

Mini-Review

Molecular Mechanisms of Arterial Stiffening

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Key Words

Arterial calcification · Arterial stiffening · Cardiovascular disease · Genomic studies · Vascular aging

Abstract

Stiffening of large arteries is a hallmark of vascular aging and one of the most important determinants of the age-related increase in blood pressure and cardiovascular disease events. Despite a substantial genetic component, the molecular mechanisms underlying phenotypic variability in arterial stiffness remain unknown. Previous genetic studies have identified several genetic variants that are associated with measures of arterial stiffness. Here, we review the relevant advances in the identification of pathways underlying arterial stiffness from genomic studies.

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Arterial Stiffness as a Risk Factor and Target for Treatment

Stiffening of the aorta and large elastic arteries is a hallmark of vascular aging [1]. It has a number of adverse haemodynamic consequences, including a major contribution to isolated systolic hypertension [2–4]. When measured by aortic pulse wave velocity (aPWV), it is highly predictive of clinical cardiovascular disease events independent of blood pressure, both in the general population and in groups with additional risk factors [5, 6]. Formerly thought to be simply a marker of atherosclerosis [7], the pathology of aortic stiffening may differ, at least in part, from that of atherosclerosis [8–10]. Thus, in primate models of atherosclerosis, aPWV is reduced compared to non-atherosclerotic controls, at least in the early stages of atheroscle-

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rosis [10]. In humans, aPWV is largely independent of risk factors other than age and blood pressure [11] and is not elevated in the presence of non-calcified atheromatous plaque [8, 9]. The prognostic importance of arterial stiffening and the fact that it may be driven by a specific pathology distinct from atherosclerosis makes it an appealing target to prevent cardiovascular disease events.

Arterial Calcification as a Cause of Arterial Stiffening

In older subjects, calcification occurs in the media of the arterial wall around elastin fibres ('elastocalcinosis') [12] and within atherosclerotic plaque in the intima [13]. Although often regarded as distinct entities, intimal and medial calcifications often coexist. Arterial stiffening is closely associated with calcification, an association that could be explained by coexistent atherosclerosis [7]. However, animal models show that medial calcification (in the absence of atherosclerosis) increases arterial stiffness, suggesting a direct causal relation between calcification and stiffening [14]. Using combined computed tomography and magnetic resonance imaging to measure calcification and atheroma in the Twins UK population, we have shown that even though calcification often colocalises with atherosclerotic plaque, the association of stiffness with calcification is not explained by coexistent atheromatous plaque [8]. Furthermore, the correlation between calcification and stiffness is explained by shared genetic factors distinct from those responsible for atherosclerosis [8, 9].

Arterial calcification is now known to be an active process resembling osteogenesis in which vascular smooth muscle cells undergo osteoblastic differentiation, expressing many of the proteins associated with bone formation [13] and releasing vesicles into the extracellular matrix which serve as nucleation sites for the accumulation of hydroxyapatite crystals [15, 16]. In culture media, high concentrations of calcium and phosphate induce osteoblastic differentiation of vascular smooth muscle cells [16, 17]. Pre-dialysis- and dialysis-dependent patients with chronic kidney diseases have high circulating calcium phosphate products and develop extensive calcification and arterial stiffening [18]. Precipitation of calcium in tissue in healthy subjects is inhibited by numerous regulatory factors including matrix gla protein, fetuin, klotho and fibroblast growth factor 23 [13]. It is likely that initiation and progression of calcification is dependent on the complex interactions between promoters and inhibitors of calcification.

