The genetic basis for altered blood vessel function in disease: large artery stiffening

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Abstract: The progressive stiffening of the large arteries in humans that occurs during aging constitutes a potential risk factor for increased cardiovascular morbidity and mortality, and is accompanied by an elevation in systolic blood pressure and pulse pressure. While the underlying basis for these changes remains to be fully elucidated, factors that are able to influence the structure and composition of the extracellular matrix and the way it interacts with arterial smooth muscle cells could profoundly affect the properties of the large arteries. Thus, while age and sex represent important factors contributing to large artery stiffening, the variation in growth-stimulating factors and those that modulate extracellular production and homeostasis are also being increasingly recognized to play a key role in the process. Therefore, elucidating the contribution that genetic variation makes to large artery stiffening could ultimately provide the basis for clinical strategies designed to regulate the process for therapeutic benefit.
Keywords: arterial stiffness, genes, polymorphism, extracellular matrix proteins

Introduction

Large artery stiffness is a principal determinant of pulse pressure and can increase the risk of cardiovascular morbidity and mortality via an elevation of systolic blood pressure (leading to elevations in left ventricular afterload) and a reduction in diastolic blood pressure altering coronary perfusion (Dzau and Safar 1988). Such stiffening can occur via processes that involve changes in the overall dimensions of arteries arising from medial hypertrophy and increased wall thickness – "geometry-dependent" (Benetos, Laurent, et al 1993; Jaeckel and Simon 2003); or it can result from perturbations in the amounts of arterial matrix, both elastic and extracellular, such that the orderly arrangement of elastic fibers and lamenae is degraded over time and an increase in collagenous material occurs – "geometry-independent" (Lakatta 1987; Laurent et al 2005). Although a number of factors combine to determine increases in large artery stiffening, a principal determinant is age; the central arteries stiffen progressively over time while the peripheral muscular arteries do not appear to undergo such profound structural changes (Benetos et al 2002). Indeed, by use of a variety of assessment criteria, age-dependent increases in arterial stiffening have been described in both healthy and diseased populations, predominantly occurring from the age of 10 years in both males and females (Laogun and Gosling 1982). These increases in large artery stiffness, whether they occur within the aorta or carotid artery, do so in a continuous and gradual manner (van der Heijden-Spek et al 2000), although there is some indication that a more pronounced increase occurs from the age of 55 years onwards (Nagai et al 1999). It is interesting that telomeric length, a heritable indicator of biological aging, may also be indicative of vascular aging given that it appears to inversely correlate with increased arterial stiffening (Jeanclos et al 2000; Benetos et al 2001).

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Whereas large artery stiffening increases gradually with aging independently of the occurrence of cardiovascular risk factors or other associated conditions, a number of studies clearly suggest that the superimposition of environmental and genetic factors is also likely to be critical in the process. Some of these environmental factors include nutritional status, smoking, and aerobic capacity. Increasingly, the influence of genetic factors is becoming better understood in terms of how it may affect the epidemiology of large artery stiffening. Studies carried out a decade ago in rat models of salt-sensitive hypertension (Benetos et al 1995; Levy et al 1997) and in human sodium-sensitive hypertensives (Draaijer et al 1993) seem to indicate that hemodynamic and humoral factors alone cannot fully account for differences in mechanoelastic arterial properties when compared with the situation in salt-resistant or normotensive controls. This suggests a major influence of predisposing genetic factors on arterial mechanical properties. Indeed, some studies suggest that the total genetic heritability of aortic stiffening determinants, which are independent of the influence of blood pressure, heart rate, height, and age, may make a substantial contribution, perhaps as high as 30%–40% (Snieder et al 2000). Furthermore, the results of genome-wide linkage analyses of pulse pressure indicate that while some overlap may exist between genetic components that underlie blood pressure phenotypes (Atwood et al 2001), there is also likely to be a distinct set of genes that contribute to the modulation of arterial stiffness (Camp et al 2003; DeStefano et al 2004). In this regard, a number of polymorphisms in various genes have been found to associate with increased arterial stiffening, thereby implicating these genes in this process. In this review, the current knowledge about these genetic factors is discussed, as is the potential molecular basis that underlies their involvement in arterial stiffening, particularly as it relates to the growth of arteries, the structure and remodeling of the extracellular matrix, and the interactions that occur between the extracellular matrix and the cellular components of the artery.

Nonmatrix gene polymorphisms
The renin–angiotensin system

Local hormonal factors that may play a pressure-independent role in the arterial wall via a modification of cellular growth or by influencing the extracellular matrix include angiotensin II acting via the angiotensin II type 1 receptor (AT_1-R). Animal studies using both Wistar Kyoto

and spontaneously hypertensive rats (SHR) have demonstrated that inhibition of angiotensin-converting enzyme (ACE) activity can reduce stiffness within the carotid artery independently of transmural pressure, both when the drug was administered locally (Levy et al 1990) and after acute oral administration (Benetos, Pannier, et al 1993). In both studies, changes in stiffness were seen even in the absence of significantly lowered systemic blood pressure. Experimentation using two-kidney, one-clip Goldblatt hypertensive rats, and SHR indicated that the beneficial effect of ACE inhibition may arise via effects on vascular smooth muscle cell hypertrophy (Levy et al 1988, 1993) or collagen accumulation (Albaladejo et al 1994; Benetos et al 1997).

While in humans the relationship between ACE inhibition and arterial stiffness is likely to be more complex, it has been demonstrated that long-term administration of ACE inhibitors to individuals with essential hypertension significantly reduces arterial stiffness within the brachial, radial, abdominal, and carotid arteries (Asmar et al 1988; De Luca et al 1993; Kool et al 1995). Such findings are consistent with the observations of elevated levels of ACE in humans with increased thickness of the carotid wall (Bonithon-Kopp et al 1994). Both ACE inhibition and AT_1-R blockade, in either acute or long-term treatment, can reduce the stiffness of muscular arteries independent of changes in mean blood pressure (Topouchian et al 1998, 1999; Benetos et al 2000). In view of these observations, it has been of interest to ascertain whether genetic polymorphism within the ACE and AT_1-R genes may be associated with alterations in arterial stiffness. For example, it has been shown that specific polymorphisms within the AT_1-R and ACE genes within hypertensive individuals do appear to be involved in the regulation of aortic stiffness. Specifically, in the study by Benetos, Gautier, et al (1996), in which arterial stiffness was assessed by measuring aortic pulse wave velocity (PWV), the AT_1-R $A^{1166}C$ polymorphism was the second most important determinant of aortic stiffness in hypertensives, accounting for 11.6% of PWV variance, an effect that appeared to be co-dominant. Although a strong association between the AT_1-R $A^{1166}C$ genotype and PWV was observed in both young and old hypertensives, the effect was more pronounced in the older individuals, suggesting that this polymorphism may amplify the effects of age on arterial stiffness. Some studies have provided data consistent with the C allele influencing arterial stiffness, for example the demonstration that ACE inhibition with perindopril

reduced PWV threefold in carriers of the C allele as compared with individuals homozygous for the A allele (Benetos, Cambien, et al 1996). Interestingly, the presence of the C allele has been associated with increased vasoconstriction of both the coronary arteries (Amant et al 1997) and the internal mammary artery (Henrion et al 1998).

