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Influence of Genetics and Mechanical Properties on Large Arteries in Man

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Cover picture: Ultrasound image of the popliteal artery.

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To Sargon, Ninos, Lucas and Ilan!

"Stirra dig inte blind på en stängd dörr. Det kan finnas en öppen bredvid"
Nalle Puh, Alan Alexander Milne

CONTENTS

ABSTRACT

Arterial pathology is the major contributor to cardiovascular diseases and mortality. The mechanical properties of arteries are independent factors for cardiovascular disease and mortality, where genetics influence the structure of the arterial wall, which may result in change in arterial stiffness. The aims of this thesis were to study the mechanical properties of the popliteal artery (PA) in healthy subjects and the influence of angiotensin-converting enzyme (ACE) polymorphism and Fibrillin-1 (*FBN1*) polymorphism on large arteries. Further, the impact of *FBN1* polymorphism on cardiovascular morbidity and mortality was investigated.

The PA is, after the abdominal aorta, the most common site of aneurysmal development. The PA was studied in healthy subject with ultrasound and the diameter increased and the distensibility decreased with age, with men having lower distensibility than women. This seems not to be the behavior of a true muscular artery but rather of a central elastic artery such as the aorta, and might have implications for the susceptibility to aneurysm formation, as well as the association of dilating disease between the PA and the aorta. The wall stress in the PA was low and unaffected by age, probably caused by a compensatory remodeling response with an increase in wall thickness. This indicates that other mechanisms than wall stress contribute to the process of pathological dilatation in the PA.

The ACE D allele may be associated with abdominal aortic aneurysm. Elderly men with the ACE D allele were associated with increased abdominal aortic stiffness compared to men carrying the I/I genotype. This suggests that the ACE D allele impairs arterial wall integrity, and in combination with local hemodynamic and other genetic factors it may have a roll in aneurysm formation.

The *FBN1* 2/3 genotype has been associated with increased systolic blood pressure. The *FBN1* 2/3 genotype in middle-aged men was associated with increased abdominal aortic stiffness and blood pressure which indicates an increased risk for developing cardiovascular disease. The increased presence of plaque in the carotid artery of middle-aged men with the *FBN1* 2/3 genotype indicates a pathological arterial wall remodeling with a more pronounced atherosclerotic burden, but did however not affect the risk of cardiovascular events and/or death in this population. This relationship needs to be studied further.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by their roman numbers.

I. **Debasso R**, Åstrand H, Bjarnegård N, Rydén Ahlgren Å, Sandgren T and Länne T.
The popliteal artery, an unusual muscular artery with wall properties similar to the aorta – implications for the susceptibility to aneurysm formation?
Journal of Vascular Surgery 2004; 39(4): 836-842.

II. **De Basso R**, Åstrand H, Rydén Ahlgren Å, Sandgren T and Länne T.
Low wall stress in popliteal artery – other mechanisms responsible for the predilection of aneurysmal dilatation?
Manuscript.

III. Ljungberg LU, **De Basso R**, Alehagen U, Björck HM, Persson K, Dahlström U and Länne T.
Impaired abdominal aortic wall integrity in elderly men carrying the angiotensin-converting enzyme D allele.
European Journal of Endovascular Surgery 2011; 42(3): 309-316.

IV. Powell JT, Turner RJ, Sian M, **Debasso** R and Länne T.
Influence of fibrillin-1 genotype on the aortic stiffness in men.
Journal of Applied Physiology 2005; 99(3): 1036-1040.

V. **De Basso R**, Hedblad B, Carlson J, Persson M, Östling G and Länne T.
Increased carotid plaque burden in men with the Fibrillin-1 2/3 genotype.
Manuscript.

ABBREVIATIONS

AA	Abdominal Aorta
AAA	Abdominal Aortic Aneurysm
ACE	Angiotensin-Converting Enzyme
ACEi	Angiotensin-Converting Enzyme inhibitors
Ang I	Angiotensin I
Ang II	Angiotensin II
ARBs	Angiotensin II Receptor Blockers
β	Beta Stiffness
BMI	Body Mass Index
BSA	Body Surface Area
BP	Base Pair
CAD	Coronary Artery Disease
CC	Compliance Coefficient
CA	Carotid Artery
CCA	Common Carotid Artery
CFA	Common Femoral Artery
CI	Confidence Interval
CV	Coefficient of Variation
CVD	Cardiovascular Disease
D	Deletion
D/D	Deletion/Deletion
DBP	Diastolic Blood Pressure
DC	Distensibility Coefficient
EGF	Epidermal Growth Factor
Ep	Pressure Strain Elastic Modulus
FBN1	Fibrillin-1
I	Insertion
I/D	Insertion/Deletion
I/I	Insertion/Insertion
IMT	Intima Media Thickness
LD	Lumen Diameter
MAP	Mean Arterial Pressure
MFS	Marfan Syndrome
MI	Myocardial Infarction
MMP	Matrix Metalloproteinase

PA	Popliteal Artery
PAA	Popliteal Artery Aneurysm
PCR	Polymerase Chain Reaction
PP	Pulse Pressure
PWV	Pulse Wave Velocity
SBP	Systolic Blood Pressure
TGF-β	Transforming Growth Factor-Beta
VNTR	Variable Number of Tandem Repeat
WS	Wall Stress
WTS	Wall Track System

INTRODUCTION

Cardiovascular disease and mortality

Heart diseases and stroke are the leading causes of death in the western world (Lloyd-Jones et al., 2010). The mortality rate of circulatory diseases has decreased with more than 60% from 1987 to 2011. However, still in 2011 diseases of the circulatory system were the underlying cause of death in 39% of women and in 38% of men in Sweden (Socialstyrelsen, 2012). Reduction in population levels of cholesterol, blood pressure, smoking and a wider use of effective treatments among persons with existing cardiovascular disease (CVD) accounts for the decline in coronary heart disease deaths. Unfortunately, trends of increasing obesity, increasing prevalence of hypertension and type 2 diabetes mellitus in the pediatric population, will likely result in future increases of CVD and stroke in adults (Lloyd-Jones et al., 2010).

The mechanical properties of arteries are independent factors for CVD and mortality (Laurent et al., 2001, Gasecki et al., 2012). To understand the pathology of CVD it is of interest to investigate the mechanical properties of the arterial wall in healthy subjects and its development during life as well as the genetic influence on CVD and mortality. Genetic factors may act indirectly through age, blood pressure, smoking, cholesterol levels, glycemia or directly affect the structure of the arterial wall, which may result in increased arterial stiffness (Laurent et al., 2005). Arterial pathology is the major contributor to cardiovascular diseases and mortality.

Structure of the arterial wall

The elastin and collagen ratio varies in the arterial system where elastin being the dominant component in central arteries and collagen in peripheral arteries. The arterial system is divided in two major categories dependent on the composition of the arterial wall: the elastic arteries (the aorta, common carotid artery, common iliac artery and main pulmonary artery) and the muscular arteries (e.g. the common femoral artery, renal artery, popliteal artery, brachial artery, coronary artery and cerebral artery), were the central elastic arteries have a large proportion of elastin components, larger diameter and being

located closer to the heart, and the peripheral muscular arteries contain a higher proportion of collagen and smooth muscle cells than elastic fibers and have medium-sized diameters.

The arterial wall consists of three layers: tunica intima, tunica media and tunica adventitia (Figure 1). The tunica intima is the innermost layer to the blood flow and consists of a single layer of endothelial cells, which are arranged according to the blood flow, and a sub endothelial layer of elastin and collagen fibres that anchor it to the internal elastic lamina. The internal elastic lamina consists of a fenestrated membrane of elastin and merges the tunica intima and tunica media and is more prominent in muscular arteries than in elastic arteries.

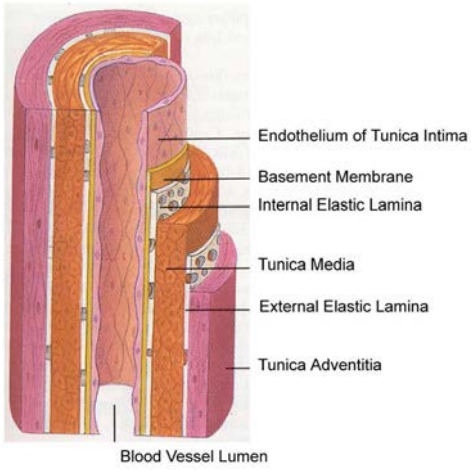

Figure 1. *Schematic illustration of the various layers of the arterial wall: tunica intima, tunica media and tunica adventitia (Illustration-1).*

The tunica media is the middle layer and the most important part of the wall and where the mechanical properties of the wall are determined. In elastic arteries the tunica media consists primarily of elastic laminae that interconnect with a network of elastic fibres. Between these laminae lie smooth muscle cells mostly parallel to the elastin, while some are oriented longitudinally. Collagenous fibres bind the smooth muscle cells to the elastic laminaes. In experimental animals and young humans the elastin, smooth muscle cells and collagenous fibres are precisely oriented and form well-defined layers at physiological pressure. In muscular arteries the tunica media consists primarily of smooth muscle cells.

The outer layer, tunica adventitia, is separated from the tunica media with the external elastic lamina and consists mostly of collagen and some elastin tissue that connects with surrounding connective tissue, small blood vessels (vasa vasorum), nerves and fibroblasts (Nichols et al., 2011).

Mechanical properties of arteries

The mechanical properties of arteries have a major impact on cardiac work. The central elastic arteries accommodate blood ejected from the left ventricle and stores a part of the stroke volume during each systole and during diastole drain this stored volume, thus permitting continuous perfusion of peripheral organs and tissue. This cushioning effect is known as the Windkessel effect.

The properties of elastin provide the conditions of the arterial wall to distend during application of force (pressure) and to retract when the force is removed. The force per unit area that causes deformation (distension) is called stress (i.e. blood pressure applies stress on to the arterial wall) and the deformation is called strain and is described as the ratio of the deformation to its original form (Figure 2). Young´s elastic modulus can be used to calculate the ratio between stress and strain in materials that have a linear stress-strain relation.

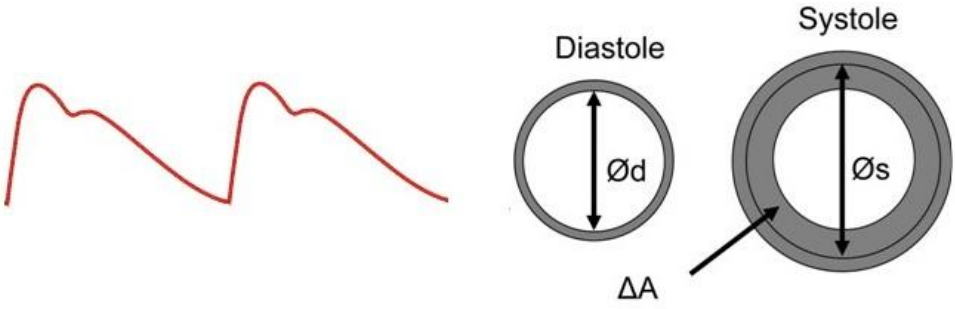

Figure 2. *Aortic distension curve illustrating the diameter change from diastole to systole. ∆A is the increase in area from diastole (Ød) to systole (Øs).*

In non-linear stress-strain relation materials, as in human arteries, it is favorable to use the slope of the stress/strain curve (i.e. the ratio between change in stress and change in strain) as an incremental elastic modulus. This is due to the properties and the ratio of elastin and collagen in the arterial wall, where elastin, which is elastic, extends and is load bearing at small distensions and collagen, being stiff, extends and is load bearing at larger distensions (Roach and Burton, 1957, Astrand et al., 2011). The arteries are distensible at low pressures and become stiffer with increasing pressure with the transition from compliant to stiff behavior occurring between 80-120 mmHg (Dobrin and Rovick, 1969) (Figure 3).

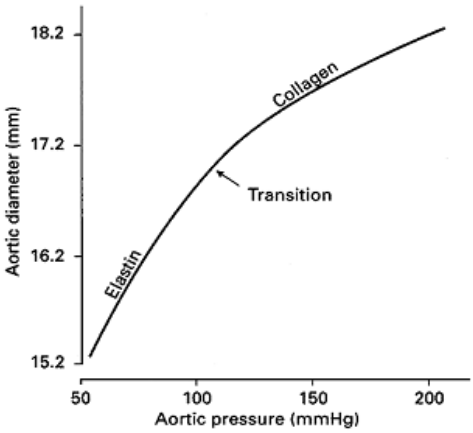

***Figure. 3**. Pressure-diameter curve of the human AA. Note the transition point indicating a stiffer wall above this point (Nichols et al. 2011: Data taken from Länne et al.1992b).*

Elastin has low stiffness and can be lengthened approximately 50-70% while collagen is 100 to 1000 times stiffer than elastin and can only be extended 2-4%. Due to the reduction of the elastin and collagen ratio in the distal arterial tree the arteries in the periphery of the circulation are generally stiffer.

