HHS Public Access

Author manuscript

Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2024 January 01.

Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2023 January ; 43(1): 15–29. doi:10.1161/ATVBAHA.122.317332.

Molecular Mechanisms of Vascular Health: Insights from Vascular Aging and Calcification

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Disclosures:
Sutton- Advisory: Philips, Abbott; Honoraria: Zoll, Abbott, Shockwave, Cordis.
Malhotra – SRA: Amgen, Bayer; Consultant: Renovocor, Third Pole, Myokardia (now BMS). Nigwekar- Consultant: Epizon Pharma, Laboratoris Sanifit, Inozyme Pharma; Grant support: Hope Pharma, Laboratoris Sanifit, Inozyme Pharma.
Goyal-Consultant: Sensorum Health. The other authors-none.

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Abstract

Cardiovascular disease is the most common cause of death worldwide, especially beyond the age of 65, with the vast majority of morbidity and mortality due to myocardial infarction and stroke. Vascular pathology stems from a combination of genetic risk, environmental factors, and the biologic changes associated with aging. The pathogenesis underlying the development of vascular aging, and vascular calcification with aging in particular, is still not fully understood. Accumulating data suggests that genetic risk, likely compounded by epigenetic modifications, environmental factors, including diabetes mellitus and chronic kidney disease, and the plasticity of vascular smooth muscle cells to acquire an osteogenic phenotype are major determinants of age-associated vascular calcification. Understanding the molecular mechanisms underlying genetic and modifiable risk factors in regulating age-associated vascular pathology may inspire strategies to promote healthy vascular aging. This article summarizes current knowledge of concepts and mechanisms of age-associated vascular disease, with an emphasis on vascular calcification.

Introduction

Cardiovascular disease is the leading cause of death worldwide and increases with age, in large part due to the cumulative effects of risk factors such as hypertension, hyperlipidemia, diabetes, tobacco use, and sedentary behavior. However, with advancing age, even individuals without traditional risk factors gradually develop vascular pathology including arterial fibrosis, stiffness, and calcification, increasing the risk of serious cardiovascular events. The importance of understanding the interplay between vascular biology and aging, independent of traditional risk factors, is of utmost importance.

Isolating vascular aging as an independent biological variable is challenging for several reasons. First, vascular aging is often accompanied by one or more cardiovascular disease risk factors. Second, there is likely a synergistic effect of both the duration and number of cardiovascular risk factors that make it challenging to fully adjust for such variables. Although studies of aging exist, they are prone to survival bias in that only individuals who survived until older age can be studied if not enrolled earlier in life. Studies such as the Progression and Early detection of Subclinical Atherosclerosis (PESA)[1] and the Asklepios Study[2] were designed to circumnavigate these challenges in order to study the interactions of age and inflammation with cardiovascular hemodynamics and development of atherosclerosis. Studies of human longevity are also challenging due to the costly and

time-consuming nature of studying an individual human over a lifespan, and long-lived individuals may have different genetic longevity variants and protein signatures.[3–5]

Improving our understanding of vascular aging and its role in cardiovascular disease progression, morbidity, and mortality is essential. The following review discusses what is currently known regarding the biology of vascular aging, clinical manifestations of age-associated vascular disease with a focus on calcification, the impact of genetic risk on vascular aging, and the environmental and molecular factors that may influence vascular aging and promote longevity (Figure 1).

Clinical manifestations of age-associated cardiovascular calcification

Arterial stiffening and calcification are characteristics of vascular aging, serve as important predictors of cardiovascular morbidity and mortality, and are exacerbated by cardiovascular disease risk factors and metabolic syndromes.[6–9] Arterial calcification is closely associated with atherosclerotic plaque evolution, and the area of coronary artery calcification (CAC) quantified on noncontrast cardiac computed tomography (CT) has a direct relationship with histopathologic coronary plaque area.[10] Autopsy studies have consistently shown a strong association between calcification of the coronary arteries and atherosclerosis.[11] Calcification is often categorized as intimal, typically associated with atherosclerotic plaque, or medial, often a more diffuse arteriosclerotic process marked by vascular stiffening and associated with conditions such as chronic kidney disease and diabetes.

CAC volume and vulnerable plaques with a lipid-rich core, thin cap, or spotty or micro calcifications are associated with the risk of future atherosclerotic cardiovascular disease (ASCVD) events.[12–15] However, within a given coronary artery, there is a wide variation between the degree of plaque calcification and severity of luminal stenosis on invasive coronary angiography due in part to individual variations in coronary artery remodeling.[16]

Noninvasive methods to evaluate coronary heart disease risk, such as exercise stress testing, typically only identify patients with advanced, obstructive atherosclerotic disease. This is of relevance as myocardial infarctions may occur when a non-obstructive atherosclerotic plaque ruptures.[17] Thus, there has been great interest in characterizing atherosclerosis in its pre-flow limiting phase, so that intensified preventive strategies can be instituted. Measurement of CAC volume on CT imaging often improves the accuracy of cardiovascular risk assessment in intermediate risk adults and may help to determine which patients may benefit from initiation of or intensification of risk factor modification strategies such as lipid lowering, aspirin, or antihypertensive therapies.[18–20]

