Structural and Genetic Bases of Arterial Stiffness

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Abstract—Arterial stiffness has independent predictive value for cardiovascular events. We review data concerning the heritability of arterial stiffness, and propose an integrated view of the structural and genetic determinants of arterial stiffness, based on a candidate gene approach and recent studies on gene expression profile. Arterial stiffness seems to have a genetic component, which is largely independent of the influence of blood pressure and other cardiovascular risk factors. In animal models of essential hypertension (SHR and SHR-SP), structural modifications of the arterial wall include an increase in the number of elastin/smooth muscle cell (SMC) connections, and smaller fenestrations of the internal elastic lamina, possibly leading to redistribution of the mechanical load toward elastic materials. These modifications may give rise to mechanisms that explain why changes in arterial wall material accompanying wall hypertrophy in these animals are not associated with an increase in arterial stiffness. In monogenic connective tissue diseases (Marfan, Williams, and Ehlers-Danlos syndromes) and the corresponding animal models, precise characterization of the arterial phenotype makes it possible to determine the influence of abnormal genetically determined wall components on arterial stiffness. Such studies have highlighted the role of extracellular matrix signaling in the vascular wall and have shown that elastin and collagen not only display elasticity or rigidity but also are involved in the control of SMC function. These data provide strong evidence that arterial stiffness is affected by the amount and density of stiff wall material and the spatial organization of that material. (Hypertension. 2005;45:1050-1055.)

Key Words: biomechanics • extracellular matrix • gene profile • hypertension • monogenic disease

Large-artery stiffness is the main determinant of pulse pressure (PP).1,2 Both have predictive value for cardiovascular events, independent of classical cardiovascular risk factors.1,2 Aortic stiffness has independent predictive value for total and cardiovascular mortality,3–6 coronary morbidity and mortality,4 and fatal stroke7 in patients with essential hypertension,3,4,7 end-stage renal failure,5 or diabetes mellitus.6 Several lines of evidence suggest that genetic factors not taken into account by classical cardiovascular risk factors could influence arterial stiffness. First, the relationship between arterial stiffness and total or cardiovascular mortality remains significant after adjustment for classical risk factors (age, sex, mean blood pressure, etc).3,4,7 Second, the predictive value of arterial stiffness has been shown to be highest in patients considered to be at low risk for cardiovascular events on the basis of cardiovascular risk scores.4 Third, the measurement of aortic stiffness takes into account changes to the arterial wall, such as intima-media thickening, which are thought to be genetically determined, at least in part.8 Genetic factors may directly influence the structure of the arterial wall or act indirectly through age, blood pressure (BP), smoking, cholesterol levels, glycaemia, and other classical risk factors, ultimately resulting in an increase in arterial stiffness.

We begin by summarizing findings concerning the heritability of arterial stiffness. We then provide an integrated view of the structural and genetic determinants of arterial stiffness, based on a candidate gene approach and recent gene expression profile studies.

Measurement of Arterial Stiffness

In clinical practice, 3 methods are commonly used to determine arterial stiffness.9 Aortic stiffness can be measured directly by determining pulse wave velocity (PWV) along the descending thoraco-abdominal aorta, using the foot-to-foot velocity method.10 Waveforms are obtained transcutaneously over the right common carotid artery and the right femoral artery, and the time delay (t) between the feet of the 2 waveforms is measured. The distance (D) covered by the waves is assimilated to the distance measured between the 2 recording sites. PWV is calculated as PWV=D(meters)/t(seconds). Carotid stiffness can be determined directly from the ratio of local PP (measured by applanation tonometry) to relative stroke change in diameter (measured by ultrasound scan).11 Aortic stiffness can be estimated indirectly from augmentation index (Alx).12 Alx is the extra pressure caused by pressure wave reflection back from the periphery. It is influenced not only by arterial stiffness but also by the intensity of wave reflections, which in turn depends on the
geometry and vasomotor tone of the arterial system.\textsuperscript{12} AIx is determined from carotid or radial artery pressure waves recorded by applanation tonometry.\textsuperscript{12}

