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PERSPECTIVES

Vascular Calcification: A Perspective On An Imminent Disease Epidemic

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Abstract

Westernized societies are on the verge of an epidemic of vascular calcification. Aging, metabolic syndrome and type II diabetes (T2DM), and chronic kidney disease increasingly characterize our populace – and promote valve and arterial calcium deposition. Epidemiology, histopathology, and pathobiology define at least 5 distinct and prevalent types of vascular calcification: calcific aortic stenosis (CAS), medial artery calcification, atherosclerotic intimal calcification, vascular calcification of chronic kidney disease, and calcific uremic arteriolopathy. The clinical impact of these 5 mineralization disorders is highly significant. For example, 2%-3% of individuals ≥ 65 years old have CAS, and require surgery to prevent precocious death and to improve quality of life. Valve calcium load is the best predictor of clinical progression; if unaddressed, interactions between longevity and T2DM prevalence will increase the future incidence of CAS. Reduced vascular compliance – arising in part from arterial calcification – impairs macrovascular Windkessel function and normal tissue perfusion; thus, medial artery calcification of T2DM conveys a 3-fold increased risk for lower extremity amputation, a debilitating and costly outcome. Once considered a passive process only, data from laboratories world-wide have now established that vascular calcification is an actively regulated type of biomineralization. As in bone, morphogenetic, mechanical, metabolic, endocrine, and inflammatory signals control vascular mineral deposition. Molecular and cellular mechanisms familiar to bone biologists are activated with disease initiation and progression. This *Perspective* briefly recounts the pathobiology of vascular calcification and conveys the urgent need to pursue the basic, translational, and outcomes-based research necessary to address an imminent epidemic of abnormal vascular calcium metabolism.
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Introduction

Westernized societies are on the verge of an epidemic of vascular calcification, a disease of disordered mineral metabolism (1). Aging, declining renal function, and the dysmetabolic milieu of type II diabetes (T2DM) are increasingly common characteristics of our populace – and these factors interact to promote aortic valve and arterial calcium deposition (2;3). Vascular calcification has been afflicting humans for least 50 centuries; computed tomographic (CT) analysis of Ötzi, the ice mummy discovered in the Tyrolean Alps, identified the presence of atherosclerotic calcification in this middle-aged man who died from homicide around 3000 B.C. (4). In the 1860s, Virchow first recognized that this form of calcified tissue metabolism exhibited features resembling skeletal ossification – and hence the "sclerosis" of "atherosclerosis" (5). Only in the past two decades has the heterogeneity of vascular calcium deposition become appreciated, largely due to enabling advances made in skeletal biology (6). Those well-versed in vertebrate development know that membranous ossification, endochondral ossification, odontogenesis, cementogenesis, and amelogenic biomineralization occur via overlapping yet distinct mechanisms (7-9). For example, membranous ossification does not require vascular invasion and osseous replacement of a precedent calcified cartilaginous template; this non-endochondral ossification mechanism absolutely requires functionally redundant programs activated by the homeodomain transcription factors Msx2 and Msx1 to direct normal craniofacial bone and tooth biomineralization (10). In contrast,

endochondral ossification organizes biomineralization around a cartilaginous template that first calcifies and then remodels to form lamellar bone; this process absolutely requires Runx2/Cbfa1 for both vascularization and matrix mineralization (11). HIF-α signaling appears more critical for endochondral bone than membranous bone formation (12). Signals provided by the zinc finger protein osterix (Osx) and the transcriptional co-adapter β-catenin globally control osteogenic differentiation and matrix mineralization (13). *In vivo*, Cbfa1/Runx2 is necessary for robust membranous ossification (11). However, *ex vivo* activation of Msx/Dlx gene regulatory programs by BMP2 (11) or forced expression of Dlx3 (14) can initiate early osteogenic differentiation – including induction of alkaline phosphatase enzyme activity (11) – in osteoprogenitors lacking Runx2. The mechanistic plurality of biomineralization, evident from studies of orthotopic calcified tissue formation, is also germane to the mechanistic underpinnings of vascular mineral deposition. Once considered a passive process, data from multiple laboratories world-wide has established that vascular calcification is in fact an actively regulated form of tissue biomineralization (1;15;16). Molecular and cellular mechanisms familiar to bone biologists (9) are activated with disease initiation and progression (1;3).

In this brief *Perspective*, the Venn diagram of osteogenic molecular biology (9) is merged with the histopathology, anatomy, and epidemiology of arterial calcification to highlight 5 clinically troublesome forms of vascular biomineralization (Table 1). More extensive discussion of vascular calcification and its pathobiology can be obtained from recent reviews (1;3;17-20). The primary goal of this manuscript is to convey the urgent need to pursue the basic, translational, and outcomes-based research necessary to address the imminent epidemic of abnormal vascular calcium metabolism.

Aortic Valve Calcification (AVC) and Calcific Aortic Stenosis (CAS)

The most robust relationship between vascular calcium load and disease pathophysiology is evident in aortic valve calcification (AVC) and its most severe form, calcific aortic stenosis (CAS) (17). The three leaflets of the normal aortic valve must be mechanically strong but also compliant (20). During systole, leaflets must flexibly bend to permit unimpeded ejection of oxygenated blood from the left ventricle into the aorta and arterial circulation. During diastole, the valve leaflets must flexibly coapt to prevent aortic regurgitation (the retrograde flow of blood from the aorta back into the left ventricle). Valve calcification impairs valve flexibility, first impeding ventricular output and subsequently generating aortic regurgitation (17). Both processes not only impair distal tissue perfusion, but increase the myocardial workload that induces ventricular hypertrophy and cardiomyopathy (17). CAS is prevalent in those ≥ 65 years of age; 25% have AVC, and 2-3% of individuals have significant CAS as quantified by echocardiography, with 80% progressing to symptoms requiring surgery to prevent death from congestive heart failure or arrhythmia (17). Rosenhek and colleagues first established that aortic valve calcium load was the single best predictor of clinical progression of CAS in initially asymptomatic but severe aortic sclerosis identified by echocardiography (21).