Prospective studies of CAC and incident cardiovascular disease risk from the Multi-Ethnic Study of Atherosclerosis and other observational cohorts show a very strong correlation between increased CAC and the risk of future cardiovascular disease events.[21] CAC scores greater than the 75th percentile for age, sex, and ethnicity or more than 100 indicate an elevated 10-year ASCVD risk and should lead to more improved lifestyle habits and strong consideration of statin therapy in intermediate-risk adults.[20] CAC percentiles based on age,

sex, and ethnicity are better predictors of lifetime risk, whereas CAC scores provide the best estimate of absolute risk in the next decade.[19]

Traditionally, CAC scores have been used to determine the need for initiating statin therapy. However, evidence suggests that while high-intensity statin therapy lowers cardiovascular event risk, it paradoxically may modestly increase CAC and potentially stabilize existing atherosclerotic plaques.[22, 23] While CAC volume has been associated with increased CVD risk, CAC density is inversely associated with CVD risk.[24] Statin-induced atherosclerotic plaque calcification has been attributed to increased plaque alkaline phosphatase activity[25] and disinhibition of the macrophage Rac (Ras-related C3 botulinum toxin substrate)–IL-1β (interleukin-1 beta) signaling axis.[26]

Emerging data has indicated that CAC scores can help in prioritizing the need for more intensive medications in higher risk individuals who have above average amounts of CAC for their age, gender, and ethnicity. These medications may include glucagon-like peptide 1 receptor agonists or sodium-glucose cotransporter 2 inhibitors in adults with diabetes or proprotein convertase subtilisin/kexin type 9 inhibitors in adults with suboptimal LDL-cholesterol lowering on maximally tolerated statin therapy. Cainzos-Achirica et al. have made a compelling case for measuring CAC to more accurately allocate medications and to use the CAC score to enrich study populations of primary prevention randomized controlled trials with participants at higher absolute risk of cardiovascular events.[27] Conversely, a CAC score of zero is a powerful negative predictor of future cardiovascular events in older patients, such that it is reasonable to consider withholding statin therapy in the absence of other risk factors.[28] Additional study of individuals who age without developing vascular calcification would be of interest.

Measurement of CAC is well-validated for risk stratification in middle to older-age adults. Incorporating CAC scores improves risk stratification for incident sudden cardiac death beyond traditional ASCVD risk factors in individuals with low-to-intermediate risk.[29] Until recently, there was limited data in adults with an age less than 40. Javaid et al recently studied the prognostic importance of CAC in nearly 20,000 asymptomatic adults aged 30–45 years without known ASCVD. They found that any CAC in this age range placed females at >90th percentile (high lifetime risk). The presence of any CAC placed White males at the 90th percentile by age 34 and Black males by age 37.[30]

Extracoronary cardiovascular calcification, including aortic[31], peripheral vascular[32], and valvular, also predicts cardiovascular risk. Calcific aortic valve stenosis is the most common valvular heart disease in the Western world, and progressive fibrocalcific changes in the valve leaflets may lead to partial aortic outflow tract obstruction.[33] However, aortic valve sclerosis (calcification and thickening of the aortic valve), even in the absence of hemodynamically significant obstruction of left ventricular outflow track, is independently associated with an increased risk of ASCVD events.[34–36] Research is ongoing to determine if aortic valve calcium scoring using cardiac CT may be useful for risk stratification and to identify those at increased risk of developing significant aortic valve stenosis.[37]

A striking demonstration of accelerated vascular calcification in adults is calciphylaxis — a rare but devastating condition that is predominantly seen among patients with end-stage kidney disease (ESKD) who have typically been dialysis-dependent for over 2–3 years.[38] The primary clinical manifestation of calciphylaxis is painful skin ulcers caused by cutaneous ischemia. These patients, almost universally, have diffuse extra-skeletal calcification. In addition to metabolic abnormalities of calcium and phosphate metabolism originating from the ESKD, over 40% of patients with calciphylaxis have diabetes mellitus, and as many as 30% have been exposed to warfarin prior to the development of calciphylaxis.[39, 40]

Warfarin, a vitamin K antagonist, may impair the gamma-carboxylation of a potent calcification inhibitor known as Matrix Gla Protein. This may further accelerate the process of vascular calcification among patients who are predisposed to it from their underlying comorbidities. At present, there is no approved treatment for calciphylaxis, although anecdotal reports of successful resolution of calciphylaxis lesions with treatments such as vitamin K supplementation and kidney transplantation provide potential insights into strategies to reduce calcification and eventually improve clinical outcomes.[41, 42]

The Biology of Vascular Aging

Vascular aging is a biological variable, conceptually distinct from chronological aging, whereby sequential and progressive changes in a cell or whole organism leads to an increased risk of dysfunction, disease, and death.[43–45] Hallmarks of biological aging include cellular dysfunction and vulnerability to cell death, and many of these hallmarks also contribute to vascular dysfunction and calcification.[46–48]

Telomeres shorten with every cellular replication cycle leading to reduced proliferative capacity of cells.[49, 50] The single strand ends of telomeres are protected to prevent the chromosomal ends from appearing as double-stranded DNA breaks, which otherwise trigger DNA damage responses.[51] Breakdown of these telomere caps can lead to age-related vascular dysfunction, including increased cellular senescence, oxidative stress, and inflammation.[52, 53]