More evidence that the AT_1-R $A^{1166}C$ polymorphism is likely to be a key determinant in the process of arterial stiffening has been provided in a recent study in which an additional cohort of individuals was also found to exhibit association between this polymorphism and arterial stiffening, although in this study (Gardier et al 2004) it was the A allele that was associated with such increased stiffening. Additional involvement of the AT_1-R gene in the process of arterial stiffening has been provided by Lajemi, Labat, et al (2001), demonstrating an age-related association between the AT_1-R $A^{153}G$ polymorphism and aortic stiffness. In the study by Benetos, Gautier, et al (1996), the ACE I/D polymorphism showed an association with aortic stiffness, albeit at a lower level, accounting for less than 2% of the PWV. This polymorphism has also been found to be associated with aortic and carotid artery stiffening in a group of 137 Japanese individuals with type 2 diabetes (Taniwaki et al 1999). In addition to these data is the study by Mattace-Raso et al (2004), demonstrating an association between this polymorphism and common carotid artery stiffness in individuals younger than 70 years. Some studies also indicate that the ability of this polymorphism either to modulate increases in pulse pressure (Safar et al 2004) or to influence diastolic blood pressure (Rudnichi et al 2004) is likely to be dependent on age and sex, with males being more susceptible to these outcomes. It should also be noted that there may be additional complexity to the relationship between the ACE I/D polymorphism and the AT_1-R $A^{1166}C$ polymorphism and modulation of arterial stiffness, as there is evidence that in some population cohorts these polymorphisms do not strongly affect the stiffness of either the carotid or radial arteries (Girerd et al 1998).

A key factor that regulates the amount of angiotensin II is its generation from angiotensinogen. Polymorphisms have been detected within the human angiotensinogen gene, which appear to correlate with plasma concentrations of angiotensinogen, specifically the variant M235T, which arises from a thymidine-to-cytosine transition at nucleotide position 704 and causes substitution of a methionine for a threonine at residue 235 in the mature angiotensinogen peptide (Jeunemaitre et al 1992). In some studies the T235 allele has been found to be associated with higher plasma concentrations of angiotensinogen in individuals homozygous for this allele (Bloem et al 1997), and it has also been associated with hypertension (Caulfield et al 1995). This polymorphism has also been found to associate with arterial structure, with hypertensive individuals homozygous for the T235 allele exhibiting a higher amount of carotid intimamedia thickness (Bozec et al 2003). More importantly, this polymorphism has also very recently been shown to be associated with arterial stiffness. In a study by Bozec et al (2004) in which 98 untreated hypertensive individuals were assessed for arterial stiffness, those who were homozygous for the T235 allele had, on average, increased carotid artery stiffness compared with the T/M235 heterozygotes and the M235 homozygotes. Such data provide further evidence that perturbation within a number of the genes encoding for components of the renin–angiotensin system is likely to play a key role in the process of large artery stiffening.

Aldosterone synthase

Aldosterone is a mineralocorticoid hormone which is involved in the regulation of blood pressure through its effects on sodium balance and intravascular volume (White 1994). Experimental studies have demonstrated that aldosterone may be capable of playing a prominent role in regulating the structure of large arteries. For example, aldosterone receptors occur within the large arteries, particularly the aorta (Lombes et al 1992). Endogenous vascular synthesis of aldosterone can also occur (Takeda et al 1995). It has also been demonstrated that aldosterone can increase the expression of both cardiac and vascular collagens (Robert et al 1994; Sun et al 1997). It has been more recently demonstrated that aldosterone can amplify the proliferative effects of angiotensin II on vascular smooth muscle cells via an increase in expression of the AT_1-R (Xiao et al 2004).

Synthesis of aldosterone occurs in the adrenal cortex from deoxycorticosterone, via the action of a mitochondrial cytochrome P450 enzyme aldosterone synthase (CYP11B2), the gene for which can be regulated by angiotensin II (White 1994). Interestingly, polymorphisms within the CYP11B2 gene that may influence its activity have also been found. A common polymorphism within the promoter region has been demonstrated to influence binding of the transcriptional regulatory protein SF-1 (White and Slutsker 1995). This C/T polymorphism (-344C/T), occurring 344 nucleotides upstream of the translational start site of the CYP11B2

protein, is within a region in which the steroidogenic transcription factor SF-1 binding occurs, and studies have found that plasma aldosterone levels or urinary aldosterone excretion can vary according to the -344 genotype (Hautanena et al 1998; Pojoga et al 1998). This polymorphism has also been found to be associated with arterial stiffening in a study in which 216 hypertensive individuals of European origin were assessed for plasma levels of renin and aldosterone, blood pressure, and PWV as a measure of arterial stiffness (Pojoga et al 1998). The presence of the -344C allele was associated with elevated levels of plasma aldosterone, with the CC homozygous individuals having on average the highest levels, and the TT homozygotes having the lowest level. Individuals carrying the C allele also exhibited a higher degree of arterial stiffening as assessed from measurements of PWV. Consistent with such polymorphisms influencing arterial stiffness via perturbations in aldosterone levels have been the findings that an increase in carotid artery stiffness in response to long-term aldosterone administration to Sprague-Dawley rats can be ameliorated by treatment with the mineralocorticoid antagonist eplerenone (Lacolley et al 2002) and that salt-dependent increases in stiffness in the mesenteric vessels of spontaneously hypertensive stroke-prone rats could also be blunted by treatment with this aldosterone antagonist (Endemann et al 2004).

Moreover, there is evidence demonstrating that gene–gene interaction between components of the rennin–angiotensin–aldosterone system act to modulate large artery properties. For example, in a study of 756 Belgian individuals ranging in age from 12–79 years, a single-gene effect was observed to occur for the ACE I/D polymorphism on compliance of the common carotid artery, with the D allele being associated with decreased compliance (Balkestein et al 2001). In multigenetic analysis, it was seen that the influence of the ACE D allele depended on both "vascular territory" and genetic background, such that the presence of the aldosterone -344T allele appeared necessary to bring about the negative association of the ACE D allele on distensibility of the common carotid artery (Balkestein et al 2001). Although such an interaction occurred at the level of the common carotid artery, it did not appear to influence properties of the femoral artery. Rather, in the latter, ACE DD homozygotes, when additionally homozygous for the α-adducin Gly460 allele, had lower cross-sectional compliance and lower distensibility (Balkestein et al 2001).

Guanine nucleotide regulatory proteins (G-proteins)

G-proteins are key components of a plethora of intracellular signaling cascades, relaying signals from more than 1000 receptors to a diversity of intracellular effector molecules, including enzymes and ion channels (Farfel et al 1999). The structural features of the G-protein are an α-subunit bound loosely to a tightly associated dimer consisting of a β- and a γ-subunit, each subunit being the product of a different gene. Diversity of G-protein composition is generated via the number of different genes that encode for these subunits, with 16 α-subunit genes, 6 β-subunit genes, and 12 γ-subunit genes (Farfel et al 1999). It is known that mutations within these trimeric proteins are involved in a number of disease states, including hypertension. Specifically, a common C-to-T polymorphism in exon 10 (C825T) of the β3 subunit whereby the T allele is associated with a splice variant (GNB3-s) in which the nucleotides 498–620 of exon 9 are deleted in-frame, thereby truncating the β3 protein by 41 amino acids, has been demonstrated to be capable of significantly enhancing signaling when in a trimeric complex with $G\alpha_{i2}$ and $G\gamma_5$ (Siffert et al 1998). Interestingly, the 825T allele has been associated with hypertension (Benjafield et al 1998; Schunkert et al 1998; Siffert et al 1998; Dong et al 1999) and higher blood pressure (Benjafield et al 1998; Schunkert et al 1998; Hengstenberg et al 2001). Moreover, the potential for this polymorphism to influence arterial structure has been demonstrated by the finding that in a French cohort of 306 individuals with no history of cardiovascular disease, a significant association existed between the 825T allele and radial artery hypertrophy, independent of age, blood pressure, sex, and body mass index (Hanon et al 2002). This polymorphism has been recently found to be associated with arterial stiffness, as measured by PWV and augmentation index (Nürnberger et al 2004). Specifically, this study compared a group of young healthy males with and without the 825T allele under resting conditions, finding that carriers of this allele exhibited a significantly higher PWV and augmentation index than individuals with the CC genotype. Although the underlying mechanistic basis linking such a polymorphism to arterial stiffening remains to be fully elucidated, it may relate to altered vascular remodeling events arising from aberrant vascular smooth muscle cell proliferation in response to enhanced Na^+/H^+ exchanger activity (Siffert 2000).

