

THE PRESENT AND FUTURE

JACC STATE-OF-THE-ART REVIEW

Medial Arterial Calcification

JACC State-of-the-Art Review



Peter Lanzer, MD,^a Fadi M. Hannan, DPHIL,^b Jan D. Lanzer, MD,^{c,d,e} Jan Janzen, MD,^f Paolo Raggi, MD,^g Dominic Furniss, DM, MBChB,^h Mirjam Schuchardt, PhD,ⁱ Rajesh Thakker, ScD,^j Pak-Wing Fok, PhD,^k Julio Saez-Rodriguez, PhD,^c Angel Millan, PhD,^l Yu Sato, MD,^m Roberto Ferraresi, MD,ⁿ Renu Virmani, MD,^m Cynthia St. Hilaire, PhD^{o,p,q}

ABSTRACT

Medial arterial calcification (MAC) is a chronic systemic vascular disorder distinct from atherosclerosis that is frequently but not always associated with diabetes mellitus, chronic kidney disease, and aging. MAC is also a part of more complex phenotypes in numerous less common diseases. The hallmarks of MAC include disseminated and progressive precipitation of calcium phosphate within the medial layer, a prolonged and clinically silent course, and compromise of hemodynamics associated with chronic limb-threatening ischemia. MAC increases the risk of complications during vascular interventions and mitigates their outcomes. With the exception of rare monogenetic defects affecting adenosine triphosphate metabolism, MAC pathogenesis remains unknown, and causal therapy is not available. Implementation of genetics and omics-based approaches in research recognizing the critical importance of calcium phosphate thermodynamics holds promise to unravel MAC molecular pathogenesis and to provide guidance for therapy. The current state of knowledge concerning MAC is reviewed, and future perspectives are outlined. (J Am Coll Cardiol 2021;78:1145-1165) © 2021 the American College of Cardiology Foundation. Published by Elsevier. All rights reserved.

Medial arterial calcification (MAC) is a systemic vascular disorder distinct from atherosclerosis that is associated with the following: an increase in arterial stiffness (1); diastolic heart failure (2); impaired perfusion of a high blood flow organs such as brain, kidney, and liver (3); and chronic limb-threatening ischemia (CLTI) (4-7). MAC is frequently, but not always (8), associated with diabetes mellitus (DM) (9,10), chronic kidney disease (CKD) (11,12), and aging (13). Furthermore, MAC is a part of more complex phenotypes in a large number of less common disorders



Listen to this manuscript's audio summary by Editor-in-Chief Dr. Valentin Fuster on JACC.org.

From the ^aMiddle German Heart Center-Bitterfeld, Bitterfeld-Wolfen Health Care Center, Bitterfeld, Germany; ^bNuffield Department of Women's & Reproductive Health, University of Oxford, Oxford, United Kingdom; ^cInstitute for Computational Biomedicine, Bioquant, Faculty of Medicine, Heidelberg University, Heidelberg, Germany; ^dDepartment of Internal Medicine II, Heidelberg University Hospital, Heidelberg, Germany; ^eFaculty of Biosciences, Heidelberg University, Heidelberg, Heidelberg, Germany; ^fVascPath Switzerland, Bern, Switzerland; ^gDivision of Cardiology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; ^hBotnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom; ⁱDepartment of Nephrology and Medical Intensive Care, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität and Humboldt Universität Berlin, Campus Benjamin Franklin, Berlin, Germany; ^jAcademic Endocrine Unit, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom; ^kDepartment of Mathematical Sciences, University of Delaware, Newark, Delaware, USA; ^lInstitute of Materials Science, University of Zaragoza, Zaragoza, Spain; ^mCVPath Institute, Gaithersburg, Maryland, USA; ⁿCardiovascular Department, Humanitas Gavazzeni, Bergamo, Italy; ^oDivision of Cardiology, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; ^pPittsburgh Heart, Lung, and Blood Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; and the ^qDepartment of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the [Author Center](#).

Manuscript received April 12, 2021; revised manuscript received June 23, 2021, accepted June 28, 2021.

ABBREVIATIONS AND ACRONYMS

AC	= arterial calcification
ATP	= adenosine triphosphate
CaP	= calcium phosphate
CaSR	= calcium-sensing receptor
CKD	= chronic kidney disease
CLTI	= chronic limb-threatening ischemia
CT	= computed tomography
DM	= diabetes mellitus
ECM	= extracellular matrix
GWAS	= genome-wide association study
HAP	= hydroxyapatite
HDAC9	= histone deacetylase 9
IAC	= intimal arterial calcification
MAC	= medial arterial calcification
MV	= matrix vesicle
PAD	= peripheral artery disease
T2DM	= type 2 diabetes mellitus
VC	= vascular calcification
VSMC	= vascular smooth muscle cell

(Supplemental Appendix A). MAC interferes with revascularization procedures and mitigates their outcomes (14-16).

MAC is characterized by accumulation of calcium phosphate (CaP) with formation of hydroxyapatite (HAP) crystals (17), resulting in progressive petrification of the medial layer of the arterial wall. In some cases ectopic vascular osteogenesis may also develop (18).

The discovery of bone morphogenetic protein in samples of calcified human atherosclerotic lesions launched the biologic hypothesis of arterial calcifications (ACs) (19). However, to date, with the exception of the monogenic disorders (20-23), the molecular pathogenesis of AC is poorly understood (24), and no causal treatment is available (25). The slow progress in unraveling the pathogenesis stems from confounding distinct AC entities, a lack of experimental models replicating MAC (26), and a deficient appreciation of the thermodynamic principles governing CaP precipitation (27).

Here, we review the clinical and research signatures of MAC, address some of the perceived current shortcomings, and suggest directions for future research.

EPIDEMIOLOGY

The true prevalence of MAC is not known. MAC frequently represents incidental findings, or it is misdiagnosed as atherosclerosis and may therefore escape clinical attention. The prevalence of MAC, on the basis of an ankle-brachial index (ABI) >1.3, has been estimated to be ~0.5% of adults, with a male-to-female ratio of 3:2 (28); however, this is not an accurate metric for diagnosis (29). MAC is found in 17% to 42% of type 2 DM (T2DM) patients (9,10), in 27% to 40% of patients with advanced CKD (11,12) and in up to 72% in patients with CLTI (30,31).

The prevalence of MAC in the coronary arteries is unknown. However, intravascular ultrasound imaging revealed isolated deep calcifications and deep combined with superficial calcifications in 28% and 24%, respectively; this finding potentially corresponds to MACs and atherosclerotic intimal arterial calcifications (IACs) (32).

The prevalence of MAC in the thoracic aorta is currently unknown, even though the distinction between MAC and atherosclerotic IAC is clinically important in patients with porcelain aorta (33). In patients with acute and chronic Stanford A or B

HIGHLIGHTS

- MAC is a systemic vascular disorder distinct from atherosclerosis that results in progressive calcification of the medial layer of the arterial wall.
- MAC is frequently associated with diabetes mellitus, chronic kidney disease, and aging, and it can result in severe limb ischemia.
- Genetic and proteomic investigations are needed to clarify the pathogenesis of MAC and develop specific therapeutic approaches.

dissection of the thoracic aorta, MAC was found in 22% and 52%, respectively (34).

CLINICAL IMPLICATIONS

In the past, MAC was considered an innocent bystander. However, studies now demonstrate that MAC can be considered the silent killer of the cardiovascular system.

In a 10-year follow-up study of 133 patients with T2DM, MAC was found to be a powerful and independent risk factor for cardiovascular mortality and substantially stronger than the impact of IAC (9). MAC was found to be a strong and independent predictor of total cardiovascular mortality, and it predicts the risk of future coronary events in diabetic patients (10). Similarly, MAC is closely associated with the duration of hemodialysis and CaP disorders (11). In patients with T2DM, the finding of MAC in foot arteries has been associated with below-the-knee artery disease and foot ulcers (35,36). A recent meta-analysis of below-the-knee MAC demonstrated a strong association between infrapopliteal MAC and lower limb amputation risk (37).

These clinical data provide definite proof that MAC is an independent cause of peripheral artery disease (PAD) shadowing the role of atherosclerotic PAD in predisposed patients, and they clearly justify systematic studies targeting detection and clinical relevance of MAC in arteries large and small.

PATHOPHYSIOLOGY

The current hypotheses of ACs, including both MAC and IAC, span a wide range of molecular biology pathways found in inflammation, apoptosis, disruptions of CaP homeostasis, matrix vesicle (MV) extrusions, osteogenic transformations, extracellular

matrix (ECM) degeneration, and genetic aberrations. Although these processes overlap, the initial drivers specific to MAC are unclear. **Figure 1** provides an overview of some of the proposed pathogenetic principles to explain AC.

CELLULAR AND MOLECULAR CONSIDERATIONS. It is assumed that, with the proper building blocks and milieu conditions, CaP ions are prone to nucleate. Thus, it has been proposed that to prevent ectopic nucleation within the ECM, certain proteins such as matrix γ -carboxyglutamic acid (MGLA) protein may neutralize the crystallization process in a vitamin K-dependent manner (38). Although studies have shown that patients with T2DM have a higher serum level of the inactive form of MGLA that correlated with below-the-knee MAC (39), a more recent systematic review found that “no single gamma-carboxyglutamic protein species has demonstrated a significant association with VC [vascular calcification]” (40).

In humans, inorganic phosphate serum levels are tightly regulated within the range of 2.5 to 4.5 mg/dL (0.81-1.45 mmol/L). In patients with CKD, profound impairment of phosphate homeostasis promotes the development of MAC (12,41). Extracellular levels of phosphate are sensed and taken up by the type III sodium-dependent phosphate cotransporters PiT1 and PiT2, as well as by the calcium-sensing receptor (CaSR). Mutations in PiT2 are found in idiopathic basal ganglia calcification, where calcification has been hypothesized to be the result of excess accumulation of extracellular phosphate (22). More recent studies have shown that these proteins not only transport phosphate, but also appear to trigger intracellular signaling events that suppress osteogenic programs (42,43) because deficiencies in PiT-mediated signaling induced the up-regulation of osteogenic genes and exacerbated MAC in a murine CKD model (44,45). Nevertheless, the applicability of the experimental data to MAC evolution in humans has been questioned (46-48), and the controversy concerning the recipe molding the ingredients into a plausible molecular pathways hypothesis persists.

The CaSR regulates CaP homeostasis and is also expressed within arteries with altered CaSR function impairing the transdifferentiation of vascular smooth muscle cells (VSMCs) to mineralizing cells (49). The CaSR is also expressed in monocytes, which have been shown to prevent arterial calcification in vitro (50). Monocyte CaSR expression is decreased in CKD, and this is associated with an impaired ability of monocytes to inhibit AC (50).

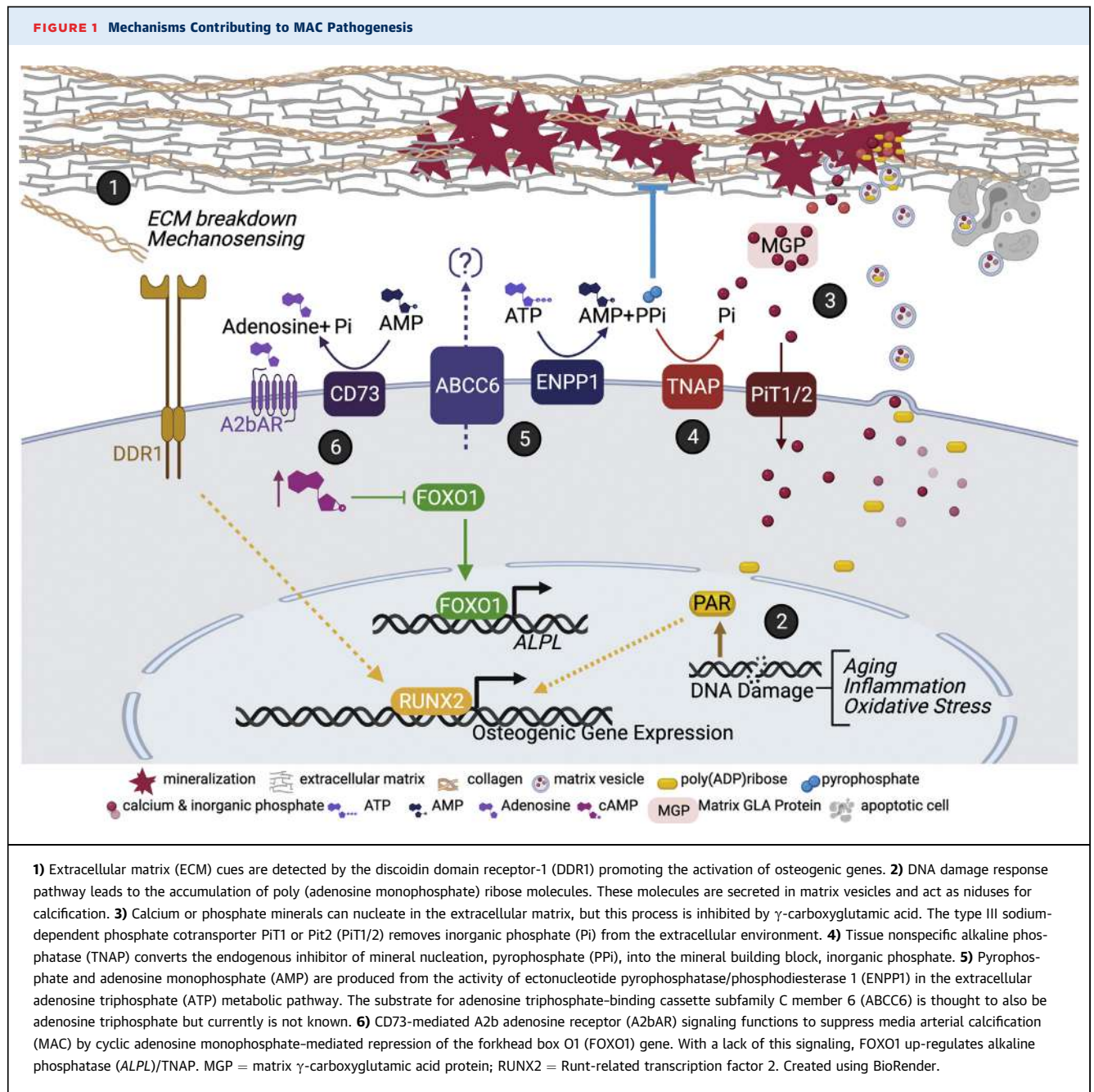
Other minerals besides CaP ions may influence the development of MAC. In animal and laboratory

experiments, magnesium has been shown to modulate the development of phosphate-induced calcification in a dose-dependent manner through the down-regulation of promoters and the up-regulation of inhibitors of calcification (51,52).