Senescent cells are not inert and may extrude chemical mediators that further propagate an inflammatory phenotype to neighboring cells.[54] Vascular smooth muscle cells (SMCs) exhibit markers of senescence and calcify in response to uptake of endothelial-derived exosomes.[55, 56] In addition, microparticles from older individuals' senescent endothelial cells induce vascular SMC calcification[57], and human vascular function *in vivo* inversely correlates with the presence of senescence markers in endothelial cells.[58] In mice, senolytic drugs, which induce death of senescent cells, restore vascular function in aged mice.[59]

Accumulation of DNA damage, whether due to exogenous factors (such as ionizing radiation), replication errors, or impaired repair, contributes to cellular dysfunction in part due to the generation of reactive oxygen and nitrogen species and may also lead to cardiovascular calcification.[60, 61] Increased oxidative stress is also a major factor promoting

loss of vascular SMC contractility and increased osteogenic differentiation and calcification, characteristics of vascular aging.[8, 62]

Inflammageing, or the age-related increase in pro-inflammatory markers in the blood and tissues[63], is likely both a biomarker of biological aging as well as cause of age-related cardiovascular pathology. Inflammageing may occur due to increased production of inflammatory mediators, such as from senescent cells, or due to impaired inflammatory resolution, [64, 65] as was recently reviewed in detail elsewhere as a target in atherosclerosis.[66] That endothelial cells stimulated with tumor necrosis factor (TNF) α released microparticles containing bone morphogenetic protein 2 (BMP2), which in turn were phagocytosed by vascular SMCs and enhanced osteogenesis supports the role for inflammageing in promoting age-associated arterial calcification.[67] BMP2 is also proinflammatory and induces endothelial activation, suggesting these local inflammatory perturbations could auto-feedback and escalate age-associated calcification.[68] C-reactive protein has been implicated in promoting age-associated vascular SMC osteogenic transdifferentiation via the Fc fragment of IgG receptor IIa and the p38 mitogen-activated protein kinase pathway.[69]

Epigenetic marks on histones can dictate global gene expression patterns.[44] These epigenetic modifications correlate with biological age and more accurately predict lifespan than chronological age.[70–72] Epigenetic programming occurs during development and informs cellular phenotypes. Recent studies show that these developmental programs, or the loss of them, help to drive vascular cell dysfunction, including calcification and the loss of the contractile phenotype of vascular SMCs.[73–79]

Genetic risk and cardiovascular disease

While many traditional risk factors become more clinically relevant in middle age, one's genetics are present from birth. Polygenic scores (PGS, also known as genetic risk scores or polygenic risk scores) build on results from genome-wide association studies (GWASs) to allow estimation of one's cumulative genetic risk for a given endpoint.[80] PGS enable identification of patients at high risk for common, complex diseases such as cardiovascular disease (CVD), much like carriers of a Mendelian mutation. PGS also allow for improved reclassification of patients with cardiovascular disease and early onset myocardial infarction and early onset coronary heart disease.[81, 82]

For example, in the United Kingdom (UK) Biobank, participants with a PGS for coronary artery disease (CAD) in the top 5% of the cohort's PGS distribution have a greater than three-fold risk for CAD compared to the rest of the population.[83] This is similar to the CAD risk conferred by mutations in genes causing familial hypercholesterolemia (FH), yet 20 times as many individuals fall into this polygenic high-risk category as carry an FH mutation.[83, 84] Moreover, PGS have stronger risk stratification power in younger populations than older ones.[85] PGS have been found to predict incident CAC[86–88] and can be useful in predicting the optimal age for CAC screening.[89] Favorable lifestyles mitigate the susceptibility to CAC even if genetic risk is elevated.[90]

The Finnish GeneRISK study, a web-based communication tool (KardioKompassi), aims to assess the clinical utility of PGS by providing personalized 10-year CVD risk to a prospective cohort of 7,342 individuals.[91] After only 1.5 years, 71% of the participants were re-assessed, and genetic risk was found to motivate positive health behavior. In another study with a prospective observational cohort of 3,800 individuals, knowledge of having a high CAD PGS was associated with earlier initiation (52 years versus 65 years) and use of a lipid lowering therapy (42.4% versus 28.5%).[92]

The potential clinical utility of PGS is often quantified with the net reclassification index (NRI), the percent of patients who would be reclassified into a different risk category upon addition of the PGS to conventional CVD risk prediction models (Table 1). An important limitation is that PGSs have mostly been derived from populations of European genetic ancestry and are generally not available at large commercial labs for clinical use. However, the AHA recently issued a scientific statement with guidance for their use.[93]

Genetics underpinnings of cardiovascular calcification

In contrast to the use of polygenic scores based on common genetic variation to predict risk, certain gene defects are responsible for rare, Mendelian disorders of premature vascular pathology (Table 2). For example, rare diseases resulting in premature vascular calcification stem from abnormalities in the extracellular ATP metabolic pathway.[94] ATP is released from cells under conditions of stress or death and can act in a paracrine manner through its cognate receptors or be metabolized to its constituent parts by a series of ectonucleotidases.[95] Several ATP metabolites regulate vascular calcification.[96] Calcium and inorganic phosphate are the building blocks of calcification, but an endogenous inhibitor of mineral nucleation is pyrophosphate, which is the product of the breakdown of ATP by ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1).[96] In a murine model of Hutchinson-Gilford progeria syndrome with accelerated vascular aging, pyrophosphate treatment inhibited arterial calcification.[97]