**Heritability of Arterial Stiffness**

A pioneering study, the Bogalusa Heart Study,\textsuperscript{13} showed that carotid artery stiffness was greater in adolescents with a parental history of myocardial infarction or diabetes than in adolescents with no such parental history. In a recent study, AIx was found to be higher in the offspring of families with hypertension than in control subjects after correction for known cardiovascular risk factors.\textsuperscript{14} Because aortic PWV was similar in the 2 groups, this study\textsuperscript{15} indicated the existence of a functional and/or structural decrease in the caliber of small arteries. The Strong Heart Study,\textsuperscript{8} performed in 950 adult men and women from 13 American Indian communities, assessed the heritability of carotid stiffness, estimated as the proportion of residual phenotypic variance caused by the additive effects of genes after accounting for the effects of covariates (sex, age, diabetes, etc). The authors showed that although classical covariates could account for up to 51\% of the variance of carotid stiffness, the proportion of residual phenotypic variance caused by the additive effects of genes was 23\%. AIx inheritance has also been studied in twins, using a co-twin case-control analysis in monozygotic and dizygotic female twins.\textsuperscript{15} A quantitative genetic modeling technique was used, based on comparison of the variance–covariance matrices for monozygotic and dizygotic twins. Up to 37\% of the variance of AIx was found to be determined by genetic factors, and only a small proportion of the total variance was caused by genes also affecting height, heart rate, and mean BP.

Thus, arterial stiffness probably has a genetic component, largely independent of the influence of BP, heart rate, height, age, and other cardiovascular risk factors. The use of more direct measurements of arterial stiffness, such as PWV, would be of value in future studies.

**Integrative Physiology of Arterial Stiffness in Essential Hypertension**

Aging and BP are the 2 major determinants of arterial stiffness. Aging has different effects on proximal, predominantly elastic arteries, such as the aorta and the carotid artery, than on distal, predominantly muscular arteries, such as the radial and the femoral arteries.\textsuperscript{16} Central arteries stiffen progressively with age, whereas the stiffness of muscular arteries changes little with age.\textsuperscript{16} In the load-bearing media of elastic arteries, the orderly arrangement of elastic fibers and laminae is gradually lost over time, and thinning, splitting, fraying, and fragmentation are observed.\textsuperscript{17} The degeneration of elastic fibers is associated with an increase in collagenous material and in ground substance, often accompanied by calcium deposition in ground substance and in degenerate elastic fibers.

Animal models of essential hypertension, such as spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHR-SP), can provide insight into the cellular and molecular determinants of arterial stiffness. The sustained increase in BP observed in essential hypertension is a trigger for the development of arterial wall hypertrophy, which in turns leads to the normalization of circumferential wall stress ($\sigma$), according to Lame’s equation ($\sigma$ = MBP/R/h, where R is the radius and h is wall thickness). According to the laws of physics, any increase in wall thickness should lead to an increase in arterial stiffness for a given BP, because of the juxtaposition of material with identical mechanical properties. Surprisingly, we and others have shown that hypertrophy in patients with hypertension is accompanied by a decrease in the stiffness of the wall material (Young’s elastic modulus), with the artery as a whole considered to display normal stiffness.\textsuperscript{18–21} Similar findings were obtained with SHR and SHR-SP for analyses of the carotid artery and abdominal aorta in comparisons of hypertensive strains and Wistar-Kyoto (WKY) rats.\textsuperscript{22,23} Thus, hypertension-induced wall thickening is not associated with an increase in arterial stiffness in patients with essential hypertension and in rat models of hypertension.

Wolinsky and Glagov\textsuperscript{24} stressed that the lamellar unit of the aortic medial structure, consisting of elastin, collagen, and smooth muscle, contributes to the viscoelastic properties accounting for many of the static and dynamic mechanical features of elastic arteries. We suggested that sustained hypertension might be associated with a rearrangement of arterial wall material, involving qualitative or quantitative changes in arterial components, leading to mechanical adaptation of the arterial wall. The 3-dimensional organization of stiff elements is probably more important than their content and density in determining the global mechanical behavior of the artery. O’Rourke and Avolio\textsuperscript{25} put forward a model in which smooth muscle, which is in series with some of the stiffer collagenous components but in parallel with the elastic lamellae, transfers stresses to collagen during contraction and to the elastic lamellae during relaxation.

