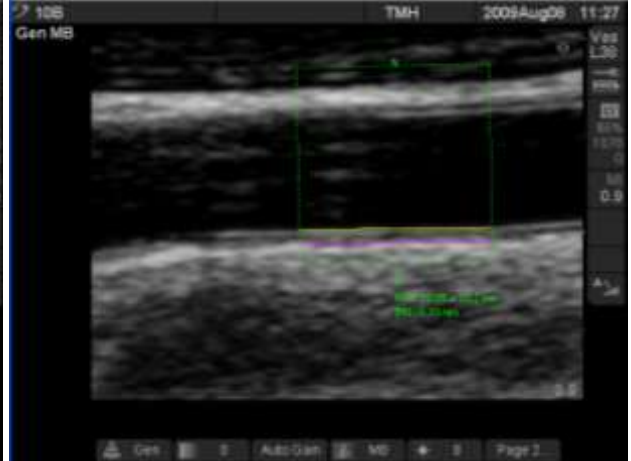
AIx, augmentation index; PWV, pulse wave velocity; IMT, intima media thickness; CCA, common carotid artery; ICA, internal carotid artery

Figure 12. Right (A) and left (B) CCA ultrasound images of a 24-year old American male MZ twin pair (twin 1 and twin 2) having optimal arterial stiffnesses (AIx_{bra} : -68.8 vs -68.2%, PWV_{ao} : 6.3 vs 6.7 m/s)

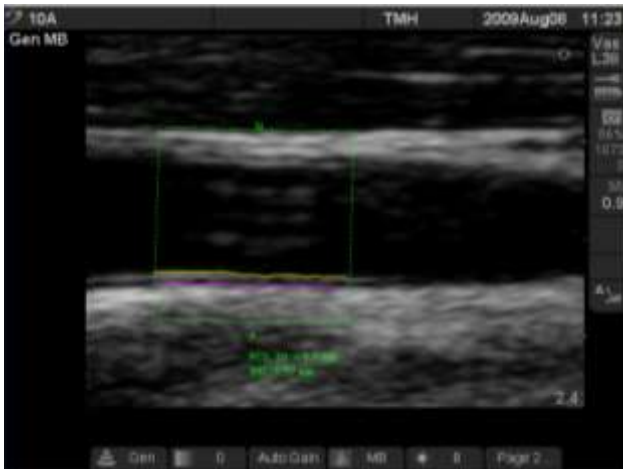
TWIN 1
A



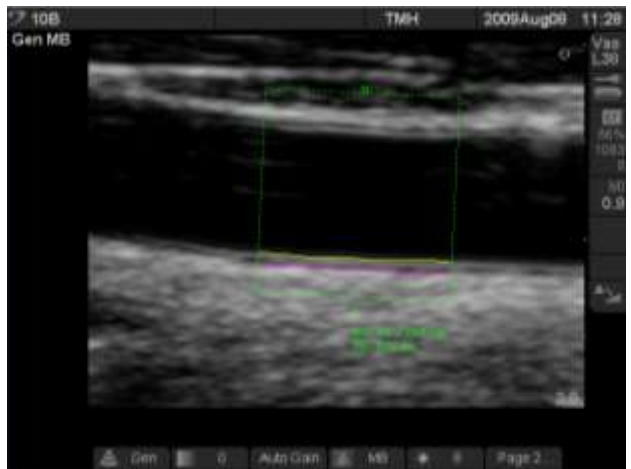
TWIN 2
A



B



B



Footnote: Mean IMT values are shown on the images.

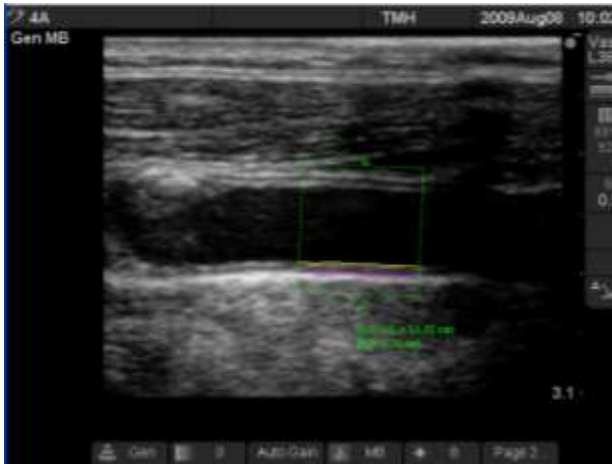
Figure 13. Right (A) and left (B) CCA ultrasound images of a 63-year old American female MZ twin pair (twin 1 and twin 2) having increased arterial stiffnesses (AIx_{bra} : 15.0 vs 11.4%, PWV_{ao} : 10.5 vs 14.5 m/s)