In generalized arterial calcification of infancy (GACI), mutations in *ENPP1* lead to a deadly disease of extensive medial arterial calcification in large arteries, which presents in infancy, and it is the lack of local production of extracellular pyrophosphate that drives this devastating phenotype.[98] The other product of ENPP1 is AMP, which is further metabolized to inorganic phosphate and adenosine by the ectonucleotidase CD73.[96] Inactivating mutations in the gene encoding for CD73 lead to medial arterial calcifications in adulthood that phenocopy the pathologies seen in patients with diabetes and ESRD.[99, 100] In this disease (termed Arterial Calcification due to Deficiency of CD73, ACDC), mechanistic studies have uncovered that the lack of adenosine signaling via the A2b adenosine receptor (A2bAR) drives the osteogenic transition of these SMCs.[101] Calcified femoropopliteal arteries from patients with non-genetic forms of medial arterial calcification exhibit signatures of this rare disease, suggesting the mechanism that operates in this pathology.[102, 103] Pseudoxanthoma elasticum is characterized by microvascular arterial calcification in childhood and is caused by mutations in the ATP binding cassette subfamily C member 6 (*ABCC6*); however, the factor being transported is debated.[104, 105] Singleton-Merton syndrome is caused by a missense mutation in interferon-induced helicase C domain

1 (*IFIH1*)[106], and Hutchinson-Gilford progeria syndrome is caused by a splice defect in lamin A (*LMNA*)[107, 108]; both disorders manifest with premature and extensive aortic and valvular calcification. The relationships of these genes to adult, age-related calcific disorders, however, remain uncertain.

GWAS have identified several seemingly unrelated genes implicated in CAC. The first GWAS for CAC identified two loci at 6p24 and 9p21.[109] The former is nearest to the phosphatase and actin regulator 1 (*PHACTR1*) gene, which plays a role in endothelial cell survival. Targeted deletion at this locus increases the expression of nearby gene endothelin 1 (*EDN1*), a potent vasoconstrictor known to promote atherosclerosis.[110] In an exome-wide association meta-analysis, protein-coding variants in apolipoprotein B (*APOB*) and apolipoprotein E (*APOE*) were also associated with CAC among patients without overt coronary heart disease, thus linking CAC, perhaps unsurprisingly, with lipid metabolism.[111]

More recently identified is the association between clonal hematopoiesis of indeterminate potential (CHIP) with CAC. CHIP carriers had 3.3 times higher CAC than non-carriers.[112] Insufficiency of tet methylcytosine dioxygenase 2 (*TET2*), a gene commonly mutated in CHIP, exaggerated atherosclerosis in mice,[112] which has been attributed to TET-2 deficient macrophages exhibiting an increase in Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing (NLRP) 3 inflammasome-mediated IL-1β secretion.[113] Finally, matrix gla protein (*MGP*) is considered to be one of the strongest endogenous inhibitors of vascular calcification, and putatively disruptive polymorphisms in *MGP* correlate strongly with subclinical CAC.[114] These findings highlight the diversity of known cell types (e.g., endothelial, SMC, hematopoietic) and signaling pathways involved in CAC development.

GWAS have also identified an abdominal aortic calcification (AAC) risk locus on chromosome 7 in the intergenic region between histone deacetylase 9 (*HDAC9*) and twist family bHLH transcription factor 1 (*TWIST1*).[115] Knockdown of *HDAC9* reduced calcification, contractility, and *RUNX2* expression of aortic SMCs induced to undergo osteogenic transformation.[115] Conversely, overexpression of *HDAC9* amplified *RUNX2* expression and increased calcification.[115] Analogously, *Hdac9*-null mice were protected from calcification and mortality compared to haploinsufficient and wild-type mice in a model of medial vascular calcification (MGP deficiency).[115] HDAC9 also promotes endothelial-to-mesenchymal transition and unfavorable atherosclerotic plaque composition.[116] In rat SMC calcification assays, *TWIST1* knockdown increased calcification, and overexpression decreased calcification.[117]

Aortic valvular (AV) calcification has been associated with the lipoprotein a (*LPA*) locus on chromosome 6 at genome-wide significance, and minor alleles in this locus confer as much as a two-fold increase in the odds of AV calcification and an increased risk for aortic stenosis.[118] Mendelian randomization analysis demonstrated a causal role for genetically determined Lp(a) levels in the development of AV calcification. In the same study, two variants near the proinflammatory gene, interleukin 36 gamma (*IL1F9*), were associated with mitral annular calcification at a genome-wide level of significance.

In a separate GWAS of calcific aortic stenosis, the association of AV disease with *LPA* was redemonstrated, and two additional risk loci near palmdelphin (*PALMD*) and testis expressed 41 (*TEX41*) were identified.[119] These loci are also associated with bicuspid aortic valve and congenital septal defects, potentially implicating cardiac developmental pathways in calcific aortic valve disease.