When measuring the relative amounts of various components of the human arterial wall, water comprises 70% of the weight. Of the dry weight, about 50% is made of elastin and collagen, the reminder consists of smooth muscle cells and non-fibrous matrix (Fischer and Llaurado, 1966).

The increase in length of an elastic material is proportional to the tension/stress applied to it and in a very thin-walled cylindrical tube the circumferential stress is proportional to the pressure and the internal radius of

the tube, described by the law of Laplace (Pierre S de Laplace, 1749-1827). If the wall has a thickness, the circumferential stress is inversely proportional to the wall thickness, often known as Lamé's equation:

$$\text{Circumferential Wall Stress} = \frac{\text{Pdiastolic} \times \text{LD}/2}{\text{IMT}} \quad (1)$$

Since wall thickness is included in the formula of incremental elastic modulus and is difficult to measure accurate in vivo, Peterson et al. (1960) established a measure of vascular stiffness that resembles the incremental elastic modulus but neglects wall thickness: the Pressure strain elastic modulus (Ep). Ep is however pressure dependent because it relates pulse pressure to relative diameter change (strain). The relation between the logarithm of relative pressure and strain were linear in vitro however. Based on the above the stiffness index (β) was established by Hayashi et al.(1980) and modified for in vivo use by Kawasaki et al. (1987). The distensibility coefficient (DC) and compliance coefficient (CC) are other equations that describes arterial wall properties (Reneman et al., 1986). The DC is the relative change in arterial diameter during a cardiac cycle for a given increase in pressure and CC of an artery is the absolute increase in cross-sectional area during a cardiac cycle for a given increase in arterial pressure, assuming that the vessel length is constant during the pulse wave. A low CC indicates a reduced buffering capacity and a decrease in DC indicates a reduced elasticity and increased stiffness of the artery.

Effects of aging on arteries

Aging leads to a number of changes in the arterial wall. The optimal proportion of elastin and collagen are at young age. This ratio changes over time and thus also changes vessel wall movement. Aging of the arterial wall involve hyperplasia in the tunica intima but affects primarily the load-bearing tunica media with loss of the orderly arrangement of elastin fibres and laminae. These undergoes thinning, splitting, fraying and fragmentation and degradation of elastin fibres is associated with an increase of collagen resulting in an increase collagen to elastin ratio with age, and deposition of calcium is often seen (Lakatta, 1989, Fonck et al., 2007, McEniery et al., 2009). Collagen is produced throughout life while elastin is not synthesized leading to increased arterial stiffness with age and a decreased arterial distensibility and compliance (Sonesson et al., 1993, Ahlgren et al., 1997). These age-related

changes are mainly seen in the central elastic arteries. Further, as shown in e.g. the human abdominal aorta (AA), the diameter and intima media thickness (IMT) increases (Länne et al., 1994, Astrand et al., 2005). Arterial stiffness leads to an increased systolic blood pressure, which increases the workload of the left ventricle, as well as decreased diastolic blood pressure which reduce coronary perfusion (Laurent et al., 2006).

There is a gender difference regarding arterial stiffness where men are affected by a higher increase in arterial stiffness and cardiovascular morbidity and mortality increases earlier in life. Since arterial disease has different manifestations in the arterial tree in men and women and arterial stiffness is an independent predictor for cardiovascular morbidity and mortality, it is of interest to study differences in the mechanical properties between arteries and gender (Laurent et al., 2001, Boutouyrie et al., 2002).

Aneurysmal disease

The general definition of an arterial aneurysm is a diameter of 1.5 times that of the normal artery (Figure 4). In the clinical setting, a diameter larger than 30 mm in the AA is considered to be an aneurysm and is a candidate for operation when it exceeds 50-55 mm due to the risk of rupture. Aneurysms are by definition focal but may in some individuals be multiple and associated with generalized arteriomegaly. The pathological process in most aneurysms includes upregulation of proteolytic pathways, inflammation and loss of arterial wall matrix, as reported in cerebral-, thoracic- and abdominal aortic aneurysms (AAA). Aneurysms dilate gradually with an increasing risk of rupture or, as seen in popliteal aneurysms, thrombosis develops with risk of embolization distally (Norman and Powell, 2010).

The most common location for aneurysm is in the AA with a prevalence of approximately 5-6% in men and 1-2% in women, for those older than 65 years of age (CASSG, 2001). About 30% of patients with popliteal artery aneurysm (PAA) have an AAA, however relatively few patients with AAA have PAA (Ravn et al., 2007, Morris-Stiff et al., 2005). The incidence of aneurysm in the coronary arteries is 1.5-5% with a predilection for the right coronary artery and the prevalence of cerebral aneurysm is about 2 % (Syed and Lesch, 1997, Ruigrok et al., 2005). Approximately 18% of patients with AAA have coronary artery ectasia or aneurysm formation (Kishi et al., 1997). Aneurysms in the

thoracic aorta are most commonly seen in the ascending or descending aorta, but are approximately five times less common than in the AA (Gillum, 1995).

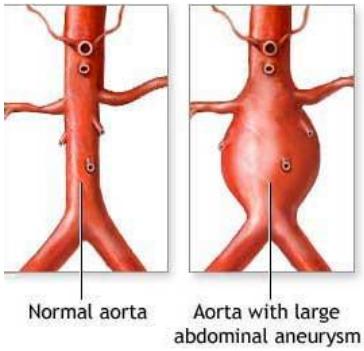

Figure 4. *A normal AA and an AAA (Illustration-2).*

Abdominal aortic aneurysm

There are some observations that suggest that the entire vascular tree is abnormal in patients with aneurysmal disease, where AAA has been associated with generalized arteriomegaly, or reduced distensibility at distant arterial sites (Johnsen et al., 2009, Sonesson et al., 1997). Other observations indicate that AAA is associated mainly by abnormalities in the central arteries (Sandgren et al., 2001).

The most common environmental risk factors for aneurysmal diseases are age, male gender and smoking, where the AA appears to be particularly susceptible for smoking (VanderLaan et al., 2004). Previously it was considered that AAA was associated with atherosclerosis, but this relationship is being questioned because aneurysms are rare in locations prone to atherosclerosis, e.g. superficial femoral artery (Norman and Powell, 2010). Furthermore, diabetes mellitus seems to be a negative risk factor for acquiring an AAA, and growth rate of AAA in these patients is lower than in non-diabetics (Sweeting et al., 2012).

There is a strong genetic influence on the development of AAA where different ***mutated candidate genes*** have been studied. 1) The mutated Fibrillin-1 (*FBN1*) gene affects microfibrils, elastogenesis and the transforming growth factor-β (TGF-β) pathway in Marfan syndrome patients with AAA. 2) The mutated collagen type III alpha 1 gene alter the extracellular matrix fibres in Ehlers-Danlos type IV patients with AAA. 3) The mutated elastin gene causes loss of elastin function and alter the extracellular matrix fibres in AAA patients with Cutis Laxa (Norman and Powell, 2010).

Susceptibility genes associated with AAA are studied widely and a linkage with AAA and a locus in chromosome 12q13.3 which contains the gene for low-density lipoprotein receptor-related protein-1 has been identified (Sakalihasan et al., 2005). Other susceptible genes associated with AAA are 1) a 4G/5G polymorphism in the plasminogen activator inhibitor promoter, 2) a locus on chromosome 19q13 and 3) a locus on chromosome 9p21 (Rossaak et al., 2000, Jones et al., 2002, Boucher et al., 2003, Helgadottir et al., 2008).

Matrix metallopreoteinases (MMP) are enzymes that degrade the extracellular matrix and are involved in normal and diseased tissue remodeling. Studies on MMP genes shows elevated levels of MMPs, especially MMP2 and MMP9, in serum and aortic wall of AAA patients, where mRNA levels of MMP3, known as an activator of MMP9, are significantly increased in AAA tissue (Sakalihasan et al., 1996, Carrell et al., 2002). Increased pressure and strain, activates MMP2 and MMP9 which in turn emphasize the possible importance of wall stress (WS) in the remodeling of the aortic wall (Chesler et al., 1999). Further, there is a relationship between blood pressure, increasing aortic aneurysm diameter and thus WS and the risk of rupture (Cronenwett et al., 1990).

Fibrillin-1

Fibrillin-1 is a glycoprotein with forty-seven epidermal growth factor (EGF)-like domains, of which forty-three are calcium-binding (Figure 5). The binding of calcium plays a structural role by protecting the molecule from proteolysis by e.g. trypsin and elastase. Since fibrillin-1 is the main component of microfibrils apart from elastin, the microfibrils are weakened when proteolysis of fibrillin-1 occurs. When the Fibrillin-1 (*FBN1*) gene is mutated the function of EGF is changed with insufficient calcium binding altering the function of fibrillin-1 and thus the strength of the microfibrils (Jensen et al., 2012). Fibrillin-1 has widespread distribution in both elastic and non-elastic connective tissue throughout the body and occurs in e.g. skin, muscles, kidneys, blood vessels. In arteries, microfibrils are associated with elastic fibres that separate smooth muscle cells and elastic lamellae. Microfibrils also function as a skeleton for the deposition of amorphous elastin (tropoelastin) and provide load-bearing function in the arterial wall (Sherratt et al., 2001). Mutations in the *FBN1* gene, located on chromosome 15, cause the connective tissue disorder Marfan syndrome with increased aortic stiffness, elevated pulse pressure and aortic root dilatation (Dietz et al., 1991, Lee et al., 1991, Jeremy et al., 1994).

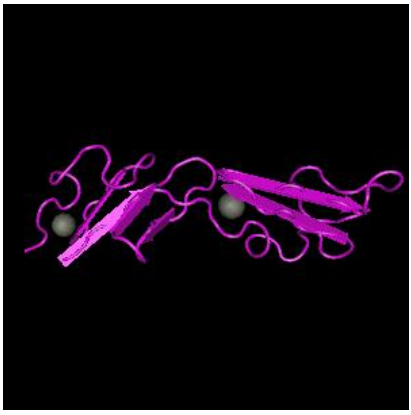

Figure 5. *3D structure of Fibrillin-1 (Illustration-3).*

Previous studies have revealed a potential connection between polymorphisms within the *FBN1* gene and aortic impedance. The 2/3 genotype has been shown to be associated with elevated arterial pulse pressure (PP) in middle-aged men and with systolic blood pressure (SBP) in men with AAA (Powell et al., 1997, MacSweeney et al., 1996). A more recent study in mainly

male patients with coronary artery disease (CAD) has shown an association with increased pulse pressure, large artery impedance and the 2/3 genotype (Medley et al., 2002). On the other hand, another study showed that the 2/3 genotype was not associated with increased aortic pulse wave velocity (PWV) in apparently healthy subjects (Yasmin et al., 2006).

Marfan syndrome

The Marfan syndrome (MFS) was first reported by the French pediatric Dr. Antoine Bernard-Jean Marfan in 1896, where he described a 5-year-old girl with skeletal features e.g. long thin limbs and fingers (Marfan, 1896). MFS is a connective tissue disorder that is characterized by abnormalities in the assembly of elastic fibers, involving the skeletal, ocular and cardiovascular system and the major cause of Marfan syndrome is mutations in the *FBN1* gene located on chromosome 15q21.1 (Tsipouras et al., 1992).

Due to structural defects and a decrease in the content of aortic elastin, the aorta degenerates prematurely and ascending aorta dilates and finally ruptures. Another mechanism related to abnormalities in synthesis of fibrillin-1 is a defect in production of the latent transforming growth factor-β (TGF-β), caused by fibrillin-1 (Pearson et al., 2008). Increased serum levels of TGF-β have been noted in MFS patients, and in mouse models, antibodies against TGF-β prevent development of mitral valve disease and aortic aneurysm (Keane and Pyeritz, 2008, Matt et al., 2009). Dilatation of the aorta and increased aortic stiffness in MFS patients has been reported and blood pressure reduction has been shown to delay aortic dilatation in Marfan syndrome (Jeremy et al., 1994, Sonesson et al., 1994a, Baumgartner et al., 2006, Yetman, 2007, Williams et al., 2008).

Prominent subjects with Marfan syndrome are Abraham Lincoln (1809-1865) US president and Nicolo Paganini (1782-1840) Italian violinist and composer (McKusick, 1991, Schoenfeld, 1978).

