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EDITORIAL COMMENT

Proteomics of Large Artery Stiffness

Digging for the Roots of Cardiometabolic and Cardiovascular Disease

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Large artery stiffness, meaning primarily aortic stiffness, plays a critical role in the pathogenesis of cardiovascular disease (CVD). Numerous studies and meta-analyses have demonstrated strong relations between measures of aortic stiffness, such as carotid-femoral pulse wave velocity (CFPWV), and various adverse CVD outcomes.[1,2] Despite clear insights into the important consequences of accelerated aortic stiffening, factors contributing to the pathogenesis of aortic stiffness and interventions that seek to prevent or reverse excessive stiffening remain incompletely elucidated.

The paradigm of aortic stiffening has evolved considerably over the past few years. An early model portrayed the aorta as an innocent victim of attack by various forms of cardiometabolic disease—hypertension, diabetes, lipid abnormalities, and obesity—that were thought to arise for various reasons, such as primary small vessel disease leading to hypertension or insulin resistance leading to glucose intolerance and diabetes. These conditions and diseases were posited to produce a state of secondary accelerated vascular aging, leading to progressive and irreversible stiffening of the aortic wall and an increase in CFPWV.[3] The increase in CFPWV resulted in premature arrival of a composite reflected wave that augmented central pressure and pulse pressure (PP = systolic pressure − diastolic pressure). However, recent studies have shown that aortic stiffness precedes and contributes to the pathogenesis of cardiometabolic disease,[4-6] suggesting that the availability of interventions that reduce aortic stiffness could provide an opportunity for primordial prevention of cardiometabolic disease well ahead of downstream adverse CVD outcomes.

The goal of preventing or reversing aortic stiffness requires further elucidation of factors that contribute to stiffening of the aorta. Standard risk factors, like smoking or elevated levels of lipids or glucose, have rather modest cross-sectional[7] and longitudinal[8] relations with aortic stiffness. Load in the aortic wall is largely borne by elastic fibers in the aortic medial layer. These fibers are deposited once during prenatal development and early life and must then last a lifetime. Therefore, insights into early life factors that modulate the complement of fibers deposited and whole life factors that modulate the degradation of these fibers or other elements of the aortic media are needed.

The study by Dib et al[9] in this issue of *JACC: Basic to Translational Science* takes a step in the right direction by presenting an analysis of relations of a large panel of plasma proteins with CFPWV, the reference standard measure of aortic wall stiffness, in the relatively healthy, middle-aged Asklepios cohort. The authors used the SomaScan platform to assay plasma levels of >7,000 proteins in 1,250 randomly selected Asklepios participants and found relations of CFPWV with 106 proteins that passed a Bonferroni-adjusted *P* value threshold. The authors then used these protein targets as the basis for a 2-sample Mendelian randomization (MR) analysis. First, they used published summary data from 2 separate cohorts to identify cis (local) protein quantitative trait loci (pQTLs) for plasma levels of the foregoing 106 proteins. Then they used summary data from a large genome-wide association study to determine whether genetic variants in the same regions were also associated with PP, a related measure of aortic stiffness.

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The author attests they are in compliance with human studies committees and animal welfare regulations of the author's institution and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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In cases where pQTLs aligned with PP loci (13 proteins), they were able to estimate a predicted difference in pulse pressure per SD difference in predicted protein level. Finally, they performed a colocalization analysis, which is a Bayesian technique that estimates a posterior probability that a specific genetic variant is associated with both protein level and PP, from which 5 proteins emerged.