Environmental and modifiable factors that accelerate calcification

There are several clinical conditions where vascular calcification is markedly accelerated, including metabolic abnormalities such as diabetes mellitus and chronic kidney disease (CKD).[120] In these conditions, calcification and mortality do not track with chronological age, as observed in the general population. Instead, patients show accelerated cardiovascular mortality, such that young adults with ESKD requiring chronic dialysis have a risk of cardiovascular mortality similar to octogenarians.[121, 122] Emerging evidence suggests that accelerated vascular aging may contribute to the development of vascular calcification and increased mortality in these patient groups.[123–126]

Using human tissue samples, several studies have documented the presence of DNA damage (gamma H2 histone family member X (gH2AX) and 8-oxo-2'-deoxyguanosine (8-oxo-Dg)) and senescence markers, such as the cell cycle regulators p21 and p16, in calcified arteries of patients with CKD and diabetes.[126] Compelling evidence comes from studies of the arteries of children with CKD on dialysis, which showed elevated oxidative DNA damage and senescence in medial vascular SMCs.[124] Numerous *ex vivo* and *in vitro* studies of SMCs have shown that a number of environmental factors contribute to oxidative stress and DNA damage in these disease states. These include elevated glucose and dysregulated mineral metabolism, with elevated phosphorus thought to be a driver of premature aging, as well as various uremic toxins and mitochondrial damage.[120, 127–131]

DNA damage signaling and cellular senescence drive a number of processes that lead to vascular SMC calcification, including osteogenic differentiation and cell death. Two key DNA damage signaling pathways upstream of vascular SMC osteogenic differentiation are ataxia telangiectasia mutated (ATM) and poly-ADP ribose polymerase (PARP), and blocking either of these pathways can alleviate mineralization both *in vitro* and *in vivo* in models that mimic the dysregulated mineral metabolism observed in CKD.[77, 132]

These signaling pathways exert their effects on osteogenic differentiation of SMCs in a number of ways, and many of them converge to the Runt-related transcription factor-2 (Runx2), the major transcription factor driving osteogenic phenotype transition.[133–139] Oxidative stress-induced Runx2 upregulation plays an essential role in vascular SMC calcification, while SMC-specific Runx2 deletion protects from the development of vascular calcification in atherosclerosis and CKD.[137–139] Multifaceted posttranslational modifications (PTMs) of Runx2, including phosphorylation, acetylation, ubiquitination, and O-GlcNAcylation, modulate Runx2 protein stability, cellular localization, and its interaction with other transcription factors and target genes that are critical for its osteogenic transcriptional activity.[131]

Recent studies have linked protein *O*-GlcNAcylation with Runx2 upregulation and SMC calcification in diabetes.[140–143] In addition, Runx2 is a component of the DNA damage response (DDR). In response to elevated calcium and phosphate, Runx2 becomes PARylated, leading to the selective activation of its downstream osteogenic targets.[139]

Another mechanism whereby metabolic changes can influence vascular calcification include epigenetic modifications to DNA or histones. In many instances, these pathways also intersect with DNA damage signaling and senescence. Sirtuins, a family of histone deacetylases, play a role in regulating the DDR and senescence in vascular SMCs and hence osteogenic differentiation and calcification. Sirt1 is reduced in the vasculature of patients with diabetes mellitus, and its activation leads to efficient DNA repair and normalizes vascular SMC phenotype.[144] Similarly, sirtuin 6 is reduced in the vessels of patients with CKD. Studies *in vitro* show that sirtuin 6acts to deactylate Runx2, leading to its nuclear export and degradation, thus preventing osteogenic differentiation.[145]

An additional feature of the persistent DNA damage and cellular senescence is activation of the senescence-associated secretory phenotype (SASP) and activation of innate immune signals by vascular SMC, including interleukin-6 (IL-6) and BMP2 as relevant to arteriosclerotic calcification.[124, 146] The connections to osteogenic BMP-Msx-Wnt signaling are presented below.

Activation of the BMP2/Msx/Wnt signaling pathways increases SMC calcification *in vitro* and *in vivo*.[147–150] Wnts are secreted, fatty acylated glycoproteins that signal through G-protein coupled receptors of the Frizzled (Fzd) family or via GPR124.[151–153] Signaling is modulated by co-receptors including low density lipoprotein related proteins LRP5 and LRP6 and several transmembrane receptor tyrosine kinase-like proteins.[154, 155] Wnts are fatty acylated and very hydrophobic -- associated with membranes, extracellular vesicles, and lipoprotein particles.[156] The vertebrate genome encodes 19 Wnt ligands and 10 Fzd receptors with downstream signaling relays characterized as either canonical (requiring β–catenin) or noncanonical (calcium/NFAT, Jun kinase, planar cell polarity).[157, 158] Alternative Wnt signaling through transcriptional coactivators YAP and TAZ resulting in osteogenic differentiation has also been described.[157, 159]

The first robust clue that Wnt signaling might be involved in vascular aging phenotypes came from the work of Mani and colleagues. They identified that a missense mutation in LRP6 (R611C) resulted in precocious osteoporosis and coronary artery disease in an Iranian kindred.[160] This hypomorphic allele causes dysregulated signaling bias between canonical and noncanonical Wnt relays in vascular SMCs as necessary to stabilize phenotype.[161] Consistent with this, others demonstrated that loss of SMC LRP6 increases noncanonical Wnt signals that activated SMC osteochondrogenic gene expression, and promoted vascular calcification, and arteriosclerotic stiffening in mice susceptible to atherosclerosis.[162, 163]

Interestingly, expression of non-canonical Wnt ligands is increased in calcific aortic valve disease and with cardiac fibrosis.[164–167] Age-related mitochondrial dysfunction and ER stress bias towards non-canonical Wnt signaling as well.[168, 169] These data suggest that development of LRP6 mimetics, or other strategies that restrain specific aspects of

non-canonical Wnt signaling, may help prevent or mitigate progression of cardiovascular fibrocalcific disease processes with aging.[170]