Nitric oxide synthase

In addition to factors that can influence the growth of vascular smooth muscle cells or the expression of extracellular matrix proteins within the arterial wall, a degree of functional regulation of smooth muscle tone by circulating and locally produced vasoactive factors may also influence arterial stiffness. One such factor, nitric oxide, is synthesized from L-arginine in vascular endothelial cells by the action of the enzyme endothelial nitric oxide synthase (eNOS) and has a profound effect on both vascular tone and blood pressure (Palmer et al 1987; Rees et al 1989). In this regard it has been demonstrated that both exogenous and endogenous eNOS inhibitors, which decrease basal endothelial cell eNOS, can cause an increase in carotid artery intimamedia thickness (Lacolley et al 1998; Wilkinson et al 2002). Within the human eNOS gene, a polymorphic transversion of a G-to-T nucleotide at position 894 (G894T) within exon 7 results in a substitution of Glu by Asp at amino acid residue 298 (Glu298Asp) (Marsden et al 1993). This polymorphism has been shown to influence vascular responsiveness to vasoconstricting hormones, with the 894T allele being associated with an enhancement of the hemodynamic response to phenylephrine in a cohort of individuals undergoing cardiac pulmonary bypass surgery (Philip et al 1999). Interestingly, a relationship between this polymorphism and blood pressure level was found to occur, with the T allele being associated with lower blood pressure in a young adult cohort aged 19–38 years in the Bogalusa Heart Study (Chen et al 2001). In a recent study examining the independent effect of the G894T eNOS polymorphism on arterial stiffness in 118 African Americans and 285 white young adults (aged 25–37 years), the T allele was associated with lower systolic blood pressure and a lower degree of carotid artery stiffening within the African American cohort (Chen et al 2004), after adjusting for insulin, heart rate, and mean arterial pressure. The genotype effect was not significant within the white male cohort, an observation consistent with a previous study utilizing a French cohort in which the T allele was not found to be significantly associated with PWV (Lacolley et al 1998). It appears likely that the eNOS gene may be one of the key underlying factors that contribute to the observed ethnic differences in both hypertension and arterial stiffness. The mechanistic basis for how the G894T eNOS polymorphism could be linked with effects on arterial stiffness remains unclear, but is likely to relate to the ability of nitric oxide not only to be involved in the maintenance of vascular tone but also to exert an effect on arterial mechanical properties via inhibition of vascular smooth muscle cell proliferation (Garg and Hassid 1989; Jeremy et al 1999). At this stage, the relationship between the G894T eNOS polymorphism and eNOS enzyme activity and subsequent nitric oxide production is yet to be clarified, with the polymorphism having no significant effect on eNOS activity in a study in which this parameter was tested (Kamitani et al 1998). Another possibility is that the G894T polymorphism either is in strong linkage disequilibrium with another, as yet unidentified, eNOS polymorphism(s) that has an effect on enzyme activity or is linked with another gene that influences arterial stiffness.

Endothelin and endothelin receptors

Endothelins -1, -2, and -3 are peptides of 21 amino acids, produced from different genes in a diverse array of cells, with endothelin-1 (ET-1) – the only member of the family to be expressed in endothelial cells and vascular smooth muscle cells (Levin 1995) – being a potent paracrine vasoconstrictor that can modulate vasomotor tone, cell proliferation, and vascular remodeling (Komuro et al 1988; Levin 1995). These peptides exert their biological responses by binding to two types of receptors (ET_AR and ET_BR), which are G-protein linked and range in size from 45 kDa to 50 kDa in various cells and tissues, including vascular smooth muscle cells and endothelial cells (Sakurai et al 1990; Seo et al 1994). Although this is somewhat controversial, a relationship may exist between the expression of endothelin/endothelin receptors and hypertension, with some studies showing overexpression of both ET-1 and its ET_AR genes in arteries of hypertensive patients (Hasegawa et al 1994; Schiffrin et al 1997) and others finding elevated ET-1 levels in some hypertensive animal models (Larivière, Day, et al 1993; Larivière, Thibault, et al 1993) and in some patients with hypertension (Yokokawa et al 1991). A number of polymorphisms have also been identified within both the ET-1 and ETR genes that have shown associations with either hypertension or elevated pulse pressure (Nicaud et al 1999; Jin et al 2003). As well, an association has been found between specific ETR polymorphisms and arterial stiffness. In a study by Lajemi, Gautier, et al (2001) 528 untreated hypertensive individuals of European origin (314 men, 214 women) were assessed for aortic stiffness using PWV measurement and association with the ET_AR −231 A/G and +1363 C/T polymorphisms, the ET_BR +30 G/A polymorphism, and the ET-1 138 I/D polymorphism. In women, these analyses

showed that the age-adjusted PWV was associated with both the ET_AR −231 A/G polymorphism and the ET_BR +30 G/A polymorphism, with the −231 G and +30 G alleles being associated in a co-dominant manner with higher PWV, both alleles being significant independent determinants of PWV. In men, no association was seen for any of these polymorphisms and PWV, although the presence of the ET_BR 30A allele was associated with higher levels of radial artery wall cross-sectional area (Lajemi, Gautier, et al 2001). The mechanistic basis for such sex-specific association between the ET_AR polymorphisms and arterial stiffening has yet to be elucidated.

Extracellular matrix gene and matrix metalloproteinase gene polymorphisms

Matrix homeostasis is also a critical determinant of the mechanical properties of the blood vessel, and the mechanisms whereby matrix proteins are regulated in their accumulation and deposition are therefore likely to play key roles in the process of arterial stiffening (Safar et al 2003). In view of the information pertaining to polymorphism in the genes described above, it is critical to understand the extent to which polymorphism within extracellular matrix genes themselves and genes that encode for the proteins that regulate matrix homeostasis (the matrix metallo-proteinases [MMPs]) plays a role in relation to the process of arterial stiffening. Evidence is emerging to indicate the contribution of genetic variation to both the composition and accumulation of the arterial extracellular matrix and to the activity of specific MMPs (Ye 2000).

Elastin and fibrillin-1

Major determinants of the arterial distensibility of large blood vessels include desmin (Lacolley et al 2001) and elastin. The latter, a highly insoluble extracellular matrix protein synthesized as a soluble precursor, tropoelastin, by vascular cells (including smooth muscle cells), is the principal component of elastic fibers from the arterial media (Wolinsky and Glagov 1967). Interaction between the elastic fibers and vascular proteoglycans may also influence arterial geometric and elastic properties (Germain et al 2003). Although no studies have yet been carried out to assess the potential association of polymorphisms within the desmin gene and arterial stiffness, a number of polymorphisms have been identified within the elastin gene. One of these, an A-to-G substitution resulting in a serine-to-glycine

replacement at amino acid 422 in the elastin protein, has been demonstrated to be associated with carotid artery distensibility, independent of age and blood pressure (Hanon et al 2001).