Angiotensin-converting enzyme

Angiotensin-converting enzyme (ACE), or kininase II, plays an important role in blood pressure regulation and electrolyte balance by converting angiotensin I (Ang I) to angiotensin II (Ang II), a potent vasoconstrictor. Ang II has significant impact on several important processes in the human body. It stimulates release of aldosterone from the adrenal gland which constricts the renal arterioles leading to an increasing salt and water retention in the kidneys and in the blood vessels. Ang II affects vascular remodeling through induction of smooth muscle cell growth, up-regulation of growth factors and the synthesis of extracellular matrix proteins (Campbell-Boswell and Robertson, 1981, Naftilan et al., 1989, Tokimitsu et al., 1994).

The somatic ACE is expressed in many tissues including cardiovascular system, kidneys, intestine, adrenal glands, liver etc and testicular ACE is only expressed in sperm (Ehlers et al., 1989). In the cardiovascular system ACE is found either bound to the cell membrane of different cell types or in a soluble form in the blood. ACE is cleaved from the endothelial cell membrane and circulates in the blood (Oppong and Hooper, 1993, Beldent et al., 1993). It is still unknown if the circulating level of ACE reflects the level of ACE in tissues.

The ACE gene is located on chromosome 17q23 and there is a variation within the gene, consisting of a 287 base pair insertion/deletion (I/D) polymorphism located in intron 16 which generates three different ACE genotypes: I/I, I/D and D/D (Rigat et al., 1990). The studies on the effect that the ACE I/D genotypes have on CVD are numerous with different conclusions. The first published study that found an association between ACE I/D genotypes and CVD, reported that ACE D/D genotype was more frequent among patients that had suffered myocardial infarction compared to healthy subjects (Cambien et al., 1992). The D/D genotype has also been associated with CAD, hypertension and heart failure, but other studies have failed to confirm these conclusions (Leatham et al., 1994, Lindpaintner et al., 1995, Wang et al., 1996, O'Donnell et al., 1998, Kario et al., 1999, Schut et al., 2004, Agerholm-Larsen et al., 1997). Thus there is no consensus regarding the impact of the ACE genotype on cardiovascular disease.

AIMS

The aims of this thesis were to investigate:

I. The mechanical properties of the popliteal artery in relation to age and gender in healthy subjects.

II. If wall stress of the popliteal artery differs from the adjacent common femoral artery, not being affected by pathological dilatation to the same extent as the popliteal artery.

III. If there are significant associations between the ACE I/D polymorphism, circulating ACE and mechanical properties of the abdominal aorta in elderly subjects.

IV. If variations in Fibrillin-1 genotype are associated with aortic stiffness in male subjects.

V. If the Fibrillin-1 2/3 genotype is associated with presence of carotid plaques and cardiovascular morbidity and mortality in middle-aged subjects.

MATERIALS

Ethical approval

The studies were approved by the Ethic committee of Lund University (Paper I, II, IV and V) and the Ethic committee of Linköping University (Paper III), Sweden and each participant gave informed consent accordingly to the Helsinki declaration.

Studied subjects

Table I shows a summary of the studied populations in Paper I-V.

Paper I and II

108 subjects were investigated (52 men, range 9-78 years and 56 women, range 9-82 years) in Paper I and of those, 94 subjects were studied in Paper II (45 men, range 10-78 years and 49 women, range 10-83 years). 14 subjects were excluded due to low quality of ultrasound measurements. The subjects were recruited from medical staff, friends and advertising. They were all non-smokers, free from medication, did not have any history of hypertension, cardiopulmonary or renal disease, cerebro-vascular events, diabetes or intermittent claudicatio. The ankle-brachial index was >1 in all subjects. Pregnancy was an exclusion criterion.

Paper III

In 1999, 1130 inhabitants, aged 65-82 years, were invited to participate in an ongoing longitudinal study of elderly people from a rural community in southeast Sweden (Alehagen et al., 2007) were 876 subjects accepted. During 2003 and 2005 a follow-up was performed and 672 subjects were asked to participate in a study regarding mechanical properties of the AA. 452 subjects agreed to participate and ultrasound examinations were successfully performed in 406 subjects (212 men and 194 women) and were included in Paper III. 46 subjects were excluded due to low quality of the ultrasound examination, irregular heart rate, difficulties in obtaining blood samples. Those who choose not to participate in the study indicated as a main reason transportation problem and that they had a long distance to the clinic.

Paper IV

79 healthy men (range 28-81 years), first degree relatives of patients with AAA, but not affected to an AAA, were investigated. Subjects with peripheral arterial disease with an ankle brachial index <0.9 and an AA diameter >25 mm were excluded.

A questionnaire was administered to determine the history of previous myocardial infarction, angina, hypertension, diabetes and smoking, and the use of any regular medication was documented.

Paper V

The subjects were recruited from the Malmö Diet and Cancer Study (MDCS) that is a population based prospective cohort study, designed to investigate the association between diet and cancer (Berglund et al., 1993). In short, all inhabitants in the city of Malmö, Sweden, born 1926-1945 (aged 45-69) were invited by mail or by newspaper advertisement to participate. Of an eligible population of 74000, 28449 subjects participated and underwent a health examination and completed a self-administered questionnaire (Manjer et al., 2001). Between November 1991 and February 1994 every second subject that entered the MDCS (n=12445) was invited to participate in a study on the epidemiology of carotid disease, known as the MDCS-Cardiovascular Cohort (MDCS-CC) (Rosvall et al., 2005b). Of the 6103 subjects who participated in the MDCS-CC, 5765 (2424 men, 3341 women) were successfully analyzed regarding their *FBN1* genotype. 338 were excluded due to low quality signals during capillary electrophoresis analysis.

Table I. *Study populations used in paper I-V.*

	N	Men/Women	Age (range years)	Investigated artery	Comments
Paper I	108	52/56	9-82	PA	Healthy subjects.
Paper II	94	45/49	10-83	PA and CFA	Healthy subjects, mainly the same subjects as in paper I.
Paper III	406	212/194	70-88	AA	Population based study with elderly subjects.
Paper IV	79	79/-	28-81	AA	First degree relatives of patients with AAA.
Paper V	5765	2424/3341	46-68	CA	Randomized population based prospective cohort study.

METHODS

For detailed information about the methods, see Method sections of Paper I-V, respectively.

Ultrasound measurements

Paper I, II and IV

Pulsatile diameter changes during the cardiac cycle in the AA, PA and common femoral artery (CFA) were registered with an ultrasound echo-tracking system (Diamove, Teltec AB, Lund, Sweden) interfaced with a 3.5 MHz and a 5 MHz B-mode real-time linear scanner (EUB 240, Hitatchi, Tokyo, Japan). The ultrasound echo-tracking system was designed with two electronic markers that were aligned with and locked on the echoes from the posterior interface of the anterior wall and the anterior interface of the posterior wall. The markers followed the pulsate movements of the vessel wall. The repetition frequency of the echo-tracking loops was 870 Hz, the time resolution was 1.2 ms and the smallest detectable movement was <10 μm (Lindström et al., 1987, Benthin et al., 1991). The echo-tracking system was used in Paper I, II and IV. The pulsatile diameter change during a cardiac cycle together with blood pressure was used to calculate arterial stiffness.

For measuring the IMT and the lumen diameter (LD) of the PA and CFA in Paper I and II, a Philips P700 ultrasound device was used (Philips Ultrasound, Santa Ana, California, US) with a 7,5 MHz linear transducer to visualize the CFA and a 5 MHz linear transducer to visualize the PA. A longitudinal perpendicular image of the vessel was insonated and recorded on a video monitor, three images of good quality were frozen in diastole, according to the prevailing standard of IMT measurements, and the IMT of the far wall as well as the LD were measured manually by tracing a cursor along the echo edges on a section of 10 mm with the aid of the digitizer. This provides approximately 100 boundary points from which the mean value of IMT and LD is automatically calculated (VAP version 2.0, Dept of Appl Electronics, Chalmers University of Technology, Gothenburg, Sweden) (Wendelhag et al., 1991). The accuracy of the technique have showed a good correlation between ultrasound and histology measurement of the arterial wall

(Pignoli et al., 1986). The reproducibility in the PA and CFA measurements were acceptable, coefficients of variation (CV) were 10 and 2 % for IMT and LD respectively (Astrand et al., 2003). The mean value of IMT and LD was calculated based on three images with good recording quality.

Paper III

The Wall Track System (WTS2, Pie Medical, Maastricht, Netherlands) was used on the AA. It was installed in a PC connected to an ultrasound scanner (Esaote AU5, Esaote Biomedica, Florence, Italy), equipped with a 7.5 MHz linear transducer. The Wall Track System (WTS) enables measurements of lumen diameter, pulsatile diameter changes and IMT (Hoeks et al., 1997) (Figure 6).

ECG electrodes were connected to the subject and the artery was visualized in M-mode. The WTS automatically positions two anchors at the posterior and the anterior arterial wall, where manual adjustment could be made, and pulsatile arterial wall movements recorded. Arterial distension waveforms were generated and lumen diameter and pulsatile diameter changes calculated. Using radio frequency signal the WTS can automatically determine end-diastolic IMT of the posterior arterial wall from the interface between the lumen and the tunica intima to the interface between the tunica media and the tunica adventitia.

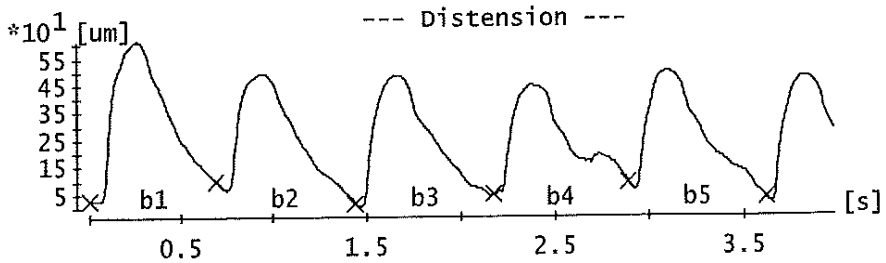

Figure 6. *Distension curves from the AA determined by the Wall Track System.*

The measurements were evaluated and compared with the ultrasound image by visual inspection and measurements that were in agreement with the visual estimation were included. The ultrasound examinations of the AA were performed by two experienced and skilled ultrasonographers. CV was 5% for absolute lumen diameter, 21% for pulsatile diameter change and 17% for IMT.

Paper V

B-mode ultrasound (Acuson 128 CT system, Mountain View, VA, US) of the right carotid artery (CA) was used to measure atherosclerosis by a trained certified ultrasonographer (Berglund et al., 1994). IMT was measured 1 cm proximal to the bifurcation in the far wall of the right distal common carotid artery (CCA) according to the leading edge principle, using a specially designed computer-assisted analyzing system (Wendelhag et al., 1991). The right CA was scanned within a predefined window compromising 3 cm of the distal CCA, the bulb and 1 cm of the internal and external CA for occurrence of plaques defined as a focal thickening of the IMT>1.2 mm. The intra- and interobserver variation between two measurements of the IMT was 9.0% and 8.7% respectively (Rosvall et al., 2000).

Examination

In Paper I-V the subjects rested in supine position in a darkened and quiet room for 10-15 minutes before blood pressure measurements and ultrasound examination were performed. Differences in blood pressures between the left and right arm were excluded before the investigation.

The right PA was examined in prone position at the site of the popliteal fossa (Paper I and II), the right CFA was examined in supine position at the site of the inguinal fossa (Paper II), with the hip joint as a landmark and the AA was examined in supine position 3-4 cm proximal to the aortic bifurcation (Paper IV). The arteries were visualized in the longitudinal section and care was taken to minimize pressure from the transducer to the skin. A sequence of five representative consecutive diameter cycles was manually chosen and the diameters and the diameter changes were calculated as means of the selected diameter cycles. Each artery was examined three times and the mean value was then calculated. Brachial blood pressure was recorded immediately after ultrasound examination.

In Paper III the AA was examined 3-4 cm proximal to the aortic bifurcation with WTS. Mean values from three consecutive recordings were used.

In Paper V the right distal CCA, the bulb and 1 cm of the internal and external CA was examined.