A common theme in all of the age-associated cardiovascular "Wnt-opathies" is activation of innate immunity, a key feature of inflammation, and some features of cell senescence (vide infra).[170–172] Pathogen- and senescence –associated programs elevate the expression of Wnt genes either directly or indirectly via TNF, IL1-β, or receptor for AGE (RAGE) ligands including oxylipids.[170] Importantly, senescent cells that accrue in aging tissues actively contribute to the inflammatory phenotypes.[173–176] A gene set containing numerous direct BMP/Wnt modulators (e.g., Bmp2, Wnt2, Wnt16, Dkk1, etc.) and targets of noncanonical Wnt action was shown to demarcate senescent cells in multiple tissues.[174] However, the conflicting literature on the role of Wnt agonists in promoting or preventing cell senescence suggests that canonical-noncanonical signaling bias and duration of signal exposure deserves additional investigation.[177, 178]

Therapeutic considerations

There are no currently approved therapies specifically targeting prevention or promoting regression of vascular or valvular calcification for the general population at any age. Metformin is associated with reduced coronary calcification in animal and human studies[179, 180]; possible mechanisms included reduced osteoprotegerin[181] production and decreased oxidative stress.[182, 183] Senolytic combinations of desatinib and quercetin were shown to reduce vascular calcification in animal models, attributed to reduced oxidative stress.[59, 184] Also in animal models, PARP inhibition with specific inhibitors or minocycline[185] reduced vascular calcification, as has pyrophosphate administration.[97] No therapy is available to treat valvular calcification except surgical and transcatheter interventions. Ample opportunities remain to apply known mechanisms of aging and calcification to clinical cardiovascular care.

Conclusions and perspective

In this review, we provide a high-level summary of the current knowledge of vascular aging, emphasizing the clinical manifestations, genetic diatheses, environmental risk factors, and emerging molecular mechanisms of cardiovascular calcification. Age-associated pathways critical to the development of vascular calcification are highlighted, including DNA damage repair and senescence signals, innate immunity, activating BMP2-Msx-Wnt pathways, and the Runx2 transcription factor. Arterial SMC phenotypic switching contributes significantly to vascular aging -- manifested as abnormal conduit vessel physiology and mechanical integrity due to arteriosclerotic calcification, fibrosis, matrix remodeling, and impaired contractile functions. Even though key discoveries have been made, much remains to be learned concerning the regulation of arteriosclerotic calcification and its relationship to the vascular SMC phenotype with aging.

For instance, both Runx2 and Msx2 directly reduce the expression of SMC contractile markers and promote the osteogenic phenotype, and Runx2 and Msx2 proteins interact to form a transcriptional complex.[137, 186–188] On the other hand, *O*-GlcNAcylation via

Ogt has also emerged as an important regulator for the master SMC transcription factors, including myocardin, serum response factor (SRF), and KLF4.[141, 142] However, the reasons why activities of Runx2, Msx2, and Ogt in the SMC lineage – absolutely required for osteogenic differentiation and matrix deposition – can become dissociated from arterial matrix mineralization in some settings remains to be determined.[189] Incorporation of multi-omics, systems biology, single cell sequencing, and computational studies are novel approaches for the identification of new pathways, candidate drug targets, and repurposing of old drugs to treat vascular and valvular calcification.[187, 190, 191]

Endothelial cell dysfunction, with or without the endothelial-mesenchymal transition, also impacts the SMC phenotype via juxtacrine/paracrine signals that controls osteogenic potential, and may be one such determinant.[192, 193] Likewise, key components of the vascular extracellular matrix such as nitogen-2 also control SMC plasticity, and matricrine cues in cardiovascular aging are poorly characterized.[194] Of note, in utero or childhood environmental exposures impair endothelial functions decades later in adulthood.[195, 196]

Therefore, a better understanding of the vascular epigenetic landscape that regulates vascular SMC phenotypic plasticity during health span and lifespan will be needed to mitigate age-associated vascular dysfunction. Finally, it has become abundantly apparent that duration of cardiometabolic insult exposure[197] and sex significantly impact age-dependent responses, and women experience a much steeper increase in cardiovascular disease severity with age, later in life.[198] Other age-related vasculopathies exhibit sex dimorphism as well, including aneurysmal remodeling, that is determined by sex chromosome content.[199] Thus, additional studies are warranted to uncover in even greater detail the mechanisms controlling vascular SMC phenotypic stability vs. plasticity, phenotypic switching with osteogenic re-programming, and vascular mineralization as a function of environment, cardiometabolic insult, matricrine cues, (epi)genetics, age, and sex. Insights from these studies will afford novel targets and therapeutic strategies necessary to halt, or potentially reverse, processes of age-associated vascular calcification.

Acknowledgments:

Funding sources:

R.M. was supported by the National Heart, Lung, and Blood Institute (R01HL142809), the American Heart Association (18TPA34230025), and the Wild Family Foundation.

E.A. is supported by research grants from the National Institutes of Health R01HL136431, R01HL141917 and R01HL147095.

C.S.H. holds grants from the National Institutes of Health (HL142932) and the American Heart Association (20IPA35260111).

B.N.W. is funded by the EU H2020 Research and Innovation programme INTERVENE, grant nr. 101016775.