TWIN 1

TWIN 2

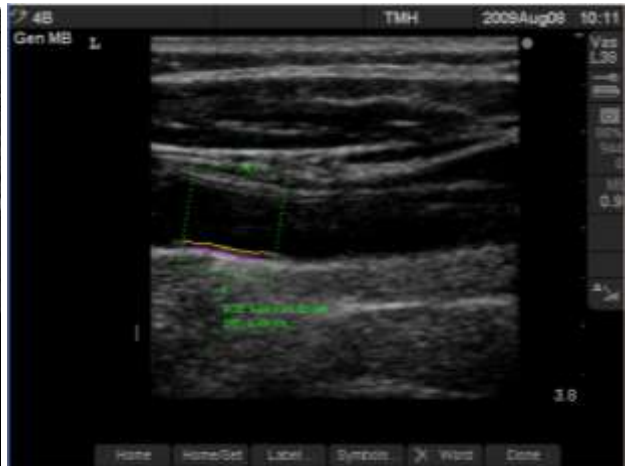
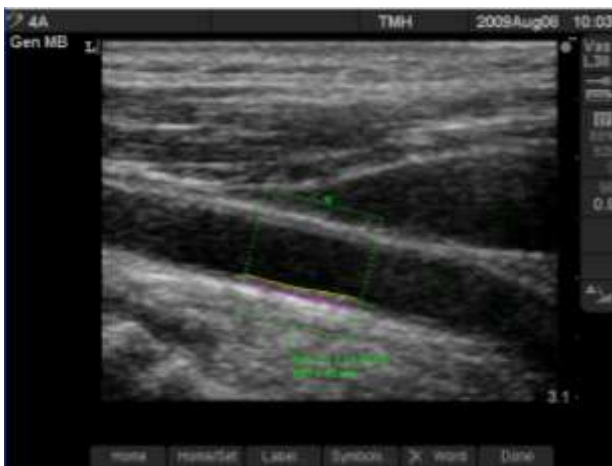
A

A



B

B



Footnote: Mean IMT values are shown on the images. „Edited” means if the software failed the correct marking of the intima layer it was manually corrected.

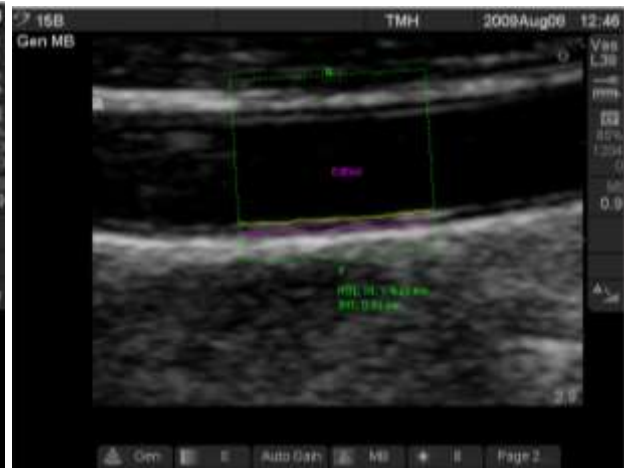
Figure 14. Right (A) and left (B) CCA ultrasound images of a 52-year old American female MZ twin pair (twin 1 and twin 2) having abnormal arterial stiffnesses (AIx_{bra} : 41.8 vs 40.5%, PWV_{ao} : 12.8 vs 15.4 m/s)

TWIN 1

TWIN 2

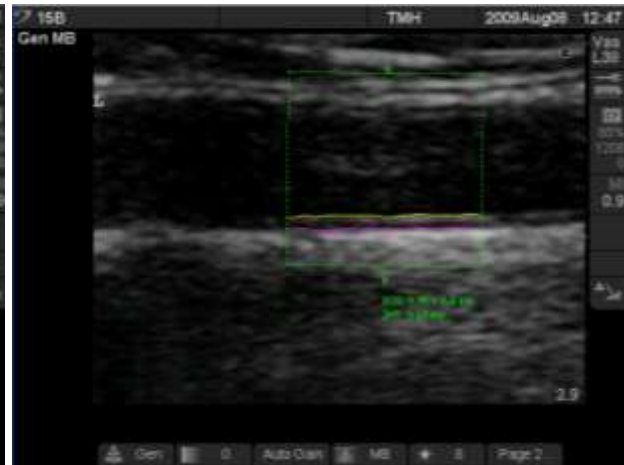
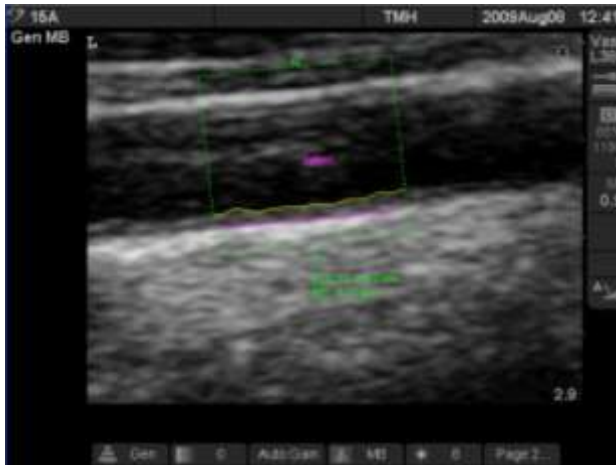
A

A



B

B



Footnote: Mean IMT values are shown on the images. „Edited” means if the software failed the correct marking of the intima layer it was manually corrected.

4.4 Results of anthropometric twin study

4.4.1 Univariate model describing the heritability of body composition

Intraclass correlation coefficients of all investigated body composition parameters were higher among MZ than DZ pairs, suggesting that genetic factors may be important contributors to these traits. Results combined from all twin data on genetic and environmental variance estimates with 95% confidence intervals are presented in **Table 14**. A large proportion of the total variance for all parameters is attributable to genetic factors between 74% and 82%. No role of non-additive genetic variance was found. Furthermore, a lower Akaike information Criteria value was shown in the AE model than in the ACE model, proving that the AE model is the best model (**Table 14**). Therefore, common environmental effects had no impact on total variance of all the parameters considered; unique environmental effects instead were estimated as between 19% and 26% with regard to each parameter.