Certain genetic diseases with complex phenotypes, including MAC, originate from inactivating mutations of genes participating in the adenosine triphosphate (ATP) metabolic pathway and shed light on the initiating steps in MAC pathogenesis. ATP is released from cells under numerous metabolic stresses and insults, with ATP degradation products generally assisting cells and tissues to resist injury and to maintain homeostasis (53). It has been assumed that mutations in ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) and ATP-binding cassette subfamily C member 6 (ABCC6) drive MAC by the reduction in extracellular pyrophosphate, a key endogenous inhibitor of ectopic mineralization. Deficiency of ABCC6 is considered pathogenetically linked to the development of disseminated calcifications in pseudoxanthoma elasticum (54). ENPP1 breaks down ATP to adenosine monophosphate and pyrophosphate, and Ecto-5'-nucleotidase (CD73) converts extracellular adenosine monophosphate into phosphate and adenosine, which can signal through the 4 adenosine receptors. Lack of adenosine signaling promotes AC secondary to CD73 deficiency in affected patients (20).

Lack of CD73-mediated adenosine signaling causes up-regulation of tissue nonspecific alkaline phosphatase (TNAP), a key component of ectopic mineralization (55). The lack of CD73-mediated adenosine signaling has led to increased levels of the transcription factor FOXO1, which binds the TNAP promoter and induces TNAP transcription (56). Importantly, this mechanism was also found in popliteal arteries collected from non-CD73-deficient patients presenting with MAC. This observation suggests that the mechanisms underlying CD73 deficiency—loss of adenosine signaling and up-regulation of FOXO1 activity—may also have a role in a common MAC variety. Future studies should explore how CD73 and adenosine receptor protein levels change with age and whether their induced expression and activation may protect against MAC.

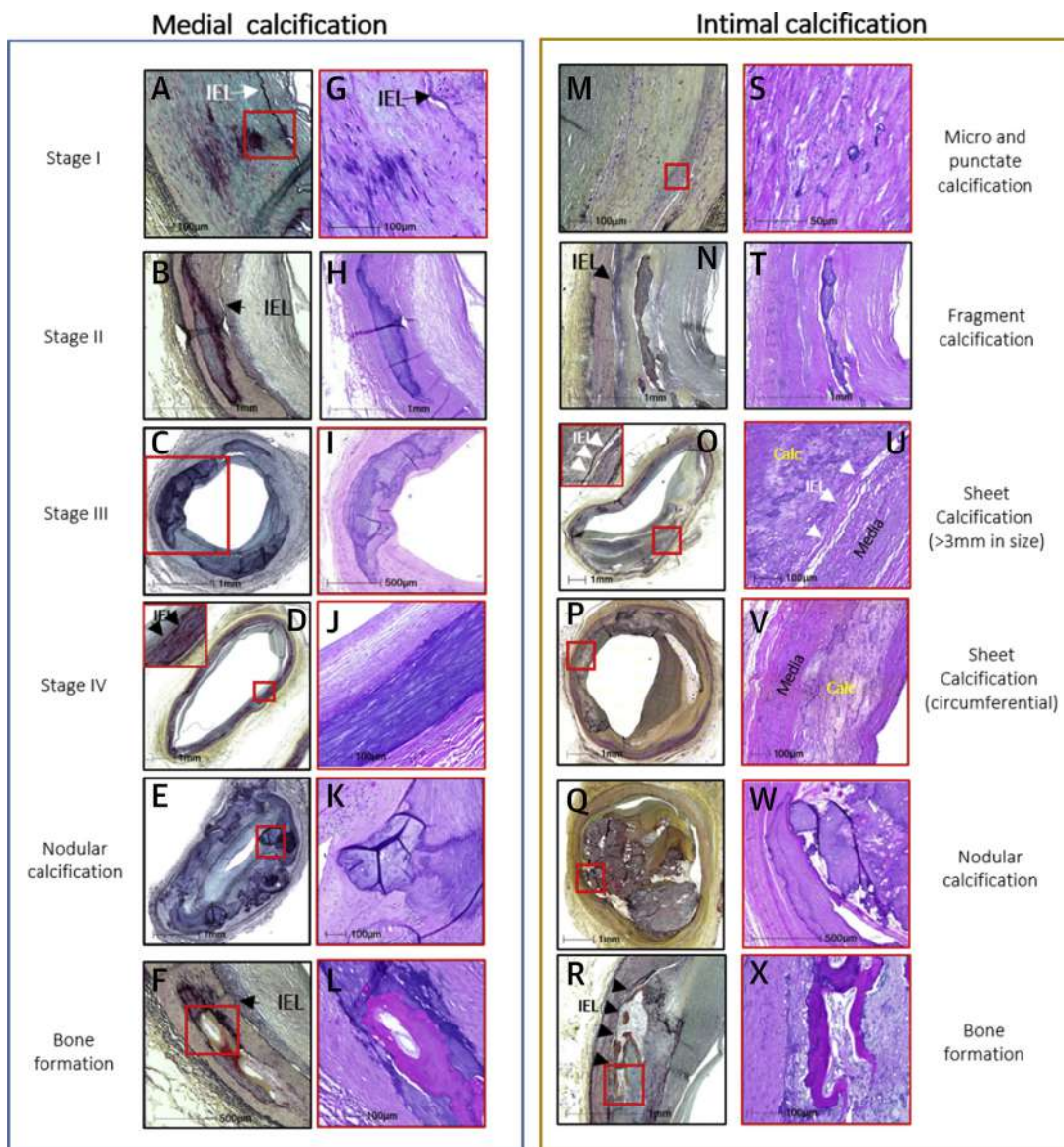
Disruption of the ECM contributes to the development of MAC. This effect is appreciated in aneurysms, whereby calcification and disorganized and fragmented aortic elastic lamina lead to dilatation and tortuosity (57). Patients with connective tissue disorders, such as Marfan syndrome and several types of Loey-Dietz syndrome, also exhibit calcification in tortuous vessels localized along damaged elastic



lamina, strikingly similar to the pathologic vascular features of MAC observed in genetic diseases (58,59). These genetic aneurysmal diseases share increased activity of the transforming growth factor- β signaling pathway, and it has been proposed that the calcification seen in these vessels is subsequent to the aberrant remodeling of the vessel wall (60-63). Indeed, it is well known that cells are able to sense the stiffness of their environment and that, conversely, the environment itself has may induce

switches in cell phenotypes (64). Stiffening of ECM transduced to VSMCs may trigger signaling events through the collagen-binding discoidin domain receptor 1, which was found to promote osteogenic differentiation and calcification (65). Exploration of the significance of epigenetic imprinting stemming from the developmental origin of individual vascular beds and their propensity to calcify the medial layer provides an interesting example of prospective comprehensive disease modeling (66).

FIGURE 2 Histology of MAC and Intimal (Atherosclerosis) Calcification



Medial arterial calcification (MAC) and the intimal atherosclerotic calcifications (Calc) are shown. The legends to each medial and intimal calcification pattern are summarized in Table 1. The histologic sections shown in the left and right columns of the medial and intimal calcifications were stained with (A to F and M to R) Movat pentachrome and (G to L and S to X) hematoxylin and eosin. The red boxes in the Movat pentachrome sections indicate areas of magnifications shown in the hematoxylin sections and in stage IV (medial) and sheet (intimal) Movat pentachrome sections. IEL = internal elastic lamina (black arrows, black and white arrowheads). Modified and reproduced with permission from <https://doi.org/10.1016/j.jcmg.2018.08.039> and <https://doi.org/10.1016/j.ejvs.2020.08.037>.

Another mechanism driving MAC pathogenesis is related to DNA damage response pathways. On DNA damage, the ataxia telangiectasia mutated protein phosphorylates gH2AX, which marks the DNA lesion and triggers DNA repair proteins (67). Among these response proteins are poly[adenosine diphosphate-ribose]polymerases (PARPs) which trigger events

such as senescence or cell death, but the product of PARPs, poly[adenosine diphosphate-ribose] (PAR), itself can promote the MAC by 2 means: 1) activation of osteogenic gene expression; and 2) amalgamation and packaging of PAR in MVs that are delivered to the ECM, where PAR and calcium and phosphate ions nucleate (65,68).

TABLE 1 Stages of Progression: Comparison Between MAC and Intimal Calcifications

	Medial Arterial Calcification		Intimal Calcification
I	Calcification of the internal elastic membrane with or without extension into the media (A and G)		Microcalcification (includes micro and punctate) is identified by calcium particles ranging from >0.5 μm to <1 mm in diameter (M and S)
II	Calcification coalescence and becomes confluent (varying in size from 1 to 3 mm), forming fragments of calcification (B and H)		Small calcification is often accompanied by inflammation, areas of microcalcification coalescence forming fragments of calcification that are >1 mm but <3 mm in diameter (N and T)
III	Calcification length > 3 mm and/or extending to involve >90° of the circumference (C and I)		Calcification of the intima >3 mm or >90° (O and U) Calcification can extend and become circumferential (P and V)
IV	Calcifications of the media, spanning the entire circumference (D and J)		
Nodular calcification	Nodular calcification is rarely seen in medial wall, which is composed of nodules of calcification often accompanied by fibrin (E and K)		Nodular calcification is composed of nodules of calcification often accompanied by fibrin with a fibrous cap (Q and W)
Bone formation	Bone formation may be observed in fragmented and areas of sheet calcification (F and L) Bone formation and rarely cartilaginous metaplasia may be seen in late stages, most frequently in stages III and IV, but rarely also in stage II		Bone formation can be observed within the regions of calcification (R and X)

A to X correspond to [Figures 2A to 2X](#). Modified and reproduced with permission from <https://doi.org/10.1016/j.jcmg.2018.08.039>; <https://doi.org/10.1016/j.ejvs.2020.08.037>.

The role of MVs in mineralization was first noticed in cartilage (69), but it is now appreciated to contribute to both MAC and IAC. In addition to delivering packets of the mineral building blocks of HAP, MVs contain lipids, metabolites, microRNAs, and proteins that promote the osteogenic switch of cells (70). Although MVs play a role in atherosclerosis and MAC, the similarities and differences of their biogenesis, accumulation, and cargo have not been fully explored.

In T2DM patients with sympathetic fiber damage, and in patients with sympathetic denervation following sympathectomy, MAC is a common finding; however, the mechanism causing calcification in such patients remains to be clarified (71,72).

In the setting of CKD, the potential role of microbiome dysbiosis-derived uremic proteins in AC pathogenesis has been proposed (73), although the proof of concept and confirmation in clinical studies of MAC are lacking.

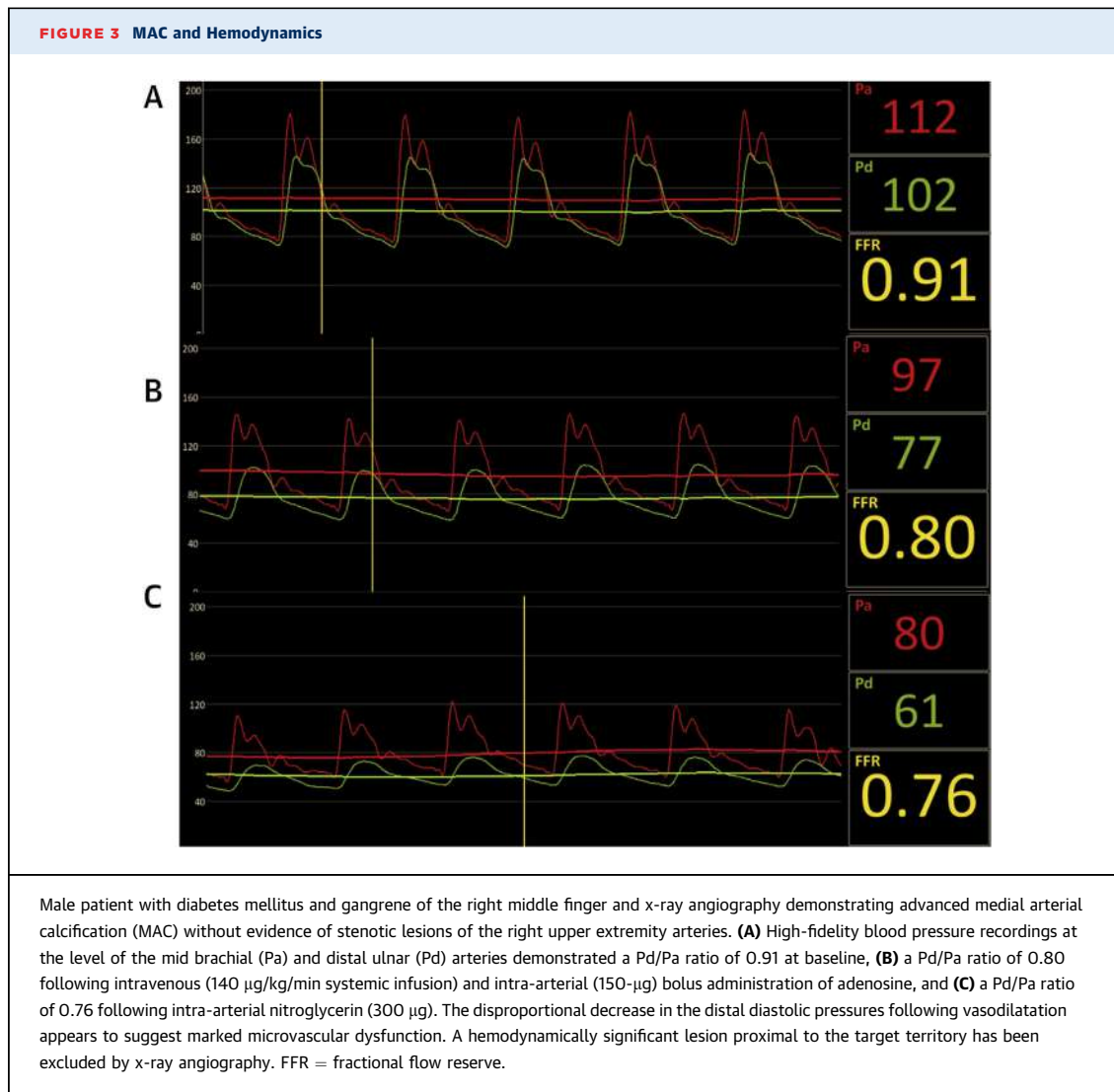
GENETIC CONSIDERATIONS. For rare mendelian disorders, the rare variants with large effect size (mutations) directly cause the disease. In contrast, AC associated with common clinical conditions such as CKD and DM appear to have a more complex etiology, with multiple low-frequency or common variants with small effect size interacting with nongenetic factors. For example, in patients with an ankle-brachial index >1.3 suggestive of PAD, a meta-analysis of genome-wide association studies (GWASs) from 21 population-based cohorts identified a single locus on chromosome 9 of genome-wide

significance (74). In a study of the UK Biobank participants whose arterial stiffness was measured, the arterial stiffness index was the subject of 2 GWAS analyses. These studies revealed 4 significant loci (near *TEX41*, *FOXO1*, *C1orf21*, and *MRVI1*), with gene-based analysis implying *COL4A2* (75). Thus, although these studies provide preliminary evidence of a genetic contribution to AC phenotypes, studies with specific well-defined cohorts of patients with MAC are lacking.