In a classic cross-sectional study, Otto and colleagues defined a sequence of histopathological changes during initiation and progression of aortic valve "degenerative" sclerosis (22). Bounded by endothelium on both aortic and ventricular faces, three layers (laminae) characterize the aortic valve: the collagen-rich aortic fibrosa, the interstitial spongiosa, and the elastin-rich ventricularis (20). Subendothelial thickening and fatty expansion with inflammation of the lamina fibrosa were initially observed, notably characterized by intracellular and extracellular lipid accumulation. Stippled calcification in this fibro-fatty material often paralleled collagen fibrils of the fibrosus as the base of lesions, *i.e.*, towards the fibrosa-spongiosa junction (22). Displacement and re-duplication of the valve intimal elastic lamina also occurs, indicating elastinolysis and matrix remodeling (22). Advanced

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TYPE	CLINICAL SETTING & SOME DEFINING CHARACTERISTICS	RISK FACTORS	HISTOPATHOLOGY & UNIQUE SERUM BIOCHEMISTRY	PATHOBIOLOGY	REF.
Aortic valve calcification (AVC)	• Calcification of the aortic valve leaflets. • With progression to Calcific Aortic Stenosis (CAS), causes increased afterload, variable regurgitation, left ventricular hypertrophy, congestive heart failure, syncope, and sudden cardiac death.	• Advanced age. • Bicuspid aortic valve. • Type II diabetes. • Metabolic syndrome parameters*. • Hypercholesterol-emia.	• Fibro- fatty expansion and inflammation of the lamina fibrosa. Intracellular and extracellular lipid accumulation. • Displaced and/or split elastic lamina. • Woven bone with marrow elements seen in 13% of specimens. • Nodular amorphous calcium phosphate also accumulates via epitaxial deposition.	• Mixed picture, both membranous and endochondral programs elaborated by valve interstitial myofibroblasts that undergo osteo/chondrogenic differentiation. • Wnt3a/LRP5/beta-catenin osteogenic programs activated. Msx2, Runx2, and Sox9 expressed in valve myofibroblasts.	17, 20, 25, 26, 32,
Medial Artery Calcification (AMC)	• Calcification of the tunica media of large and medium-sized arteries. • Reduced vascular compliance (arteriosclerosis) with calcification, and impaired Windkessel physiology. • Increased pulse-pressure. • Increased afterload. • Increased lower extremity amputation, MI, stroke risk.	• Type II diabetes. • Metabolic syndrome parameters*. • Advanced age.	• Circumferential calcium deposition in the arterial tunica media. Contiguous. • Adventitial inflammation. • Adventitial fibro-fatty expansion. • Elevated lipid markers of oxidative stress such as 8-F2alpha-Isoprostanes. • Elevated circulating OPG indicates low grade vascular inflammation. • Matrix vesicle mediated calcification by electron microscopy.	• Activation of BMP2/Msx2/Wnt "membranous" ossification programs. Adventitial-medial Wnt (Wnt3a, Wnt7a) signaling initiated in adventitial myofibroblasts directs osteogenic differentiation of CVCs in the tunica media. • Dependent upon beta-catenin and induction of alkaline phosphatase. • Entrained to adventitial TNF-alpha & vascular inflammatory redox signaling.	1, 15, 33, 35 40, 43
Atherosclerotic intimal calcification (AIC)	• Atherosclerotic calcification. • Increased risk of acute thrombotic occlusion, acute coronary syndrome. • Increased myocardial infarction, stroke risk. • Reduced vascular compliance (arteriosclerosis) due to fibrosis and calcification-induced impedance mismatch.	• Hypercholester-olemia. • Hypertension. • Type I diabetes.	• Eccentric lumen-deforming type Vb atherosclerotic plaque. Patchy. • Several calcification mechanisms. - Lipid core calcification - Fibrous calcification / apoptotic bodies - Endochondral ossification with matrix vesicles. • Vessel remodeling, elastinolysis. • Increased oxidized LDL.	• Mostly an "endochondral" ossification picture. BMP2 expressed in CVCs (a mural pericytic myofibroblast). • Runx2, Sox9 > Msx2 gene expression programs upregulated. • TNF-alpha and oxidized LDL promote release of osteogenic signaling from macrophages.	1, 47, 71-74
Vascular calcification of Chronic Kidney Disease	• Patients on hemodialysis or peritoneal dialysis (CKD5), or CKD prior to dialysis (CKD4, GFR < 30 cc/min but >15 cc/min). • Approximately 40% of patients with CKD5 have diabetes as the underlying cause of end-stage renal disease. • In general, prior to CKD4 of CKD5, some form of vascular calcification will have already initiated due to diabetes, or dyslipidemia, or hypertension. CKD dramatically worsens vascular disease. • At any level of CKD, patients with diabetes have greater vascular calcium loads.	• Any of the above. • End-stage kidney disease / CKD5. • Renal osteodystroph -Low-turnover bone disease. -High turnover bone disease. • Hyperphosphate-mia /phosphate retention. •Excessive calcium-based phosphate binders. • Excessive calcitriol, PTH suppression.	• All of the above can be present. • AMC initially predominates if type II diabetes is the etiology of CKD. • Hyperphosphatemia & hypercalcemia. • Reduced circulating serum pyrophosphate and fetuin. • Severe hyperparathyroidism or hypoparathyroidism. • Reduced circulating BMP7/BMP2 ratio.	• Enhanced vascular smooth muscle cell matrix vesicle production due to elevated serum calcium and phosphate. • Runx2/Cbfa1 osteogenic programs also activated in vascular smooth muscle cells by elevated serum calcium and phosphate, signaled via Pit1. • Loss of skeletal mineral deposition to "buffer" calcium phosphate transients worsens inflammation and vascular calcium load.	19, 78-82
Calcific uremic arteriolopathy (CUA) a.k.a. "calciphylaxis"	• Mercifully less common. Starts as violaceous painful nodules, evolve to dermal necrosis. • Most often in lower extremity – thigh, groin. Untreated, mortality approaches 100% within the year following presentation.	• CKD4 or CKD5. • Coumadin anti-coagulant therapy. • Type II diabetes. • Very severe hyperparathyroid-ism.	• Arteriolar (~ 0.6 mm diameter) medial calcification of dermal > pulmonary and mesenteric vessels. • Fibroproliferative occlusion, secondary skin and dermal fat necrosis. • Peri-arteriolar inflammation and BMP4 expression. • Reduced Gla-modified MGP. • Greatly elevated CRP and ESR.	• Few studies, but BMP4 expressed in peri-arteriolar dermal tissue. • Loss of MGP-dependent (a) internalization of vascular smooth muscle cell matrix vesicles; and (b) inhibition of BMP4-dependent alkaline phosphatase induction.	86-89