Table 14. Intraclass correlation and ACE/ADE model of body composition variables

	n	Zygoty	Intraclass correlation		Best model analysis							
			value	95%CI	A	95%CI of A	E	95%CI of E	χ^2 *	Δ df	P	AIC
BMI	229	MZ	0.81	0.77-0.85	0.81	0.77-0.85	0.19	0.15-0.23	0.19	1	0.66	-1.81
	150	DZ	0.44	0.29-0.55								
Waist circumference	227	MZ	0.74	0.68-0.79	0.74	0.68-0.79	0.26	0.21-0.31	0.37	1	0.54	-1.63
	149	DZ	0.41	0.27-0.53								
Hip circumference	227	MZ	0.74	0.68-0.79	0.74	0.69-0.79	0.26	0.21-0.31	3.09	1	0.08	1.09
	149	DZ	0.49	0.35-0.59								
Body fat / Fat-free mass	228	MZ	0.76	0.71-0.81	0.76	0.71-0.81	0.24	0.19-0.29	0.02	1	0.89	-1.98
	148	DZ	0.39	0.24-0.52								

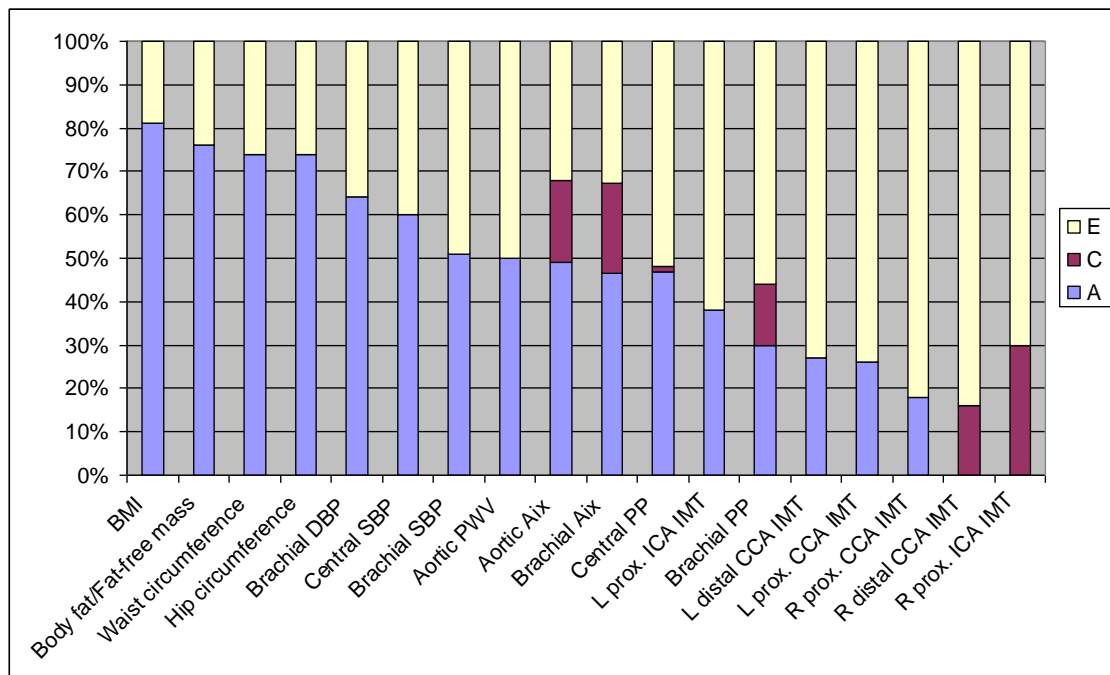
MZ, monozygotic; DZ, dizygotic; A, additive genetic effects; E, unique environmental effects; χ^2 , chi-square ($\chi^2 = [-2 \log\text{-likelihood submodel}] - [-2 \log\text{-likelihood full model}]$); Δ df, df submodel – df full model; P, probability; AIC, Akaike Information Criteria, $\chi^2 - 2$

Δ df.

4.5 Summary of the univariate model results of the three substudies

The anthropometric parameters showed the highest genetic influence (74-82%). Moderate genetic influence was observed concerning the hemodynamic variables (blood pressure and arterial stiffness components; 30-60%). Interestingly, the heritability of central variables was always higher than one of the brachial (peripheral) values (eg., SBP, PP, AIx). Genetics had the lowest proportion of total phenotypic variance in carotid IMT parameters ranging between 0% and 38% (*Figure 15*).

Figure 15. Mean values of additive genetic (A, blue), common (C, dark red) and unique environmental (E, yellow) influences on cardiovascular and anthropometric phenotypes of interest (structural equation model [ACE] analysis)



BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; PWV, pulse wave velocity; Aix, augmentation index; PP, pulse pressure; L, left; R, right; ICA, internal carotid artery; CCA, common carotid artery; IMT, intima-media thickness

5 Discussion

We aimed to determinate the influence of genetic and environmental factors on several key cardiovascular and anthropometric traits. If a certain heritable phenotype predisposing to a disease is mainly determined by genetic factors, the development or the progression of the certain trait could be avoided or postponed by preventive screening. In contrary, if the unique environmental factors determine a phenotype, prevention (eg., lifestyle intervention) should be highlighted. In addition, we aimed to investigate the relationship of certain hemodynamic and carotid IMT variables, and if a relation exists, whether genetic or environmental factors are responsible for that. Although no such comprehensive study exists in the literature and the heritability of some hemodynamic traits has never been studied, we hypothesized that antropometric variables will be highly, while the cardiovascular traits will be moderately heritable. The investigations were carried out by novel techniques (oscillometric measurement, body impedance analysis).