We investigated the role of wall components, focusing on fibronectin, dense plaques, and fenestrations. Fibronectin (FN) plays an important role in cell–matrix interactions by interacting with specific cellular integrin receptors, such as the $\alpha$5$\beta$1-integrin.\textsuperscript{26,27} We found that total FN, cellular FN (EIIIA FN isoform), and $\alpha$5-integrin levels were high in the aortas of SHR\textsuperscript{28} and SHR-SP.\textsuperscript{23} Dense plaques of smooth muscle are a major site of anchorage between smooth muscle cells (SMCs) and the extracellular matrix.\textsuperscript{26} We demonstrated by electron microscopy\textsuperscript{28} that the percentage of the cell surface occupied by dense plaques and connected to the elastic lamellae in the aorta was twice as high in SHR as in Wistar rats. Thus, the elastin network may contribute to the mechanical adaptation of the arterial wall in SHR through variations in the total amount of elastin and in the extent of anchorage to muscle cells. This anchorage, which concerns the internal elastic lamina and the elastic lamellae involved in the musculo-elastic fascicles described by Wolinsky and Glagov,\textsuperscript{24} may thus play a role in both medium-sized muscular arteries and large elastic arteries. Mechanical stress is concentrated in the regions close to the fenestrations of elastic lamellae. We showed by confocal microscopy\textsuperscript{23} that the mean area of fenestrations and the proportion of the area occupied by fenestrations of the internal elastic lamina were smaller in SHR-SP and SHR than in WKY rats. Thus, decreases in the
stress concentration effects within the internal elastic lamina may represent an adaptive mechanism protecting the elastin network against increases in mean wall stress.

Figures 1 and 2 summarize the structural modifications to the arterial wall in SHR and SHR-SP that may be involved in mechanical adaptation to a high level of wall stress. These changes, which include increases in the number of FN/α5β1 integrin complexes and elastin/SMC connections, and smaller internal elastic lamina fenestrations, may result in the redistribution of mechanical load toward elastic materials. They provide mechanisms explaining that the changes in arterial wall material that accompany wall hypertrophy in animal models of essential hypertension are not associated with an increased stiffness and mechanical strength.

Candidate Gene Approach
A candidate gene approach can be used to characterize the cellular and molecular determinants of arterial stiffness. We begin by reviewing genotype–phenotype studies, including studies attempting to confirm human data in genetically modified animals. We then deal with monogenic diseases of the arterial wall in humans and related animal models, which constitute interesting models for increasing our understanding of the structure–function relationship of the arterial wall, including the influence of abnormal genetically determined wall components on arterial stiffness.

Genetic Polymorphisms and Animal Models
The relationships between polymorphisms of several candidate genes and arterial stiffness have been investigated. Studies initially focused on candidate genes of the renin-angiotensin-aldosterone system, which is involved in BP control, cell proliferation, matrix production, and vascular hypertrophy, and plays a key role in arterial stiffness. In hypertensive patients, Benetos et al found a positive association between PWV and both the A1166C polymorphism of the angiotensin II type 1 receptor and the angiotensin-converting enzyme (ACE) I/D polymorphism. In a larger population, including hypertensive subjects who had never been treated and hypertensive subjects who had been treated in the past, the same group showed that the A153G and A1166C polymorphisms affected the increase in aortic stiffness with age, but that the ACE I/D and AGT T747C gene polymorphisms had no effect. The T344C polymorphism of the aldosterone synthase (CYP11B2) gene has been shown to be associated with an increase in PWV in some studies but not in others. Carotid artery stiffness was increased in never treated essential hypertensive patients homozygous for the T allele of the M235T polymorphism of the angiotensinogen gene. In subjects of the FLEMENGHO study, 77% of whom had hypertension, carotid artery stiffness was associated with the ACE I/D polymorphism. The contribution of a given gene polymorphism to the variance of a specific phenotype is limited. Studies have therefore been performed to determine the interactions between aging, genetic variants, and arterial stiffness or between 2 or more gene polymorphisms. For instance, femoral artery distensibility has been shown to be lower than the population mean in ACE DD subjects homozygous for α-adducin Gly460.