• *Per NHLBI -modified ATPIII: Fasting blood glucose >100 mg/dL, fasting triglycerides > 150 mg/dL, hypertension, viz. blood pressure >130/85, HDL < 50 mg/dL in man or HDL < 40 mg/dL woman, waist circumference > 40 inches in man or >35 inches in woman indicative of abdominal obesity.

• Abbreviations: BMP, bone morphogenetic protein; CKD, chronic kidney disease; CRP, C-reactive protein; CVC, calcifying vascular cell of Demer; ESR, erythrocyte sedimentation rate; GFR, glomerular filtration rate. Gla, gamma-carboxy glutamic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; LRP, LDL receptor related protein; MGP, matrix Gla Protein; OPG, osteoprogerin; Pit1, Sodium Phosphate co-transporter, a.k.a. SLC20A1, Glvr1.

Table 1. Clinically significant types of vascular calcification.

calcified nodules also contain apatitic calcium phosphate and lipid with much less collagen accumulation (17). The work of Giachelli *et al*. (23), Fitzpatrick *et al*. (24), and Rajamannan *et al*. (25) identified the molecular signature of active osteogenic programs in all calcifying human aortic valve specimens. Recent data have pointed to the importance of canonical Wnt signals. Expression and activation of Wnt3a-LRP5 osteogenic cascades – indicated by the valvular accumulation of β-catenin protein in concert with Wnt3a-LRP5 upregulation – is significantly induced in calcifying aortic valve specimens as compared to non-diseased human aortic valves (26). The osteo/chrondogenic transcription factor Runx2 is co-expressed (26), as is the osteoblast homeoprotein Msx2 (our unpublished observations, and see below). The temporospatial patterns of β-catenin, Runx2, Msx2, and alkaline phosphatase protein expression during the initiation and progression phases of *human* calcific aortic valve disease have not been thoroughly investigated (26).

By histopathology, ectopic woven bone formation containing hematopoietic marrow elements is seen in ~13% of calcifying aortic valves (27). However, unlike other forms of vascular calcification, largely acellular nodules of amorphous calcium phosphate also form with CAS (22). The genesis of these valvular concretions is not well understood, but is presumed to arise from passive epitaxial growth of mineral with phospholipids from the actively regulated early phases of mineral deposition (27). This latter feature may help explain why later implementation of aggressive lipid-lowering and anti-inflammatory statin therapy has little impact on aortic valvular disease once initiated (28), while earlier aggressive intervention may help retard disease progression (29).

At the moment, no medical therapy has been established to prevent CAS or induce disease regression (17;20) – although early implementation of aggressive and potent statin therapy holds promise (29). Serial echocardiographic assessment with surgical management at the stage of critical CAS or with symptoms is the current mainstay of therapy. (17) Pre-clinical animal models of CAS such as those recently developed by Heistad *et al*. (30) are crucial to identifying the most important disease components to target (see below) to prevent cardiovascular compromise. Moreover, the elegant optical imaging techniques characterized by Aikawa and colleagues (31) can now spatially localize the elaboration of active osteogenic programs and mineral deposition during disease initiation and progression in such models.

Recently, the Multiethnic Study of Atherosclerosis (MESA) has provided insights that may prove useful for designing future strategies to medically prevent and treat CAS (32). O'Brien and colleagues have identified that, after adjusting for confounding factors, aortic valve calcification risk progressively increases with accrual of the 5 metabolic syndrome parameters (32). Hyperglycemia, abdominal obesity, hypertriglyceridemia, hypertension, and low HDL levels increase aortic valve calcification risk in a step-wise fashion, with the greatest risk observed in those individuals with overt type II diabetes (T2DM) (32). Thus, targeting these characteristics along the metabolic syndrome–T2DM continuum may hold promise for reducing the future burden of clinically significant CAS (32). At a minimum, MESA points to an impending increase in CAS. With our progressively aging and obese society, the percentage of the population ≥ 65 with diabetes will continue to increase from the current 18% figure, and these factors are predicted to increase the future prevalence of CAS (2;17).