Our results showed that central systolic and pulse pressures, brachial pulse pressure, AIX, aortic PWV are moderately heritable and a moderate genetic covariance among aortic PWV and central PP, central SBP and brachial SBP exists. Our results indicate that the investigated carotid IMT parameters appeared to be only moderately or negligible influenced by genetic factors and mostly the environmental variance dominated. Furthermore, bioelectrical impedance analysis applied to this twin cohort provided additional evidence to the heritability of anthropometric attributes related to obesity.

5.1 Role and importance of moderate heritability on hemodynamic variables

To the best of our knowledge, this is the first study which investigates heritability of central blood pressure and its phenotypic correlation with hemodynamic measures in a relatively large, international twin sample.

Blood pressure has a multifactorial origin arising from an interaction between susceptibility genes and environmental factors. Genetics of hypertension is complex with no known single gene playing a major role, but rather many genes each with mild effects on blood pressure, each encoding for a variety of enzymes, ion channels, receptors, and proteins involved in hormonal regulation and in the structure and/or regulation of vascular tone (Butler 2010). The heritable component of BP has been documented in familial and twin studies suggesting that 30-50% of the variance of blood pressure readings is attributable to genetic heritability and about 50% to environmental factors (Luft 2001, Weitz 1925, Stocks 1930, Borhani et al 1976, Hong et al 1994, Rao et al 1993, Somes et al 1995, Grim et al 1990, Vinck et al 2001, Snieder et al 2000, Snieder et al 2003). Heritability estimates of the proportion vary from 25% in pedigree studies to 65% in twin studies for sitting diastolic BP (Knuiman et al 1996, Mitchell et al 1996, An P et al 1999, Rotimi et al 1999, Livshits and Gerber 2001, North et al 2003). In these studies, shared family environment (eg., salt intake, alcohol use, amount of exercise) was responsible for only 7% of the total variance of diastolic BP (Williams et al 1991).

In our study, age-, sex- and country-adjusted heritability of central BP components was generally higher than the corresponding brachial pressures (SBP, 60% central vs 51% peripheral; PP, 47% central vs 30% peripheral). A possible explanation for the greater heritability of central BP is its being influenced by major determinants with potential genetic components, including wave reflections, vascular stiffness, left ventricular contractility and heart rate (Roman 2009).

Cecelja et al had estimated the heritability of PP to 43% after adjustment for mean arterial pressure and heart rate, although only female twin pairs were examined in that study (Cecelja et al 2009). Of note, we reported higher central PP in females compared to males which had been observed in previous studies as well. PP is lower in women before 50 years of age, and becomes higher in women than in men after 60 years because of accelerated aortic wall stiffening, which may partially contribute to the higher prevalence of cardiovascular disease in older women as compared to men (Franklin et al 1997, Mitchell et al 2008). Accordingly, reduced aortic diameter and impaired matching between diameter and flow accounted for

the sex difference in PP in elderly people (Mitchell et al 2008). In contrast, a previous study reported no difference between genders (Segers et al 2007).

Snieder et al reported that the heritability of AIx was 37%, whereas heritabilities for blood pressure traits varied between 13% and 25% in females (Snieder et al 2000). The heritable component of AIx was largely independent from the influence of BP, heart rate, height, and age (Snieder et al 2000). Significant intrafamilial concordance and heritability for brachial and central AIx and for central PP and mean arterial pressure ranging between 37% and 41% was reported in a family-based population study measured sphygmomanometrically (Seidlerová et al 2008). To our knowledge, no previous twin study has reported the relationship between direct measures of arterial stiffness and wave reflections in healthy individuals in both genders so far. Age- and gender-adjusted heritability for carotid-femoral PWV was 36% in a genetically isolated Dutch population connected in a single pedigree (Sayed-Tabatabaei et al 2005). The heritabilities of aorto-radial and aorto-dorsalis-pedis PWV were reported to be 43% and 53%, respectively, in a sample of young American twins (Ge et al 2007). On the background of these studies, our findings show a moderate genetic effect on arterial stiffness in a relatively large wide-age ranged twin sample, comparable to other smaller or restricted age-ranged studies (Snieder et al 2000, Seidlerová et al 2008, Sayed-Tabatabaei et al 2005, Ge et al 2007). Of note, model fit of aortic PWV was not good which casts doubts on this heritability result.

Overall, heritability of aortic stiffness was found to be of the same magnitude as that of BP, although arterial stiffness is influenced by the cumulative effects of blood pressure and additional risk factors on the stiff and elastic components of the arterial wall. Heritability of arterial stiffness might be related to its structural and genetic determinants. A relationship between individual gene expression and arterial stiffness has been investigated in genome-wide association linkage and candidate gene polymorphism association studies (Laurent et al 2005, Durier et al 2003, Lacolley et al 2009). Further analysis of the molecules associated with vascular wall stiffening and their signaling pathways is essential for the development of future drug treatments for arterial stiffness (Laurent et al 2005).

Potential therapeutic targets include cytokines and their receptors, transcription factors and network-forming collagen type VIII (Lacolley et al 2009).

A worldwide epidemic of cardiovascular and cerebrovascular disease has been anticipated (Husten 1998), and prevention of hypertension and related complications in high-risk subjects would be highly desirable. Our findings suggest that unshared environmental factors are moderately responsible for the investigated variables which can be preventable in the high-risk patients. Antihypertensive medication may have a protective effect on cognitive sequelae of hypertension, as suggested by a recent twin study which showed that in middle-aged men, untreated, but not treated hypertension suppresses genetic influences on certain domains of cognition (Vasilopoulos et al 2012). There is an emerging current evidence that BP-lowering drug treatments can differentially affect aortic pressure and stiffness parameters relative to brachial BP which may effect the arterial stiffness independently of BP-changes (Williams 2012). A future challenge is to put emphasis on individual BP lowering therapy along with the favorable modification of aortic pressure and stiffness (Williams 2012). It must be underlined that lifestyle measures, such as healthy diet, increase of physical activity, and reduction of stress, have an important role (32-56%) in the management and these factors should be also taken into consideration as possible destiffening measures in addition to drugs. Therapeutic strategies including hypertension management proved beneficial in preventing, delaying or attenuating vascular aging by “de-stiffening” (Lee et al 2010).