Measuring mechanical properties of arteries

From the diameter, pulsatile diameter change during a heart cycle and blood pressure the mean diameter, fractional diameter change (strain), pressure strain elastic modulus (Ep), stiffness (β), compliance coefficient (CC) and distensibility coefficient (DC) were calculated based on the following equations (Kawasaki et al., 1987, Reneman et al., 1986, van der Heijden-Spek et al., 2000):

$$Strain = \frac{Dsystolic - Ddiastolic}{Ddiastolic} \quad (2)$$

$$Ep = K \frac{Psystolic - Pdiastolic}{(Dsystolic - Ddiastolic)/Ddiastolic} \quad (3)$$

$$\text{Siffness}\,(\beta) = \frac{\ln(Psystolic / Pdiastolic)}{(Dsystolic - Ddiastolic)/Ddiastolic} \quad (4)$$

$$CC = \frac{\pi \times (2 \times Ddiastolic \times \Delta D + \Delta D^2)}{4 \times \Delta P} \quad (5)$$

$$DC = \frac{2 \times Ddiastolic \times \Delta D + \Delta D^2}{\Delta P \times Ddiastolic^2} \quad (6)$$

Where Dsystolic and Ddiastolic are the maximum systolic and minimum diastolic diameter (mm). Psystolic and Pdiastolic are the systolic and diastolic blood pressure (mmHg). Ep in measured in N/m^2, K is the converting factor for mmHg to N/m^2 and equals 133.3. ΔD is the diameter change (Dsystolic – Ddiastolic) (mm) and ΔP is the pressure change (Psystolic – Pdiastolic) (mmHg). The unit for CC is mm^2/kPa and for DC $10^{-3}/kPa$.

The circumferential wall stress (WS) was calculated according to the Lamé's equation, an extended equation from the law of Laplace:

$$\text{Wall Stress} = \frac{Pdiastolic \times LD/2}{IMT} \quad (7)$$

Diastolic pressure (Pdiastolic, dyne/cm²) was used since IMT measurements were performed in diastole. 1 mm Hg equals 1333 dyne/cm². LD, the lumen diameter (cm). IMT, intima-media thickness (cm).

Body surface area (BSA) was estimated according to Du Bois formula (Du Bois and Berlington, 1916):

$$BSA\ (m^2) = weight^{0.425}(kg) \times height^{0.725}(cm) \times 71.84 \quad (8)$$

Noninvasive blood pressure measurements

Blood pressure was measured by the auscultatory method with a sphygmomanometer on the upper arm. A cuff with appropriate size was chosen and blood pressure registration was performed with the subjects in supine position immediately after the registration of the pulsatile diameter changes in the AA, PA and CFA (Paper I, II and IV). In Paper III the blood pressure was recorded with an oscillometric method (Dinamap PRO 200 Monitor, Critikon, Tampa, Fl, US). In Paper V, blood pressure was measured auscultatory with a sphygmomanometer on the right upper arm. Blood pressure was measured in both arms to exclude blood pressure differences. Mean arterial pressure (MAP) was defined as the diastolic blood pressure plus one third of the pulse pressure.

When using blood pressure measurements in the calculation of arterial stiffness, it would be favorable to measure it invasively "in situ", since the blood pressure undergoes transformation in the arterial tree with pressure differences between central and peripheral arteries due to the pulse wave travel and reflections in the arterial tree. Pulse pressure and systolic pressure increase towards the periphery although these differences seem to become less marked later in life due to elastic artery degeneration, decreased distensibility and timing of wave reflection from the lower body (Nichols et al., 2011). Direct invasive measurement of blood pressure in the arteries may be performed, but seems unethical and difficult to use in larger population studies. Instead, we

used the upper arm, and the brachial artery for blood pressure measurements and this could induce an error in the calculations of stiffness/distensibility. However, when comparing invasive pressure in the AA with auscultatory brachial pressure, this showed that auscultatory brachial pulse pressure values were only slightly lower than the invasive aortic pulse pressure leading to a systematic underestimation of Ep and β by 15-20% (Sonesson et al., 1994c). Invasive CFA blood pressure comparison with auscultatory brachial blood pressure shows slightly higher systolic blood pressure and lower diastolic blood pressure in the femoral artery (Ahlgren et al., 2001). There are no available comparisons between invasive pressure in the PA and auscultatory brachial pressure.

Classification of cardiovascular events (Paper V)

Baseline cardiovascular characteristics were assessed by the self-administered questionnaire and the procedure of follow up in cardiovascular events has been described in detail (Rosvall et al., 2005a, Rosvall et al., 2005b). The National Inpatient Register, the Swedish Hospital Discharge Register, the Stroke Register of Malmö and the National Cause of Death Register were used. The ascertainment of cases and validity of these register has been shown to be high (Socialstyrelsen, 2000, Engstrom et al., 2001). The subjects were followed from baseline examination until first occurring CVD event, emigration from Sweden, or death until December 31st 2008.

CVD events and cause of death were coded in accordance with the 9th version of the International Classification of Diseases, ICD-9. A CVD event was defined as fatal or nonfatal myocardial infarction (ICD-9: 410), fatal or nonfatal stroke (ICD-9: 430, 431 and 434), or death attributable to CVD (ICD-9: 412 to 414). ICD-9: 390-459 were used to classify CVD death and ICD-9: 140-239 to classify death attributed by tumors. The mean follow up time was 13.2 years.

Measurement of ACE level (Paper III)

ACE level was determined in plasma using enzyme-linked immunosorbent assay (ELISA) (Quantikine, Human ACE Immunoassay, R&D Systems, Minneapolis, US). The procedure is as follows: monoclonal antibodies specific for ACE are coated on the bottom of a 96-well microplate. Samples are added in to the wells and the available ACE in the samples binds to the antibodies. The unbound substances are removed by washing and biotinylated polyclonal antibodies directed against ACE are added, followed by addition of streptavidine-horseradish peroxidase, which adheres to the polyclonal antibody. A substrate is added, which is converted to a coloured compound by horseradish peroxidase. With a spectrophotometer the intensity of the colour is measured and is proportional to the amount of ACE in the sample. The samples were diluted 1:200. Standards with known concentration of ACE were included in each assay and used to calculate the concentration of ACE in samples. Samples were analyzed in duplicate and re-analyzed if variation from the mean value exceeded 15%. The lower limit of detection was 0.05 ng/ml and intra-assay variation for the analysis of ACE was 6.5%.

Since ACE level was measured in plasma stored at -70° C for 3-5 years degradation of protein might occur, thus the stability of ACE in frozen samples was studied. Plasma from 23 men, recruited from a screening program for AAA at Linköping University Hospital, were stored and frozen at -70° C and ACE level measured after 3 weeks and 12 months. No difference in ACE level was found indicating no degradation of proteins during the first year of freezing.

Genotyping ACE (Paper III)

DNA was isolated from peripheral blood cells using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacture's protocol for preparation for polymerase chain reaction (PCR) and according to the standard method of genotyping ACE (Cheon et al., 2000, Ljungberg and Persson, 2008). Three primers were used, for the deletion (D) gene the primers detect a 238 base pair (bp) fragment and for the insertion (I) gene two fragments are detected, 525 bp and 155 bp. The amplified DNA was separated by gel electrophoresis using a 1.5% agarose gel stained with ethidium bromide and visualized by ultra violet light (Figure 7).

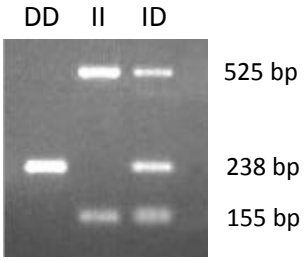

Figure 7. *Gel electrophoresis was used to separate the amplified DNA and visualized by UV-light. One band represents D/D genotype, two bands I/I genotype and three bands I/D genotype.*

Genotyping Fibrillin-1 (Paper IV-V)

DNA from peripheral blood cells was prepared for PCR by typing for the variable tandem nucleotide repeat (VNTR) $(TAAAA)_n$ in intron 28 of the *FBN1* gene on chromosome 15 using the forward primer 5' 6FAM - CAG AGT ACA TAG AGT GTT TTA GGG AGA -3' and the reverse primer 5'- GTT TCT TCC TGG CTA CCA TTC AAC TCC C-3'. 1 µl portion of the PCR product was diluted with 9 µl highly deionized formamide (GeneScan™ 500ROX™ Size Standard) used for electrokinetic injection on the capillary electrophoresis system. Each of the DNA fragments was labeled with the ROX™ fluorophore which results in a single peak when run under denaturing conditions. The alleles were then identified by the number of bp corresponding to each peak (2/2: 162 bp, 162 bp, 2/3: 157bp, 162 bp and 2/4; 152 bp, 162 bp) (Paper V).

The forward primer CCT GGC TAC CAT TCA ACT CCC and reverse primer GAG TAC ATA GAG TGT TTT AGGG were used in Paper IV and the *FBN1* genotype was identified as described above.

Statistics

Data are presented as means ± SD and P<.05 was considered as significant.

Paper I and II

Pearson´s correlation coefficient was used to assess the relationship between age and diameter, strain, stiffness, DC, CC, WS and IMT. Differences between genders were tested using analysis of covariance. A multiple exponential regression model was performed. Paired student t-test was used to calculate differences between studied vessels. A multiple regression model was performed on IMT and LD.

Paper III

Comparisons were performed by χ^2 tests for categorical variables and one-way analysis of variance (ANOVA) or Student´s t-test was used to compare continuous data between groups, adjusted for potentially confounding factors. Multiple regression analyses were made to assess the effect of ACE genotype and ACE level on DC, CC and stiffness (β), adjusted for potentially confounding factors. Hardy-Weinberg equilibrium was assessed by χ^2 tests.

Paper IV

Comparisons were performed by χ^2 tests for categorical variables. Analysis of variance was used for the three-group analysis of continuous variables. Associations amongst the three *FBN1* genotypes and aortic stiffness were adjusted for age, body mass index, mean blood pressure and heart rate (HR) in a regression model.

Paper V

Comparisons were performed by χ^2 tests. Analysis of variance was used for continuous variables with post-hoc tests. The Kaplan-Meier method was used to estimate and plot the first CVD event and survival distribution amongst the three *FBN1* genotypes. Differences in incident CVD and survival were assessed using the Log-Rank test.

RESULTS

For detailed information about the results, see Result sections of Paper I-V, respectively.

Mechanical properties of the popliteal artery

108 subjects were successfully investigated and the mean diameter of the PA was 7.43 mm (confidence interval (CI); 7.16-7.71 mm) and 6.33 mm (CI; 6.11-6.56 mm) for men and women respectively (P<.001). The diameter increased with age in both men and women, with men having 17% larger diameters than women (P<.001). The diameter was also correlated with age in both men (r=0.66, P<.001) and women (r=0.51, P<.001). The fractional diameter change, strain, of the PA decreased exponentially with age in both men (r=-0.64, P<.001) and women (r=-0.67, P<.001), with women having higher strain values than men (P<.01). The CV regarding arterial diameter was 4% and fractional diameter change 24%. Figure 8 shows that the stiffness (β) of the PA increased exponentially with age in both men and women (P<.001 respectively) and that men had higher stiffness (β) values than women (25%, P<.01). Stiffness (β) was mainly influenced by age (45% males and 56% females, P<.01) while systolic blood pressure (SBP) and MAP had minor importance.

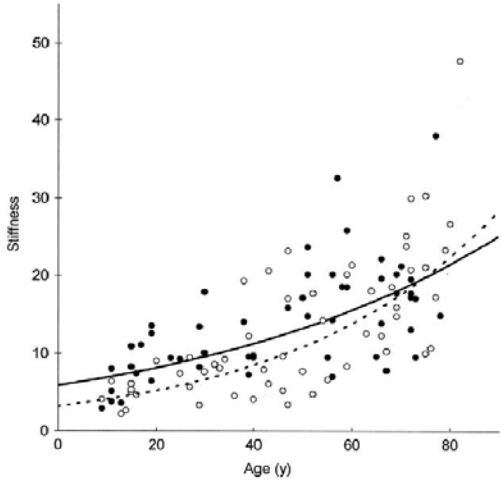

Figure 8. *Stiffness (β) of the PA in healthy men (filled circles, solid line) and women (open circles, dashed line) in relation to age. There was a significant correlation between the increase in stiffness (β) and age in men and women, with higher stiffness values in men.*

The DC values decreased with age in both men and women (r=-0.71 and r=-0.80, P<.001 respectively), with men having 24% lower DC values than women (P<.01). DC was mainly influenced by age, 51% and 64% in men and women respectively (P<.01). SBP and diameter were of minor importance, 7% and 4%, (P<.01 and P<.05 respectively). MAP and IMT did not influence DC. The CC decreased with age in men and women (r=-0.40, P<.01 and r=-0.69, P<.001 respectively), without any gender differences.