Interestingly, recent genotype-phenotype studies have revealed a potential link between polymorphisms within the fibrillin-1 gene and aortic stiffness and coronary artery disease severity (Medley et al 2002). Fibrillin-1 is the principal constituent of 10-nm microfibrils and functions as a scaffold for the deposition of tropoelastin, thereby participating in both load-bearing and anchoring within the artery. In Marfan syndrome, in which aortic stiffening and elevated pulse pressure are the primary determinant of aortic root dilation (Jondeau et al 1999), mutations in the fibrillin-1 gene leading to abnormalities in elastic fibers are known to occur (Tsipouras et al 1992). In addition, within the fibrillin-1 gene, a polymorphic (variable) tandem nucleotide repeat (VNTR) sequence, TAAAA, found within intron 28, has been shown to be associated with elevated arterial pulse pressure in men older than 50 years (Powell et al 1997). More recently, this polymorphism has been found to modulate large artery stiffness and pulse pressure within a group of 145 individuals with moderate-severity coronary artery disease (Medley et al 2002). Moreover, individuals heterozygous for fibrillin-1 alleles in which the number of TAAAA repeats was 2 and 3 (2–3 genotype) had stiffer large arteries and higher pulse pressure than other genotypes for this VNTR. Although the precise molecular mechanism linking this intronic polymorphism to large artery stiffening has yet to be elucidated, it may relate to an effect on gene expression or occur via an influence on RNA splicing, as the VNTR is located near the 3′ splicing boundary for exon 28 of the fibrillin-1 gene (Pereira et al 1993).

Type I collagen

Collagen is a primary constituent of extracellular matrix and is abundant in bone, connective tissues, and the arterial wall (Bedalov et al 1994). There is evidence to suggest an interplay between the levels of elastin and collagen and the mechanical properties of arteries with regard to stiffness (Lakatta 1987; Laurent et al 2005). Polymorphism has been shown to exist in the gene encoding for type I collagen, which appears to be associated with arterial stiffness (Brull et al 2001). This polymorphism (2046G/T) is located within the promoter region of the collagen type I-α1 gene (COL1A1) at the first base of a consensus site for the binding of the transcription factor Sp1 and has previously been shown to be associated with reduced bone density and

osteoporotic fracture (Grant et al 1996). In a study of 489 Northern Irish individuals (251 men and 238 women) the T allele was associated with a higher aortic PWV, independent of age, sex, and mean arterial pressure (Brull et al 2001). Presumably, this polymorphism alters Sp1 binding and therefore affects transcription rates of the COL1A1 gene, suggesting that altered collagen deposition is directly related to the effects on arterial stiffening.

Matrix metalloproteinase-3 (MMP-3)

With regard to matrix homeostasis, MMP-3 (stromelysin-1) is likely to be a critical regulator of arterial stiffness, given its wide range of substrates that include most major matrix components within arteries, such as the fibronectins, collagens, gelatins, laminins, elastin, and various proteoglycans (Wilhelm et al 1987). In addition, MMP-3 is capable of activating other MMPs, such as collagenases (Milner et al 2001), matrilysin (Imai et al 1995), and gelatinase B (Dreier et al 2004). The level of MMP-3 is regulated primarily by its gene transcription, and its promoter is known to contain sequences that mediate induction by various stimuli, including growth factors and cytokines such as platelet-derived growth factor (Kanaki et al 2002) and interleukin-12 (Monteleone et al 1999), and suppression by agents such as dexamethasone (Hoshino et al 2001). A common polymorphism has been found within the MMP-3 promoter, giving rise to one allele that has a run of six adenosines (6A) and another having a run of five adenosines (5A) at nucleotide position −1171 relative to the transcriptional start site (Ye et al 1995). The ability of this polymorphism to influence expression of MMP-3 via effects on transcription factor binding within the MMP-3 promoter has been demonstrated from cell culture experiments, in which the 5A allele has a higher promoter activity (Ye et al 1996). Moreover, this allele has been associated with acute coronary events (Terashima et al 1999) and aortic aneurysms (Yoon et al 1999), most likely via an increase in matrix proteolysis. Conversely, the 6A allele has been found to be associated with carotid intimamedia thickening (Gnasso et al 2000; Rauramaa et al 2000), presumably via a mechanism arising from an increase in matrix accumulation. The relationship between MMP-3 allelic variation and age-related large artery stiffening appears complex, with a recent study demonstrating that both the 5A/5A and 6A/6A homozygotes are associated with age-related aortic stiffening, as compared with the 5A/6A hetero-zygotes exhibiting delayed large artery stiffening (Medley et al 2003), thereby implying that either high

(5A/5A) or low (6A/6A) MMP-3 activity has an adverse effect on the outcome over time on aortic stiffening. In the situation of high MMP-3 activity, the mechanism underlying the adverse increase in aortic stiffening may arise from the augmented degradation of elastin leading to dilatation of the vessel lumen and a subsequent increase in stiffness as the elastin is replaced with stiffer collagen (O'Rourke 1995). Subsequently, adverse effects regarding aneurysmal formation could occur via the thinning of the atherosclerotic vessel wall as collagen is then degraded by high MMP-3 activity, either directly or via its activation of other collagenases (Carrell et al 2002). Alternatively, plaque instability could occur via a similar degradation mechanism leading to weakening of the fibrous cap in the advanced atherosclerotic lesion (Terashima et al 1999; Nojiri et al 2003).

Matrix metalloproteinase-9 (MMP-9)

More recently, a potential involvement of MMP-9 polymorphisms on large artery stiffening has also been reported. MMP-9, otherwise known as gelatinase B, is a 92 kDa type IV collagenase, which has been shown to be highly expressed in the rupture-prone regions of human atherosclerotic plaques (Galis et al 1994). As its name suggests, it is particularly active at degrading gelatins, but its substrate specificity is sufficiently broad to render it capable of also degrading type IV collagen, a primary substituent of the basement membrane that underlies the endothelium and is found enveloping smooth muscle cells (Birkedal-Hansen et al 1993). The expression of MMP-9 is controlled in large part at the level of its gene transcription, and it has been shown that factors such as interleukin-1 (Huhtala et al 1991), platelet-derived growth factor, tumor necrosis factor-α (Fabunmi et al 1996), and epidermal growth factor (Kondapaka et al 1997) can influence the expression of MMP-9 via stimulation of its promoter region. In this regard it has been shown that several *cis*-acting elements in the MMP-9 promoter are critical in the regulation of its transcription, including two AP-1 sites bound by c-*fos* and c-*jun* transcription factors, a PEA3 motif mediating regulation by the ETS transcription factor, and a potential binding site for nuclear factor-κB (Huhtala et al 1991; Gum et al 1996). Additionally, sites mediating transcriptional repression of the MMP-9 gene have been identified within its promoter (Zhang et al 1999). Within this 9-bp sequence a C-to-T polymorphism exists (GCGCA*C/T*GCC), such that repression is minimal when the T substitution (C1562T) occurs, as shown from transient

transfection experiments in cultured macrophages (Zhang et al 1999). It has also been shown that individuals of the T/T genotype tend to have a higher serum level of MMP-9 than those of either the C/T or C/C genotype (Blankenberg et al 2003); furthermore, in a sample of 374 individuals from France for whom coronary angiographic data were available, there was a significant association between this polymorphism and the severity of coronary atherosclerosis as assessed by the number of coronary arteries that had a stenosis > 50% (Zhang et al 1999). Using this criterion, the number of patients with triple-vessel disease was higher in the T/T and C/T genotypes than in the C/C genotypes. Interestingly, this polymorphism has recently been shown to associate with large artery stiffness in a group of individuals with angiographically defined coronary artery disease. Specifically, individuals carrying the T allele (either T/T or C/T genotype) had stiffer large arteries (higher input and characteristic impedance) and higher carotid pulse and systolic blood pressure than individuals with the C/C genotype (Medley et al 2004). Such a relationship remained significant after age, sex, mean arterial pressure, total cholesterol, low-density lipoprotein cholesterol, and triglycerides were considered as covariates.