Professors Laurent and Parati commented on our published paper whether central blood pressure and arterial stiffness assessments in high-risk patients could be a Holy Grail in the future (Laurent et al 2012). In their editorial they negotiated whether these measurements could be used in clinical practice to detect patients at high cardiovascular risk and to intensify preventive measures (Laurent et al 2012). Furthermore, these interventions could guide the appropriate therapeutic strategies at an early stage in order to reverse damage by „de-stiffening” (Laurent et al 2012). Beyond the morphological assessment of vascular damage, such as carotid ultrasound examination, also vascular function tests will be necessary besides the risk factor assessment for the management of cardiovascular diseases (Tomiyama

and Yamashina 2010). Nowadays, the spectrum of the clinical application of central blood pressure and arterial stiffness assessment is progressively enlarging and better standardized measurement procedure are more frequent available (Laurent et al 2012). The wide clinical application of arterial stiffness will likely contribute to cardiovascular assessment and management in future clinical practice by simultaneous measurements of different parameters of vascular function and structure could improve risk stratification (Palatini et al 2011). In this context, our study could be an additional argument for the more widespread measurement of arterial stiffness and central BP in the general population in order to detect individuals with abnormally high values due to the moderate heritability of central and peripheral hemodynamic traits (Laurent et al 2012).

Future studies are necessary to investigate how certain epigenetic factors can modify the actions of genes influencing the hemodynamic measures. For instance, an European family-based epidemiological survey demonstrated that individuals with the same genetic predisposition had different arterial stiffness depending on whether a high-sodium or a low-sodium diet was eaten (Wojciechowska et al 2004). Thus, large population studies which take into account gene-environment and gene-gene interactions are expected in the future in order to study complex cardiovascular phenotypes.

The observed correlation between AIx and blood pressure is obvious since AIx is calculated by these blood pressure variables. In addition, our study also reports that the genetic covariances of central SBP, PP, brachial SBP and aortic PWV are moderate. The common genetic influence implies that the above parameters of central hemodynamics are influenced by shared genes. Vascular aging includes all the above mentioned morphologic changes leading to increased aortic PWV, systolic blood pressure and pulse pressure (Lee et al 2010). Vascular aging is accelerated by coexisting cardiovascular risk factors, such as hypertension, metabolic syndrome and diabetes (Lee et al 2010). In this setting, our findings confirm the key importance of prevention, because of the moderate genetic and environmental influencing effects in preventing, delaying or attenuating vascular aging. Besides, this finding should encourage performance of further genetic studies on the structural components of the arterial wall involved in arterial

stiffening (Laurent et al 2012). Possible new molecular targets could aid to the preventive treatment of accelerated vascular aging (Laurent et al 2012).

Our findings, that strong genetic covariance is present between aortic PWV and central PP ($p < 0.001$) rather than brachial PP ($p = 0.08$) indicate that genetic factors may affect small arteries, large arteries, and the aorta to a common extent. This mechanistic aspect has rarely been investigated (Laurent et al 2012). In contrary, Cecelja et al found indirectly that the increase in central BP is mainly related to the geometrical changes or vasomotor tone of peripheral large arteries, rather than to aortic stiffening (Cecelja et al 2009). Thus, the contradictory results should stimulate additional investigations to determine whether the supposed common genetic background for aortic stiffness and central BP is strong enough (Laurent et al 2012). Of note, genetic covariance decomposition is a technique that traditionally call for larger samples. A larger study could produce more precise results for all the insignificant covariance results in Table 11.

Potential limitations and strengths of our study should be considered. All the arterial stiffness tests were performed by the same trained researchers (ADT and DLT) using the same device. Our results were obtained in healthy adult twins within a wide age range (from 18 to 82), and therefore may not be extended to younger subjects or to populations with clinically manifest cardiovascular disease. In our study, aortic PWV was estimated from a single-site determination of the suprasystolic waveform. While the principle underlying aortic PWV determination with Arteriograph has been called into question in a computer model (Trachet et al 2010), clinical data from an invasive study support the good agreement of aortic PWV as estimated by Arteriograph with direct intra-arterial measurement (Horváth et al 2010). Any potential lack of precision of Arteriograph in estimating aortic PWV is expected to result in an underestimation of the genetic components of aortic PWV. The editorial comment highlighted that the significant relationship of aortic PWV with central SBP and PP shows that the oscillometric device (Arteriograph) measures true arterial properties influencing wave reflection and augmentation pressure (Laurent et al 2012). Since Hungarian and American twins were not recruited from a population-based twin registry (as it is the case in Italy), volunteering twins were mainly females who are more interested in health-related

researches and are more willing to participate in a twin study e.g. at a twin festival. Furthermore, monozygotic twins tend to volunteer more than dizygotic twins. However, gender differences were taken into account in multivariate analyses and in twin models. Finally, we did not exclude twins taking antihypertensive medication since it removes an important part of the population variance of interest and thereby reduced heritability estimates directly lead to a loss of power (Palmer 2003, Kupper et al 2005).