HISTOPATHOLOGY

MAC affects predominantly muscular arteries. In large arteries, early stages are characterized by fine granulations of the CaP precipitates located within the media in the proximity of the internal elastic membrane, later progressing in size and distribution. In advanced stages, large precipitates forming sheaths replacing large areas of the healthy media and fragmentation of the internal elastic membrane are seen (7). In some cases, osteogenic transformation and invasions of the intima can be seen (18). The stages and differences between MAC and IAC have been summarized in [Figures 2A to 2X](#) and in the corresponding [Table 1](#).

It is not known how far distally on the arterial tree MAC can spread. Some studies suggest that in small arteries, numerous changes may occur, including severe intimal hyperplasia and thrombotic occlusions (7,31,76), along with CaP deposits (77). In amputated limbs of CLTI patients, a strong correlation was found between MAC of the foot arteries and metatarsal artery



obstruction. The most common obstructive lesion was a combination of intimal thickening, advanced MAC, and thrombosis that was striking “for the relatively small size of the metatarsal arteries” (78).

HEMODYNAMIC IMPAIRMENTS

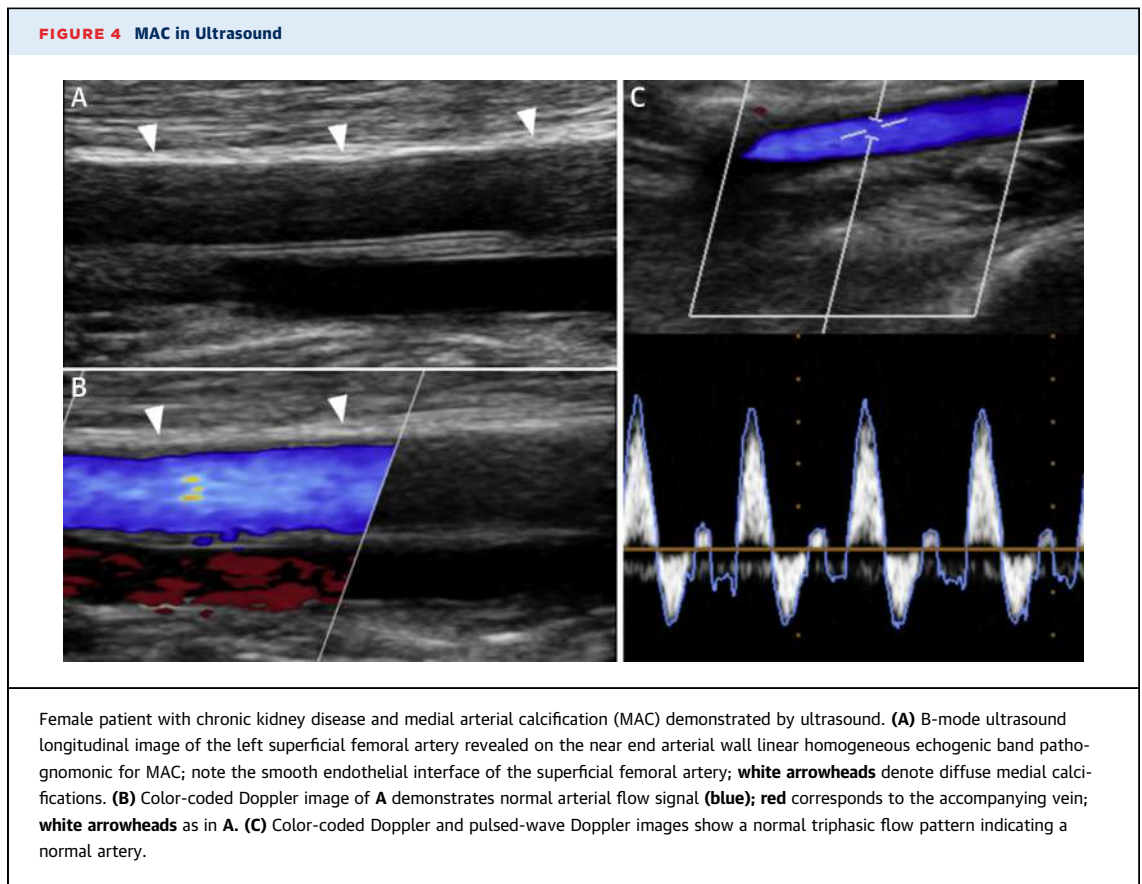
MAC causes arterial wall stiffening, which is associated with fundamental changes in central hemodynamics: impaired windkessel function, increase in left ventricular afterload, decrease in coronary artery perfusion, and increase in blood flow pulsatility (1,79,80). The limited studies of hemodynamics in peripheral arteries of patients with MAC revealed marked impairments suggestive of peripheral shunting (81).

On the basis of our preliminary observations in patients with CLTI, following vasodilatation,

advanced MAC appears to cause an accentuated fall in mean and diastolic arterial pressures. These data appear to suggest a decrease in the driving force resulting from the arterial wall rigidity and pathologic peripheral vasodilatation (Figures 3A to 3C) (Lanzer et al, unpublished data, 2020). These early data seem to corroborate the clinical observations in patients with CLTI secondary to MAC resulting in severe limb ischemia (7).

INTERACTIONS WITH ATHEROSCLEROSIS

The exact nature of interactions between MAC and atherosclerosis appears to be predominantly biomechanical. In healthy media, when atheroma develops, the luminal area is initially maintained by circumferential expansion (82). It is thought that this compensatory stage lasts until the vessel’s cross-



sectional area expands by approximately 40%. Beyond 40%, inward remodeling occurs, leading to a decreasing lumen area. However, in the presence of MAC, the compensatory stage may be prematurely disrupted. MAC may encourage the inward phase of remodeling to start earlier, thereby giving rise to an acceleration of luminal stenosis related to the progression of atherosclerosis. How the enhanced inward remodeling occurs physiologically remains to be fully characterized, but 1 hypothesis suggests a competition in stiffness between the intima and the media (83). If the stiffness of the media is much greater than that of the intima, the media will act as a stiff band surrounding a relatively compliant intima. With further growth, the intima is forced to encroach into the lumen, thus leading to a reduction in blood flow. A stiffer media could also interfere with the vessel's ability to stretch and recoil, disrupting the normal development of pressure gradients. A long-term disruption in hemodynamics may also promote an earlier onset of disease because low endothelial shear stress is thought to be atherogenic (84).

Although IAC typically occurs in advanced atherosclerotic lesions often promoted by localizing

biomechanical factors (85), MAC occurs early in the course of the disease along elastic lamellae. Thus, whereas IACs of atherosclerosis occur in advanced disease and threaten patients with focal or multifocal plaque evolution, rupture, and thromboembolic complications, MAC occurs early in the disease's course, at least in the peripheral arteries, and it is the main risk factor for progressive shutdown of circulation secondary to small artery disease.

DIAGNOSTIC EVALUATIONS

MAC is most commonly seen in the arteries of the lower and upper extremities, but it has been reported in all major vascular territories (86), including the temporal (87), facial (88), and mammary (89) arteries. The diagnosis of MAC is frequently incidental in the course of other diagnostic evaluations or a result of systematic screening in patients with PAD. In PAD, the ankle-brachial index has been the preferred first-line method to screen for MAC; however the validity of this index to determine MAC has been questioned (29). Recently, a simple MAC score was proposed on the basis of a planar radiograph of the foot in 2

projections. Looking at 5 vascular sites and evaluating the length of the “rail-tracking,” patients can be divided into 3 MAC categories: absent, moderate, and severe (90).

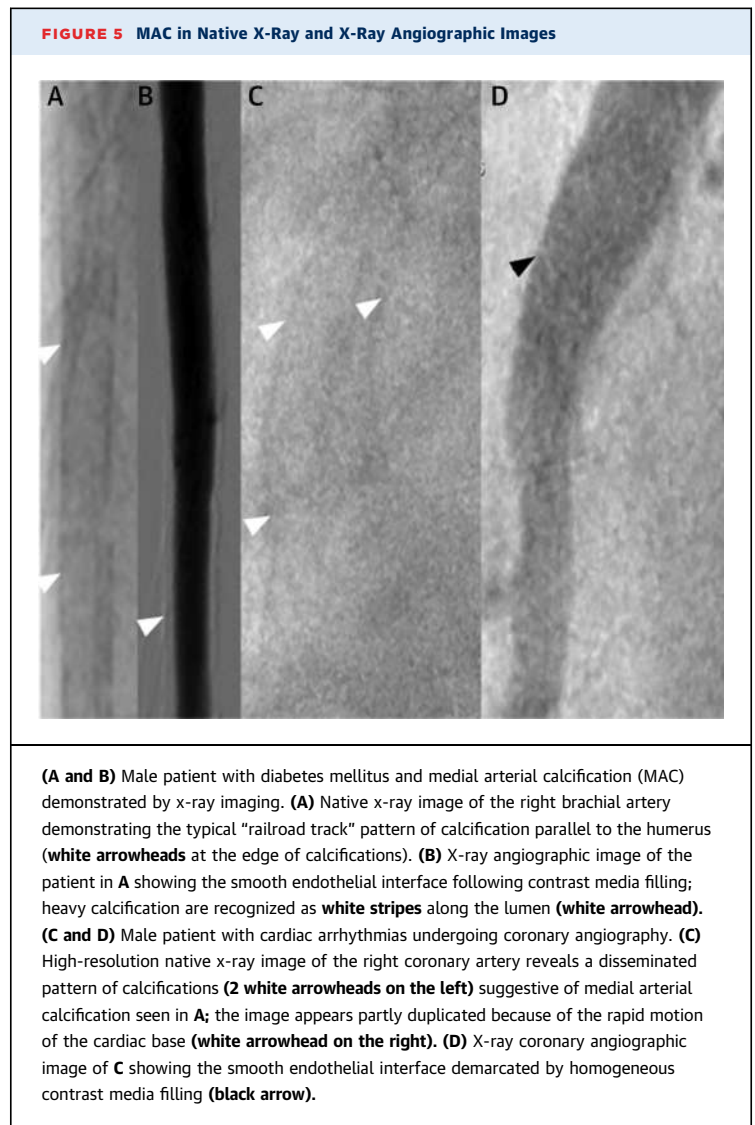
Multiple imaging modalities allowing differentiation between the intimal and medial layers of the arterial walls are suitable for MAC detection. In peripheral arteries, MAC and IAC can be clearly differentiated by transcutaneous ultrasound imaging. MAC is recognized on longitudinal views by echogenic abluminal bands and typically smooth endothelial interfaces (Figures 4A to 4C), whereas IAC appears granular and spotty (91). On conventional x-ray radiography, MAC radiopaque “rail-tracking” type shadows are typical, in contrast to the irregular and discrete “plaquelike” IAC (92). If conventional x-ray radiography is combined with angiography, smooth endothelial interfaces can be appreciated (Figures 5A to 5D). Computed tomography (CT) exquisitely depicts peripheral and coronary artery calcifications as high-density signals, yet because of its limited spatial resolution, MAC and IAC cannot be differentiated on conventional CT images. A distinction between these 2 types of calcification on the basis of differences in patterns has been proposed on thin-slice CT images (93). Compared with other imaging modalities, CT may provide whole body estimates of AC burden, as shown in Figures 6A to 6F.

Coronary artery calcifications can be reliably visualized by CT, and coronary calcium scores have been used for detection and prognostication of coronary artery disease for more than 2 decades (94). However, to allow a distinction between MAC and IAC, either intravascular ultrasound or, even more optimally, optical coherence tomography with axial resolution of approximately 100 and 10 μm , respectively, is needed (95). By using optical coherence tomography, the artery wall layers containing calcifications are clearly visualized (Figures 7A to 7C). However, given their invasiveness, both techniques are applied only if clinically indicated in selected patients. Figures 8A to 8F demonstrate the typical native and angiographic x-rays and clinical findings of MAC in the limb arteries.

The typical laboratory test values and biomarkers for MAC are summarized in Supplemental Appendix B.