Medial Artery Calcification (AMC)

The medial artery calcification of T2DM (Fig. 1) has emerged as an important predictor of foot amputation (33). Unlike atherosclerotic calcification (see below), AMC circumferentially and contiguously afflicts the muscular medial layer of arteries (Fig. 2). Of note, the CT-based methodologies routinely used to detect and quantify vascular calcium load cannot discriminate between AMC and atherosclerotic intimal calcification (AIC) (18); high quality plain radiographs or histopathology are currently required for this categorization (33;34). Using femoral artery

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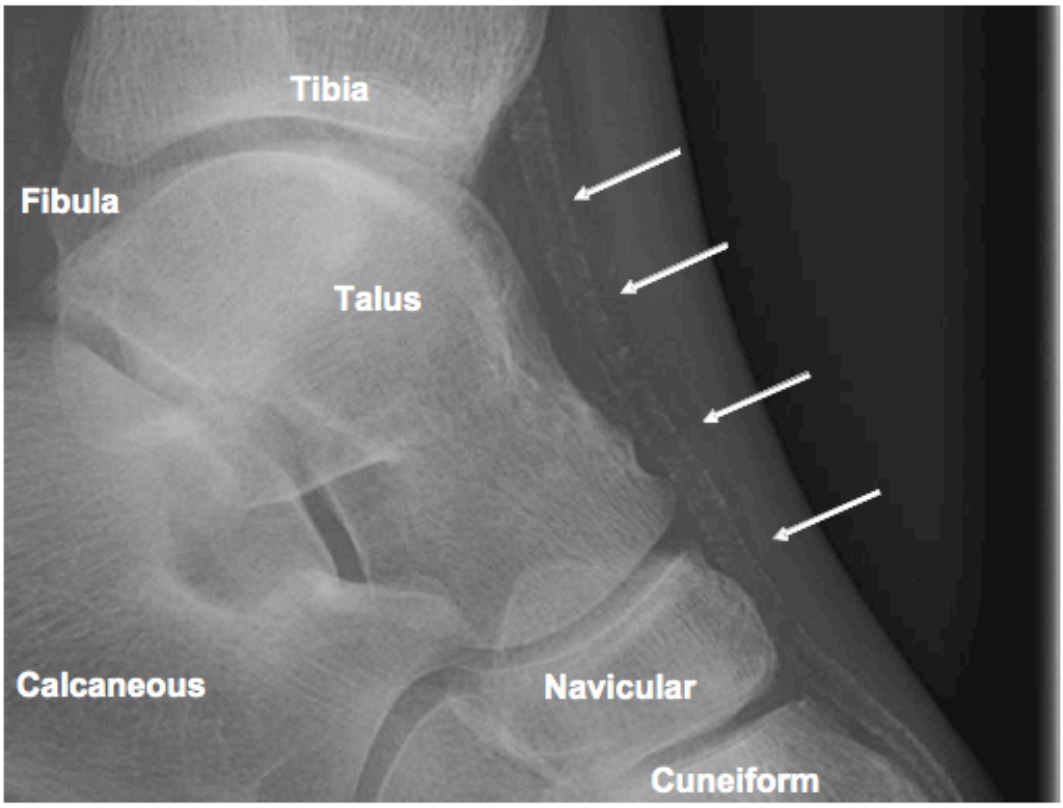

Fig. 1. Medial artery calcification (AMC). This lateral foot X-ray of a man with type II diabetes reveals the presence of medial artery calcification of the dorsalis pedis artery (arrows). Lower extremity AMC was bilateral, and femoral and popliteal artery medial calcification was also present (not shown).

radiographs, studies from Lehto *et al*. revealed that AMC – not AIC – presaged a 3-fold increased risk for lower extremity amputation (33). Similar results in a smaller study of Pima Indians also demonstrated this risk (35). These data begin to highlight that biological and histoanatomic differences in arterial calcification translate into meaningful differences in clinical disease.

It is hard to overstate the significance of lower extremity amputation in T2DM. Recently, the United Kingdom Prospective Diabetes Study showed that the costs of lower extremity amputation were equivalent to the combined costs of fatal and non-fatal MI or fatal and non-fatal stroke (36) – over three times the costs of managing congestive heart failure in the setting of T2DM (Figure 3). The global burden of diabetic foot disease is heavy, and the annual costs of treating diabetic lower extremity ulcer with or without amputation (37) are beginning to approximate the annual costs of osteoporotic fractures in Westernized societies (38). Once again, the burgeoning population of aging patients with T2DM is predicted to augment the prevalence of AMC, and the attendant lower extremity amputation risk (33;35). Along with osteoarthritis and osteoporosis, diabetic foot disease will increasingly demand the attention of those physicians and scientists dedicated to addressing unmet needs in musculoskeletal medicine (39).