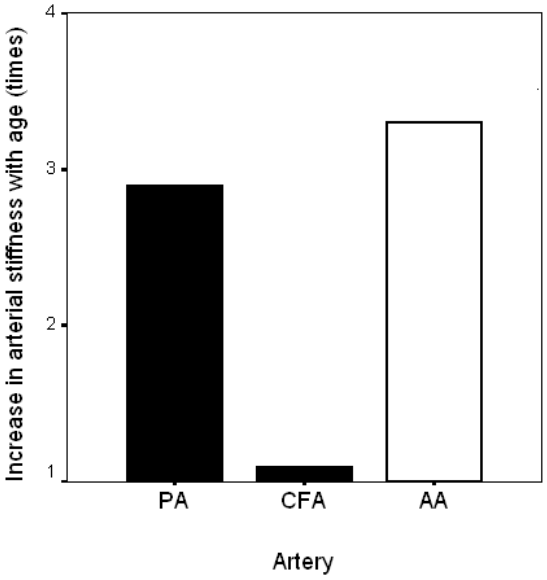

Figure 9. *Increase in stiffness (β) between ages 20 and 70 years (times the measured value at 20 years of age) in the PA (muscular artery, present study), and earlier published data regarding the CFA (muscular artery) and the AA (elastic artery).*

Figure 9 shows a comparison of the increase in stiffness (β) between ages 20 and 70 years (times the measured value at 20 years of age) in the PA (muscular artery, present study), and earlier published data regarding the CFA (Ahlgren et al., 2001), with subjects from the present investigation included (muscular artery) and the AA (elastic artery) (Sonesson et al., 1993). The increase in stiffness (β) in the PA was similar to the increase seen in the AA. Note the lack of increase in the CFA.

Ultrasound examinations on diameter and IMT were successfully performed in 44 males and 45 females of the PA and in 36 males and 42 females in the CFA. The IMT of the PA increased exponentially with age in men and women (r=0.82 and r=0.62, P<.001 respectively), with men having

14% higher IMT values than women (P<.001). In adults between the ages 25 to 70 years, the IMT of the PA increased from 0.42 to 0.63 mm (50%) in men and from 0.41 to 0.54 mm (32%) in women. When comparing IMT of the PA and CFA there was no difference seen in women but the IMT of the PA was larger than the IMT of the CFA in men (P<.001).

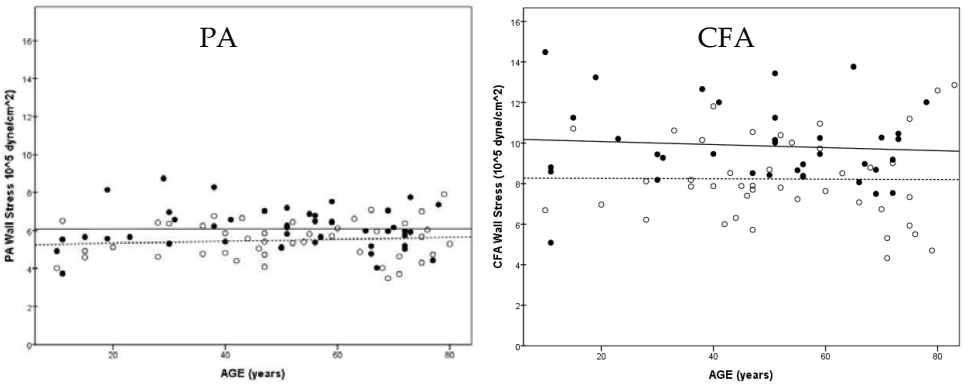

Figure 10. *Wall stress of the PA and CFA in relation to age (men: black circles solid line, and women: open circles dashed line).*

Figure 10 shows the WS at different ages of the PA and CFA. The WS did not increase during age in PA or in CFA. Men had 11 and 20% higher WS values than women in the PA and the CFA (P<.01 and P<.001 respectively). Figure 11 shows the difference in WS in CFA and PA in men and women, where WS was higher in the CFA than in the PA in both men and women (P<.001).

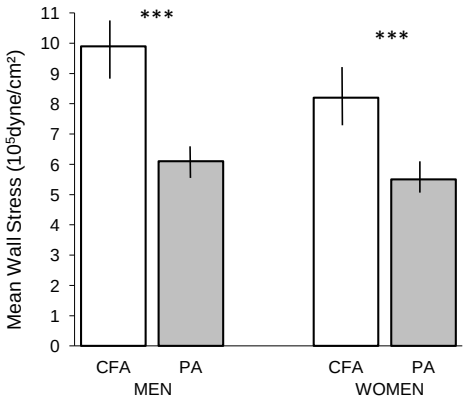

Figure 11. *Mean WS in the PA (grey bars) and in the CFA (white bars) in men and women. The PA WS is lower than the CFA in both men and women.*

Angiotensin-converting enzyme and abdominal aortic stiffness

406 subjects (212 men and 194 women) were successfully investigated. The use of anti-hypertensive drugs was high (64%) and 23% were using lipid lowering drugs in this elderly study population without gender differences regarding treatment (20% ACE inhibitors (ACEi), 4% angiotensin II receptor blockers, 36% beta blockers and 36% diuretics).
Since ACEi stimulates up-regulation of ACE, subjects with ongoing ACEi treatment (43 men and 40 women) were excluded from the statistical analysis on ACE level.

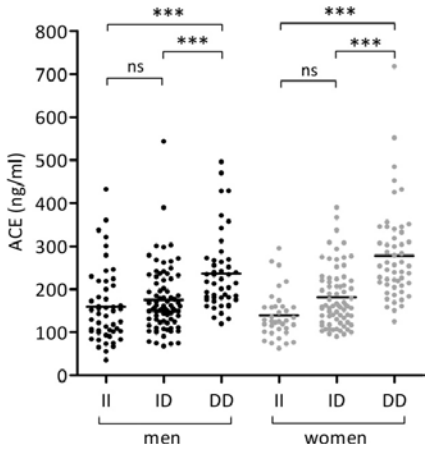

Figure 12. *Plasma ACE level according to ACE I/D polymorphism in men (black circles) and women (grey circles).*

The frequency of the D allele was 0.50 (0.48 men and 0.53 women) and in accordance with the Hardy-Weinberg equilibrium. Figure 12 shows that the plasma levels of ACE were influenced by ACE I/D polymorphism. The ACE D allele had an impact of 17% (9% in men and 27% in women) of the circulating plasma ACE level, without gender differences.

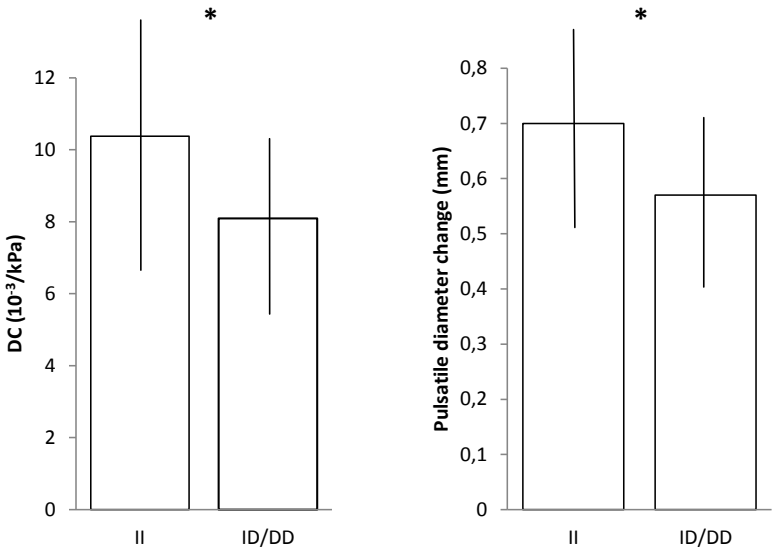

Figure 13. *Distensibility coefficient and pulsatile diameter change of the AA according to ACE I/D polymorphism in elderly men.*

Figure 13 shows that in elderly men there were significant associations in the subjects carrying the ACE D allele, with lower DC (P=.017) and lower pulsatile diameter change (P=.014) in the AA than in those carrying the ACE II allele.

18 men and two women were identified with an AAA, of these, 10 men were excluded due to low quality of the ultrasound measurements leaving eight men and two women with an AAA in the study population of 406 subjects. When excluding these subjects with an AAA, the DC was still low in men carrying the ACE D allele (P=.036). Further, multiple regression analysis showed in men an association between the ACE D allele and reduced DC (P=.003), CC (P=.045) and increased stiffness (β) (P=.048) in the AA, but no association with circulating ACE levels.

Fibrillin-1 and abdominal aortic stiffness

The distribution of *FBN1* genotypes in the 79 men was: 1/2 (n=4), 1/3 (n=1), 1/4 (n=1), 2/2 (n=50), 2/3 (n=10), 2/4 (n=11), 3/3 (n=1) and 3/4 (n=1) and associations between *FBN1* genotypes and AA stiffness were calculated in the three common genotypes: 2/2, 2/3 and 2/4. Table II shows the demographics and measured data according to the three genotypes, where subjects of 2/3 genotype had higher SBP (P=.04), PP (P=.04) and MAP (P=.03) than subjects of 2/2 and 2/4 genotypes. Seven subjects were being treated for hypertension (four 2/2, two 2/3 and one 2/4).

Table II. *Characteristics according to FBN1 genotype.*

	FBN1 genotype			
	2/2 (n=50)	2/3 (n=10)	2/4 (n=11)	P
Age (years)	54.7±3.2	56.0±1.7	55.4±2.4	0.91
BMI (body mass index, kg/m^2)	25.3±2.1	26.9±2.2	25.3±1.8	0.25
Current smokers (%)	10(20)	2(20)	4(36)	0.42
Systolic pressure (mm Hg)	140±15	158±9	139±16	0.04
Diastolic pressure (mm Hg)	88±10	95±5	87±7	0.08
Mean pressure (mm Hg)	105±11	117±8	105±11	0.03
Pulse pressure (mm Hg)	52±9	61±9	51±11	0.04
Aortic diameter (cm)	1.84±0.22	1.86±0.15	1.87±0.16	0.81
Pressure strain elastic modulus x 10^{-5} (N/m^2)	1.76±0.69	3.04±0.72	1.66±0.55	0.001

The 2/3 genotype subjects also had the highest pressure strain elastic modulus (Ep) (P<.001), highest HR (P<.01) and AA stiffness (β) (P<.001) compared to 2/2 and 2/4 genotype (Figure 14).
After adjusting stiffness (β) for age, body mass index (BMI), MAP and HR the association between AA stiffness and *FBN1* 2/3 genotype still remained highly significant (P=.005). Due to an age related increase in stiffness of the AA, 47 middle-aged men (40-59 years) were analyzed separately (2/2 n=33, 2/3 n=7 and 2/4 n=7) where there were no difference between the groups regarding mean age, smoking habits, BMI and AA diameter. In this subgroup, subject of 2/3 genotype had the highest MAP, Ep and stiffness (β), but not PP. Stiffness (β) remained significant even after adjustment for age, BMI, MAP and HR (P=.008).

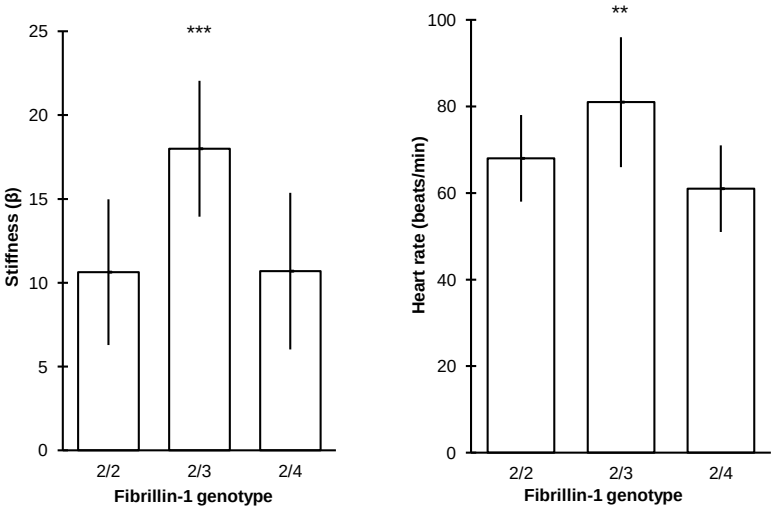

Figure 14. *The AA stiffness (β) and HR according to the three common FBN1 genotypes.*

Fibrillin-1 and cardiovascular morbidity and mortality

Four alleles and 10 combinations of *FBN1* genotypes were identified in 5765 subjects in the Malmö Diet and Cancer Study (MDCS), i. e. 1/1 (0.02%, n=1), 1/2 (1.1%, n=65), 1/3 (0.1%, n=8), 1/4 (0.3%, n=16), 2/2 (56.4%, n=3254), 2/3 (12.1%, n=698), 2/4 (23.7%, n=1365), 3/3 (0.8%, n=47), 3/4 (2.7%, n=155) and 4/4 (2.7%, n=156), where the most common genotypes were 2/2, 2/3 and 2/4 and accounted for 92.2% (n=5317) of the studied population.