THERAPY

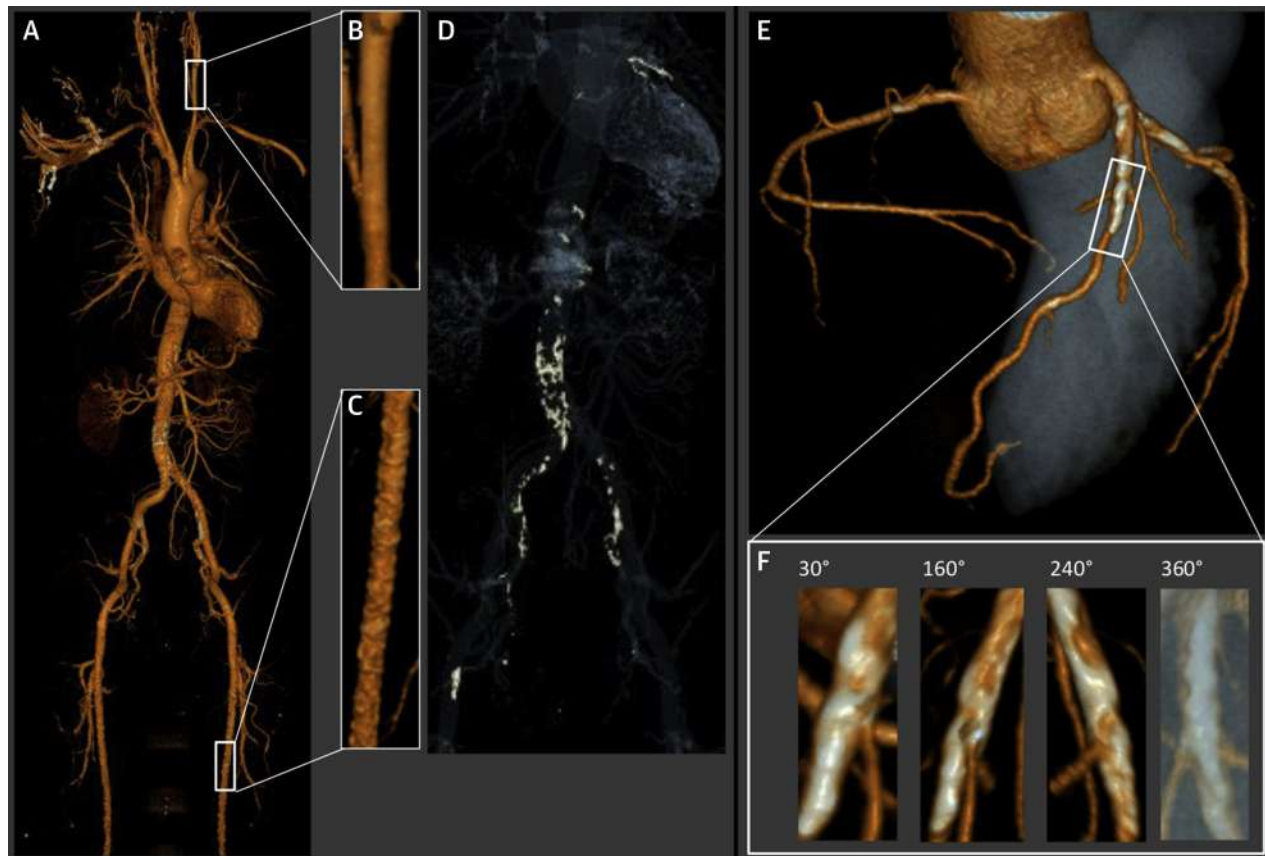
PHARMACOLOGIC THERAPY. Slowing of MAC progression in patients with CKD has been demonstrated with the use of phosphate binders and calcimimetic agents (96,97). An extensive body of published



reports on patients undergoing long-term dialysis suggests that the benefit of non-calcium-based phosphate binders is not limited to phosphate reduction, but rather it extends to reduction in the calcium load that patients receive from calcium-based binders and an effective control of parathyroid hormone (98). Indeed, parathyroid hormone was shown to have direct procalsifying effects in animal experiments (99). Additionally, these binders enhanced bone mineral density while slowing AC progression (100).

Other experimental therapeutic approaches included vitamin K supplementation, pyrophosphate, tenapanor, and acetazolamide. The Valkyrie study demonstrated a nominal but nonsignificant slower progression of calcifications in patients with atrial fibrillation who were undergoing hemodialysis and

FIGURE 6 MAC in CT



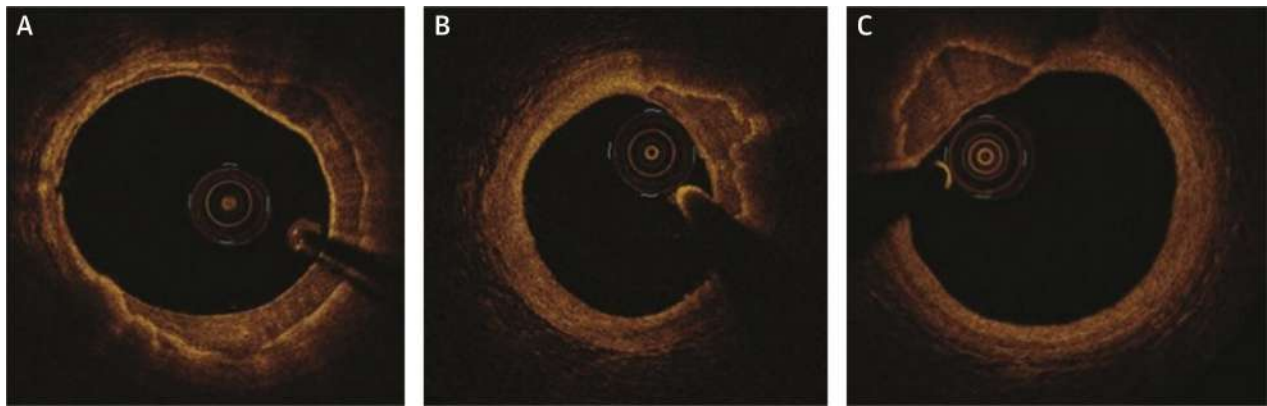
A 3-dimensional computed tomography (CT) reconstruction surface rendering in a male patient with medial arterial calcification (MAC). **(A)** Whole body scan reveals calcifications (**white spots**) within the abdominal aorta and the pelvic and leg arteries. **(B)** Enlarged section of the internal carotid artery from the image in **A** appears relatively free from calcifications. **(C)** Enlarged section of the superficial femoral artery with heavy diffuse calcification typical of medial arterial calcification. **(D)** Calcium windowing highlights the calcifications of the vasculature. **(E)** Coronary computed tomography angiography shows extensive calcifications of the proximal left anterior descending coronary artery and lesser calcifications of the proximal right and left circumflex coronary arteries. **(F)** Enlarged section of the proximal left anterior descending artery shows heavy confluent, partly circumferential calcifications.

who were randomized to rivaroxaban with or without vitamin K supplementation (101).

Tenapanor, an inhibitor of the sodium-hydrogen isoform 3 protein that regulates paracellular absorption of sodium and phosphate in the intestine, demonstrated several beneficial effects, such as reduction in sodium and phosphorus absorption, reduction in serum levels of phosphorus and fibroblast growth factor-23, and reduced heart mass in rats with chemically induced CKD. Additionally, it significantly decreased ectopic aortic calcification (102). In 2 human trials of patients undergoing hemodialysis, tenapanor showed a clear dose-dependent ability to reduce serum phosphate levels, although the trials did not measure the effect of this drug on AC (103).

Pyrophosphate inhibits calcium and phosphate deposition in bone and soft tissue. Bisphosphonates are analogues of pyrophosphate, and animal studies in the 1970s suggested the inhibitory activity of these agents on AC. Early forms of this drug (etidronate) have successfully halted the extensive calcification of newborns with generalized arterial calcification of infancy, thereby allowing their survival (104). Currently, a clinical trial (Etidronate for Arterial Calcifications Due to Deficiency in CD73 [ACDC]; NCT01585402) is under way to test whether this therapy may also be useful in patients with CD73 deficiency. Randomized controlled trials in patients before beginning dialysis and in patients undergoing dialysis failed to reaffirm this hypothesis (105), and

FIGURE 7 MAC in OCT



On optical coherence tomography (OCT), medial arterial calcification (MAC) appears as regions with homogeneous low signal intensity and sharply demarcated border zones. **(A to C)** Cross-sections of the coronary arteries. **(A)** Medial arterial calcification spans more than one-half of the circumference (12 to 7 o'clock). **(B)** Extensive, partly eccentric medial arterial calcification (1 to 3 o'clock). **(C)** Highly eccentric medial arterial calcification (10 to 12 o'clock). Note the smooth and regular intima in all 3 images. Courtesy and Copyright, Abbott, Inc.

the drug therapy appeared to promote osteomalacia (106). Etidronate, a bisphosphonate with a mechanism of action similar to that of pyrophosphate, did slow peripheral AC in a recent trial (107).

Aldosterone expands the procalcifying activity of hyperphosphatemia and facilitates osteoblastic transformation of VSMCs in vitro (108), whereas spirinolactone inhibited calcification in a Klotho-deficient mice model (109). Because patients with CKD often have hyperphosphatemia and concomitant hyperaldosteronism, human trials with mineralocorticoid antagonists are currently under way.

The anticalcifying activity of magnesium was demonstrated in 2 small human trials where magnesium supplementations slowed the progression of coronary artery calcium in predialysis patients (110) and were associated with a decreased propensity to AC development in patients undergoing hemodialysis (111).

The most interesting recent development in treatment of AC involves a direct inhibitor of HAP, the final step in all calcification processes. SNF472, myo-inositol hexaphosphate (112), was shown to slow the progression of aortic valve and coronary artery calcification significantly in patients with end-stage CKD (113).

Whether any of the modest effects described here will translate into meaningful clinical benefits in patients with MAC remains to be determined (114). Besides pharmacologic interventions, renal transplantation seems to slow the progression of MAC, but it

does not cause regression of calcification (115). Although CaP overload represents a valid target in patients with CKD, in disorders with preserved systemic CaP homeostasis, with the exception of pseudoxanthoma elasticum, no therapy targets have been established.

INTERVENTIONAL THERAPY. AC is associated with an increased risk of complications during surgical and percutaneous coronary and peripheral revascularization procedures (14-16). Numerous surgical and endovascular strategies were designed to improve outcomes. For example, in endovascular procedures, noncompliant, scoring, and cutting balloons, excimer laser ablation, rotational and orbital atherectomy, and, more recently, intravascular lithotripsy have been used to manage coronary and peripheral artery calcifications (116-118). Although these procedures do not remove calcium, they fracture the superficial and/or deep calcifications, and by modifying the lesions they improve stent placement and provide access to distal target sites. **Figures 9A to 9M** review the histologic features of calcified lesions treated with endovascular procedures.

FUTURE PERSPECTIVES

EXPERIMENTAL DATA AND PLAUSIBILITY CONSIDERATIONS. Currently, AC pathogenesis has been studied with a variety of in vitro and in vivo experimental models using numerous experimental protocols replicating some but not all of the AC

FIGURE 8 MAC in Limb Arteries



(A to C) Male patient with the gangrene of the left great toe. **(A)** Native x-ray image of the left foot; heavy calcifications of the metatarsal and toe arteries, particularly of the first metatarsal and great toe arteries, are shown (**white arrowheads**). **(B)** Angiographic x-ray image corresponding to the image in **A**; shown is the extensive destruction of the small arteries; the paucity of the distal arterial supply is highlighted in the context of the great toe (**oval broken line**). **(C)** Photograph of the left foot corresponding to the image in **A**; shown is extensive necrosis of the left great toe. **(D to F)** Male patient with the gangrene of the left middle finger. **(D)** Native x-ray image of the left hand; heavy calcifications of the metacarpal and finger arteries are shown (**white arrowheads**). **(E)** Angiographic x-ray image corresponding to the image in **A**; shown are multiple occlusions of the metacarpal and finger arteries and virtual absence of arterial blood flow to the fingers (exemplified by the middle finger, **oval broken line**). **(F)** Photograph of the left hand corresponding to the image in **A**; shown is distal necrosis of the left middle finger. MAC = medial arterial calcification.

features encountered in human diseases ([Supplemental Appendix A](#)). Albeit providing important insights to date, these studies have not uncovered novel and targetable molecular machinery that may be used to design pharmacologic prevention and therapy for AC.

Given the extreme sensitivity of the CaP precipitation processes to the ionic strength of calcium and phosphorus ions modified by promoters and inhibitors of mineralization, aberrations of *in vivo* conditions in experimental protocols bear the risk of factitious results and errors in interpretations ([27](#))

([Supplemental Appendix C](#)). To avoid these inconsistencies, experimental designs conforming to the laws of thermodynamics governing the CaP precipitation must be observed, and first principles thinking should be applied ([Supplemental Appendix D](#)). Identification of the triggers of CaP nucleation, along with the potentially reversible stage of the amorphous phosphate formation, and the irreversible point of no return following HAP crystallization will be critical to define the targets of pharmacologic therapy ([27](#)). Replication or direct observation of the *in vivo* conditions, while conserving the principles of

thermodynamics and focusing on the medial layer, will allow insights into the pathobiology of MAC, and it holds the potential for developing causal treatments.

GENETICS. Genetic analysis provides an important direction of research to unravel MAC molecular pathogenesis. Future genetic analyses of MAC will depend on the availability of whole exome sequencing and the identification of appropriate patient cohorts, such as multigenerational families with affected members, to help identify monogenic disorders (119,120). The genes identified in monogenic disorders may also be relevant for polygenic disease. However, robust characterization of genetic variants contributing to polygenic forms of MAC requires the establishment of large cohorts excluding secondary causes of AC, as outlined later in this section.

Genetic variants that promote the development of MAC as a function of comorbidities such as DM and CKD are not easily identified by familial studies. Thus, discovery of genetic variants influencing MAC risk should follow several related paths. First, to discover low-frequency variants of high effect size, whole exome sequencing or whole genome sequencing should be undertaken using large and diverse groups. Both of these techniques are limited by the ability to parse variants easily into benign or pathogenic categories (121).

Second, GWASs could be undertaken to look for common variants of small effect size that could nevertheless yield important insights into the biology of AC. For example, a GWAS of >9,400 individuals identified an association with the histone deacetylase 9 (*HDAC9*) gene and atherosclerotic aortic calcification. This study showed *HDAC9* to increase expression of RUNX family transcription factor 2 (*RUNX2*), which promotes the differentiation of VSMCs into cells with an osteogenic phenotype (122). Moreover, *HDAC9* overexpression was shown to increase calcification in cultured VSMCs of mice, whereas deficiency of *HDAC9* decreased calcification (122). Thus, whereas *HDAC9* was demonstrated as a potential therapeutic target for atherosclerotic AC, comparable data on MAC are not available. A major limitation of the GWAS is the need for well-phenotyped cohorts with a sample size in the thousands. As mentioned earlier, discerning between IAC and MAC is not often determined routinely. Furthermore, associations should be replicated in an independent cohort, again necessitating the collection of thousands more cases and control subjects. It is therefore clear that a large, international, multidisciplinary team would be required to gather sufficient cases, analyze the

genetic data, and conduct in silico and laboratory studies to discover causal variants and identify candidate genes for MAC.