Moreover, in a subsample of eight aortic tissues from a separate population of cardiac transplant patients, MMP-9 gene expression was found to be fivefold higher in T allele carriers than in individuals with the C/C genotype (Medley et al 2004); within these samples there was a trend towards increased total MMP-9 protein in the T allele carriers, and MMP-9 activity was significantly higher in this group. The mechanism linking increased MMP-9 expression to large artery stiffening could potentially occur via processes involving vascular remodeling events. In this regard it is interesting that in MMP-9-deficient mice, arterial geometrical remodeling within the carotid artery has been observed to be impaired subsequent to flow cessation (Galis et al 2002), an effect most likely exerted through a modulation of collagen metabolism and organization. A relationship between MMP-9 expression and large artery stiffening has also been seen in Marfan syndrome (Segura et al 1998).

Conclusions

It has become increasingly recognized that the stiffening of large arteries, a critical independent predictor of cardio-vascular risk, is likely to be profoundly influenced by variation within a diverse array of genes, the proteins for which are involved in the modulation of arterial growth and extracellular matrix properties. An expanding array of genetic polymorphisms has been identified to associate with arterial stiffness. This "candidate gene" approach has yielded significant information about the identity of various genes that are likely to be involved in human arterial stiffening, and for some of these polymorphisms the mechanistic link to this process has been suggested from structure-function studies and supported by studies in appropriate animal models. However, limitations to some of the studies must be acknowledged, particularly where the mechanistic link between the identified polymorphism(s) and the process of arterial stiffening remains to be elucidated or in cases for which specific polymorphisms have not been consistently associated with arterial stiffness between different population groups. In this regard, the use of complementary approaches such as gene array profiling between individuals with increased arterial stiffening as compared with individuals with more distensible arteries has the potential to discern genes in which polymorphisms result in perturbations in protein expression, rather than direct effects on protein function. The applicability of such an approach has already been shown in a study in which a diverse set of genes, including those involved in cellular signaling and the mechanical regulation of vascular structure, were seen to be differentially expressed in aortas with increased stiffness (Durier et al 2003). The situation is likely to be even more complex when the influence of linkage disequilibrium and the combination of various poly-morphisms, or haplotypes, is additionally considered. This has been shown by studies indicating that when combinatorial arrays of specific polymorphisms, either from the same gene or between different genes, are used to evaluate relationships to arterial stiffening, associations exist that either were not evident or were less apparent when considering one polymorphism alone (Balkestein et al 2001; Mourad et al 2002). Clearly, future research in which there is an integration of genetic, molecular, biochemical, clinical, and epidemiological studies (as similarly advocated by Staessen et al [2003]) to elucidate the genetic and environmental factors that interact to cause human hypertension should contribute significantly to a greater understanding of the heritable factors that act to modulate arterial stiffness. Ultimately, the clarification of these genetic influences will allow for the targeting of key factors for therapeutic benefit, particularly in individuals with either increased or premature arterial stiffening.

References

Albaladejo P, Bouaziz H, Duriez M, et al. 1994. Angiotensin converting enzyme inhibition prevents the increase in aortic collagen in rats. *Hypertension*, 23:74–82.

Amant C, Hamon M, Bauters C, et al. 1997. The angiotensin II type 1 receptor gene polymorphism is associated with coronary artery vasoconstriction. *J Am Coll Cardiol*, 29:486–90.

Asmar RG, Pannier B, Santoni JP, et al. 1988. Reversion of cardiac hypertrophy and reduced arterial compliance after converting enzyme inhibition in essential hypertension. *Circulation*, 78:941–50.

Atwood LD, Samollow PB, Hixson JE, et al. 2001. Genome-wide linkage analysis of pulse pressure in Mexican Americans. *Hypertension*, 37:425–8.

Balkestein EJ, Staessen JA, Wang JG, et al. 2001. Carotid and femoral artery stiffness in relation to three candidate genes in a white population. *Hypertension*, 38:1190–7.

Bedalov A, Breault DT, Sokolov BP, et al. 1994. Regulation of the α1(I) collagen promoter in vascular smooth muscle cells: comparison with other α1(I) collagen-producing cells in transgenic animals and cultured cells. *J Biol Chem*, 269:4903–9.

Benetos A, Bouaziz H, Albaladejo P, et al. 1995. Carotid artery mechanical properties of Dahl salt-sensitive rats. *Hypertension*, 25:272–7.

Benetos A, Cambien F, Gautier S, et al. 1996. Influence of the angiotensin II type 1 receptor gene polymorphism on the effects of perindopril and nitrendipine on arterial stiffness in hypertensive individuals. *Hypertension*, 28:1081–4.

Benetos A, Gautier S, Lafleche A, et al. 2000. Blockade of angiotensin II type 1 receptors: effect on carotid and radial artery structure and function in hypertensive humans. *J Vasc Res*, 37:8–15.

Benetos A, Gautier S, Ricard S, et al. 1996. Influence of angiotensin-converting enzyme and angiotensin II type 1 receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients. *Circulation*, 94:698–703.

Benetos A, Laurent S, Hoeks AP, et al. 1993. Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. *Arterioscler Thromb*, 13:90–7.

Benetos A, Levy BI, Lacolley P, et al. 1997. Role of angiotensin II and bradykinin on aortic collagen following converting enzyme inhibition in spontaneously hypertensive rats. *Arterioscler Thromb Vasc Biol*, 17:3196–201.

Benetos A, Pannier B, Brahimi M, et al. 1993. Dose-related changes in the mechanical properties of the carotid artery in WKY rats and SHR following relaxation of arterial smooth muscle. *J Vasc Res*, 30:23–9.

Benetos A, Okuda K, Lajemi M, et al. 2001. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension*, 37:381–5.

Benetos A, Waeber B, Izzo J, et al. 2002. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am J Hypertens*, 15:1101–8.

Benjafield AV, Jeyasingam CL, Nyholt DR, et al. 1998. G-protein β3 subunit gene (GNB3) variant in causation of essential hypertension. *Hypertension*, 32:1094–7.

Birkedal-Hansen H, Moore WG, Bodden MK, et al. 1993. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med*, 4:197–250.

Blankenberg S, Rupprecht HJ, Poirier O, et al. AtheroGene Investigators. 2003. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation*, 107:1579–85.

Bloem LJ, Foroud TM, Ambrosius WT, et al. 1997. Association of the angiotensinogen gene to serum angiotensinogen in blacks and whites. *Hypertension*, 29:1078–82.