Genotype–phenotype studies have also focused on matrix proteins, mainly elastin and collagens. An increase in carotid stiffness has been reported in subjects carrying the A allele of the Ser422Gly polymorphism of the elastin gene. In patients with coronary artery disease, the 2 to 3 genotype of the fibrillin-1 gene has been shown to be associated with a higher characteristic impedance and central PP (ie, greater aortic stiffness), than the 2 to 2 and 2 to 4 genotypes. Matrix metalloproteinases (MMPs) are potential candidate proteins, because they are involved in matrix homeostasis and arterial wall remodeling. This is particularly true of MMP-3 (stromelysin-1), which acts on various substrates, including fibronectin, elastin, and collagens. Aortic stiffness has been shown to be greater in subjects older than 60 years and homozygous for the 5A promoter polymorphism of MMP-3 than in age-matched subjects homozygous for the 6A polymorphism.

Monogenic Disease and Related Knockout Mice
Monogenic diseases of the arterial wall constitute interesting models for increasing our understanding of the structure–
function relationship of the arterial wall, and the effect on arterial stiffness of abnormal genetically determined wall components in particular. These diseases are complicated by major cardiovascular events, such as dilatation, dissection, and rupture (Marfan syndrome, Ehlers–Danlos syndrome) or hypertrophy and stenosis (Williams syndrome, pseudoaxanthoma elasticum).

Marfan Syndrome

Marfan syndrome (MFS) is a connective tissue disorder inherited as an autosomal-dominant trait and is characterized by abnormalities involving the skeletal, ocular, and cardiovascular systems. MFS results from mutations in the gene encoding fibrillin-1 (FBN1), leading to abnormalities in the assembly of elastic fibers. In MFS patients, the increase in arterial stiffness is confined to the aorta, with no change in stiffness observed for the carotid, femoral, and radial arteries.

A clinical hallmark of MFS and the major cause of morbidity and premature death from this syndrome is aortic root dilatation and associated aortic regurgitation, dissection, and rupture. The exact mechanisms leading to dilatation are not fully understood, but steady and pulsatile stresses are probably important, leading to the mechanical fatigue of abnormal elastic fibers and microdissections. Central PP, which is influenced by aortic stiffness, is a powerful determinant of ascending aorta diameter in MFS patients, regardless of age and body surface area, whereas mean BP is not.

Similar findings have been reported in hypertensive subjects: carotid PP is a major independent determinant of carotid internal diameter, whereas mean BP is not. Thus, aortic dilatation probably results from the failure of abnormal elastic fibers to sustain physiological pulsatile stress by analogy with aging.

In a rodent model of MFS in which FBN1 is underexpressed, aortic wall stiffness was found to be 4-times higher than that in wild-type strains. Electron microscopy showed that elastic laminae had an unusually smooth surface and lacked the cell attachments normally mediated by FBN1. Bunton et al. suggested that the loss of cell attachments triggers signaling initiating a nonproductive program for the synthesis and remodeling of an elastic matrix. Thus, FBN1 appears to be a key element in the normal spatial organization of the arterial wall, ensuring adequate loading of elastic components, thereby maintaining physiological arterial stiffness. Similar findings have been reported for mice lacking desmin. In desmin knockout mice, arterial SMCs lose some of their connections to the extracellular matrix: finger-like SMC projections to elastic lamellae are less frequent and the carotid artery is stiffer than in wild-type mice. The arteries of desmin-deficient mice also have a lower in vitro breaking pressure. The less solid arterial wall in MFS and in mice lacking desmin suggests that connections between the extracellular matrix and vascular SMCs are probably involved in both arterial elasticity and mechanical strength.

Williams Syndrome

Williams syndrome is a genetic disorder characterized by mental and statural deficiencies and cardiovascular abnormalities including peripheral arterial stenoses and hypertension. These features may be related to the deletion of 1 allele of the elastin gene. In mice, the absence of elastin has been shown to be sufficient to induce the subendothelial proliferation of SMCs and to contribute to obstructive arterial disease. Thus, elastin is not purely structural and fulfills a regulatory function during arterial development, controlling the proliferation of smooth muscle and stabilizing arterial structure.

Aggoun et al. showed that carotid intima-media thickness and distensibility were significantly higher in children with Williams syndrome than in controls. We obtained similar results for 3 adult patients. Electron microscopy of renal artery stenosis material showed major abnormalities of the elastic fibers and of immunohistochemical staining, indicating a low level of differentiation of SMCs. The abnormally distensible and thick carotid artery wall may result from abnormal elastic fiber assembly within the media. Smooth muscle cell dedifferentiation, leading to arterial wall hyper trophy, may be a major factor responsible for increases in distensibility.