Third is the need for functional validation of GWAS discoveries. The International Knockout Mouse Consortium, whose objective is to establish knockout mouse lines for each of the protein-coding genes (123), may provide an opportunity to functionally validate candidate genes identified from GWASs and whole exome sequencing or whole genome sequencing studies. Systematic phenotyping for AC in these knockout mouse lines could yield novel genes involved in VSMC function and the pathogenesis of MAC. However, it is established that mice are not good models for human MAC (41). The challenge lies in establishing noninvasive in vivo imaging modalities for the high-throughput detection and quantification of AC in mice. Fluorine-18-sodium fluoride positron emission tomography has potential as a sensitive in vivo imaging technique (124). If greater resolution is required, ex vivo micro-CT analysis could also be considered to detect microcalcification (55).

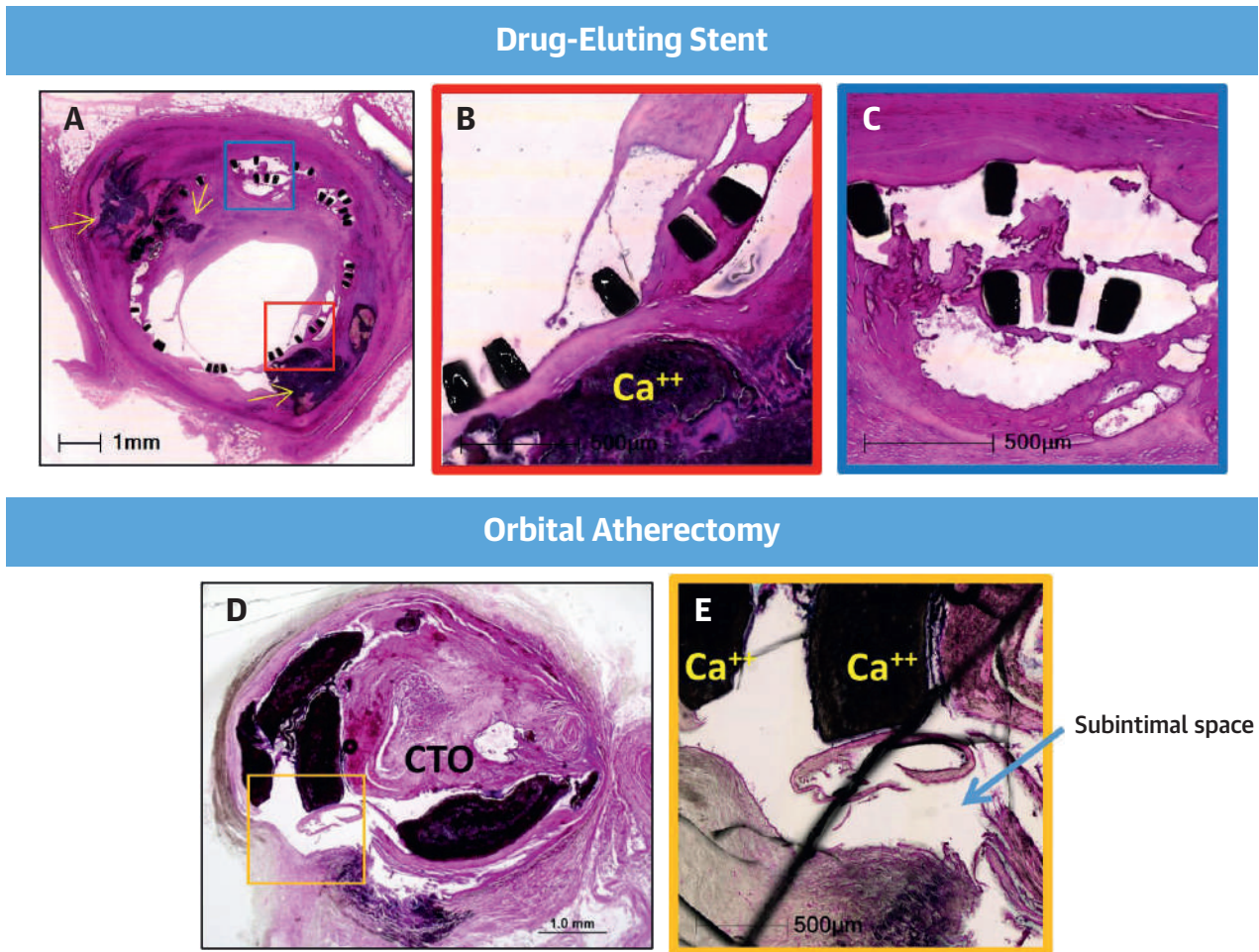
In addition to MAC manifesting as a comorbidity, primary systemic MAC characterized by the absence of predisposing factors has been reported (8). In the absence of known risk factors for MAC, the possibility of inherited genetic causes is likely, thereby providing an opportunity to search for additional monogenic causes of MAC in these patients. The availability of blood samples and vascular tissues from patients with primary MAC would greatly simplify research protocols by providing valuable opportunities to identify the causes of MAC in such patients.

Furthermore, the striking differences in the topographic distribution of MAC among individual vascular beds with strong predisposition for the arteries of the extremities appear to coincide with the differences in the embryologic origins of VSMCs (125). This finding potentially points toward an epigenetic predisposition worthy of being explored.

OMICS. Although applications of omics approaches to VC are still in their infancy, the research into MAC and IAC will benefit from a variety of studies using different omics, including proteomics (126), metabolomics (127), and, most recently, single-cell transcriptomics (128).

The in-depth characterization of calcifying blood vessels or cells could yield important insights into the evolving disease processes, mainly by providing unbiased tissue profiles. As depicted in **Figure 10**, omics-based tissue profiles can have different resolutions.

FIGURE 9 Histology of Calcified Plaques and Endovascular Interventions



(A to M) Examples of histologic findings of intimal and medial calcifications (Ca) following endovascular interventions. **(Top)** Common femoral artery and superficial femoral artery following drug-eluting stent placement and orbital atherectomy device in an ex vivo setting, respectively. **(Bottom)** Coronary artery treated by a rotational atherectomy device, superficial femoral artery (SFA) treated with an intravascular lithotripsy device, and plaque debris obtained from directional atherectomy device. **(A)** Histologic image of a stented common femoral artery with intimal nodular calcification. **(B)** A high-power image of the **red-boxed area in A**. Some stent struts remain uncovered, others are partially covered, and a few are surrounded by fibrin. **(C)** A high-power image of the **blue-boxed area in A**. Stent struts are surrounded by fibrin. **(D)** Histologic image of a superficial femoral artery chronic total occlusion (CTO) lesion treated with an orbital atherectomy device in an ex vivo setting. **(E)** Note that calcified plate (Ca^{++}) is broken into multiple pieces and the **blue arrow** shows subintimal space. **(F and G)** Histologic images of coronary artery sections stained with Movat pentachrome showing sheet calcium and an area of nodular calcification. The lesion was treated 12 hours earlier by a rotational atherectomy device; note the sharp edge of the calcified plaque (**red arrowheads**) where the burr interacted with the plaque. Medial dissection is observed (**blue arrowheads**). **(H)** A histologic image of SFA treated with an intravascular lithotripsy device. **(I)** A high-power image of the **green-boxed area in H** shows fracture of the medial calcification resulting from lithotripsy. **(J)** A coregistered micro-CT image with the histologic image **H**. **Red arrows** show the fracture sites in MAC. **(K)** A gross image of plaque tissues removed by directional atherectomy device. **(L and M)** A histologic image of the collected plaque with calcification (**L**) and thrombus (Th) (**M**). **A to D, H, I, L, and M** are stained with hematoxylin and eosin. **E to G** are stained with Movat pentachrome. Ca^{++} = calcium; CT = computed tomography; MAC = medial arterial calcification. Modified and reproduced with permission from <https://doi.org/10.1016/j.jcin.2019.10.060>. **(F and G)** Modified and reproduced with permission from [https://doi.org/10.1016/0002-8703\(95\)90384-4](https://doi.org/10.1016/0002-8703(95)90384-4).

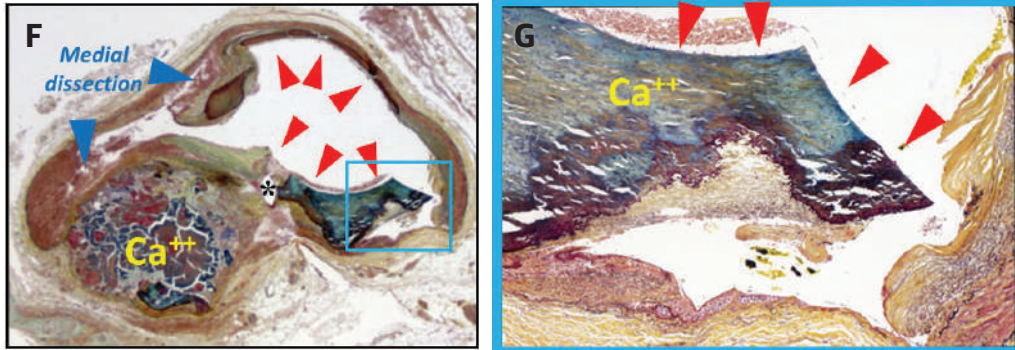
Continued on the next page

To date, a few important bulk-omics-based studies on AC have been published. Long-noncoding RNAs (LncRNAs) are gene regulatory transcripts longer than 200 bases that have been associated with major

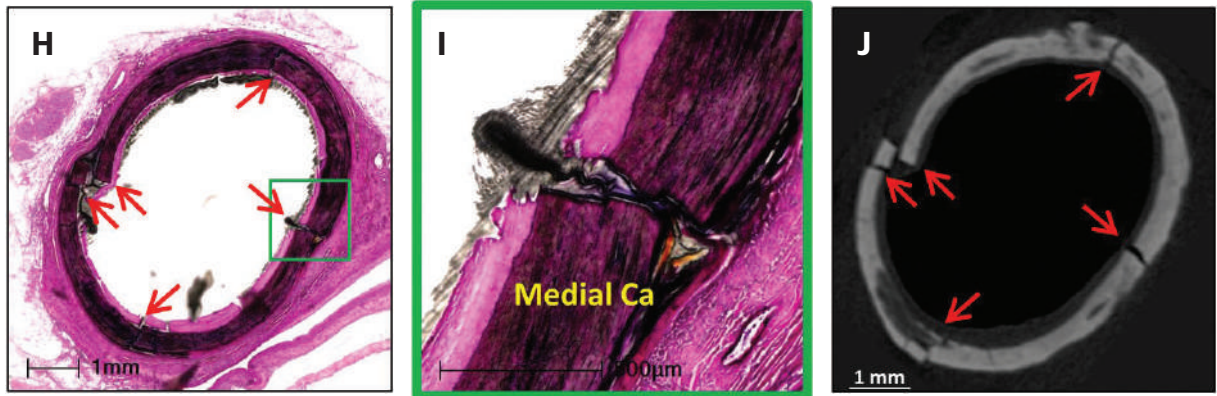
cardiovascular diseases, including heart failure (129) and atherosclerosis (130). Using an in vitro model, key regulatory LncRNAs were detected by transcriptomics in calcifying rat VSMCs (131), and the identified