Bonithon-Kopp C, Ducimetiere P, Touboul PJ, et al. 1994. Plasma angiotensin-converting enzyme activity and carotid wall thickening. *Circulation*, 89:952–4.

Brull DJ, Murray LJ, Boreham CA, et al. 2001. Effect of a COL1A1 Sp1 binding site polymorphism on arterial pulse wave velocity: an index of compliance. *Hypertension*, 38:444–8.

Bozec E, Fassot C, Tropeano AI, et al. 2003. Angiotensinogen gene M235T polymorphism and reduction in wall thickness in response to antihypertensive treatment. *Clin Sci (Lond)*, 105:637–44.

Bozec E, Lacolley P, Bergaya S, et al. 2004. Arterial stiffness and angiotensinogen gene in hypertensive patients and mutant mice. *J Hypertens*, 22:1299–307.

Camp NJ, Hopkins PN, Hasstedt SJ, et al. 2003. Genome-wide multipoint parametric linkage analysis of pulse pressure in large, extended Utah pedigrees. *Hypertension*, 42:322–8.

Carrell TW, Burnand KG, Wells GM, et al. 2002. Stromelysin-1 (matrix metalloproteinase-3) and tissue inhibitor of metalloproteinase-3 are overexpressed in the wall of abdominal aortic aneurysms. *Circulation*, 105:477–82.

Caulfield M, Lavender P, Newell-Price J, et al. 1995. Linkage of the angiotensinogen gene locus to human essential hypertension in African Caribbeans. *J Clin Invest*, 96:687–92.

Chen W, Srinivasan SR, Elkasabany A, et al. 2001. Combined effects of endothelial nitric oxide synthase gene polymorphism (G894T) and insulin resistance status on blood pressure and familial risk of hypertension in young adults: the Bogalusa Heart Study. *Am J Hypertens*, 14:1046–52.

Chen W, Srinivasan SR, Bond MG, et al. 2004. Nitric oxide synthase gene polymorphism (G894T) influences arterial stiffness in adults: the Bogalusa Heart Study. *Am J Hypertens*, 17:553–9.

De Luca N, Savonitto S, Ricciardelli B, et al. 1993. Effects of the single and repeated administration of benazepril on systemic and forearm circulation and cardiac function in hypertensive patients. *Cardiovasc Drugs Ther*, 7:211–16.

DeStefano AL, Larson MG, Mitchell GF, et al. 2004. Genome-wide scan for pulse pressure in the National Heart, Lung and Blood Institute's Framingham Heart Study. *Hypertension*, 44:152–5.

Dong Y, Zhu H, Sagnella GA, et al. 1999. Association between the C825T polymorphism of the G protein β3-subunit gene and hypertension in blacks. *Hypertension*, 34:1193–6.

Draaijer P, Kool MJ, Maessen JM, et al. 1993. Vascular distensibility and compliance in salt-sensitive and salt-resistant borderline hypertension. *J Hypertens*, 11:1199–207.

Dreier R, Grassel S, Fuchs S, et al. 2004. Pro-MMP-9 is a specific macrophage product and is activated by osteoarthritic chondrocytes via MMP-3 or a MT1-MMP/MMP-13 cascade. *Exp Cell Res*, 297:303–12.

Durier S, Fassot C, Laurent S, et al. 2003. Physiological genomics of human arteries: quantitative relationship between gene expression and arterial stiffness. *Circulation*, 108:1845–51.

Dzau VJ, Safar ME. 1988. Large conduit arteries in hypertension: role of the vascular renin-angiotensin system. *Circulation*, 77:947–54.

Endemann DH, Touyz RM, Iglarz M, et al. 2004. Eplerenone prevents salt-induced vascular remodelling and cardiac fibrosis in stroke-prone spontaneously hypertensive rats. *Hypertension*, 43:1252–7.

Fabunmi RP, Baker AH, Murray EJ, et al. 1996. Divergent regulation by growth factors and cytokines of 95 kDa and 72 kDa gelatinases and tissue inhibitors or metalloproteinases-1, -2, and -3 in rabbit aortic smooth muscle cells. *Biochem J*, 315:335–42.

Farfel Z, Bourne HR, Iiri T. 1999. The expanding spectrum of G protein diseases. *N Engl J Med*, 340:1012–20.

Galis ZS, Johnson C, Godin D, et al. 2002. Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. *Circ Res*, 91:852–9.

Galis ZS, Sukhova GK, Lark MW, et al. 1994. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*, 94:2493–503.

Gardier S, Vincent M, Lantelme P, et al. 2004. A[1166]C polymorphism of angiotensin II type 1 receptor, blood pressure and arterial stiffness in hypertension. *J Hypertens*, 22:2135–42.

Garg UC, Hassid A. 1989. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest*, 83:1774–7.

Germain DP, Boutouyrie P, Laloux B, et al. 2003. Arterial remodeling and stiffness in patients with pseudoxanthoma elasticum. *Arterioscler Thromb Vasc Biol*, 23:836–41.

Girerd X, Hanon O, Mourad JJ, et al. 1998. Lack of association between renin-angiotensin system, gene polymorphisms, and wall thickness of the radial and carotid arteries. *Hypertension*, 32:579–83.

Gnasso A, Motti C, Irace C, et al. 2000. Genetic variation in human stromelysin gene promoter and common carotid geometry in healthy male subjects. *Arterioscler Thromb Vasc Biol*, 20:1600–5.

Grant SF, Reid DM, Blake G, et al. 1996. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nat Genet*, 14:203–5.

Gum R, Lengyel E, Juarez J, et al. 1996. Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase kinase 1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. *J Biol Chem*, 271:10672–80.

Hanon O, Luong V, Mourad JJ, et al. 2001. Aging, carotid artery distensibility, and the Ser422Gly elastin gene polymorphism in humans. *Hypertension*, 38:1185–9.

Hanon O, Luong V, Mourad JJ, et al. 2002. Association between the G protein β3 subunit 825T allele and radial artery hypertrophy. *J Vasc Res*, 39:497–503.

Hasegawa K, Fujiwara H, Doyama K, et al. 1994. Endothelin-1-selective receptor in the arterial intima of patients with hypertension. *Hypertension*, 23:288–93.

Hautanena A, Lankinen L, Kupari M, et al. 1998. Associations between aldosterone synthase gene polymorphism and the adrenocortical function in males. *J Intern Med*, 244:11–18.

Hengstenberg C, Schunkert H, Mayer B, et al. 2001. Association between a polymorphism in the G protein β3 subunit gene (GNB3) with arterial hypertension but not with myocardial infarction. *Cardiovasc Res*, 49:820–7.

Henrion D, Amant C, Benessiano J, et al. 1998. Angiotensin II type 1 receptor gene polymorphism is associated with an increased vascular reactivity in the human mammary artery in vitro. *J Vasc Res*, 35:356–62.

Hoshino Y, Mio T, Nagai S, et al. 2001. Fibrogenic and inflammatory cytokines modulate mRNA expressions of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-3 in type II pneumocytes. *Respiration*, 68:509–16.

Huhtala P, Tuuttila A, Chow LT, et al. 1991. Complete structure of the human gene for 92-kDa type IV collagenase. Divergent regulation of expression for the 92- and 72-kilodalton enzyme genes in HT-1080 cells. *J Biol Chem*, 266:16485–90.