There were no differences between the three genotypes regarding age, blood pressure, smoking habits, lipids, CCA diameter and CCA IMT in men or women at baseline, nor in blood pressure lowering drugs, lipid lowering drugs, anti-diabetes drugs and cardiovascular events in men and women at baseline.

Figure 15 shows the occurrence of plaque in the CA where men of 2/3 genotype had higher plaque prevalence than men of 2/2 and 2/4 genotypes; 55% vs. 46% and 50% (P=.03) and after adjusting for factors that affects plaque occurrence such as age, BMI, SBP and DBP the difference remained highly significant (P=.007). There were no differences regarding *FBN1* genotype and plaque occurrence observed in women. The odds ratio (OR) for prevalent carotid plaque in men with the 2/3 and 2/2 genotype, respectively, as compared to men with the 2/4 genotype was 1.15 (95% CI: 0.86-1.53) and 0.84 (95% CI: 0.69-1.029), respectively.

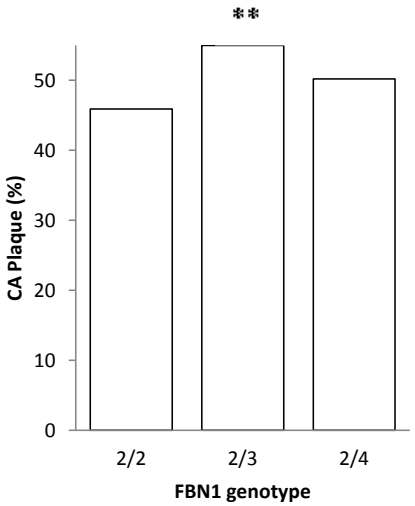

Figure 15. *The occurrence of plaque in the CA in men according to the three common FBN1 genotypes (adjusted for age, BMI, SBP and DBP).*

Incidence of first CVD events during the follow-up (mean 13.2 years) was 8.6 events per 1000 person-years (12.4 in men and 6.0 in women, P<.001). The corresponding figures for deaths per 1000 person-years were 11.5 (15.1 in men and 9.1 in women, P<.001). Men were affected by a higher number of CVD events than women (Table III).

The underlying cause of death was CVD (ICD-9: 390-459) in 35.9% of the men and 25.7% of the woman, of whom only 12 men and 9 women had the 2/3 genotype. Corresponding figures for cancer (ICD-9: 140-239) were 44.9% and 57.6%, respectively. Autopsy was performed in 24% of the deceased cases. There were no significant differences in incidence of first CVD events (2/3 vs 2/2, P=.605; 2/3 vs 2/4, P=.873; and 2/2 vs 2/4, P=.383, respectively) or all-cause mortality (2/3 vs 2/2, P=.406; 2/3 vs 2/4, P=.860; and 2/2 vs 2/4, P=.384,

respectively) between the main three *FBN1* genotypes in all subjects, in men or in women (Figure 16).

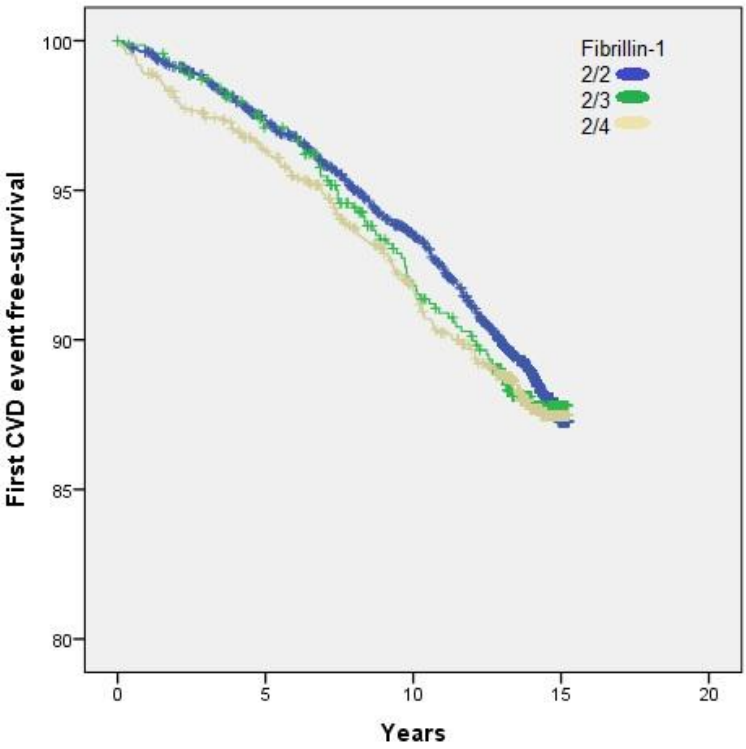

Figure 16. *Kaplan-Meier plot showing cardiovascular events (MI and stroke) and mortality according to the main three FBN1 genotypes.*

Table III. *Incidence (per 1000 person-years) in cardiovascular events (MI and stroke) and mortality according to the main three FBN1 genotypes.*

	Men						Women					
Variables	2/2 genotype		2/3 genotype		2/4 genotype		2/2 genotype		2/3 genotype		2/4 genotype	
	n	Inc	n	Inc	n	Inc	n	Inc	n	Inc	n	Inc
Cardiovascular event	206	11.9	47	12.5	91	12.4	143	5.7	32	6.0	67	6.4
MI	117	6.8	25	6.7	59	8.0	63	2.5	15	2.8	32	3.1
Stroke	89	5.1	22	5.9	32	4.4	80	3.2	17	3.2	35	3.4
Mortality	279	16.1	53	14.1	108	14.7	234	9.3	47	8.8	94	9.0
Cardiovascular mortality	77	3.1	12	3.2	34	4.6	46	1.8	9	1.7	23	2.2

n = number of subjects
Inc = Incidence (per 1000 person-years)

DISCUSSION

The mechanical properties of arteries have a major impact on cardiac work, where the Windkessel effect (buffering function) plays an important role in permitting continuous perfusion of peripheral organs and tissue also during diastole. The composition of the arterial wall influences the mechanical properties of arteries, where the elastin and collagen ratio varies in the arterial system and with age. In central arteries, the dominant component is elastin and in peripheral arteries collagen, with optimal proportions of elastin and collagen at young age, but with an increasing amount of collagen and less elastin with age thus changing the vessel wall movement resulting in increased arterial stiffness, decreased distensibility and compliance (Ahlgren et al., 1997, Sonesson et al., 1993). These age-related changes are mainly seen in the central elastic arteries. Large-artery stiffness is the main determinant of pulse and systolic pressure, and aortic stiffness has independent predicted value for cardiovascular mortality, coronary morbidity and mortality and stroke (Safar et al., 1987, Laurent et al., 2001, Boutouyrie et al., 2002, Laurent et al., 2005, Lacolley et al., 2009).

Popliteal artery, an unusual muscular artery

The most common site of aneurysm development, after the AA, is the PA where almost 30% of patients with popliteal artery aneurysm (PAA) have an AAA (Ravn et al., 2007). Why the PA is more susceptible than other peripheral muscular arteries to aneurysmal disease is at present unknown. One underlying factor of importance may be differences in arterial wall composition compared with other peripheral muscular arteries, which in turn may affect the wall properties. Several studies of mechanical properties on central elastic arteries have shown an age-related increase in stiffness, arterial diameter and intima media thickness (Sonesson et al., 1994c, Hansen et al., 1995, Astrand et al., 2005). However, studies on peripheral muscular arteries are sparse and show no increase in stiffness, indicating less of age-related arterial wall degeneration, e.g. CFA and distal brachial artery (van der Heijden-Spek et al., 2000, Ahlgren et al., 2001, Bjarnegard and Lanne, 2010).

The absence of an age-related increase in arterial stiffness in muscular arteries may seem surprising, because it is well known that the arterial wall structure changes with an increase in collagen and thickening of the arterial wall. There is no reason to believe that age-related histological changes and subsequent remodeling are absent in muscular arteries, inasmuch as age-related dilatation of muscular arteries of similar magnitude to that in elastic arteries has been found (Kawasaki et al., 1987, Sonesson et al., 1993, Hansen et al., 1995, Ahlgren et al., 2001).

The diameter of the muscular PA in healthy subjects increased with age in both men and women with men having larger diameter than women due to larger body size. This gender difference was also observed in the CFA. The age-related arterial dilatation might be influenced by the distending force acting on the arterial wall, the blood pressure. Consequently we found a correlation between blood pressure and arterial dilatation.

The PA strain decreased exponentially with age in both gender, with lower strain values in men. The stiffness of the PA increased exponentially with age, with increased stiffness in men compared to women (Figure 8), as would have been expected from the strain values. Interestingly, when the increase in stiffness (β) between ages 20 and 70 of the PA (muscular artery) was compared to CFA (muscular artery) and AA (elastic artery) the increase in stiffness (β) in the PA was similar to the increase observed in the AA, while the CFA showed no increase at all (Figure 9). Earlier studies in stiffness of the PA in healthy subjects are sparse. Tai et al. (1999) investigated the PA in 11 young and 12 elderly subjects and found an increased stiffness with age. However, the number of subjects included was small and there was no separation between genders. Brodszki et al. (2002) studied the PA in young women but without any attempt to define the relation between stiffness and age or gender. The increased stiffness of the PA differs from the near superficial and common femoral artery, as well as other regions of muscular arteries, such as the radial artery and brachial artery, where the age-related increase in stiffness seems to be absent (Tai et al., 1999, Ahlgren et al., 2001, Benetos et al., 1993, Bortolotto et al., 1999, van der Heijden-Spek et al., 2000, Bjarnegard and Lanne, 2010). Furthermore, the stiffness values of the PA show similarities to the AA with an age-related increase, and with men having higher stiffness values than females (Länne et al., 1992, Sonesson et al., 1993). The increased stiffness of the popliteal wall, as in the aorta, probably reflects a decreased elastin to collagen ratio. The amount of elastin and collagen changes

during life, with a fragmentation of elastin without neosynthesization, while collagen is produced throughout life (Lakatta, 1989, Fonck et al., 2007, Nichols et al., 2011).

Incipient atherosclerosis might also lead to an increase in stiffness (Lind et al., 2009). The literature concludes however that the effect of atherosclerosis on arterial stiffness is minor. Another factor that might be of importance is wall thickness, according to the equation of Young´s modulus (Nichols et al., 2011). Thus increased arterial wall thickness may increase stiffness. The IMT of the PA increased with age in both gender, with men having higher IMT than women and in accordance with findings in the femoral artery. Further, men had higher IMT in the PA compared to CFA. IMT was correlated to age but did however not affect the stiffness of the PA wall. Thus, it seems reasonable to assume that the composition of the PA wall may be more similar to central elastic arteries such as the aorta, than to other peripheral muscular arteries. In view of the fact that the stiffness, diameter and IMT increases with age to a similar extent in both PA and AA it may be speculated that this might be the reason for the similar pathology in both regions with a tendency to aneurysm formation.

An increase in blood pressure and diameter leads to increased wall stress (WS) according to the law of Laplace, which in turn activates smooth muscle cells of the arterial wall, leading to an increase in matrix and wall thickness (Ben Driss et al., 1997). Since blood pressure and diameter increases with age, an increased WS in the PA would have been expected. However, no increase was found neither in the PA or nor in the CFA (Figure 10). Further, men had higher WS than women (Figure 11). The absence of WS increase in PA and CFA was probably caused by a compensatory remodeling response with an increase in arterial wall thickness. Interestingly, WS was lower in PA than in the CFA in both genders, despite the fact that aneurysms are more common in the PA than in the CFA (Norman and Powell, 2010). This indicates that other mechanisms than WS are involved in the process of pathological arterial dilatation in the PA. In the AA the WS has been shown to be high, with an age-related increase in WS observed in men but not in women, which may be related to the increased prevalence of AAA in males (Astrand et al., 2005). In diabetic patients however, the WS of the AA is reduced compared to healthy controls, probably due to increased IMT and might be the reason for the reduced risk of aneurysmal disease in diabetic patients (Astrand et al., 2007).