Contribution of Changes in the Extracellular Matrix

Whilst calcification may represent the later stages of a degenerative arteriosclerotic process that can be detected macroscopically, it is likely to be initiated by elastin degradation and a change in the type of collagen, which may also contribute to arterial stiffening independent of calcification [14]. Such a degenerative process may relate to repetitive mechanical stress (heart rate \times blood pressure product) [19]. It is thought to promote calcification through elastin-derived soluble peptides (matrikines or elastokines) which activate smooth muscle cell osteogenic differentiation [20] and increase matrix affinity for nucleating mineral deposition [21]. Matrix metalloproteinases (MMPs) degrade components of the extracellular matrix including elastin, and in vivo, MMP-mediated elastin degradation is closely associated with both medial calcification and increased arterial stiffness [22, 23]. MMPs are also implicated in cutaneous elastin degradation that may parallel changes in the arterial wall [24, 25].

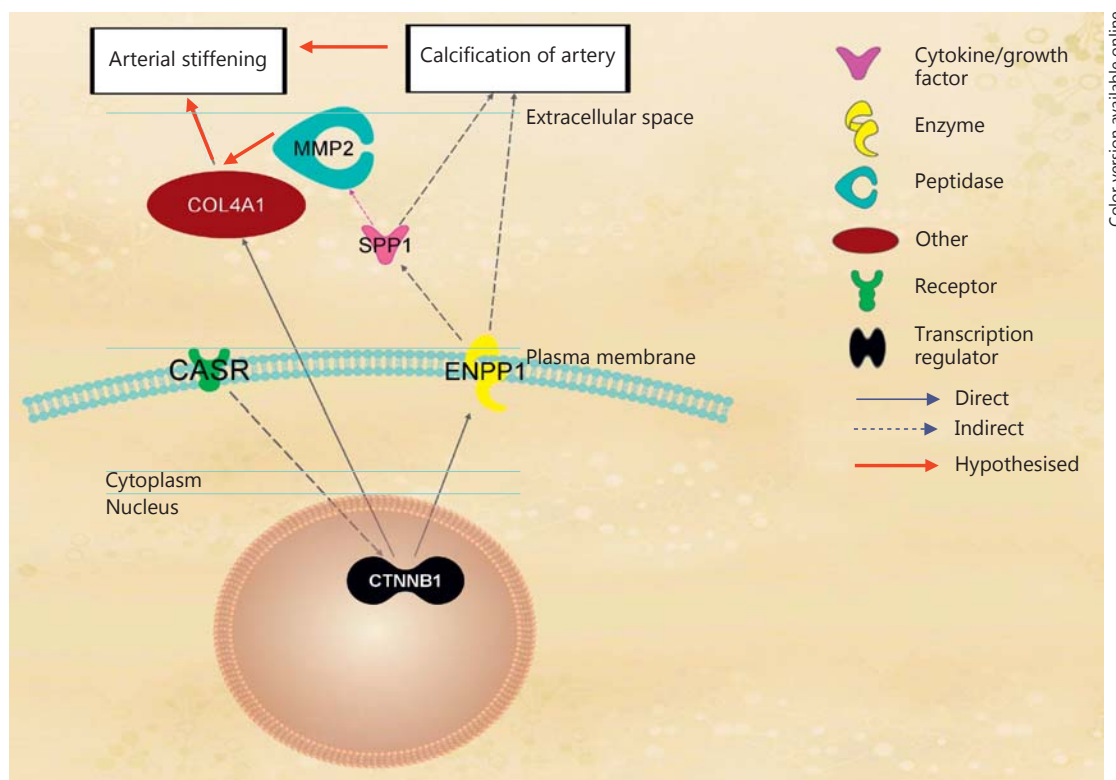
Genetics of Arterial Stiffness

Heritability of arterial stiffness is approximately 40% as estimated from twin and family studies [26–28]. Candidate gene approaches have identified single-nucleotide polymorphisms related to arterial stiffness on genes potentially implicated in the regulation of the extracellular matrix and calcification: MMPs [29, 30], collagen 1 [31], fibrillin 1 [32] and ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*, an inhibitor of calcification) [33] in addition to genes relating to blood pressure regulation [34–36], and inflammation [37, 38]. Genome-wide association studies (GWAS) have only identified a small number of genetic variants reaching genome-wide significance. The collagen type IV alpha 1 (*COL4A1*) gene polymorphism on chromosome 13 (collagen type 4) was significantly associated with higher arterial stiffness [39] in one study, but the association failed to be replicated in a recent GWAS meta-analysis of 11 community-based cohorts. A significant association in the 3'-B-cell lymphoma/leukaemia 11B (3'-BCL11B) gene desert located on chromosome 14 did achieve genome-wide significance, but its potential role in arterial stiffening remains to be established [40].