5.2 Role and importance of low to moderate heritability on carotid IMT variables

Our results indicate that most of the carotid IMT parameters appeared to be only moderately or negligible influenced by genetic factors thus mostly influenced by environmental factors. These environmental factors of relevance appeared not to be shared within family but related to individual experience (eg., smoking habits, diet, physical activity). Accordingly, shared environmental factors do not contribute significantly to carotid IMT. Increased carotid IMT could be prevented or postponed if the underlying unshared environmental factors, such as active and passive smoking, diet, stress, physical activity could be appropriately managed in high-risk patients.

Most studies reported that genetic factors account for 24%-59% of carotid IMT variation in families after adjustment for traditional cardiovascular risk factors (Zhao et al 2008, Swan et al 2003, Jartti et al 2002). A very high heritability estimate of carotid IMT (92%) was reported in a Mexico population, but the sample size of the study was very small (Duggirala et al 1996). Swan et al. estimated the heritability of carotid IMT of 31% in a Scottish twin sample (Swan et al 2003). A Finnish study reported a modest heritability of carotid IMT (36%) (Jartti et al 2002). Zhao et al performed a classical twin study involving 98 middle-aged male twin pairs from the Vietnam Era Twin Registry demonstrating a significant heritability for carotid IMT (59%), but due to the twin cohort characteristics of military veterans (elderly male subjects), the generalizability to other populations was uncertain (Zhao et al 2008). The Healthy Twin Study reported also moderate

heritability of intima-media thickness (38%-48%) for common, carotid bifurcation, and internal carotid artery (Lee et al 2012). In addition, a shared genetic influence was found between the three carotid segments (Lee et al 2012). As regards the absence of heritability for the IMT of distal right CCA and right proximal ICA, no former studies have had these findings. The possible explanation why no genetic influence was found on these two segments could be the measurement error which may be reflected in the increased E variance component. Heterogeneity of the investigators (eg., experience, right/left handedness) and ultrasound devices could play in the role in this finding. Right handedness of most of the investigators could have influenced the larger genetic effects on the left side.

Statistically significant differences were observed across countries (between Hungary and Italy, between USA and Italy) for some IMT measurements, which may be attributable to the different subject characteristics of involved Italian twins (eg., older age and higher smoking prevalence).

The relationship of carotid IMT, arterial stiffness and augmentation index is poorly investigated in a healthy sample. Previous studies focused on subjects with coexisting cardiovascular diseases. Tu et al tested subjects with a history of stroke or myocardial infarction and found that CCA and bifurcation IMTs correlated with stiffness (Tu et al 2010). Accordingly, carotid IMT and arterial stiffness are considered as independent risk factors for atherosclerotic diseases (Tu et al 2010). In our study, we substantially confirmed these findings in a healthy twin cohort. The insignificant correlation between the carotid IMT, arterial stiffness and AIX variables indicates that increased arterial stiffness and pulse wave amplification is not accompanied with increased carotid IMT. Endothelial dysfunction is an early phase of atherosclerosis which does not necessarily accompanies with morphological changes in our healthy cohort. Of note, increased carotid IMT correlates with the extent and severity of coronary artery disease (Adams et al 1995). Of note, the observed inverse correlation between right proximal CCA IMT and aortic PWV before and following adjustment for age may originate from measurement error as described above.

The genetic influence on most of carotid IMT traits (18-38%) may play a role in early detection of initial atherosclerosis. Consequently, patients with a positive

family medical history of (early) cardiovascular diseases could be screened by carotid ultrasound already in young adulthood in high-risk individuals in order to prevent or postpone serious consequences related to increased carotid IMT (plaque formation, etc.). Although it is important to note that the estimated low correlation between the carotid IMT and arterial stiffness parameters indicates that the morphologically detectable thickening of carotid intima-media layers will only develop if the increased AIx and/or aortic PWV is already present. In addition, taking into account that the heritability of carotid IMT was much lower than that of arterial stiffness, preventive carotid ultrasound screening may bear with less magnitude. Accordingly, in order to prevent unnecessary and increased number of carotid ultrasound screenings, an appropriate arterial stiffness test could be performed first in high-risk patients if further studies confirm its necessity. In case of increased arterial stiffness, carotid ultrasound could be performed. Several articles have suggested that carotid IMT measurement, besides the assessment of plaques in extracoronary arteries, coronary calcification, wall rigidity in aorta and peripheral arteries, abnormal flow-mediated endothelium-dependent vasodilation and blood rheology may optimize the management of hypertension (Ramsay et al 1996, Simon et al 1997). Cost-effectiveness of atherosclerosis scanning has been priorly investigated by several studies. Spence et al found that carotid plaque measurement and progression of plaque may be useful for targeting preventive therapy and may improve cost-effectiveness of secondary preventive treatment (Spence et al 2002).

Strength of our study was that all arterial stiffness tests and carotid IMT measurements were conducted on the same day. Our results were derived from healthy adult twins between age of 18 and 82, and therefore may extend to younger subjects or populations with clinically manifest cardiovascular disease, respectively. Additionally, no large (over 100 twin pairs) international twin study with both-gender twin population has ever investigated the heritability of the carotid IMT parameters.

There are some limitations to our study. First, the ultrasound measurements were conducted by different devices and by different operators in four research centers. Second, the overall sample was not homogenous because of the different

characteristics of involved Italian twins (eg., older age and higher smoking prevalence).