FIGURE 9 Continued

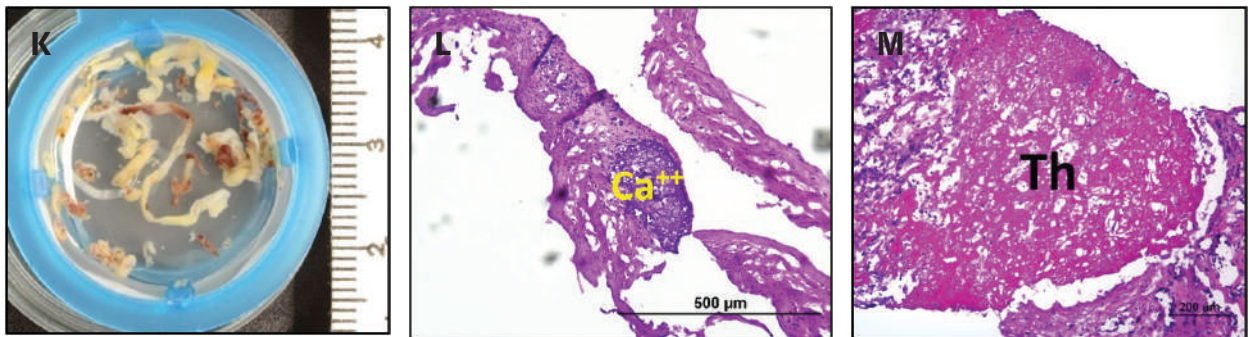
Rotational Atherectomy



Lithotripsy

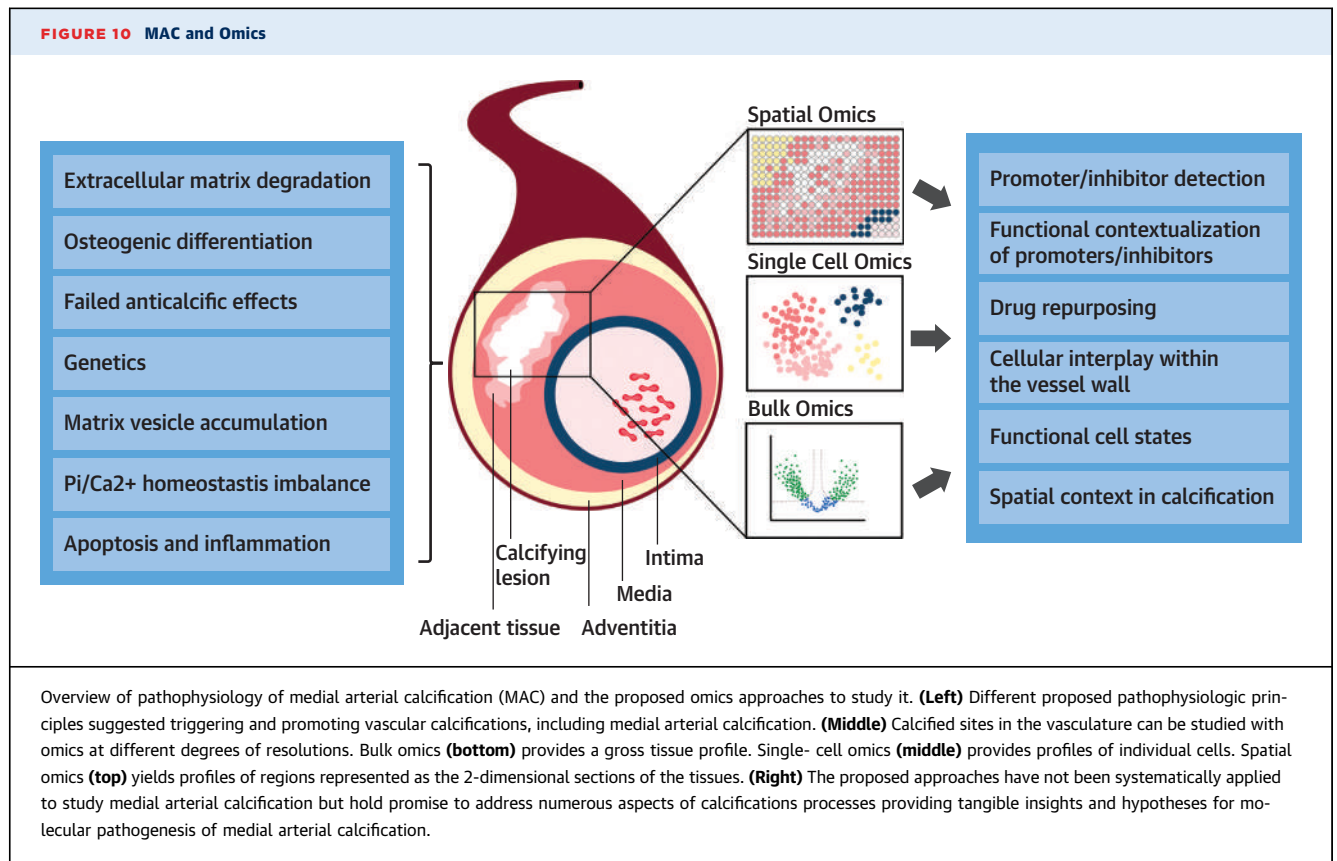


Directional Atherectomy



candidates were assayed on the basis of earlier knowledge. In this experimental set-up, LncRNA *Lrrc75a-as1* was confirmed to have a mechanistic impact on in vitro calcification. A similar pipeline was applied by Schanstra et al (119), who analyzed the proteomic difference between human arterial tissue

samples in patients with early and late stages of a broad spectrum of cardiovascular diseases. To identify potentially beneficial drugs, unbiased drug signatures (Connectivity MAP) (132) were applied to match the proteomic disease signature, assuming that the drug could reverse the pathogenic phenotype. By



using this approach, cytosolic phospholipase A₂ inhibitor was predicted to prevent AC, a prediction that was subsequently confirmed experimentally *in vivo*.

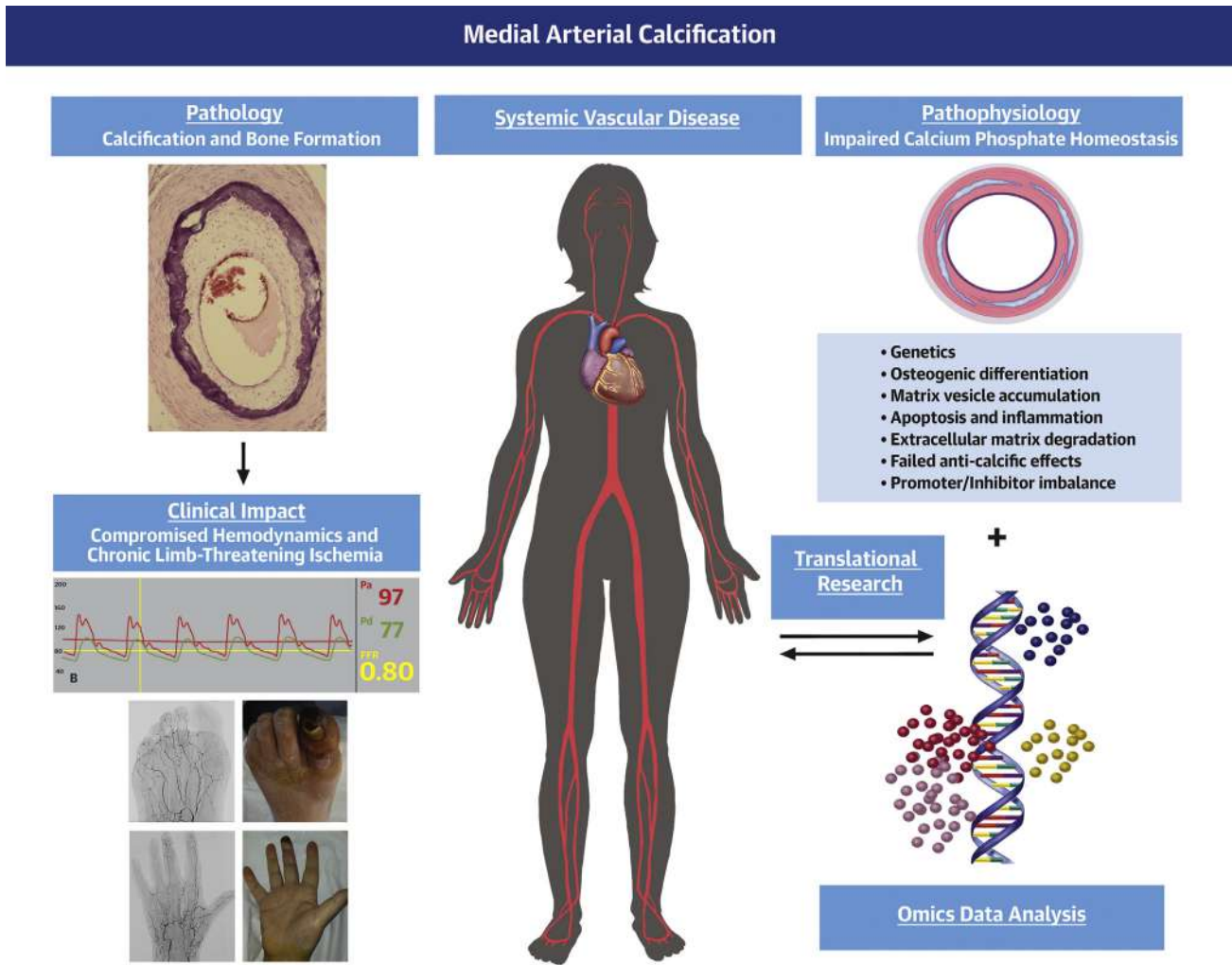
Regarding the evaluation of complex biologic systems and the integration molecular cascades by network analysis, Song et al (133) assembled a network from protein-protein interactions in VSMCs. Known proteins involved in AC were used as seed genes and mapped onto the resulting network. This was repeated for other endophenotypes such as fibrosis and inflammation to evaluate their relationship with the AC endophenotype through the protein-protein interaction network. The network was further used for drug repurposing by assessing the network distance between drug targets and the calcification module. Three candidates, including the mammalian target of rapamycin (mTOR) inhibitor everolimus, were experimentally validated to reduce VSMC calcification.

Single-cell omics allows analysis across the complexity spectrum down to a single cell. This appears to be of particular value to research on AC because it has enabled insights into vessel wall biology (128), especially into cell type and cell state composition in atherosclerotic plaques (134,135). The medial layer of arteries contains mainly VSMCs,

fibroblasts, pericytes, and transitional cell types termed myofibroblasts (136). Single-cell technologies are likely to provide important insights into the state of cellular heterogeneity of VSMCs and transitional cells in their functional evolution, along with their calcifying potential.

ACs exhibit a strong topographic aspect. They begin locally restricted to specific loci of the vasculature and are also present within specific layers of the vessel wall. In MAC, calcification sites occur predominantly in the arteries of the extremities, and here, within the media as the characteristic single target. Thus, spatial aspects of pathogenesis are expected to be highly influential and should be addressed with proper sampling strategies, as demonstrated for calcific aortic valve disease (137) and by using spatially resolved omics. These novel technologies assess molecular profiles while retaining their spatial information. Although novel technologies are under development, spatial transcriptomics are the most advanced at present (138,139). The blood vessels, small and well-structured organs, constitute excellent study subjects for these technologies. Here, the functional analysis of calcifying sites could substantially improve our understanding of how and why calcification occurs at certain sites while other sites are spared.

CENTRAL ILLUSTRATION Medial Arterial Calcification: A Systemic Vascular Disorder Devastating Peripheral Circulation



Lanzer, P. et al. *J Am Coll Cardiol.* 2021;78(11):1145-1165.

In health, calcium phosphate homeostasis is maintained, and crystallization is prevented. In medial arterial calcification calcium phosphate homeostasis breaks down, resulting in progressive mineralization and, in more advanced stages, bone formation within the medial layer. Certain pathogenetic principles, including smooth muscle cell osteogenic differentiation, apoptosis, inflammation, and molecular defects of matrisome, have been reported to regulate the calcification process. Medial arterial calcification impairs hemodynamics and often causes chronic limb-threatening ischemia. Omics approaches hold distinct promise to define medial arterial calcification molecular pathogenesis and design treatments.

Although omics have the potential to reshape research on AC, important limitations should be considered. The major limitations include reproducibility batch effects, cost, and coverage. Single-cell and spatially resolved omics and the computational methods used for data analysis are still in early stages of development, and mechanistic insight from omics studies relying on biologic

assumptions may not always reflect biologic reality. Nevertheless, omics holds a great promise providing data-driven hypotheses requiring validation experiments and allowing causal conclusions, and as such, omics needs to be systematically used to support biochemical, genetic, and experimental approaches to understand AC (24) and specifically MAC.

The definition of the MAC molecular biology will ultimately need to be addressed by interdisciplinary teams with complementary expertise (24,140,141).

The **Central Illustration** summarizes the key points pertaining to the pathophysiology and clinical manifestation of MAC.

SUMMARY

MAC is a chronic systemic vascular disorder distinct from atherosclerosis that is frequently but not always associated with DM, CKD, and aging. It is part of several complex phenotypes observed in numerous less common diseases. The hallmarks of MAC include disseminated and progressive precipitation of CaP within the medial layer, compromise of hemodynamics, a prolonged and clinically silent course, and CLTI. The pathogenesis of MAC is unknown. We review the current state of knowledge about MAC and briefly outline directions for future research.

ACKNOWLEDGMENT Dr Lanzer acknowledges fruitful discussions on coronary calcifications with Dr Gary Mintz of Cardiovascular Research Foundation, New York, during the late phases of drafting the manuscript.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr Furniss is supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre. Dr Schuchardt has received support from Charité 3R and the Bundesministerium für Bildung und Forschung. Dr J.D. Lanzer has received support from Informatics for Life funded by the Klaus Tschira Foundation. Dr Thakker has received support from a Wellcome Trust Senior Investigator Award, an NIHR Senior Investigator Award, and the NIHR Oxford Biomedical Research Centre Programme. Dr Saez-Rodriguez has

received support from Informatics for Life funded by the Klaus Tschira Foundation; has received funding from GSK and Sanofi; and expects consultant fees from Traverre Therapeutics. Dr Millan has received financial support from the Spanish Ministry of Science, Innovation, and Universities (grant no: PGC2018_095795_B_I00). Dr Sato has received institutional research support from NIH-HL141425, Leducq Foundation Grant, 480 Biomedical, 4C Medical, 4Tech, Abbott, Accumedical, Alivas, Amgen, Biosensors, Boston Scientific, Canon USA, Cardiac Implants, Celonova, Claret Medical, Concept Medical, Cook, CSI, DuNing, Inc, Edwards LifeSciences, Emboline, Endotronix, Envision Scientific, Lutonix/Bard, Gateway, Lifetech, Limflo, MedAlliance, Medtronic, Mercator, Merill, Microport Medical, Microvention, Mitraalign, Mitra assist, NAMSA, Nanova, Neovasc, NIPRO, Novogate, Occulotech, OrbusNeich Medical, Phenox, Profusa, Protembis, Qool, ReCor Medical, Senseonics, Shockwave, Sinomed, Spectranetics, Surmodics, Symic, Vesper, W.L. Gore, and Xeltis. Dr Virmani has received institutional research support from NIH-HL141425, Leducq Foundation Grant, 480 Biomedical, 4C Medical, 4Tech, Abbott, Accumedical, Alivas, Amgen, Biosensors, Boston Scientific, Canon USA, Cardiac Implants, Celonova, Claret Medical, Concept Medical, Cook, CSI, DuNing, Inc, Edwards LifeSciences, Emboline, Endotronix, Envision Scientific, Lutonix/Bard, Gateway, Lifetech, Limflo, MedAlliance, Medtronic, Mercator, Merill, Microport Medical, Microvention, Mitraalign, Mitra assist, NAMSA, Nanova, Neovasc, NIPRO, Novogate, Occulotech, OrbusNeich Medical, Phenox, Profusa, Protembis, Qool, ReCor Medical, Senseonics, Shockwave, Sinomed, Spectranetics, Surmodics, Symic, Vesper, W.L. Gore, and Xeltis; is a consultant for Abbott Vascular, Boston Scientific, Celonova, OrbusNeich Medical, Terumo Corporation, W.L. Gore, Edwards Lifesciences, Cook Medical, CSI, ReCor Medical, SinoMedical Sciences Technology, Surmodics, and Bard BD; and is a Scientific Advisory Board Member for Medtronic and Xeltis. Dr St. Hilaire has received support from National Institutes of Health grants HL142932 and HL117917. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr Peter Lanzer, Mitteldeutsches Herzzentrum-Standort Bitterfeld, Health Care Center Bitterfeld-Wolfen gGmbH, Friedrich-Ludwig-Jahn-Strasse 2, D-06749 Bitterfeld-Wolfen, Germany. E-mail: lanzer.peter@web.de.