The limitations of GWAS in identifying causal genes in hypertension, arterial stiffness and other common conditions are increasingly recognised and include limited power to identify common variants with a modest effect size, lack of coverage of rare variants, genetic interactions and gene × environment interactions [41, 42]. In addition, identified single-nucleotide polymorphisms are often located in intergenic or non-coding regions, and it is not clear which gene is the causal gene or the regulatory pathway/network. Thus, it is of interest to examine whether gene expression, an intermediate step between genetic variation and structural and functional variation, may be related more closely to the phenotype.

Blood-based gene expression tests have previously been associated with a wide range of non-haematological disorders including coronary artery disease [43–46] and hypertension [47]. While the exact nature of these associations remains unknown, they may either reflect parallel changes in gene expression across tissues or participation of haematological factors in the disease process. Where similar pathways may operate in parallel in vascular and non-vascular tissues, there may be a direct correlation of gene expression in sample tissues with arterial tissue. MMP9 expression in the skin, for example, has been shown to relate to arterial stiffness [24]. Alternatively, there may be a direct correlation between gene expression in circulating leukocytes that participate in the pathogenesis of arteriosclerosis. In agreement with this, previous microarray data from Huang et al. [48] have identified the differential expression of several genes in circulating cells, including calcium- and bone-related genes, in individuals with and without vascular calcification.

Using the Twins UK cohort, we have investigated the association of gene expression levels with cross-sectional and longitudinal changes in arterial stiffening [49]. Genes were selected if they had previously been associated with arterial stiffening in genome-wide or candidate gene studies. We found that expression levels of *ENPP1*, a transmembrane glycoprotein enzyme that generates inorganic pyrophosphate and is associated with vascular calcification, were independently associated with PWV in a cross-sectional analysis. These findings were successfully replicated in a longitudinal analysis where *ENPP1* was a significant predictor of aPWV progression. We additionally found expression levels of *COL4A1*, which encodes for one component of type IV collagen, to be significantly associated with progression of PWV. Possible biological pathways and gene networks linking *ENPP1* and *COL4A1* to arterial stiffness and calcification were identified using the Ingenuity Knowledge Base (fig. 1) [49].



Color version available online

Fig. 1. Conceptual pathway linking *COL4A1* and *ENPP1* with arterial stiffness using the Ingenuity Pathway Analysis knowledge database. Activation of the calcium-sensing receptor (CASR) acts on the transcriptional regulator catenin (cadherin-associated protein) beta 1 (CTNNB1) which initiates the transcription of *ENPP1* and *COL4A1*. Increased *ENPP1* expression may lead to arterial stiffening through its effect on calcification, or it may trigger osteopontin (SPP1) and influence the extracellular matrix through its effects on MMP2/9 [49].

Conclusion

Despite the prognostic importance of arterial stiffness, very little is known regarding the molecular mechanisms of stiffening. Unique approaches utilising both genetic variants and gene expression data and their associations with particular phenotypes may help to identify novel pathways, likely involving calcification/extracellular matrix degradation, for interventions to prevent or reverse arterial stiffening. In particular, combining this with longitudinal studies may elucidate different mechanisms determining arterial stiffness at different vascular ages that are specific to the human aorta. Due to the unique characteristics of the human aorta with the predominance of extracellular matrix and coexistent atheromatous disease, animal models may be of limited use in identifying such mechanisms but could be important for validating the findings of genetic studies to test the role of genes in pathways influencing both muscular and elastic arteries.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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