Imai K, Yokohama Y, Nakanishi I, et al. 1995. Matrix metalloproteinase 7 (matrilysin) from human rectal carcinoma cells. Activation of the precursor, interaction with other matrix metalloproteinases and enzymic properties. *J Biol Chem*, 270:6691–7.

Jaeckel M, Simon G. 2003. Altered structure and reduced distensibility of arteries in Dahl salt-sensitive rats. *J Hypertens*, 21:311–19.

Jeanclos E, Schork NJ, Kyvik KO, et al. 2000. Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension,* 36:195–200.

Jeremy JY, Rowe D, Emsley AM, et al. 1999. Nitric oxide and the proliferation of vascular smooth muscle cells. *Cardiovasc Res*, 43:580–94.

Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al. 1992. Molecular basis of human hypertension: role of angiotensinogen. *Cell*, 71:169–80.

Jin JJ, Nakura J, Wu Z, et al. 2003. Association of endothelin-1 gene variant with hypertension. *Hypertension*, 41:163–7.

Jondeau G, Boutouyrie P, Lacolley P, et al. 1999. Central pulse pressure is a major determinant of ascending aorta dilation in Marfan syndrome. *Circulation*, 99:2677–81.

Kamitani S, Saito Y, Miyamoto Y, et al. 1998. Effect of Glu298Asp missense mutation of human endothelial nitric oxide synthase gene on its enzyme activity [abstract]. *Circulation*, 97(Suppl):I-537.

Kanaki T, Bujo H, Mori S, et al. 2002. Functional analysis of aortic endothelial cells expressing mutant PDGF receptors with respect to expression of matrix metalloproteinase-3. *Biochem Biophys Res Commun*, 294:231–7.

Komuro I, Kurihara H, Sugiyama T, et al. 1988. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. *FEBS Lett*, 238:249–52.

Kondapaka SB, Fridman R, Reddy KB. 1997. Epidermal growth factor and amphiregulin up-regulate matrix metalloproteinase-9 (MMP-9) in human breast cancer cells. *Int J Cancer*, 70:722–6.

Kool MJ, Lustermans FA, Breed JG, et al. 1995. The influence of perindopril and the diuretic combination amiloride + hydro-chlorothiazide on the vessel wall properties of large arteries in hypertensive patients. *J Hypertens*, 13:839–48.

Lacolley P, Challande P, Boumaza S, et al. 2001. Mechanical properties and structure of carotid arteries in mice lacking desmin. *Cardiovasc Res*, 51:178–87.

Lacolley P, Gautier S, Poirier O, et al. 1998. Nitric oxide synthase gene polymorphisms, blood pressure and aortic stiffness in normotensive and hypertensive subjects. *J Hypertens*, 16:31–5.

Lacolley P, Labat C, Pujol A, et al. 2002. Increased carotid wall elastic modulus and fibronectin in aldosterone-salt-treated rats: effects of eplerenone. *Circulation*, 106:2848–53.

Lajemi M, Gautier S, Poirier O, et al. 2001. Endothelin gene variants and aortic and cardiac structure in never-treated hypertensives. *Am J Hypertens*, 14:755–60.

Lajemi M, Labat C, Gautier S, et al. 2001. Angiotensin II type 1 receptor-153A/G and 1166A/C gene polymorphisms and increase in aortic stiffness with age in hypertensive subjects. *J Hypertens*, 19:407–13.

Lakatta EG. 1987. Human aging: changes in structure and function. *J Am Coll Cardiol*, 10:42A–47A.

Laogun AA, Gosling RG. 1982. In vivo arterial compliance in man. *Clin Phys Physiol Meas*, 3:201–12.

Larivière R, Day R, Schiffrin EL. 1993. Increased expression of endothelin-1 gene in blood vessels of deoxycorticosterone acetate-salt hypertensive rats. *Hypertension*, 21:916–20.

Larivière R, Thibault G, Schiffrin EL. 1993. Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. *Hypertension*, 21:294–300.

Laurent S, Boutouyrie P, Lacolley P. 2005. Structural and genetic bases of arterial stiffness. *Hypertension*, 45:1050–5.

Levin ER. 1995. Endothelins. *N Engl J Med*, 333:356–63.

Levy BI, Benessiano J, Poitevin P, et al. 1990. Endothelium-dependent mechanical properties of the carotid artery in WKY and SHR. Role of angiotensin converting enzyme inhibition. *Circ Res*, 66:321–8.

Levy BI, Michel JB, Salzmann JL, et al. 1988. Effects of chronic inhibition of converting enzyme on mechanical and structural properties of arteries in rat renovascular hypertension. *Circ Res*, 63:227–39.

Levy BI, Michel JB, Salzmann JL, et al. 1993. Long-term effects of angiotensin-converting enzyme inhibition on the arterial wall of adult spontaneously hypertensive rats. *Am J Cardiol*, 71:8E–16E.

Levy BI, Poitevin P, Duriez M, et al. 1997. Sodium, survival, and the mechanical properties of the carotid artery in stroke-prone hypertensive rats. *J Hypertens*, 15:251–8.

Lombes M, Oblin ME, Gasc JM, et al. 1992. Immunohistochemical and biochemical evidence for a cardiovascular mineralocorticoid receptor. *Circ Res*, 71:503–10.

Marsden PA, Heng HH, Scherer SW, et al. 1993. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. *J Biol Chem*, 268:17478–88.

Mattace-Raso FU, van der Cammen TJ, Sayed-Tabatabaei FA, et al. 2004. Angiotensin-converting enzyme gene polymorphism and common carotid stiffness. The Rotterdam study. *Atherosclerosis*, 174:121–6.

Medley TL, Cole TJ, Dart AM, et al. 2004. Matrix metalloproteinase-9 genotype influences large artery stiffness through effects on aortic gene and protein expression. *Arterioscler Thromb Vasc Biol*, 24: 1479–84.

Medley TL, Cole TJ, Gatzka CD, et al. 2002. Fibrillin-1 genotype is associated with aortic stiffness and disease severity in patients with coronary artery disease. *Circulation*, 105:810–15.

Medley TL, Kingwell BA, Gatzka CD, et al. 2003. Matrix metallo-proteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. *Circ Res*, 92: 1254–61.

Milner JM, Elliott SF, Cawston TE. 2001. Activation of procollagenases is a key control point in cartilage collagen degradation: interaction of serine and metalloproteinase pathways. *Arthritis Rheum*, 44: 2084–96.

Monteleone G, MacDonald TT, Wathen NC, et al. 1999. Enhancing lamina propria Th1 cell responses with interleukin 12 produces severe tissue injury. *Gastroenterology*, 117:1069–77.

Mourad J-J, Ducailar G, Rudnicki A, et al. 2002. Age-related increase of pulse-pressure and gene polymorphisms in essential hypertension: a preliminary study. *J Renin Angiotensin Aldosterone Syst*, 3:109–15.

Nagai Y, Fleg JL, Kemper MK, et al. 1999. Carotid arterial stiffness as a surrogate for aortic stiffness: relationship between carotid artery pressure-strain elastic modulus and aortic pulse wave velocity. *Ultrasound Med Biol*, 25:181–8.

Nicaud V, Poirier O, Behague I, et al. 1999. Polymorphisms of the endothelin-A and -B receptor genes in relation to blood pressure and myocardial infarction: the Etude Cas-Temoins sur l'Infarctus du Myocarde (ECTIM) Study. *Am J Hypertens*, 12:304–10.