Application of the foregoing cascaded statistical sieves to the various CFPWV pQTLs and genome-wide PP loci produced a final list of proteins (CDH55, NOTCH1, RARRES2, CILP2, and INHBC) that includes some interesting candidates, as detailed by the authors. However, a few potential limitations of the statistical approach require careful consideration. The authors have attempted to relate levels of 7,000 proteins with CFPWV in 1,250 participants, meaning that they are performing around 6 statistical tests per participant. To control the type I error rate, they adjusted their *P*-value threshold for multiple comparisons by using a principal components analysis of the proteins to determine the number of independent principal components (PCs) required to explain 95% of the variance in the various individual proteins. The adjustment that was applied (a factor of ~1,000) raises the interesting question of how variability in levels of 7,000 proteins can be explained by 1,000 PCs. Is this true biologic coexpression of proteins, or is it a manifestation of crosstalk in the SomaScan aptamer technology? In addition, when examining pQTLs, it is important to note that associations could represent either a change in circulating plasma levels or a change in the configuration of the protein that modifies aptamer affinity. Finally, associations of CFPWV or PP with circulating plasma levels of (fragments of) predominantly intracellular or matrix proteins can be a consequence, rather than a cause, of aortic stiffening. The authors acknowledge these limitations and underscore the need for independent validation of identified associations using antibody or mass spectrometry methods and interpretation of relations in the context of known function.

The use of MR analysis of PP to validate candidate pQTLs identified by CFPWV-protein relations potentially limited the ability of the authors to replicate their protein associations. PP is a measure of aortic stiffness that is closely related to characteristic impedance (Zc) of the aorta. CFPWV and Zc have similar direct relations with aortic wall stiffness, but they differ dramatically in their inverse relations with aortic diameter. CFPWV is relatively insensitive, whereas Zc is very highly sensitive to aortic lumen diameter. Despite their shared dependency on aortic wall stiffness, considerable modulation of aortic diameter across the lifespan in response to various factors, such as somatic growth, weight gain, and smoking, can result in divergent changes in CFPWV and PP in various settings. For example, CFPWV increases but PP falls with age in young adults because of a predominant effect of aortic diameter remodeling, leading to a reduction in Zc and PP. By contrast, both CFPWV and PP increase after midlife, when diameter remodeling plateaus and wall stiffening accelerates. Disparate effects of diameter on CFPWV as compared with Zc and PP result in only moderate correlation between CFPWV and PP. As a result, one can speculate that proteins and genetic loci that affect only aortic wall stiffness might be replicated using this mixed phenotype discovery and validation approach. However, loci that modulate both wall stiffness and diameter could be missed.

Although the limitations of the approach used by Dib et al[9] are important, the study represents a step forward in the quest to identify potentially modifiable root causes of aortic stiffening and resulting downstream cardiometabolic disease and major CVD events. The concept that the aorta is an innocent victim of the ravages of cardiometabolic disease, resulting in inevitable and irreversible aortic stiffening, is no longer tenable. When the aorta stiffens, the increase in Zc erodes the impedance mismatch between aorta and stiff first-generation conduit vessels, resulting in increased transmission of harmful levels of pulsatile energy into the conduits and distal microcirculation.[10] Considerable evidence suggests that aortic wall stiffening, and the resulting increase in CFPWV, Zc and PP, precede and contribute to the pathogenesis of cardiometabolic disease.[4-6] Isolated or predominant systolic hypertension (ie, wide PP hypertension) is the most prevalent form of hypertension, particularly in treatment-resistant cases. Perhaps this trend is not surprising, given that the development of hypertension drugs over the past half century has focused exclusively on mean arterial pressure and microvascular function. The current study is a first step in a necessary redirection of effort toward the identification and implementation of targeted interventions that prevent or mitigated aortic stiffening—a key element in the pathophysiology of cardiometabolic and cardiovascular disease.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr Mitchell is the owner of Cardiovascular Engineering, Inc, a company that designs and manufactures devices that measure vascular stiffness. The company uses these devices in clinical trials that evaluate the effects of diseases and interventions on vascular

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stiffness. He also reports receiving grants from the National Institutes of Health, Novartis and deCODE genetics and consulting fees from Novartis, Bayer, Merck, Servier and deCODE genetics and is an inventor on pending patent applications that disclose methods for estimating carotid-femoral pulse wave velocity and other measures of organ age by using convolutional neural networks.

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