N.R.S. National Institute on Aging 1K76AG064426-01A1

P.G. is supported by American Heart Association grant 20CDA35310455, National Institute on Aging grant K76AG064428, and Loan Repayment Program award L30AG060521

S.U.N. is supported by the National Institute of Biomedical Imaging and Bioengineering (1R01EB031813-01A1) and by the National Institute of Diabetes and Digestive and Kidney Diseases (1U01DK123818-01).

C.S. is supported by grants from the British Heart Foundation (RG/17/2/32808).

D.A.T. is supported by grants from the National Institutes of Health (HL069229-21), American Diabetes Association (1-18-IBS-224), and the Pak Center for Mineral Metabolism Research.

Y.C. is supported by grants from the National Institutes of Health (HL136165, HL146103 and HL158097) as well as United States Department of Veterans Affairs Basic Sciences R&D Service (BX005800 and BX004426).

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Highlights

- The pathogenesis underlying the development of vascular aging, and vascular calcification with aging in particular, is still not fully understood.
- Genetic risk, likely compounded by epigenetic modifications, environmental factors, including diabetes mellitus and chronic kidney disease, and the plasticity of vascular smooth muscle cells to acquire an osteogenic phenotype are major determinants of age-associated vascular calcification.
- Arterial SMC phenotypic switching contributes significantly to vascular aging -- manifested as abnormal conduit vessel physiology and mechanical integrity due to arteriosclerotic calcification, fibrosis, matrix remodeling, and impaired contractile functions.

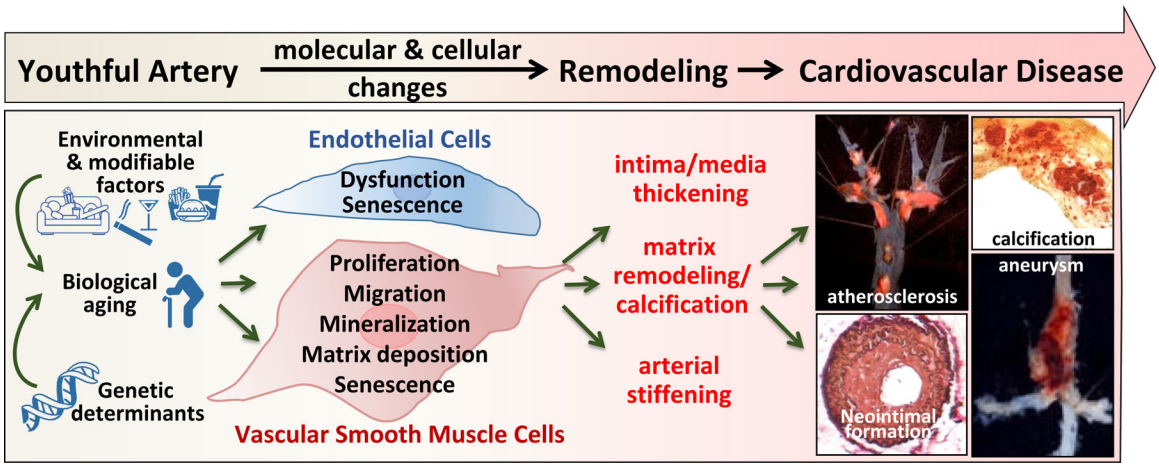

Figure 1.

Vascular aging and aging-accelerated vascular disease. Genetic and environmental factors induced endothelial cell dysfunction and vascular smooth muscle cell phenotypic modulation that leads to vascular remodeling and development of cardiovascular disease.

Table 1.

Net Reclassification Index of Polygenic Risk Scores

Publication	Score description	Outcome	Group (Ancestry)	# of samples	Continuous NRI (%)	Categorical NRI (%)
Elliot et al., JAMA 2020[a][200]	Lassosum	Incident CAD in UKBiobank	Events (EUR)	6272	15.4 (13.0, 17.9)	4.4 (3.5, 5.3)
			Non-events (EUR)	346,388	15.8 (15.5, 16.1)	−0.4 (−0.5, −0.4)
			All (EUR)	352,600	31.2 (28.7, 33.7)	4.0 (2.1, 4.9)
			All (EUR)	4,168		1.8 (−0.2, 3.6)
			All (EUR)	2,101		0.1 (−3.8, 7.6)
Mars et al., Nature Medicine 2020[82]	LDpred from external GWAS	Incident CHD in FINRISK	Events (FIN)	1,209		0.9 (−0.02, 2.0)
			Non-events (FIN)	18,956		0.2 (−0.1, 0.5)
			All (FIN)	20,165		1.1 (−0.1, 2.2)
Hindy et al., ATVB, 2020[201]	LDpred from Khera et al	Incident CAD in Malmö Diet and Cancer Study	Events (EUR)	815		17.3 (8.8, 19.9)
			Non-events (EUR)	4,870		−0.9 (−1.8, −0.2)
			All (EUR)			16.5 (7.6, 18.2)
	LDpred from Khera et al	Incident CAD in UKBiobank	Events (EUR)	7,708		9.1 (7.7, 10.5)
			Non-events (EUR)	317,295		−0.6 (−0.7, −0.6)
			All (EUR)	325,003		8.5 (7.1, 9.8)
Riveros-McKay, Circ Gen & Prec Med, 2021[a][202]	Novel PRS	Incident CAD in UKBiobank	Events	4,122		5.97 (4.83–7.12)
			Non-events	24,434		−0.09 (−0.26, 0.08)
			All	186,451		5.88 (4.73,7.04)
Sun et al., PLOS Med, 2021[b][203]	metaPRS	Incident CVD in UKBiobank	Events (EUR)	5680	10.2 (7.2, 13.2)	0.3 (−0.7,1.2)
			Non-events (EUR)	300,974	12.6 (12.2,13.0)	2.2 (1.8, 2.6)
Weale et al., American Journal of Cardiology, 2021[204]	LDpred of custom GWAS summary statistics	Incident CVD combined across UKBiobank, MESA, ARIC	Events (EUR)	2096		2.7 (1.17–4.22)
			Events (AFR)	309		2.24 (0.39–4.08)
Lu et al., European Heart Journal, 2022[205]	Custom PRS of CAD and CAD-related traits in EAS and EUR	Incident CAD in China-PAR	Events (EAS)	840	15.7 (7.7, 22.2)	3.2 (0.9–5.8)
			Non-events (EAS)	32,859	10.1 (9.1, 11.1)	0.3 (0.1–0.5)
			All (EAS)	33,699	25.8 (18.5, 32.5)	3.5 (1.2–6.0)
Stienfeldt, et al. Lancet Digital Health, 2022 [c][206]	6 PGS from PGS Catalog	Incident MACE in UKBiobank	Non-events (majority EUR)	371,909		0.05 (0.03, 0.12)
			Events (majority EUR)	23,790		1.12 (0.62,1.54)