The pathobiology of medial calcification in T2DM is emerging, with a better understanding of disease afforded via detailed study of diet-induced animal models (3;40). High fat diets typical of Westernized societies (42% of calories from fat, 0.15% cholesterol by mass) cause obesity, type II diabetes, hypertriglyceridemia and hypercholesterolemia in male *Ldlr*(-/-) mice – with concomitant induction of both aortic valve and aortic medial calcification (3;40;41). With protracted high fat feeding,

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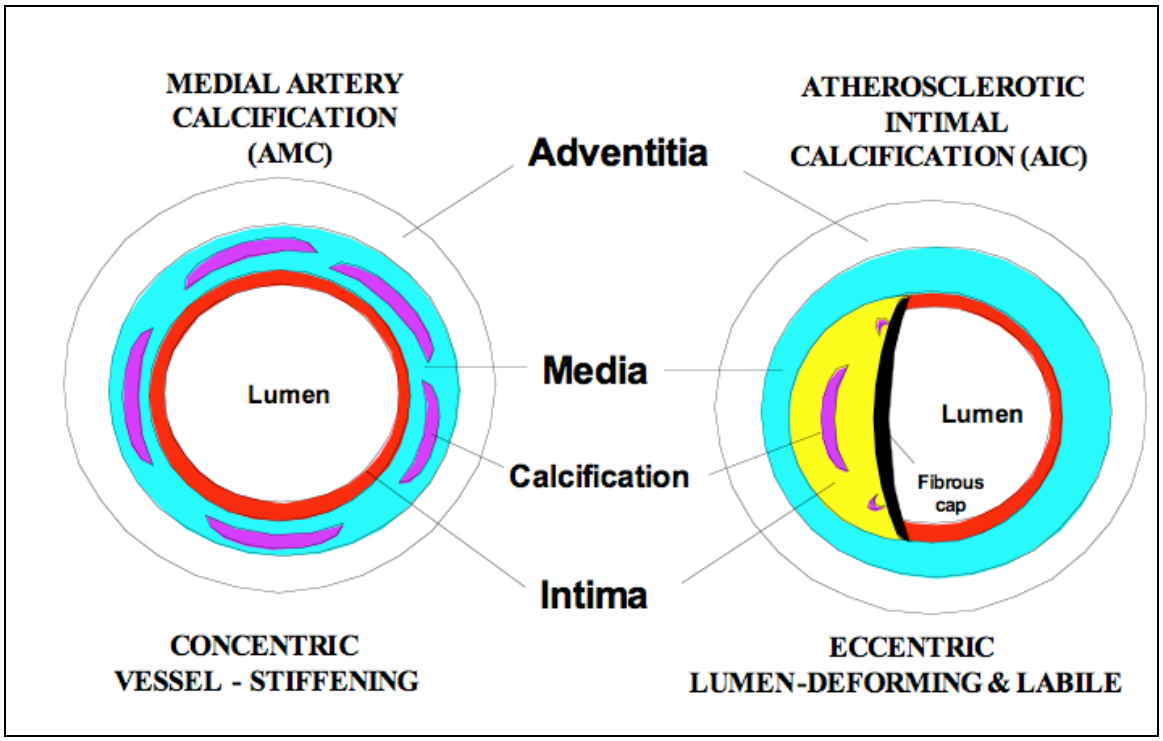

Fig. 2. Medial artery calcification (AMC) and atherosclerotic intimal calcification (AIC). The differences in the spatial distribution of vascular calcification observed by histology in cross-sections of arteries afflicted with AMC (left panel) or AIC (right panel) are depicted – emphasizing the concentric, vessel-stiffening nature of AMC vs. the eccentric, lumen-deforming nature of AIC. Acute atherothrombosis occurs in the setting of AIC. See text for details.

atherosclerosis subsequently evolves in this model, as does the accrual of atherosclerotic calcium deposition that is *preceded* by medial artery calcification (40). We identified that at the very earliest stages of disease, two molecules, Msx2 and osteopontin (OPN), were upregulated in aortic tissues in response to high fat diabetogenic diets (42). *In situ* hybridization and immunohistochemistry localized expression of both Msx2 – an osteogenic transcription factor – and OPN – a matrix cytokine – in myofibroblasts of the fibro-fatty periaortic adventitia at the stage of disease initiation (42;43). Similarly, expression of both genes was induced in aortic valve interstitial myofibroblasts. In addition, accumulating macrophages throughout the arterial vasculature elaborate OPN expression. Like the fibro-fatty expansion and inflammation observed in the aortic valve fibrosa and spongiosa mentioned above (22), fatty expansion and inflammation of the adventitia figures prominently in diabetic vascular disease (3;44-46) – and likely contributes to the concentric nature of arterial involvement (Fig. 2).

Studies of the osteogenic actions of Msx2 identified that Msx2-expressing adventitial myofibroblasts elaborate an osteogenic Wnt milieu (40;43); Wnt3a and Wnt7a/b production by these vascular myofibroblasts induces alkaline phosphatase (ALP) expression and β-catenin-regulated osteogenic mineralization via calcifying vascular cells (CVCs) (43). (CVCs are the multipotent pericytic myofibroblast of the tunica media (47)). Paracrine Wnt signaling between these vascular layers presumably occurs via the vasa vasorum, a specialized microvasculature generated in the relatively hypoxic environment of the adventitia that circumferentially provides oxygenation and nutrient supply to the outer tunica media (3;46;48). Of note, the activation of aortic Wnt-β-catenin signaling by diabetogenic diets in the *Ldlr*(-/-) mouse (40;43) is in good agreement with the more recent and important data of (a) Nalini Rajamannan,