5.3 Clinical implications of high heritability on body composition

Various twin studies provided evidence that genetic factors play a considerable role in body weight regulation (Bouchard et al 1990, Stunkard et al 1990). In an early study of twins reared apart, heritability estimates proved to be similar to those where twins were raised together (Stunkard et al 1990). Twin studies also corroborate previous conclusions that the strong predictive value of parental BMI mainly stems from genetic rather than environmental factors (Stunkard et al 1993, Maes et al 1997). Analysis of self-reported BMI collected from a large international twin cohort found a heritability of 58% to 63% for BMI, 59% to 63% for body fat percentage, 48% to 61% for waist circumference, and 52% to 58% for hip circumference (Schousboe et al 2003). In a recent review of genetic longitudinal studies on childhood and adult obesity, heritability estimates reported in twin studies ranged from 57% to 86% for the trend of BMI as followed from early adulthood to late middle age (Silventoinen and Kaprio 2009).

Consistent with earlier reports discussed above, we provide evidence that genetic effects primarily account for high concordance of the investigated anthropometric parameters indicating increased risk for obesity. Specifically, our heritability estimates range between 74% and 81% for the investigated body composition variables. Importantly, we found that only environmental influences unique to the individual and not those shared by family members affect the phenotype of body composition, contributing 19% to 26% of the variance, while shared environmental effects have no effect on the variance in line with earlier reports (Schousboe et al 2004, Stunkard and Sorensen 1990, Hanish et al 2004, Jermendy et al 2011). For instance, BMI variations in the large GenomEUtwin study were mostly influenced by additive genetic and unique (unshared) environmental factors, with the genetic contribution of BMI varying between 45% and 85% according to different countries (Schousboe et al 2003).

Several studies have reported major gene effects in the determination of body fat distribution and the relative proportion of subcutaneous and visceral fat depots (Perusse 2000). Abnormally high body fat and visceral fat content are two obesity-related phenotypes linked to various metabolic complications. Two studies conducted in male monozygotic twins have shown that variations in upper body fat and visceral fat correlate more within pairs than between pairs (Pritchard et al 1998, Bouchard et al 1996). The familial transmission of abdominal visceral fat accounted for >50% of the variance adjusted for age, sex, and total body fat content (Bouchard 1997). A more recent Danish twin study reported high genetic variance on the total (83% to 86%) and regional fat percentages (trunk, 82% to 85%; lower body, 81% to 83%; trunk:lower body, 71% to 83%) by investigating young and elderly twins (Malis et al 2005). Heritability of the amount of upper body fat or its proportion relative to lower body fat in these studies ranged from approximately 30% to 50%, which is lower compared to our heritability result of 76%.

Our high heritability estimates confirm the key importance of screening. Accordingly, detecting individuals with a positive family history of obesity is necessary to be monitored during their young adulthood to detect body composition attributes that indicate the development of obesity. Furthermore, these high-risk individuals need life-style changes to reduce the impact of environmental challenges and prevent obesity-associated co-morbidities. For instance, increased body fat and visceral fat accumulation is associated with co-morbidities including type 2 diabetes mellitus and other metabolic abnormalities, respiratory disturbances, cardiovascular conditions, and increased risk for cancer in particular for malignancies of the digestive system (Scott et al 2007, Calle et al 2003, Kannel et al 1991, Haslam and James 2005). Interestingly, obesity-associated adverse health effects may not develop in 'metabolically benign' obesity (i.e., obesity unaccompanied by hypertension, dyslipidemia, and diabetes) when body fat exerts a beneficial effect by toxic fatty acids and protecting from deleterious effects primarily associated with visceral obesity (Stefan et al 2008). However, women with metabolically benign overweight or obesity were reported to have a greater subclinical cardiovascular disease burden compared to normal weight women (Khan et al 2011). Therefore, cardiovascular screening for these co-morbidities

must be considered, regardless of adipose distribution and based on the strong heritability of body composition traits, as corroborated by our findings.

Bioelectrical impedance analysis provides good estimates of body fat mass and fat free mass as a widely available, low-cost approach by which body composition parameters can be assessed in a variety of health care settings. Interestingly, estimates on the contribution of genetic and environmental factors to body composition as determined by bioelectrical impedance analysis in a large twin cohort with a wide age range and from different geographical areas have not been available. A small study consisting of 30 twins in Germany applied tetrapolar bioelectrical impedance analysis to study anthropometric parameters and found that genetic factors amounted to a variance of 65% to 82% (Hanisch et al 2004). Our present study demonstrates the utility of bioelectrical impedance in a large international cohort of twins with a wide age range.

The strength of our study is that all body composition tests were performed by the same protocol, same personnel, and same device at all international locations, involving a relatively large number of twins with a wide age range from different geographical areas. The study is limited in that our modeling of heritability assumes equal common environmental influences on both MZ and DZ pairs. As true for all twin studies, if this assumption does not hold, then our estimate of heritability may be biased upwards. Notably, statistical differences between males and females in the sample size would not influence our results since these have been adjusted before considered into the models. While the accuracy of BIA has been called into question (Trutschnigg et al 2008), clinical data from a recent large study support the value of BIA in assessing total body and segmental body composition in the general middle-aged population, particularly for estimating body lean mass (Ling et al 2011). Several authors also observed that BIA systems provide good agreement with reference methods at a population level although BIA may be less accurate at the individual level (Jaffrin 2009, Leal et al 2011).

6 Conclusions

6.1 Central systolic and pulse pressures, brachial pulse pressure, augmentation index, aortic pulse wave velocity are moderately heritable. Unique environmental factors account for the moderate proportion of variance. A moderate genetic covariance among aortic PWV and central PP, central SBP and brachial SBP was found. The shared genetic background was stronger between aortic PWV and central PP rather than brachial PP.