Repetitive longitudinal deformation of an artery caused by flexion, has been correlated to subsequent lesion progression as shown in the coronary arteries and may also be of relevance for the preponderance of pathological dilatation in the PA (MacIsaac et al., 1993, Falk et al., 1995). Anecdotal evidence from cavalry officers indicate that tight boots together with repeated flexion and extension while riding may traumatize the PA and cause an aneurysm (Sedge et al., 1961, Stein et al., 1994). Further, the propagating arterial pulse wave causes cyclic changes in size and shape of the arterial wall. For the CFA the mean fractional diameter change is about 6% in both men and women and is constant during ageing (Ahlgren et al., 2001). In the PA however, it decreased exponentially with age, probably due to the increase in wall stiffness. Thus, a zone of compliance mismatch between the femoral artery and PA occur. In combination with stiff atherosclerotic plaques bending at the junction between stiff plaques and vessel wall, the wall constituents may weaken, in accordance with findings in the coronary tree.

Another factor to consider is that the PA below the knee is divided into three much smaller arteries which may be of relevance for increased pressure wave reflections, possibly making the PA more susceptible to aneurysmal dilatation, in analogy with the increased risk of AAA in patients with traumatic above-knee amputations (Vollmar et al., 1989). Finally, at daytime, the PA is repetitively exposed to upright posture with increased local hemodynamic burden (Gemignani et al., 2008).

Genetics and arterial stiffness

Arterial stiffness has a genetic component that is independent of the influence of blood pressure, HR, height, age and other cardiovascular risk factors. The Bogalusa Heart study showed increased CA stiffness in adolescents with a parental history of myocardial infarction or diabetes (Riley et al., 1986). Another study, The Strong Heart Study showed in 950 adults from 13 American Indian communities that although classical covariates accounted for up to 51% of the variance of carotid stiffness, the proportion of genetic influence was 23% (North et al., 2002). A study comparing monozygotic and dizygotic twins showed that 37% of the variance of augmentation index was determined by of genetic factors (Snieder et al., 2000).

Genotype studies on matrix proteins (*FBN1* gene and elastin gene) have shown that the 2/3 genotype of the *FBN1* gene is associated with higher characteristic impedance and pulse pressure in patients with CAD (Medley et al., 2002). The A allele of the Ser422Gly polymorphism of the elastin gene was associated with increased carotid stiffness (Hanon et al., 2001). In subject older than 60 years and homozygous for the 5A promoter polymorphism of MMP-3, an increased aortic stiffness has also been observed (Medley et al., 2003).

ACE and mechanical properties of the abdominal aorta

The relationship between the ACE I/D polymorphism and CVD has earlier been studied, however no consensus regarding the impact of ACE genotypes on CVD has been reached. The ACE D allele have been associated with CAD and MI in type 2 diabetic patients (Narne et al., 2012). Recently, increased circulating ACE levels have been correlated to left ventricular dysfunction (Ljungberg et al., 2012).

The mechanical properties of arteries are affected by the composition of connective tissue of the arterial wall that might be genetically influenced by the ACE gene. The ACE D allele has been associated with increased carotid stiffness in a general population and in older subjects (Mattace-Raso et al., 2004, Balkestein et al., 2001). However, other studies have shown the opposite with no relation between ACE I/D polymorphism and carotid-femoral PWV, while others have shown that the I allele was associated with higher PWV in hypertensive patients and in healthy middle-aged subjects (Mattace-Raso et al., 2004, Sie et al., 2009, Benetos et al., 1996, Dima et al., 2008). The mechanical

properties of the AA studied in the elderly subjects was measured with ultrasound technique resulting in arterial stiffness values obtained locally, while PWV on the other hand reflect mean arterial stiffness values of both elastic and muscular arteries determined over a length of the arterial tree. Thus, PWV may reflect other aspects of vascular function since elasticity varies in the arterial tree and different genetic determinants may have alternative outcomes depending on the studied artery.

Interestingly, elderly men carrying the ACE D allele had lower pulsatile diameter change and increased stiffness in the AA compared to men carrying ACE II allele, indicating that the ACE D allele is associated with impaired integrity of the AA wall (Figure 13). There were no differences observed in women regarding the genotypes and AA wall mechanics.

The effect of circulating ACE on arterial stiffness is unknown. A possible mechanism could be that carriers of the D allele have high levels of circulating ACE which increases the converting of Ang I to Ang II (Figure 12). Ang II acts as a potent vasoconstrictor and has impact on vascular homeostasis, vascular tone and vascular smooth muscle cell growth (Campbell-Boswell and Robertson, 1981, Geisterfer et al., 1988). In experimental studies, Ang II influences the production of vessel wall collagen and inhibits the production of elastin, resulting in an increase in arterial stiffness (Kato et al., 1991, Tokimitsu et al., 1994). The possible relationship between circulating ACE and stiffness is strengthened by the fact that ACE inhibitors (ACEi) and Angiotensin II receptor blockers (ARB) not only reduce blood pressure, but also arterial stiffness (London et al., 1996, Mahmud and Feely, 2002a, Mahmud and Feely, 2002b). Even though an association between the ACE I/D polymorphism and circulating ACE levels was found, there was a large variation of circulating ACE levels between carriers of the same genotype (Figure 12) (Cambien et al., 1994). We hypothesized that circulating ACE levels would be associated with arterial stiffness rather than with ACE I/D polymorphism, since ACEi reduces arterial stiffness. However, no association between circulating ACE and AA stiffness was found even though the ACE D allele was associated with increased stiffness in the AA. It is still unknown to what extent circulating ACE levels correlates with levels in the tissues. Accordingly, there might be an association between AA stiffness and ACE in the arterial wall, and the beneficial effects that ACEi and ARBs have on arterial stiffness may result from local effects within the wall. Alternatively, the D

allele may be in linkage disequilibrium with genetic variations in other genes, which in turn may influence AA stiffness.

In the studied elderly subjects, the prevalence of AAA was 8% and 1% in men and women respectively, which is similar to other reports (CASSG, 2001). Men with AAA showed a trend to higher stiffness values than those without AAA. However, even when excluding subjects with AAA, stiffness remained increased in men carrying the ACE D allele. The gender difference in the association between ACE D allele and increased AA stiffness, with an association found only in men is interesting regarding the fact that AAA is more common in men than in women. An association between the ACE D allele and AAA has earlier been found, indicating that ACE influences the development of aneurysmal disease (Pola et al., 2001, Fatini et al., 2005). Further, accumulations of ACE and Ang II-forming enzymes have been found in the arterial wall in AAA patients, and increased Ang II has also been suggested to be associated with AAA (Nishimoto et al., 2002).
Since AAA patients have been shown to have an increased AA stiffness, it would be of interest to explore the relationship between AA stiffness, ACE I/D polymorphism, circulating ACE level and AAA in future studies (Länne et al., 1992, MacSweeney et al., 1992).

Fibrillin-1 and the cardiovascular system

Fibrillin-1 is the major constitutive element of extracellular microfibrils and provide load-bearing function in the arterial wall (Sherratt et al., 2001). Mutations in the *FBN1* gene cause the connective tissue disorder Marfan syndrome with increased aortic stiffness, elevated pulse pressure and aortic root dilatation (Jeremy et al., 1994, Sonesson et al., 1994a, Jondeau et al., 1999). In the study on middle-aged men, four alleles and 10 combinations of *FBN1* genotypes were identified and the three most common genotypes were: 2/2, 2/3 and 2/4. The aortic stiffness, as measured by Ep, was higher in subjects of the 2/3 genotype compared to 2/2 and 2/4. Since the pressure-diameter relationship in the arterial walls is non-linear, Ep is pressure dependent and could explain the higher values in subjects with the 2/3 genotype. However, the association remained highly significant after adjustment for mean pressure, indicating the possibility of a structural difference in the aortic wall between the groups (Table II). Furthermore, also the stiffness index (β) was higher in the middle-age men carrying the 2/3 genotype (Figure 14). Stiffness index (β) is based on the observed linear relation between the logarithm of

relative pressure and distension ratio and has been shown to be less pressure dependent.

Subjects of 2/3 genotypes were associated with increased HR (Figure 14). High resting HR has been related to the development of coronary atherosclerosis, CVD and mortality in several studies (Gillman et al., 1993., Palatini and Julius, 1997, Thomas et al., 2001). The findings of increased aortic stiffness, blood pressure, and HR in subjects with 2/3 genotype, indicate the possibility of synergistic mechanisms increasing their risk of CVD events. Moreover, CAD patients with 2/3 genotype may have increased disease severity assessed by previous angioplasties and/or a maximum stenosis >90% (Medley et al., 2002).

Studies of the effects of *FBN1* genotypes on arterial wall integrity are sparse. Powell el al. (1997) investigated healthy middle-aged men and found an association between the 2/3 genotype and increased PP. Medley et al. (2002) studied mainly middle-aged men with CAD, where those with the 2/3 genotype were associated with higher aortic impedance and PP, and had a higher disease severity regarding number of angioplasties and/or CAD stenosis. MacSweeney et al. (1996) studied patients with AAA, where the 2/3 genotype was associated with increased systolic blood pressure. However Yasmin et al. (2006) on the other hand, found no difference regarding the *FBN1* genotype and blood pressure or PWV in apparently healthy subjects. The diverging results between our study and the one by Yasmin et al. (2006) may be explained by the fact that they determined PWV over a length of the arterial tree and we studied the arterial stiffness of the AA locally. Fibrillin-1 rich elastic fibres may have a larger impact on arterial stiffness in the AA than in other parts of the arterial tree, as have been shown in patients with Marfan syndrome (Jondeau et al., 1999).

FBN1 genotypes have been investigated in several different populations and the frequency of the genotypes in our study is in accordance with what others have reported in healthy subjects (Powell et al., 1997, Yasmin et al., 2006) and CAD patients (Powell et al., 1997, Yasmin et al., 2006, Medley et al., 2002). However, the frequency of the 2/3 genotype was more than twofold in a population with both AAA and PAA and increased in a Marfan syndrome (MFS) population (Table IV). This indicates that the 2/3 genotype may be overrepresented in the AAA and MFS populations, and also has an impact on the structure of the aortic wall as shown by the increased aortic stiffness

(Figure 14). TGF-β levels are increased in MFS patients, and antibodies against TGF-β seem to prevent development of mitral valve disease and AAA in experimental models (Keane and Pyeritz, 2008, Matt et al., 2009). Since fibrillin-1 has a functional role in the production of TGF-β, this indicates that fibrillin-1 is not only a structural protein but also has an important functional role in the complex TGF-β signaling pathway (Pearson et al., 2008).

Table IV. *Distribution of FBN1 genotypes in different populations.*

	2/2 (%)	2/3 (%)	2/4 (%)	Populations
MacSweeney et al. 1996	55	16	20	AAA N=208 (171 M/37 W; 53-89 years)
	54	**38**	0	AAA+PAA N=24 (22 M/2 W; 52-93 years)
Powell et al. 1996 and 1997	64	16	15	Healthy M, N=245 (50-61 years)
Medley et al. 2002	58	13	15	CAD N=145 (113 M/32 W; 62±9 years)
	63	9	17	Age-matched healthy controls N=170 (61±9 years)
Powell et al. 2005	63	13	14	Healthy M, AAA-relatives N=79 (28-81 years)
De Backer et al. 2006	54	**27**	15	MFS N=67 (31 M/36 W; 32±10 years)
Yasmin et al. 2006	52	16	19	Healthy subjects N=742 (16-83 years; 61±9 years)
De Basso et al. (in manuscript)	56	12	24	Randomized N=5765 (2424 M/3341 W;46-68 years)

AAA= Abdominal Aortic Aneurysm; PAA=Popliteal Artery Aneurysm; CAD= Coronary Artery Disease; MFS; Marfan Syndrome; M=Men; W=Women

Since large-artery stiffness is the main determinant of pulse and systolic pressure and aortic stiffness has independent predictive value for cardiovascular mortality, coronary morbidity and mortality and stroke, a larger cohort of middle-aged subjects from the Malmö Diet and Cancer Study (MDCS) were investigated (Safar et al., 1987, Laurent et al., 2001, Boutouyrie et al., 2002, Laurent et al., 2005, Lacolley et al., 2009). The incidence of first CVD events and mortality was monitored during 13.2 years.

Men of *FBN1* 2/3 genotype had a higher presence of carotid plaques compared to men with the 2/2 and 2/4 genotypes (Figure 15). That men and not women, with 2/3 genotype were affected, indicates a gender difference as

also observed by others (MacSweeney et al., 1996, Powell et al., 1997, Medley et al., 2002). Since carotid plaques are indicators of CVD, an increased CVD burden would have been expected (Johnsen and Mathiesen, 2009). However, no association was found between 2/3 genotype and blood pressure, CVD events and mortality (Figure 16 and Table III). A confounder could be the limited number of subjects affected by CVD events in each genotype group.