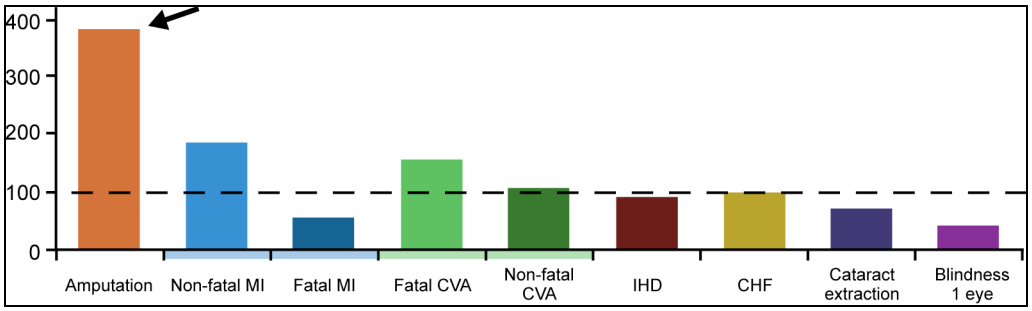

Fig. 3. Relative costs of low extremity amputation as a consequence of type II diabetes. The relative costs of treating important complications of type II diabetes is presented as a percent of the cost for treating CHF (congestive heart failure in this disease). Data are derived from Clarke *et al*. (36). Note that the costs of lower extremity amputation are greater than the combined costs of treating either MI (myocardial infarction) or stroke (CVA). IHD, ischemic heart disease. Of note, AMC in type II diabetes conveys a strong risk for lower extremity amputation (33;35;49).

demonstrating activated Wnt3a-β-catenin signaling in calcifying human aortic valves (17;26), and (b) results from MESA that identify metabolic syndrome parameters as key contributors to human aortic valve calcification (20) as well as medial calcification (33;35;49;50). Of note, Msx2 expression has now been identified in calcifying arterial specimens from patients, many of whom were diabetic (15).

What are the proximal signals that control vascular expression of Msx2 and OPN in T2DM? The "diabesity" on the metabolic syndrome–T2DM continuum promotes a low-grade systemic inflammation as well as inflammation in the fibrofatty arterial adventitia (3;45;51). TNF-α is the prototypic inflammatory cytokine mediating this inflammatory response (52). Pharmacologic and genetic studies have now demonstrated that diabetes-induced TNF-α signals are necessary and sufficient to activate osteogenic Msx2-Wnt signaling in aortas of the *Ldlr*(-/-) mouse (40). NADPH oxidase (Nox)-dependent hydrogen peroxide generation conveys the TNF-α-generated signals that induce Msx2-Wnt signaling (53). The specific Nox isoforms mediating this induction during adventitial-medial signaling have yet to be determined.

OPN expression is also upregulated by TNF-α in the arterial wall (40;54). However, hyperglycemia directly induces vascular OPN expression (55), and OPN is required for the diabetes-induced arterial oxidative stress elaborated in the *Ldlr*(-/-) mouse (54). Although intact OPN inhibits calcification and in fact promotes ectopic calcium egress (56), the cleaved form of vascular OPN is pro-inflammatory (57). Indeed, vascular OPN enhances MMP-dependent matrix remodeling (58), cardiac fibrosis (59), and myofibroblast differentiation (60) – key regulatory components that promote vascular calcification (1). Thus several groups, including our own, are now investigating the contributions of OPN in the AMC of diabetes and hyperglycemia vs. the AIC of atherosclerosis and hypercholesterolemia.

Of note, the extent of medial calcification in the diabetic foot significantly correlates with sympathetic vasomotor abnormalities in the upper extremity (61) as well as the lower extremity (49;62). The inability to regionally control blood flow in the lower extremity gives rise to shunt and tissue hypoxia, both of which track well with medial artery calcification and peripheral neuropathy (49) and thus amputation risk (33;35;49;63). Autonomic innervation of resistance arteries is provided via the tunica adventitia. The relative contributions of medial calcification and adventitial inflammation – with concomitant reductions in vasomotor responses – vs. the loss of autonomic innervation to generate the hypoxic "shunt" causing diabetic foot lesions has yet to be determined (50).

As occurs in the vast majority of patients afflicted with T2DM, the vascular calcification in the *Ldlr*(-/-) mouse model arises in response to the relevant stimuli of diet-induced diabetes, obesity, and dyslipidemia. However, vascular calcification

afflicting the tunica media can also occur in other murine models. In the apoE-deficient mouse, aortic chondroid metaplasia, calcification and atherosclerosis are induced by fatty diets in the absence of obesity, diabetes or other metabolic syndrome parameters (64). Genetic deficiencies of FGF23 (65) and OPG (66) impact phosphate homeostasis, and RANKL-dependent bone turnover and vascular inflammation, respectively (1); severe arterial calcification occurs spontaneously in both of these models. Massive arterial chondroid metaplasia also occurs with genetic deficiency in either MGP (67) or ENPP1 (68), key vascular defenses that inhibit mineralization. Moreover, warfarin treatment and vitamin D intoxication also induce extensive elastin-based medial artery calcium deposition (69;70). The relationship between mechanisms of medial artery calcification of type II diabetes vs. medial calcification induced by clinically less prevalent toxic or genetic insults remains unknown. Medial calcification observed in CKD and CUA is briefly discussed below (19).

Atherosclerotic Intimal Calcification (AIC)

The most familiar form of vascular calcification, atherosclerotic intimal calcification (AIC), is well-appreciated and has dominated the attention of most individuals interested in vascular disease. This intimally-oriented patchy calcification characterizes the lumen deforming, eccentric type Vb atherosclerotic plaque (Fig. 2); however, with the progression accentuated by uncontrolled dyslipidemia, hypertension, or uremia (Fig. 4 and see above), AIC can coalesce to extensively afflict all muscular arteries, including the coronary vascular tree. As first shown by Demer and colleagues (71) and Shanahan *et al*. (15), BMP2-dependent osteo/chondrogenic transcriptional mechanisms are activated during atherosclerotic calcification that contribute to vascular calcium load. Endochondral ossification mechanisms driven by Sox9 and Runx2/Cbfa1 transcription factors predominate in AIC osteogenic mechanisms, although Msx2 expression has been identified in arterial specimens that include samples from diabetic patients (15). Demer, Parhami, Tintut *et al*. showed that oxysterols derived from LDL oxidation provide the proximal signal that activates Runx2/Cbfa1 transcriptional processes, upregulates alkaline phosphatase expression, and promotes endochrondral ossification programs (72-74). As discussed below, the hypercalcemia and hyperphosphatemia of CKD also induce Runx2/Cbfa1 vascular mineralization programs.