Publication	Score description	Outcome	Group (Ancestry)	# of samples	Continuous NRI (%)	Categorical NRI (%)
			All (majority EUR)	394,713		1.16 (0.66, 1.59)

Net reclassification index (NRI) for ASCVD-Pooled Cohorts Equation (PCE) versus Pooled Cohorts Equation with polygenic score in several cohorts with various polygenic scores and primary outcomes used. Categorical NRI uses the 7.5% 10-year risk of ASCVD threshold unless otherwise noted.

[a] NRI multiplied by 100 as pseudo percentage with range −200 to 200

[b] Comparison made between conventional risk factors alone and with polygenic score. Categorical NRI using <5%, 5–7.5%, and ≥7.5% 10-year risk thresholds according to 2019 ACC/AHA guideline

[c] Comparison made between a neural network CVD risk predictor with and without additional of polygenic score predictors. Categorical NRI using 10% risk thresholds.

Abbreviations: AFR African genetic ancestry, CAD Coronary Artery Disease, CHD Coronary Heart Disease, CVD Cardiovascular disease, EAS East Asian genetic ancestry, EUR European genetic ancestry, MACE major adverse cardiac event

Table 2.

Genetic Determinants of Vascular and Valvular Calcification

Genomewide Significant Loci			
Calcific Disorder	**Gene/Locus (Lead Single Nucleotide Polymorphism)**	**Gene/Locus description**	**Study**
Coronary artery calcification	*PHACTR1/EDN1* (rs9349379; chr 6)	phosphatase and actin regulator 1; endothelial cell survival; upregulates endothelin 1 (*EDN1*), a vasoconstrictor	O'Donnell *et al.*, 2011[109]; Gupta *et al.*, 2017[110]
	9p21 (rs1333049; chr 9)	CDKN2A/CDKN2B	O'Donnell *et al.*, 2011[109]
	APOB (rs5742904; chr 2)	apolipoprotein B	Natarajan *et al.*, 2016[111]
	APOE (rs7412; chr 19)	apolipoprotein E	Natarajan *et al.*, 2016[111]
Abdominal aortic calcification	*HDAC9/TWIST1* (rs57301765; chr 7)	histone deacetylase 9; modulator of osteogenic phenotype; promotes endothelial-to-mesenchymal transition twist family bHLH transcription factor 1	Malhotra *et al.*, 2019[115]; Lecce *et al.*, 2021[116]; Nurnberg *et al.*, 2020[117]
Aortic valvular calcification	*LPA* (rs10455872; chr 6)	lipoprotein a; causal role for Lp(a) in AV calcification	Thanassoulis *et al.*, 2013[118]; Helgadottir *et al.*, 2018[119]
	PALMD (rs7543130; chr 1)	palmdelphin; also associated with congenital heart disease	Helgadottir *et al.*, 2018[119]
	TEX41 (rs1830321; chr 2)	testis expressed 41; also associated with congenital heart disease	Helgadottir *et al.*, 2018[119]
Mitral valvular calcification	*IL1F9* (rs17659543; chr 2)	interleukin 36 gamma; proinflammatory	Thanassoulis *et al.*, 2013[118]
Mendelian Disorders			
Calcific Disorder	**Gene(s)**	**Description**	**Study**
Generalized arterial calcification of infancy (GACI)	*ENPP1* or *ABCC6*	ectonucleotide pyrophosphatase/ phosphodiesterase 1; ATP binding cassette subfamily C member 6; purine and pyrophosphate metabolism	Rutsch *et al.*, 2003[98]
Arterial Calcification due to Deficiency of CD73 (ACDC)	*CD73* (aka *NT5E*)	Ecto-5'-nucleotidase or cluster of differentiation 73; purine metabolism	St. Hilaire *et al.*, 2011[100]
Pseudoxanthoma elasticum	*ABCC6*	ATP binding cassette subfamily C member 6; purine and pyrophosphate metabolism	Jansen *et al.*, 2013[104]