6.2 The carotid intima-media thickness parameters appeared to be only moderately or negligible influenced by genetic factors. Environmental factors of relevance for these measures appeared not to be shared within family but related to individual experience. Carotid intima-media thickness did not correlate with arterial stiffness and augmentation index.

6.3 Bioelectrical impedance analysis applied to this twin cohort provides additional evidence to the heritability of anthropometric attributes related to obesity. Heritability of weight, waist and hip circumferences, body fat percentage, fat-free mass and body mass index ranged between 74% and 82%. The completely environmental model showed no impact of shared environmental effects on the variance, while unshared environmental effects were estimated as between 18% and 26%. Moreover, our findings indicate the practical value of this relatively simple method in supporting efforts to prevent obesity-related adverse health events.

7 Summary

Study of twins can help in the better understanding of the genetic and/or environmental contribution to a disease. Our aim was the non-invasive assessment of the hemodynamic properties, carotid intima-media thickness and antropometric characteristics of adult monozygotic and dizygotic twins in an international cohort. The heritability of central systolic and pulse pressure, brachial PP, AIx and aortic PWV is moderate. Unshared environmental effects accounted for large portion of the variance. Aortic PWV had strong direct correlation with central SBP and PP. In addition, there was a moderate genetic covariance among aortic PWV, central PP, SBP and brachial SBP. These findings may further highlight the genetic and environmental etiology of vascular aging and the importance of early atherosclerosis screening, detection and prevention in high-risk patients.

The heritability of carotid IMT in an international adult twin sample is moderate or negligible. The moderate genetic influence on most of carotid IMT traits (18-38%) may play a role in early detection of initial atherosclerosis. Consequently, individuals with a positive family medical history of (early) cardiovascular diseases could be screened by carotid ultrasound already in young adulthood to prevent or postpone serious consequences related to increased carotid IMT. Increased carotid IMT could be prevented or postponed if the underlying unshared environmental factors, which are largely responsible for these traits, could be appropriately managed in high-risk patients. The not significant correlation between the carotid IMT, PWV and AIx variables indicate that increased arterial stiffness and pulse wave reflection is unaccompanied with increased carotid IMT.

Finally, our findings provide new evidence that routine use of a portable device to perform bioelectrical impedance analysis is a convenient, noninvasive way to routine assessment of key anthropometric parameters. At the very least, individuals with a positive family history of obesity may need to be monitored during their young adulthood to detect body composition attributes that indicate the development of obesity and the need for life-style changes to reduce the impact of environmental challenges and prevent obesity-associated co-morbidities.

8 Összefoglaló

Az ikrek vizsgálata egy betegség genetikai és/vagy környezeti hátterének vizsgálatát teszi lehetővé. Célunk volt, hogy az egy- és kétpetéjű ikrek hemodinamikai tulajdonságait, a carotis intima-media vastagságát, illetve az antropometriai jellemzőit vizsgáljuk egy nemzetközi kohorszban. A centrális systolés nyomás (SBP) és pulzusnyomás (PP), a brachiális pulzusnyomás, az augmentációs index (AIx) és az aortikus pulzushullám terjedési sebesség (PWV) mérsékelten örökletes. Az egyéni környezeti hatások felelősek a variancia legnagyobb részéért. Az aortikus PWV szoros direkt korrelációt mutatott a centrális SBP-vel és PP-vel. Továbbá mérsékelt genetikai kovarianciát találtunk az aortikus PWV, a centrális PP, SBP és brachiális SBP között. Ezen eredmények megvilágíthatják a vascularis öregedés genetikai és környezeti etiológiáját és a korai érlemezés szűrés, diagnosztika és prevenció jelentőségét a magas rizikójú betegek körében. A carotis intima-média falvastagság (IMT) örökletessége mérsékelt vagy elhanyagolható volt a nemzetközi felnőtt ikermintánkban. A vizsgált carotis IMT változók többsége mérsékelten örökletesnek bizonyult (18-38%), mely a kezdődő érlemezés korai felismerésében játszhat szerepet. Vagyis a (korai) kardiovaszkuláris betegségekre pozitív családi rizikóval rendelkezőket carotis ultrahanggal lehetne szűrni már akár korai felnőttkorban annak érdekében, hogy a megnövekedett carotis IMT-vel kapcsolatos szövődményeket megelőzhessük vagy késleltethessük. A megvastagodott carotis IMT megelőzhető vagy késleltethető volna, ha az alapjául szolgáló egyéni környezeti faktorokat, melyek leginkább felelősek ezen jelleg kialakulásáért, megfelelően kezelnénk a magas rizikójú betegekben. A carotis IMT, a PWV és az AIx között talált nem szignifikáns korreláció azt jelzi, hogy az emelkedett artériás stiffnesst és a pulzushullám reflexiót jelző AIx-t nem követi megvastagodott carotis IMT. Végül eredményeink új bizonyítékot szolgáltatnak arra, hogy a bioelektrikus impedanciamérés hordozható eszközzel történő rutinszerű használata a főbb antropometriai paraméterek rutin felmérésének kényelmes, nem invazív lehetősége. Az elhízásra pozitív családi anamnézissel rendelkező egyéneket szükségszerű lenne monitorozni korai felnőttkoruk során annak érdekében, hogy felfedezhessük azon

testösszetételei tulajdonságokat, melyek az obesitas kialakulását jelzik. Ez annak érdekében lenne célszerű, hogy életmódváltással a káros környezeti faktorok hatását csökkenteni lehessen és az obesitással összefüggő komorbiditásokat megelőzhetővé lehessen előzni.

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