Nojiri T, Morita H, Imai Y, et al. 2003. Genetic variations of matrix metalloproteinase-1 and -3 promoter regions and their associations with susceptibility to myocardial infarction in Japanese. *Int J Cardiol*, 92:181–6.

Nürnberger J, Saez AO, Mitchell A, et al. 2004. The T-allele of the C825T polymorphism is associated with higher arterial stiffness in young healthy males. *J Hum Hypertens*, 18:267–71.

O'Rourke M. 1995. Mechanical principles in arterial disease. *Hypertension*, 26:2–9.

Palmer RM, Ferrige AG, Moncada S. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, 327:524–6.

Pereira L, D'Alessio M, Ramirez F, et al. 1993. Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. *Hum Mol Genet*, 2:961–8.

Philip I, Plantefève G, Vuillaumier-Barrot S, et al. 1999. G849T polymorphism in the endothelial nitric oxide synthase gene is associated with an enhanced vascular responsiveness to phenylephrine. *Circulation*, 99:3096–8.

Pojoga L, Gautier S, Blanc H, et al. 1998. Genetic determination of plasma aldosterone levels in essential hypertension. *Am J Hypertens*, 11: 856–60.

Powell JT, Turner RJ, Henney AM, et al. 1997. An association between arterial pulse pressure and variation in the fibrillin-1 gene. *Heart*, 78:396–8.

Rauramaa R, Väisänen SB, Luong L-A, et al. 2000. Stromelysin-1 and interleukin-6 gene promoter polymorphisms are determinants of asymptomatic carotid artery atherosclerosis. *Arterioscler Thromb Vasc Biol*, 20:2657–62.

Rees DD, Palmer RM, Moncada S. 1989. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci U S A*, 86:3375–8.

Robert V, Van Thiem N, Cheav SL, et al. 1994. Increased cardiac types I and III collagen mRNAs in aldosterone-salt hypertension. *Hypertension*, 24:30–6.

Rudnichi A, Safar ME, Lajemi M, et al. 2004. Gene polymorphisms of the renin-angiotensin system and age-related changes in systolic and diastolic blood pressure in subjects with hypertension. *Am J Hypertens*, 17:321–7.

Safar ME, Lajemi M, Rudnichi A, et al. 2004. Angiotensin-converting enzyme D/I gene polymorphism and age-related changes in pulse pressure in subjects with hypertension. *Arterioscler Thromb Vasc Biol*, 24:782–6.

Safar ME, Levy BI, Struijker-Boudier H. 2003. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation*, 107:2864–9.

Sakurai T, Yanagisawa M, Takuwa Y, et al. 1990. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature*, 348:732–5.

Schiffrin EL, Deng LY, Sventek P, et al. 1997. Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. *J Hypertens*, 15:57–63.

Schunkert H, Hense HW, Döring A, et al. 1998. Association between a polymorphism in the G protein β3 subunit gene and lower renin and elevated diastolic blood pressure levels. *Hypertension*, 32:510–3.

Segura AM, Luna RE, Horiba K, et al. 1998. Immunohistochemistry of matrix metalloproteinases and their inhibitors in thoracic aortic aneurysms and aortic valves of patients with Marfan's syndrome. *Circulation*, 98(Suppl 19):II331–7; discussion II337–8.

Seo B, Oemar BS, Siebenmann R, et al. 1994. Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation*, 89:1203–8.

Siffert W. 2000. G protein β3 subunit 825T allele, hypertension, obesity, and diabetic nephropathy. *Nephrol Dial Transplant*, 15:1298–306.

Siffert W, Rosskopf D, Siffert G, et al. 1998. Association of a human G-protein beta3 subunit variant with hypertension. *Nat Genet*, 18: 45–8.

Snieder H, Hayward CS, Perks U, et al. 2000. Heritability of central systolic pressure augmentation: a twin study. *Hypertension*, 35:574–9.

Staessen JA, Wang J, Bianchi G, et al. 2003. Essential hypertension. *Lancet*, 361:1629–41.

Sun Y, Ramires FJ, Weber KT. 1997. Fibrosis of atria and great vessels in response to angiotensin II or aldosterone infusion. *Cardiovasc Res*, 35:138–47.

Takeda Y, Miyamori I, Yoneda T, et al. 1995. Production of aldosterone in isolated rat blood vessels. *Hypertension*, 25:170–3.

Taniwaki H, Kawagishi T, Emoto M, et al. 1999. Association of ACE gene polymorphism with arterial stiffness in patients with type 2 diabetes. *Diabetes Care*, 22:1858–64.

Terashima M, Akita H, Kanazawa K, et al. 1999. Stromelysin promoter 5A/6A polymorphism is associated with acute myocardial infarction. *Circulation*, 99:2717–19.

Topouchian J, Asmar R, Sayegh F, et al. 1999. Changes in arterial structure and function under trandolapril-verapamil combination in hypertension. *Stroke*, 30:1056–64.

Topouchian J, Brisac AM, Pannier B, et al. 1998. Assessment of the acute arterial effects of converting enzyme inhibition in essential hypertension: a double-blind, comparative and crossover study. *J Hum Hypertens*, 12:181–7.

Tsipouras P, Del Mastro R, Sarfarazi M, et al. 1992. Genetic linkage of the Marfan syndrome, ectopia lentis, and congenital contractural arachnodactyly to the fibrillin genes on chromosomes 15 and 5. The International Marfan Syndrome Collaborative Study. *N Engl J Med*, 326:905–9.

van der Heijden-Spek JJ, Staessen JA, Fagard RH, et al. 2000. Effect of age on brachial artery wall properties differs from the aorta and is gender dependent: a population study. *Hypertension*, 35:637–42.

White PC. 1994. Disorders of aldosterone biosynthesis and action. *N Engl J Med*, 331:250–8.

White PC, Slutsker L. 1995. Haplotype analysis of CYP11B2. *Endocr Res*, 21:437–42.

Wilhelm SM, Collier IE, Kronberger A, et al. 1987. Human skin fibroblast stromelysin: structure, glycosylation, substrate specificity, and differential expression in normal and tumorigenic cells. *Proc Natl Acad Sci U S A*, 84:6725–9.

Wilkinson IB, MacCallum H, Cockcroft JR, et al. 2002. Inhibition of basal nitric oxide synthesis increases aortic augmentation index and pulse wave velocity in vivo. *Br J Clin Pharmacol*, 53:189–92.

Wolinsky H, Glagov S. 1967. A lamellar unit of aortic medial structure and function in mammals. *Circ Res*, 20:99–111.

Xiao F, Puddefoot JR, Barker S, et al. 2004. Mechanism for aldosterone potentiation of angiotensin II-stimulated rat arterial smooth muscle cell proliferation. *Hypertension*, 44:340–5.

Ye S. 2000. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol*, 19:623–9.

Ye S, Eriksson P, Hamsten A, et al. 1996. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem*, 271:13055–60.

Ye S, Watts GF, Mandalia S, et al. 1995. Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. *Br Heart J*, 73:209–15.

Yokokawa K, Tahara H, Kohno M, et al. 1991. Hypertension associated with endothelin-secreting malignant hemangioendothelioma. *Ann Intern Med*, 114:213–15.

Yoon S, Tromp G, Vongpunsawad S, et al. 1999. Genetic analysis of MMP3, MMP9, and PAI-1 in Finnish patients with abdominal aortic or intracranial aneurysms. *Biochem Biophys Res Commun*, 265:563–8.

Zhang B, Ye S, Herrmann SM, et al. 1999. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation*, 99:1788–94.