REFERENCES

1. Reesink KD, Spronck B. Constitutive interpretation of arterial stiffness in clinical studies: a methodological review. *Am J Physiol Heart Circ Physiol*. 2019;316:H693-H709.
2. Weber T, Chirinos JA. Pulsatile arterial haemodynamics in heart failure. *Eur Heart J*. 2018;39:3847-3854.
3. Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease: JACC state-of-the-art review. *J Am Coll Cardiol*. 2019;74:1237-1263.
4. Mowafy KA, Soliman M, Hammada AM, Soliman RM. Bilateral lower limb disabling claudication in a young man: a case of Mönckeberg's arteriosclerosis. 2019. *Vasc Endovasc Rev*. 2019;2(1):48-52. Accessed June 6, 2020. <https://www.verjournal.com/articles/Disabling-Claudication-Monckebergs-Arteriosclerosis>
5. Pisani I, De Troia A, Allegri L, Corradi D, Vaglio A. Malignant Mönckeberg medial calcific sclerosis. *Intern Emerg Med*. 2018;13:615-617.
6. Lanzer P, Boehm M, Sorribas V, et al. Medial vascular calcification revisited: review and perspectives. *Eur Heart J*. 2014;35:1515-1525.
7. Mustapha JA, Diaz-Sandoval LJ, Saab F. Infra-popliteal calcification patterns in critical limb ischemia: diagnostic, pathologic and therapeutic implications in the search for the endovascular holy grail. *J Cardiovasc Surg (Torino)*. 2017;58:383-401.
8. Lanzer P. Mediakalkinose Mönckeberg. *Z Kardiol*. 1998;87:586-593.
9. Niskanen L, Siitonen O, Suhonen M, Uusitupa MI. Medial artery calcification predicts cardiovascular mortality in patients with NIDDM. *Diabetes Care*. 1994;17:1252-1256.
10. Lehto S, Niskanen L, Suhonen M, Rönnemaa T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 1996;16:978-983.
11. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003;18:1731-1740.
12. Nelson AJ, Raggi P, Wolf M, Gold AM, Chertow GM, Roe MT. Targeting vascular calcification in chronic kidney disease. *J Am Coll Cardiol Basic Trans Science*. 2020;5:398-412.
13. Pescatore LA, Gamarra LF, Lieberman M. Multifaceted mechanisms of vascular calcification in aging. *Arterioscler Thromb Vasc Biol*. 2019;39:1307-1316.

14. Génereux P, Madhavan MV, Mintz GS, et al. Ischemic outcomes after coronary intervention of calcified vessels in acute coronary syndromes. Pooled analysis from the HORIZONS-AMI (Harmonizing Outcomes with Revascularization and Stents in Acute Myocardial Infarction) and ACUITY (Acute Catheterization and Urgent Intervention Triage Strategy) TRIALS. *J Am Coll Cardiol*. 2014;63:1845-1854.
15. Bourantas CV, Zhang Y-J, Garg S, et al. Prognostic implications of severe coronary calcification in patients undergoing coronary artery bypass surgery: an analysis of the SYNTAX study. *Catheter Cardiovasc Interv*. 2015;85:199-206.
16. Misare BD, Pomposelli Jr FB, Gibbons GW, Campbell DR, Freeman DV, LoGerfo FW. Infrapopliteal bypasses to severely calcified, unclampable outflow arteries: two-year results. *J Vasc Surg*. 1996;24:6-15.
17. Villa-Bellostá R, Egido J. Phosphate, pyrophosphate, and vascular calcification: a question of balance. *Eur Heart J*. 2017;38:1801-1804.
18. Deneke T, Langner K, Grewe PH, Harrer E, Müller KM. Ossification in atherosclerotic carotid arteries. *Z Kardiol*. 2001;90(Suppl 3):106-315.
19. Boström K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest*. 1993;91:1800-1809.
20. St Hilaire C, Ziegler SG, Markello TC, et al. Nt5e mutations and arterial calcifications. *N Engl J Med*. 2011;364:432-442.
21. Rutsch F, Ruf N, Vaingankar S, et al. Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification. *Nat Genet*. 2003;34:379-381.
22. Wang C, Li Y, Shi L, et al. Mutations in slc20a2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. *Nat Genet*. 2012;44:254-256.
23. Leibold M, Halperin M, Phelps RG. Occult pseudoxanthoma elasticum in patients with premature cardiovascular disease. *N Engl J Med*. 1993;329:1237-1239.
24. Rogers MA, Aikawa E. Cardiovascular calcification: artificial intelligence and big data accelerate mechanistic discovery. *Nat Rev Cardiol*. 2019;16:261-274.
25. Schantl AE, Ivarsson ME, Leroux JC. Investigational pharmacological treatments for vascular calcification. *Adv Ther*. 2019;2:1800094. <https://doi.org/10.1002/adtp.201800094>
26. Herrmann J, Babic M, Tolle M, van der Giet M, Schuchardt M. Research models for studying vascular calcification. *Int J Mol Sci*. 2020;21(6):2204.
27. Millan A, Lanzer P, Sorribas V. The thermodynamics of medial vascular calcification. *Front Cell Dev Biol*. 2021;9:633465.
28. Krüger K. Epidemiologie der peripheren arteriellen Verschlusskrankheit in Deutschland; Was ist gesichert und was ist offen? *Hamostaseologie*. 2006;26:193-196.
29. Hoek AG, Sabine R, Zwakenberg SR, Petra J M Elders PJM. An elevated ankle-brachial index is not a valid proxy for peripheral medial arterial calcification. *Atherosclerosis*. 2021;323:13-19.
30. Narula N, Dannenberg AJ, Olin JW, et al. Pathology of peripheral artery disease in patients with critical limb ischemia. *J Am Coll Cardiol*. 2018;72:2152-2163.
31. O'Neill WC, Han KH, Schneider TM, Hennigar RA. Prevalence of nonatheromatous lesions in peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2015;35:439-447.
32. Mintz GS, Popma JJ, Pichard AD, et al. Patterns of calcification in coronary artery disease. A statistical analysis of intravascular ultrasound and coronary angiography in 1155 lesions. *Circulation*. 1995;91:1959-1965.
33. Abramovitz Y, Jilalawi H, Chakravarty T, Mack MJ, Makkar RR. Porcelain aorta; a comprehensive review. *Circulation*. 2020;131:827-836.
34. de Jong PA, Hellings WE, Takx RAP, Isgun I, van Herwaarden JA, Mali WPTM. Computed tomography of aortic wall calcifications in aortic dissection patients. *PLoS One*. 2014;9(7):e102036. <https://doi.org/10.1371/journal.pone.0102036>
35. Everhart JE, Pettitt DJ, Knowler WC, Rose FA, Bennett PH. Medial arterial calcification and its association with mortality and complications of diabetes. *Diabetologia*. 1988;31:16-23.
36. Smith DC, Bilmen GJ, Iqbal S, Robey S, Pereira M. Medial artery calcification as an indicator of diabetic peripheral vascular disease. *Foot Ankle Int*. 2008;29:185-190.
37. Losurdo F, Ferraresi R, Ucci A, Zanetti A, Clerici G, Zambon A. Association of infrapopliteal medial arterial calcification with lower-limb amputations in high-risk patients: a systematic review and meta-analysis. *Vasc Med*. 2021;26(2):164-173. <https://doi.org/10.1177/1358863X20979738>
38. Schurgers LJ, Teunissen KJ, Knapen MH, et al. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. *Arterioscler Thromb Vasc Biol*. 2005;25:1629-1633.
39. Dalmeijer GW, van der Schouw YT, Magdeleyns EJ, et al. Matrix Gla protein species and risk of cardiovascular events in type 2 diabetic patients. *Diabetes Care*. 2013;36:3766-3771.
40. Barrett H, O'Keefe M, Kavanagh E, Walsh M, O'Connor EM. Is matrix Gla protein associated with vascular calcification? A systematic review. *Nutrients*. 2018;10(4):415. <https://doi.org/10.3390/nu10040415>
41. Joolharzadeh P, St Hilaire C. CD73 (cluster of differentiation 73) and the differences between mice and humans. *Arterioscler Thromb Vasc Biol*. 2019;39:339-348.
42. Bon N, Couasnay G, Bourgine A, et al. Phosphate (Pi)-regulated heterodimerization of the high-affinity sodium-dependent Pi transporters PIT1/Slc20a1 and PIT2/Slc20a2 underlies extracellular Pi sensing independently of Pi uptake. *J Biol Chem*. 2018;293:2102-2114.
43. Voelkl J, Lang F, Eckardt KU, et al. Signaling pathways involved in vascular smooth muscle cell calcification during hyperphosphatemia. *Cell Mol Life Sci*. 2019;76:2077-2091.
44. Chavkin NW, Chia JJ, Crouthamel MH, Giachelli CM. Phosphate uptake-independent signaling functions of the type III sodium-dependent phosphate transporter, PIT-1, in vascular smooth muscle cells. *Exp Cell Res*. 2015;333:39-48.
45. Yamada S, Leaf EM, Chia JJ, Cox TC, Speer MY, Giachelli CM. PIT-2, a type III sodium-dependent phosphate transporter, protects against vascular calcification in mice with chronic kidney disease fed a high-phosphate diet. *Kidney Int*. 2018;94:716-727.
46. Hortells L, Sosa C, Guillén N, Lucea S, Millán A, Sorribas V. Identifying early pathogenic events during vascular calcification in uremic rats. *Kidney Int*. 2017;92:1384-1394.
47. Komaba H, Fukagawa M. Phosphate: a poison for humans? *Kidney Int*. 2016;90:753-763.
48. Villa-Bellostá R, Millán A, Sorribas V. Role of calcium-phosphate deposition in vascular smooth muscle cell calcification. *Am J Physiol Cell Physiol*. 2011;300:C210-C220.
49. Alam M, Kirton JP, Wilkinson FL, et al. Calcification is associated with loss of functional calcium-sensing receptor in vascular smooth muscle cells. *Cardiovasc Res*. 2009;81:260-268.
50. Mary A, Objois T, Brazier M, et al. Decreased monocyte calcium sensing receptor expression in patients with chronic kidney disease is associated with impaired monocyte ability to reduce vascular calcification. *Kidney Int*. 2021;99:1382-1391.
51. Louvet L, Büchel J, Steppan S, Passlick-Deetjen J, Massy ZA. Magnesium prevents phosphate-induced calcification in human aortic vascular smooth muscle cells. *Nephrol Dial Transplant*. 2013;28:869-878.
52. Xu J, Bai Y, Jin J, et al. Magnesium modulates the expression levels of calcification-associated factors to inhibit calcification in a time-dependent manner. *Exp Ther Med*. 2015;9:1028-1034.
53. Newby AC. Adenosine and the concept of 'retaliatory metabolites'. *Trends Biochem Sci*. 1984;9:42-44.
54. Li Q, van de Wetering K, Uitto J. Pseudoxanthoma elasticum as a paradigm of heritable ectopic mineralization disorders: pathomechanisms and treatment development. *Am J Pathol*. 2019;189:216-225.
55. Jin H, St Hilaire C, Huang Y, et al. Increased activity of TNAP compensates for reduced adenosine production and promotes ectopic calcification in the genetic disease ACDC. *Sci Signal*. 2016;9(458):ra121.
56. Moorhead 3rd WJ, Chu CC, Cuevas RA, et al. Dysregulation of FOXO1 (forkhead box O1 protein) drives calcification in arterial calcification due to deficiency of CD73 and is present in peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2020;40:1680-1694.
57. Schlatmann TJ, Becker AE. Pathogenesis of dissecting aneurysm of aorta. Comparative histopathologic study of significance of medial changes. *Am J Cardiol*. 1977;39:21-26.
58. Wanga S, Hibender S, Ridwan Y, et al. Aortic microcalcification is associated with elastin