A confounder that might have affected the results as well as conclusions in our study on aortic stiffness could be the fact that the men were first-degree relatives of patients with AAA, although not affected by an AAA. It might be argued that an underlying genetic predisposition to aneurysmal disease would be present, since AAA have a higher prevalence in near relatives with AAA than in a general population (van Vlijmen-van Keulen et al., 2002, Linné et al., 2012). This could impact the possibility of structural changes of the AA wall influenced by the *FBN1* gene. MacSweeney et al. (1996) showed e. g. that patients with both AAA and PAA and carrying the 2/3 genotype, had higher systolic and pulse pressure, and AAA patients have stiff aortic walls (Länne et al., 1992). Further, the frequency of the 2/3 genotype was twofold in the AAA and PAA population (Table IV). However, the frequencies of the FBN1 genotypes in paper IV and V were similar. Further, the frequencies resembled those earlier described, both in healthy middle-aged men and in patients with CAD (Table IV). Furthermore, none of the subjects in Paper IV had a dilated aorta (Table II) (Sonesson et al., 1994b). This relationship needs to be studied further.

Methodological considerations and limitations

When measuring arterial wall motion in relation to intra arterial pulse pressure variation in order to calculate the mechanical properties, it would be favorable to measure blood pressure at the site of wall motion detection, because the blood pressure undergoes transformation in the arterial tree (Nichols et al., 2011). Invasive blood pressure measurement may be performed, but seems unethical and difficult in larger population studies. Instead we used auscultatory blood pressure from the brachial artery. When comparing invasive blood pressure in the AA with auscultatory brachial pressure, the auscultatory brachial pulse pressure were only slightly lower than the invasive aortic pulse pressure leading to a systematic underestimation of Ep and β by 15-20% (Sonesson et al., 1994c). To our knowledge, there are no available comparisons between popliteal and brachial pressure. Invasive CFA blood pressure comparison with auscultatory brachial blood pressure shows however slightly higher invasive CFA systolic blood pressure and lower diastolic blood pressure compared to auscultatory brachial blood pressure (Ahlgren et al., 2001). The differences in blood pressure within the arterial tree are reduced at older ages, but in younger ages this might introduce an error in calculations of arterial stiffness. However, since the error is systematic and no age- and gender-related differences have been seen, this systematic bias should not affect comparative studies between groups.

Based on the arterial diameter, blood pressure and wall thickness, WS was calculated according to the law of Laplace. IMT was used in the measurements as arterial wall thickness, even though the adventitia layer is not included in IMT. However, evidence point out that the major part of the total wall thickness is included as shown e.g. in the abdominal aorta, iliac and coronary arteries. Further, since the relation between adventitial thickness and the IMT does not seem to be affected by age and gender this approximation is acceptable. Accordingly, IMT has during recent years been used as a surrogate for wall thickness in the calculation of WS (Carallo et al., 1999, Astrand et al., 2005, Holzapfel et al., 2005, Holzapfel, 2006, Astrand et al., 2007, Holzapfel and Ogden, 2010).

In Paper III the subjects were elderly and there might have been a survival bias due to the fact that some subject died before invitation to the study. However, the frequency of ACE genotype was in accordance with the Hardy Weinberg equilibrium which minimizes the risk of selection bias.

The subjects included in Paper IV were first-degree relatives of patients with AAA but were not affected by an AAA and had normal AA diameters. The genetic predisposition for AAA could however affect the results of increased arterial stiffness observed within the subjects carrying the 2/3 genotype. However, the frequencies of the *FBN1* genotypes in both our studies were similar. Further, the frequencies resembled those earlier described, both in healthy middle-aged men and in patients with CAD (Table IV).

The study of Paper V was based on a randomized community-based sample of a general population, which makes it less sensitive to selection bias than samples based on workplaces or populations in clinical settings. However, people who participate in public health surveys are generally healthier than the nonparticipants, which might lead to an underestimation of the true associations between the measures of atherosclerosis and CVD events. Further, the absence of increased CVD in those with the 2/3 genotype may be affected by the limited number of subjects affected by CVD events in each genotype group.

CONCLUSIONS

- The mechanical properties of the PA are affected by age and gender, not only with an increase in diameter, but also with an age-related decrease in distensibility, with men having a lower distensibility than women. This seems not to be the behavior of a true muscular artery but rather of a central elastic artery such as the aorta, and might have implications for the susceptibility to aneurysm formation, as well as the association of dilating disease between the PA and the aorta.

- The WS in the PA and CFA is unaffected by age, probably caused by a compensatory remodeling response with an increase in arterial wall thickness. The absence of high WS in the PA, and instead lower stress than in the CFA, a peripheral muscular artery not affected by aneurysmal dilatation to the same extent, indicates that other mechanisms than WS contribute to the process of pathological arterial dilatation in the PA.

- Men with the ACE D allele are associated with increased AA stiffness compared to men carrying the I/I genotype. The effect of the D allele seems dependent on other mechanisms than elevated levels of circulating ACE, since no association with circulating ACE level and AA stiffness is found. The increased stiffness in men with ACE D allele suggests impaired arterial wall integrity and in combination with local hemodynamic and other genetic factors it may have a role in aneurysm formation.

- The *FBN1* 2/3 genotype in middle-aged men is associated with increased AA stiffness, blood pressure and heart rate. This indicates an increased risk of CVD. The increased AA stiffness in men with *FBN1* 2/3 genotype indicates an impaired arterial wall integrity, thereby affecting the structure of the aortic wall and might influence aneurysmal formation.

- The increased presence of plaques in the carotid artery of middle-aged men with the *FBN1* 2/3 genotype indicates a pathological arterial wall remodeling with a more pronounced atherosclerotic burden. However, we could not find an increased risk of cardiovascular events and/or death. This relationship needs to be studied further.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Kärlväggens funktion mätt som väggstyvhet, påverkar hjärtarbetet och har en koppling till hjärtsjukdom och dödlighet. Likaså påverkar olika gener sammansättningen av kärlväggen vilket kan leda till en förändring av styvheten i kärlet. Ökad styvhet i kärlet indikerar en störd kärlväggsintegritet orsakad av en förändrad struktur av kärlväggen vilken skulle kunna leda till sjukliga förändringar i kärlet, med till exempel utveckling av kärlvidgning.

Syftet med avhandlingen har varit att 1) undersöka väggfunktionen och väggstressen i knäveckspulsådern (poplitea artären), i relation till ålder och kön hos friska försökspersoner, att 2) undersöka sambandet mellan genen ACE I/D och väggfunktionen på stora kroppspulsådern (bukaorta) hos äldre och att 3) studera om variationer av Fibrillin-1 genen (*FBN1*) är förknippade med styvhet i bukaorta samt ökad hjärt- kärlsjukdom och dödlighet hos medelålders personer.

Poplitea artären är efter bukaorta den vanligaste lokalisationen för sjuklig vidgning av artärer. Varför poplitea artären är mer känslig för sådan utveckling än andra muskulära artärer är okänt. En faktor av betydelse kan vara att sammansättningen av kärlväggen skiljer sig jämfört med andra muskulära artärer, vilken i sin tur påverkar kärlväggsfunktionen. Kärlväggsfunktionen i poplitea artären är tidigare okänd. I **delarbete I** studerades kärlväggsfunktionen i poplitea artären på 108 friska individer (9-82 år) med hjälp av ultraljud. Både diameter och väggtjocklek i poplitea artären ökade med stigande ålder, med större diameter hos män. Kärlväggens styvhet ökade exponentiellt, var högre hos män än kvinnor och påverkades inte av väggtjockleken. Ökningen av styvheten i poplitea artären liknar inte andra muskulära artärer som inte förändrar sin väggstyvhet med ökande ålder, utan poplitea artären beter sig snarare som en central elastisk artär såsom bukaorta. Denna likhet kan ha betydelse för både förekomsten av sjuklig vidgning av poplitea artären samt för sambandet som finns mellan vidgning i bukaorta och poplitea artären.

Varför en sjuklig vidgning av kärlet utvecklas i högre grad i poplitea artären och inte i andra perifera muskulära artärer är okänt. En möjlig delförklaring kan vara att väggstressen som påverkar artärväggen skiljer sig åt jämfört med andra muskulära artärer. I **delarbete II** studerades väggstressen i poplitea artären med hjälp av ultraljud på 94 friska individer (10-83 år) och jämfördes med den väggstress som påverkar den närliggande ljumskpulsådern (femoralis artären). Väggstressen i både poplitea artären och femoralis artären förändrades inte med ålder, sannolikt beroende på kompensatorisk ombyggnad av kärlväggen, med en ökning av väggtjockleken. Vidare var väggstressen som påverkar poplitea artären lägre än i femoralis artären, hos både män och kvinnor vilket tyder på att andra mekanismer än väggstress bidrar till förekomsten av sjuklig kärlvidgning i poplitea artären.

Angiotensin-converting enzyme (ACE) påverkar regleringen av blodtrycket genom att omvandla den inaktiva angiotensin I till den aktiva angiotensin II. Angiotensin II har en betydande påverkan på flera viktiga processer i människokroppen och är involverad i regleringen av hjärt-kärlsystemet och troligtvis även i utvecklingen av hjärt- kärlsjukdom. ACE finns i blodkärlens väggar samt även fritt cirkulerande i blodet. Genen för ACE är ACE I/D och den styr till viss del hur mycket av ACE som cirkulerar fritt i blodet. Tidigare studier om vilken påverkan ACE I/D genotyper (varianter av genen) har på hjärt-kärlsjukdom har lett till olika slutsatser. En ACE-genotyp har kopplats till förekomsten av sjuklig vidgning av stora kroppspulsådern. I **delarbete III** studerades kopplingen mellan ACE-genotypen, cirkulerande ACE och styvheten i stora kroppspulsådern på 406 individer (70-88 år) med hjälp av ultraljud. Män bärande på ACE D-genotypen hade högre aortaväggstyvhet än de med II-genotypen, vilket visar en defekt aortaväggs integritet, som tillsammans med lokala faktorer kan vara av betydelse för utvecklandet av sjuklig kärlvidgning.

FBN1 genen styr uppbyggnaden av proteiner som är en viktig del av de elastiska bindvävsfibrer som finns i artärväggen, vars funktion är att klara av den tänjning som uppstår i kärlväggen vid en tryckförändring. Tidigare studier av *FBN1* genen har visat ett samband mellan 2/3 genotypen (variant av genen) och ökat blodtryck hos medelålders män och män som har drabbats av hjärtinfarkt. I **delarbete IV** studerades *FBN1* genotypernas inverkan på styvheten i stora kroppspulsådern på 79 män (28-81 år) med hjälp av ultraljud. De tre vanligaste genotyperna studerades: 2/2 (64%), 2/3 (13%) och 2/4 (14%). Män med 2/3 genotypen hade högre aortaväggsstyvhet än de andra

genotyperna, vidare var hjärtfrekvensen och pulstrycket stegrat i samma genotyp indikerande en ökad risk för utvecklande av hjärt- kärlsjukdom.

I **delarbete V** studerades om *FBN1* 2/3 genotypen kunde associeras med ökad hjärt- kärlsjukdom och dödlighet på 5765 individer (46-68 år). Förekomst av åderförkalkning och väggtjocklek studerades i halspulsådern (carotis artären) med hjälp av ultraljud, och förekomsten av hjärt- kärlsjukdom och dödlighet (hjärtinfarkt och stroke) följdes upp under drygt 13 år. Män med 2/3 genotypen hade ökad förekomst av åderförkalkning i carotis artären jämfört med de andra genotyperna. Den skillnaden kunde inte återfinnas hos kvinnor. Någon relation mellan genotyp och förekomst av hjärt- kärlsjukdom eller dödlighet kunde dock inte ses. Den ökade förekomsten av åderförkalkning i carotis artären hos medelålders män med 2/3 genotypen talar för en sjuklig förändring i kärlväggen. Ytterligare studier behövs för att definiera förhållandet mellan *FBN1* genen och risken för hjärt- kärlsjukdom.

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ILLUSTRATION-1. *Schematic illustration of the various layers of the arterial wall.* http://bme.ccny.cuny.edu/faculty/jtarbell/SMC%20images.htm [Online].

ILLUSTRATION-2 http://heartstrong.wordpress.com/2010/08/24/risk-factors-for-abdominal-aortic-aneurysm-studied.

ILLUSTRATION-3 *Entrez´s 3D structure database MMDB (ID 22599)* http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=22599.

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