The Armed Forces Institute of Pathology (AFIP) identified several types of calcium deposition within atherosclerotic plaques, including lipid core calcification, fibrous calcification, and endochondral ossification (75). With more advanced lesions, endochondral ossification can generate ectopic vascular bone replete with hematopoietic elements (1). Detailed clinical correlation with histopathology by the AFIP identified that both lipid core calcification and fibrous cap calcification are associated with positive remodeling lesions (75) that are at high risk for acute rupture and acute coronary events (76).

At this point, it should be highlighted that vascular ossification is not equivalent to vascular calcification – even though both processes accrue calcium deposition via osteogenic signaling mechanisms. The specific effect of vascular ossification vs. AIC on cardiovascular disease is not known. In a very recent and excellent overview, Cathy Shanahan has noted that, although macrovascular compliance becomes progressively impaired, the risks of *acute* vascular embarrassment conveyed by microcalcifications early on may in fact diminish with progressive calcium accumulation (16). Until non-invasive methods are available to characterize AIC micro- vs AIC macro-calcification vs. vascular ossification – and prospectively ascertain the risk of acute arterial occlusion events in afflicted vascular beds – clinical correlates and patient management will remain equivocal. As certain technological hurdles are overcome, novel and sensitive optical imaging agents derived from calcium-binding bisphosphonates may prove useful for identifying vascular micro-calcifications (31;77).

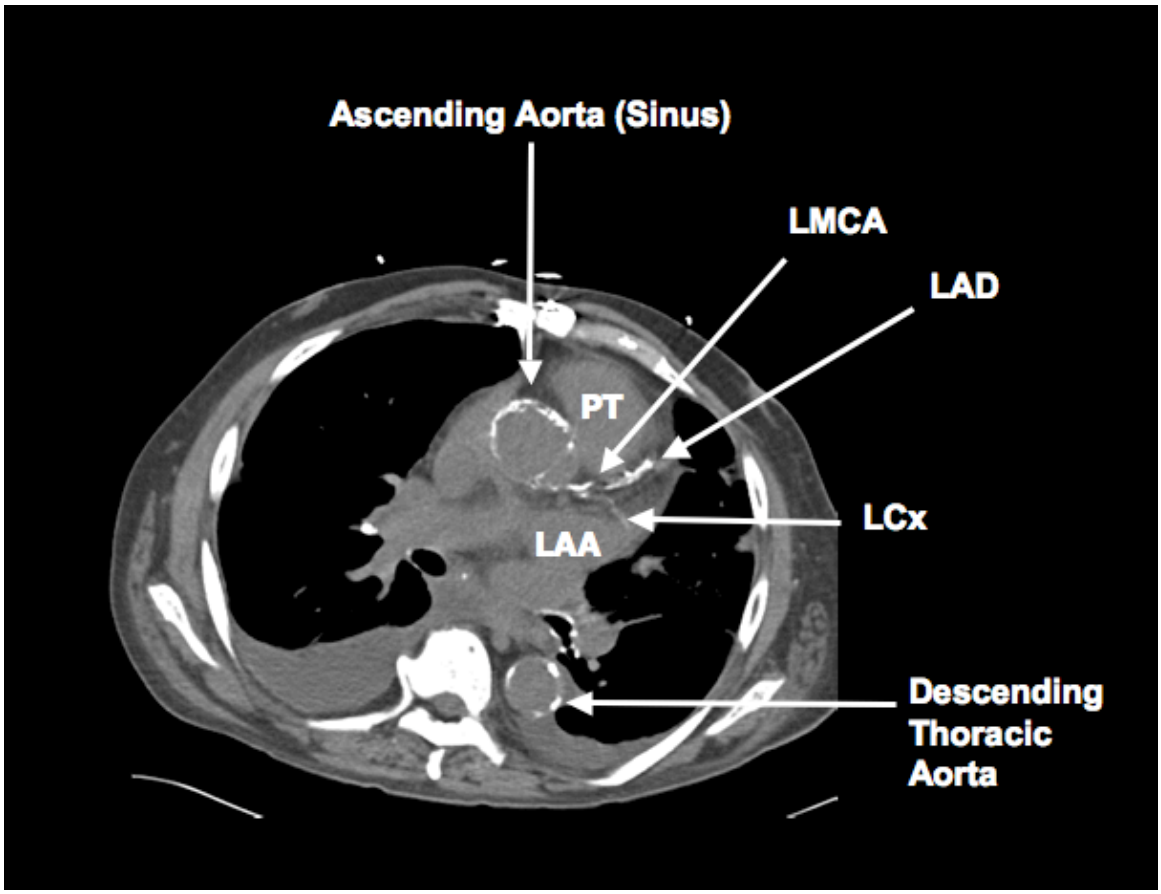

Fig. 4. Atherosclerotic calcification (AIC) of the coronary arteries and the aorta visualized on cross section by chest CT. Notice the eccentric nature of calcification in the ascending aorta at the level of the aortic sinus, and the patchy nature of calcium deposition. Also note that the extensive disease in the coronary arteries begins to coalesce, and cannot unambiguously be categorized as AIC vs. AMC. LAA, left atrial appendage. LAD, left anterior descending coronary artery. LCx, left circumflex coronary artery. LMCA, left main coronary artery. PT, pulmonary trunk.