Ehlers-Danlos Syndrome

Ehlers–Danlos syndrome type IV, the vascular type, results from mutations in the gene for type III procollagen. It is a rare connective tissue disorder inherited as an autosomal-dominant trait, characterized mainly by the spontaneous rupture of arteries, and a gravid uterus or intestines. In a recent cross-sectional study, we compared the arterial phenotype of vascular-type Ehlers–Danlos syndrome patients with that of age-, sex-, and BP-matched control subjects. We observed no significant difference in carotid–femoral PWV and arterial distensibility (carotid and radial arteries). However, carotid intima-media thickness was 32% lower and circumferential wall stress was 43% higher than in matched controls. The higher circumferential wall stress is probably a major risk factor for the dissection and rupture of fragile arterial tissue. The fragility of the arterial wall in mice with mutations affecting type I and type III collagens has been attributed to a large decrease in the number of collagen type I fibrils in the aortic media and adventitia.

In conclusion, precise characterization of the arterial phenotype in monogenic diseases of connective tissue provides insight into the effect of abnormal genetically determined wall components on arterial stiffness. The data obtained also highlight the role of extracellular matrix signaling in the vascular wall and show that elastin and collagen are not simply passive compounds that can be elastic or rigid, they are also involved in the control of SMC function: migration, proliferation, adhesion, and cytoskeletal rearrangement.

Gene Expression Profile

Because the 3-dimensional organization of arterial wall components plays an important role in arterial stiffness and in adaptive mechanisms in polygenic and monogenic diseases, we used a global approach to clarify the relationship between individual gene expression and arterial stiffness in humans. We used microarray technology to identify novel transcriptional biomarkers of arterial stiffening. Our working hypothesis was that patients with abnormal aortic stiffness have a
specific gene expression profile in aortic wall tissue (genes abnormally overexpressed or underexpressed) different from that in patients with normal arterial stiffness. Aortic biopsy samples were taken from patients with coronary heart disease during a coronary artery bypass operation. RNA samples were analyzed with Affymetrix GeneChip technology. We found that 151 probe sets, of the 12 588 transcripts studied, were differentially expressed between stiff and distensible aortas, and that 32 of these probe sets were significantly positively or negatively associated with PWV. Most of the genes identified were related to the cytoskeleton, with the remainder distributed between matrix and membrane. Differentially expressed genes involved in the mechanical regulation of vascular structure included integrins (α2b, α5, β1, and β3), proteoglycans (decorin, osteomodulin, etc), fibrin-1, and fascin. Almost half the abnormal transcripts could be classified as involved in signaling/communication or cell structure/motility. We identified 2 distinct groups of genes: those associated with cell signaling and those associated with the mechanical regulation of vascular structure (cytoskeletal, cell membrane, extracellular matrix). Many studies have concentrated on the contribution of the extracellular matrix to arterial stiffness, but these data suggest that changes in the expression of signaling molecules plays an equally important role. Changes in the profiles of signaling molecules may be involved in the regulation of cell cytoskeletal organization, cell–matrix interactions, or the contractile state of the cell.

**Therapeutic Applications**

The identification of genes differentially expressed in normal and pathological aortic tissue would facilitate the identification of potential pharmacological targets for decreasing arterial stiffness after the optimal reduction of BP. The most interesting targets for drug candidates should theoretically fulfill the following conditions: (1) proteins encoded by genes found to be overexpressed or underexpressed in stiff aortas; (2) proteins found to be underproduced or overproduced in aortic tissue; (3) for those corresponding to a genetic polymorphism, genotype–phenotype analysis should have revealed abnormally high or low levels of arterial stiffness in carriers of the specific polymorphism; and (4) for those corresponding to a monogenic disease, genotype–phenotype analysis should have demonstrated abnormally high or low levels of arterial stiffness in these patients. Candidate proteins could be known targets of an existing drug (collagen I, elastin), an identified target for which there is currently no drug (FBN1, fibrillin), or a target never previously identified as involved in arterial stiffness (decorin, osteomodulin).

**Conclusion**

Recent studies in animals and humans have investigated the structural and genetic bases of arterial stiffness. They have shown that several genes and molecules are associated with vascular stiffening and have illustrated the consequences of changes to these genes and molecules in various clinical conditions. We need to identify these molecules and their signaling pathways for the development of future drug treatments for arterial stiffness.

**References**

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