- fragmentation in Marfan syndrome. *J Pathol.* 2017;243:294-306.
59. Markello TC, Pak LK, St Hilaire C, et al. Vascular pathology of medial arterial calcifications in NTSE deficiency: implications for the role of adenosine in pseudoxanthoma elasticum. *Mol Genet Metab.* 2011;103:44-50.
60. Canadas V, Vilacosta I, Bruna I, Fuster V. Marfan syndrome. Part 1: pathophysiology and diagnosis. *Nat Rev Cardiol.* 2010;7:256-265.
61. Neptune ER, Frischmeyer PA, Arking DE, et al. Dysregulation of TGF- β activation contributes to pathogenesis in Marfan syndrome. *Nat Genet.* 2003;33:407-411.
62. Gallo EM, Loch DC, Habashi JP, et al. Angiotensin II-dependent TGF- β signaling contributes to Loey's-Dietz syndrome vascular pathogenesis. *J Clin Invest.* 2014;124:448-460.
63. Callewaert BL, Willaert A, Kerstjens-Frederikse WS, et al. Arterial tortuosity syndrome: clinical and molecular findings in 12 newly identified families. *Hum Mutat.* 2008;29:150-158.
64. Jaalouk DE, Lammerding J. Mechano-transduction gone awry. *Nat Rev Mol Cell Biol.* 2009;10:63-73.
65. Ngai D, Lino M, Rothenberg KE, Simmons CA, Fernandez-Gonzalez R, Bendeck MP. DDR1 (discoidin domain receptor-1)-RhoA (Ras homolog family member A) axis senses matrix stiffness to promote vascular calcification. *Arterioscler Thromb Vasc Biol.* 2020;40:1763-1776.
66. Hou YC, Lu CL, Yuan TH, Liao MT, Chao CT, Lu KC. The epigenetic landscape of vascular calcification: an integrative perspective. *Int J Mol Sci.* 2020;21(3):980.
67. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature.* 2009;461(7267):1071-1078.
68. Wang C, Xu W, An J, et al. Poly(ADP-ribose) polymerase 1 accelerates vascular calcification by upregulating Runx2. *Nat Commun.* 2019;10(1):1203. <https://doi.org/10.1038/s41467-019-09174-1>
69. Anderson HC. Electron microscopic studies of induced cartilage development and calcification. *J Cell Biol.* 1967;35:81-101.
70. Blaser MC, Aikawa E. Roles and regulation of extracellular vesicles in cardiovascular mineral metabolism. *Front Cardiovasc Med.* 2018;5:187. <https://doi.org/10.3389/fcvm.2018.00187>
71. Edmonds ME, Morrison N, Laws JW, Watkins PJ. Medial arterial calcification and diabetic neuropathy. *Br Med J (Clin Res Ed).* 1982;284:928-930.
72. Bourron O, Aubert CE, Liabeuf S, et al. Below-knee arterial calcification in type 2 diabetes: association with receptor activator of nuclear factor κ B ligand, osteoprotegerin, and neuropathy. *J Clin Endocrinol Metab.* 2014;99:4250-4258.
73. Filipka I, Winiarska A, Knysak M, Stompór T. Contribution of gut microbiota-derived uremic toxins to the cardiovascular system mineralization. *Toxins (Basel).* 2021;13(4):274. <https://doi.org/10.3390/toxins13040274>
74. Murabito JM, White CC, Kavousi M, et al. Association between chromosome 9p21 variants and the ankle-brachial index identified by a meta-analysis of 21 genome-wide association studies. *Circ Cardiovasc Genet.* 2012;5:100-112.
75. Zekavat SM, Aragam K, Emdin C, et al. Genetic association of finger photoplethysmography-derived arterial stiffness index with blood pressure and coronary artery disease. *Arterioscler Thromb Vasc Biol.* 2019;39:1253-1261.
76. Goldenberg S, Alex M, Joshi RM, Blumenthal HAT. Nonatheromatous peripheral vascular disease of the lower extremity in diabetes mellitus. *Diabetes.* 1959;8:261-273.
77. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification. *Circ Res.* 2006;99:1044-1059.
78. Ferrier TM. Comparative study of arterial disease in amputated lower limbs from diabetics and non-diabetics (with special reference to feet arteries). *Med J Aust.* 1967;1:5-11.
79. McEniery CM, McDonnell BJ, So A, et al. Aortic calcification is associated with aortic stiffness and isolated systolic hypertension in healthy individuals. *Hypertension.* 2009;53:524-531.
80. Mitchell GF. Aortic stiffness, pressure and flow pulsatility, and target organ damage. *Appl Physiol.* 2018;125:1871-1880.
81. Edmonds ME, Roberts VC, Watkins PJ. Blood flow in the diabetic neuropathic foot. *Diabetologia.* 1982;22:9-15.
82. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Koletlis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med.* 1987;316:1371-1375.
83. Fok PW, Lanzer P. Media sclerosis drives and localizes atherosclerosis in peripheral arteries. *PLoS One.* 2018;13(10):e0205599. <https://doi.org/10.1371/journal.pone.0205599>
84. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling. *J Am Coll Cardiol.* 2007;49:2379-2393.
85. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. *Circulation.* 1995;92:1355-1374.
86. Lachman AS, Spray TL, Kerwin DM, Shugoll GI, Robert WC. Medial calcinosis of Mönckeberg. *Am J Med.* 1977;63:615-622.
87. Castillo BV, Torczynski E, Edward DP. Mönckeberg's sclerosis in a temporal artery biopsy specimen. *Br J Ophthalmol.* 1999;83:1091-1092.
88. Lee SJ, Choe YS, Lee JC, Park BC, Lee WJ, Kim DW. Two cases of Mönckeberg's medial sclerosis on the face. *Ann Dermatol.* 2007;19:31-34.
89. Kim HJ, Greener JS, Javitt MC. Breast calcification due to Mönckeberg medial calcific sclerosis. *Radiographics.* 1999;19:1401-1403.
90. Ferraresi R, Ucci A, Pizzuto A, et al. A novel scoring system for small artery disease and medial arterial calcification is strongly associated with major adverse events in patients with chronic limb-threatening ischemia. *J Endovasc Ther.* 2021;28(2):194-207. <https://doi.org/10.1177/1526602820966309>
91. Liu KH, Chu WC, Kong AP, et al. US assessment of medial arterial calcification: a sensitive marker of diabetes-related microvascular and macrovascular complications. *Radiology.* 2012;265:294-302.
92. Lidbom A. Arteriosclerosis and arterial thrombosis in the lower limb: a roentgenological study. *Acta Radiol Suppl.* 1950;80:1-80.
93. Konijnen LCD, van Overhagen H, Takx RAP, de Jong PA, Veger HTC, Mali WPTM. CT calcification patterns of peripheral arteries in patients with radiologically known peripheral arterial disease. *Eur J Radiol.* 2020;128:108973. <https://doi.org/10.1016/j.ejrad.2020.108973>
94. Greenland P, Blaha MJ, Budoff MJ, Erbel R, Watson KE. Coronary calcium score and cardiovascular risk: JACC state-of-the-art review. *J Am Coll Cardiol.* 2018;72:434-447.
95. Wang X, Matsumura M, Mintz GS, et al. In vivo calcium detection by comparing optical coherence tomography, intravascular ultrasound, and angiography. *J Am Coll Cardiol Img.* 2017;10:869-879.
96. Chen NC, Hsu CY, Chen CL. The strategy to prevent and regress the vascular calcification in dialysis patients. *BioMed Res Int.* 2017;2017:9035193. <https://doi.org/10.1155/2017/9035193>
97. Raggi P, Chertow GM, Torres PU, et al. The ADVANCE study: a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. *Nephrol Dial Transplant.* 2011;26:1327-1339.
98. Jamal SA, Vandermeer B, Raggi P, et al. Effect of calcium-based versus non-calcium-based phosphate binders on mortality in patients with chronic kidney disease: an updated systematic review and meta-analysis. *Lancet.* 2013;382(9900):1268-1277.
99. Neves KR, Gracioli FG, Reis LM, et al. Vascular calcification: contribution of parathyroid hormone in renal failure. *Kidney Int.* 2007;71:1262-1270.
100. Raggi P, James G, Burke SK, et al. Decrease in thoracic vertebral bone attenuation with calcium-based phosphate binders in hemodialysis. *J Bone Miner Res.* 2005;20:764-772.
101. De Vriese AS, Caluwé R, Pyfferoen L, et al. Multicenter randomized controlled trial of vitamin K antagonist replacement by rivaroxaban with or without vitamin K2 in hemodialysis patients with atrial fibrillation: the Valkyrie Study. *J Am Soc Nephrol.* 2020;1:186-196.
102. Leadbetter MR, Kozuka L, Kohler K, et al. Gastrointestinal inhibition of sodium-hydrogen exchanger 3 reduces phosphorus absorption and protects against vascular calcification in CKD. *J Am Soc Nephrol.* 2015;26:1138-1149.
103. Block GA, Rosenbaum DP, Yan A, Chertow GM. Efficacy and safety of tenapanor in patients with hyperphosphatemia receiving maintenance hemodialysis: a randomized phase 3 trial. *J Am Soc Nephrol.* 2019;30:641-652.
104. Rutsch F, Böyer P, Nitschke Y, et al. Hypophosphatemia, hyperphosphaturia, and bisphosphonate treatment are associated with survival beyond infancy in generalized arterial

- calcification of infancy. *Circ Cardiovasc Genet*. 2008;1:133-140.
105. O'Neill C, Lomashvili KA. Recent progress in the treatment of vascular calcification. *Kidney Int*. 2010;78:1232-1239.
106. Nitta K, Ogawa T. Vascular calcification in end-stage renal disease patients. *Contrib Nephrol*. 2015;185:156-167.
107. Kranenburg G, de Jong PA, Bartstra JW, et al. Etidronate for prevention of ectopic mineralization in patients with pseudoxanthoma elasticum. *J Am Coll Cardiol*. 2018;71:1117-1126.
108. Jaffe IZ, Tintut Y, Newfell BG, Demer LL, Mendelsohn ME. Mineralocorticoid receptor activation promotes vascular cell calcification. *Arterioscler Thromb Vasc Biol*. 2007;27:799-805.
109. Lang F, Ritz E, Voelkl J, Alesutan I. Vascular calcification-is aldosterone a culprit? *Nephrol Dial Transplant*. 2013;28:1080-1084.
110. Sakaguchi Y, Hamano T, Obi Y, et al. A randomized trial of magnesium oxide and oral carbon adsorbent for coronary artery calcification in predialysis CKD. *J Am Soc Nephrol*. 2019;30:1073-1085.
111. Bressendorff I, Hansen D, Schou M, Pasch A, Brandt L. The effect of increasing dialysate magnesium on serum calcification propensity in subjects with end stage kidney disease: a randomized, controlled clinical trial. *Clin J Am Soc Nephrol*. 2018;13:1373-1380.
112. Grases F, Costa-Bauza A. Key aspects of myo-inositol hexaphosphate (phytate) and pathological calcifications. *Molecules*. 2019;24:4434. <https://doi.org/10.3390/molecules24244434109>
113. Raggi P, Bellasi A, Bushinsky D, et al. Slowing progression of cardiovascular calcification with SNF472 in patients on hemodialysis: results of a randomized phase 2b study. *Circulation*. 2020;141:728-739.
114. Hedayati SS. A novel treatment for vascular calcification in patients with dialysis-dependent chronic kidney disease: are we there yet? *Circulation*. 2020;141:740-742.
115. Alappan HR, Vasanth P, Manzoor S, O'Neill WC. Vascular calcification slows but does not regress after kidney transplantation. *Kidney Int Rep*. 2020;5(12):2212-2217. <https://doi.org/10.1016/j.ekir.2020.09.039>
116. Mintz GS. Intravascular imaging of coronary calcification and its clinical implications. *J Am Coll Cardiol Img*. 2015;8:461-471.
117. Madhavan MV, Tarigopula M, Mintz GS, Maehara A, Stone GW, Généreux P. Coronary artery calcification: pathogenesis and prognostic implications. *J Am Coll Cardiol*. 2014;63:1703-1714.
118. Dini CS, Tomberli B, Mattesini A, et al. Intra-vascular lithotripsy for calcific coronary and peripheral artery stenoses. *EuroIntervention*. 2019;15:714-721.
119. Schanstra JP, Luong TTD, Makridakis M, et al. Systems biology identifies cytosolic PLA2 as a target in vascular calcification treatment. *JCI Insight*. 2019;4(10):e125638. <https://doi.org/10.1172/jci.insight.125638>
120. Stranneheim H, Wedell A. Exome and genome sequencing: a revolution for the discovery and diagnosis of monogenic disorders. *J Intern Med*. 2016;279:3-15.
121. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet*. 2015;47:717-726.
122. Malhotra R, Mauer AC, Lino Cardenas CL, et al. HDAC9 is implicated in atherosclerotic aortic calcification and affects vascular smooth muscle cell phenotype. *Nat Genet*. 2019;51:1580-1587.
123. International Mouse Knockout Consortium; Collins FS, Rossant J, Wurst W. A mouse for all reasons. *Cell*. 2007;128:9-13.
124. Rucher G, Cameliere L, Fendri J, et al. Molecular imaging of endothelial activation and mineralization in a mouse model of accelerated atherosclerosis. *EJNMMI Res*. 2019;9:80. <https://doi.org/10.1186/s13550-019-0550-5>
125. Sinha S, Santoro MM. New models to study vascular mural cell embryonic origin: implications in vascular diseases. *Cardiovasc Res*. 2018;114:481-491.
126. Herrington DM, Mao C, Parker SJ, et al. Proteomic architecture of human coronary and aortic atherosclerosis. *Circulation*. 2018;137:2741-2756.
127. Chen X, Liu L, Palacios G, et al. Plasma metabolomics reveals biomarkers of the atherosclerosis. *J Sep Sci*. 2010;33:2776-2783.
128. Williams JW, Winkels H, Durant CP, Zaitsev K, Ghosheh Y, Ley K. Single cell RNA sequencing in atherosclerosis research. *Circ Res*. 2020;126:1112-1126.
129. Gomes CPC, Schroen B, Kuster GM, et al. EU-CardioRNA COST Action (CA17129). Regulatory RNAs in heart failure. *Circulation*. 2020;141:313-328.
130. Zhang Z, Salisbury D, Sallam T. Long non-coding RNAs in atherosclerosis: JACC review topic of the week. *J Am Coll Cardiol*. 2018;72:2380-2390.
131. Jeong G, Kwon DH, Shin S, et al. Long non-coding RNAs in vascular smooth muscle cells regulate vascular calcification. *Sci Rep*. 2019;9:5848.
132. Lamb J, Crawford ED, Peck D, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science*. 2006;313:1929-1935.
133. Song JS, Wang RS, Leopold JA, Loscalzo J. Network determinants of cardiovascular calcification and repositioned drug treatments. *FASEB J*. 2020;34(8):11087-11100. <https://doi.org/10.1096/fj.202001062R>
134. Fernandez DM, Rahman AH, Fernandez NF, et al. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med*. 2019;25:1576-1588.
135. Depuydt MAC, Prange KHM, Slenders L, et al. Microanatomy of the human atherosclerotic plaque by single-cell transcriptomics. *Circ Res*. 2020;127(11):1437-1455.
136. Liu M, Gomez D. Smooth muscle cell phenotypic diversity. *Arterioscler Thromb Vasc Biol*. 2019;39:1715-1723.
137. Schlotter F, Halu A, Goto S, et al. Spatiotemporal multi-omics mapping generates a molecular atlas of the aortic valve and reveals networks driving disease. *Circulation*. 2018;138:377-393.
138. Zhang M, Sheffield T, Zhan X, et al. Spatial molecular profiling: platforms, applications and analysis tools. *Brief Bioinform*. 2021;22(3):bbaa145. <https://doi.org/10.1093/bib/bbaa145>
139. Asp M, Bergenstr hle J, Lundeberg J. Spatially resolved transcriptomes-next generation tools for tissue exploration. *Bioessays*. 2020;42(10):e1900221.
140. Epple M, Lanzer P. How much interdisciplinaryity is required to understand vascular calcifications? Formulation of four basic principles of vascular calcification. *Z Kardiol*. 2001;90(Suppl 3):2-5.
141. Gourgas O, Marulanda J, Zhang P, Murshed M, Cerruti M. Multidisciplinary approach to understand medial arterial calcification. *Arterioscler Thromb Vasc Biol*. 2018;38:363-372.

KEY WORDS atherosclerosis, chronic limb-threatening ischemia, genetics, medial arterial calcification, omics, peripheral artery disease, vascular calcification

APPENDIX For supplemental appendices and tables, please see the online version of this paper.