Vascular Calcification of Chronic Kidney Disease

The dysmetabolic milieu of chronic kidney disease provides the "perfect storm" for vascular calcium accumulation (19). First, as indicated in Table 1, vascular calcification – AMC, AIC, or AVC – has already initiated prior to development of CKD4 or CKD5, when phosphate retention and aberrant bone turnover begin to worsen vascular calcium load. Currently, type II diabetes (40%) and hypertension (25%) account for two-thirds of the cause for dialysis–dependent CKD5 (19). At any level of chronic kidney insufficiency, patients with diabetes have more extensive vascular calcification. The relative distribution of AMC vs. AIC has not been systematically studied with CKD prior to dialysis. However, in patients on dialysis, Gerard London has demonstrated that patients with either AMC or AIC have increased cardiovascular mortality as compared to those that lacked significant calcium load (34). Mortality with AIC was greater than that of AMC (34), again highlighting that biological and histoanatomic differences in arterial calcification translate into meaningful differences in disease outcomes – and, most likely, clinical management strategies.

Mechanisms of enhanced vascular calcium load in CKD are multifactorial (19) (Table 1), and related to: (a) pre-existing diseases such as T2DM; (b) calcium- and phosphate-induced vascular smooth muscle cell matrix vesicle formation (78); (c) declines in the serum pyrophosphate and fetuin defenses that inhibit vascular mineral deposition (79); (d) phosphate-stimulated Pit1 signals that promote vascular smooth muscle osteogenic

differentiation via Runx2/Cbfa1 induction (80); (e) tissue iron loading and other oxidative stressors that generate oxidized LDL (73); and (f) severe reductions in the beneficial BMP7 signals that help maintain normal skeletal mineralization and stabilize the healthy vascular smooth muscle cell phenotype (81). Bone turnover clearly plays an important role in the vascular calcification of CKD. In an elegant histomorphometric study, London and colleagues demonstrated that individuals with inappropriately normal or low PTH levels – and thus low-turnover uremic bone disease – have the most extensive arterial calcification (82). The maintenance of sufficient numbers of skeletal basic multicellular units (BMUs) that can "buffer" serum calcium and phosphate fluxes with dietary calcium phosphate loading is a function of PTH-dependent bone anabolism. Thus over-aggressive pharmacotherapy with calcitriol- or calcium-based phosphate binders will have significant vascular consequences in CKD (82). Recently, Chertow and colleagues published a small but very enlightening prospective study that also highlights the need to carefully consider the changes in calcium homeostasis that occur with renal failure (83). CKD5 patients supplemented with oral calcium carbonate – implemented as a phosphate binder – actually *lose* vertebral bone mineral density; bone loss was not observed when calcium carbonate was avoided, and another phosphate binder (sevelamer) was utilized that did not increase serum calcium levels (83). In this setting, calcium carbonate treatment was associated with suppression of both circulating PTH and bone specific alkaline phosphatase (83) – features consistent with the down-regulation of bone formation in these patients. Metrics of vascular calcium load were too variable to be conclusive, and a larger study is needed. Based upon these results, serum markers (*e.g.*, bone-specific ALP) and direct metabolic imaging methods that globally quantify skeletal bone formation activity may prove to be much better guides for preventing bone-vascular disease while titrating phosphate, calcium, and PTH levels. However, since bone ALP is elaborated by vascular cells of AMC and AIC (1;3), it will be important to determine the extent to which vascular vs. skeletal compartments contribute to serum bone-specific ALP.

AVC is also increased in patients with CKD (19). Deposition of amorphous calcium phosphate is likely to be in great part epitaxial, exacerbating the pre-existing AVC arising from diabetes and hypertension; however this remains to be proven. One small study identified that etidronate, a first generation bisphosphosphate that inhibits mineralization as well as bone resorption, reduces aortic valve calcium accrual in CKD5 (84). The relationship between bone turnover and calcific vascular disease in CKD is precarious (82) – and bisphosphonates are renally cleared and long-lived once in the skeleton (85). Thus, it will be important to carefully determine the best management of mineral metabolism in CKD via larger prospective studies that account for contributions of skeletal remodeling space and bone formation vs. direct inhibition of vascular tissue mineralization to any potential therapeutic strategy implemented in this complex setting.

Calcific Uremic Arteriolopathy (CUA)

The use of the word calciphylaxis to denote calcific uremic arteriolopathy (CUA) should be abandoned as suggested by Janigan (86); it has been applied indiscriminately to multiple types of vascular tissue calcification in the setting of CKD. As Janigan notes (86), the Selye animal model used to codify the nomenclature "calciphylaxis" does not accurately recapitulate key histopathologic features of CUA seen in our patients with CKD5 – namely, the medial calcification and intimal fibroproliferative acute occlusion of dermal *arterioles* < 1 mm in diameter, with secondary dermal necrosis (Figure 5), stippled calcification of subcutaneous fat, and cutaneous pathergy (86). Similar lesions can form in mesenteric and pulmonary vascular beds (87). The pathobiology is not well understood; however, two key features are recurrent and highly prevalent: the presence of CKD4 or CKD5, and a recent (< 2 year) history of treatment with the anticoagulant coumadin (warfarin) (86-88). Because of the relationships with CKD, diabetes, hyperphosphatemia, and hyperparathyroidism are frequent, but not

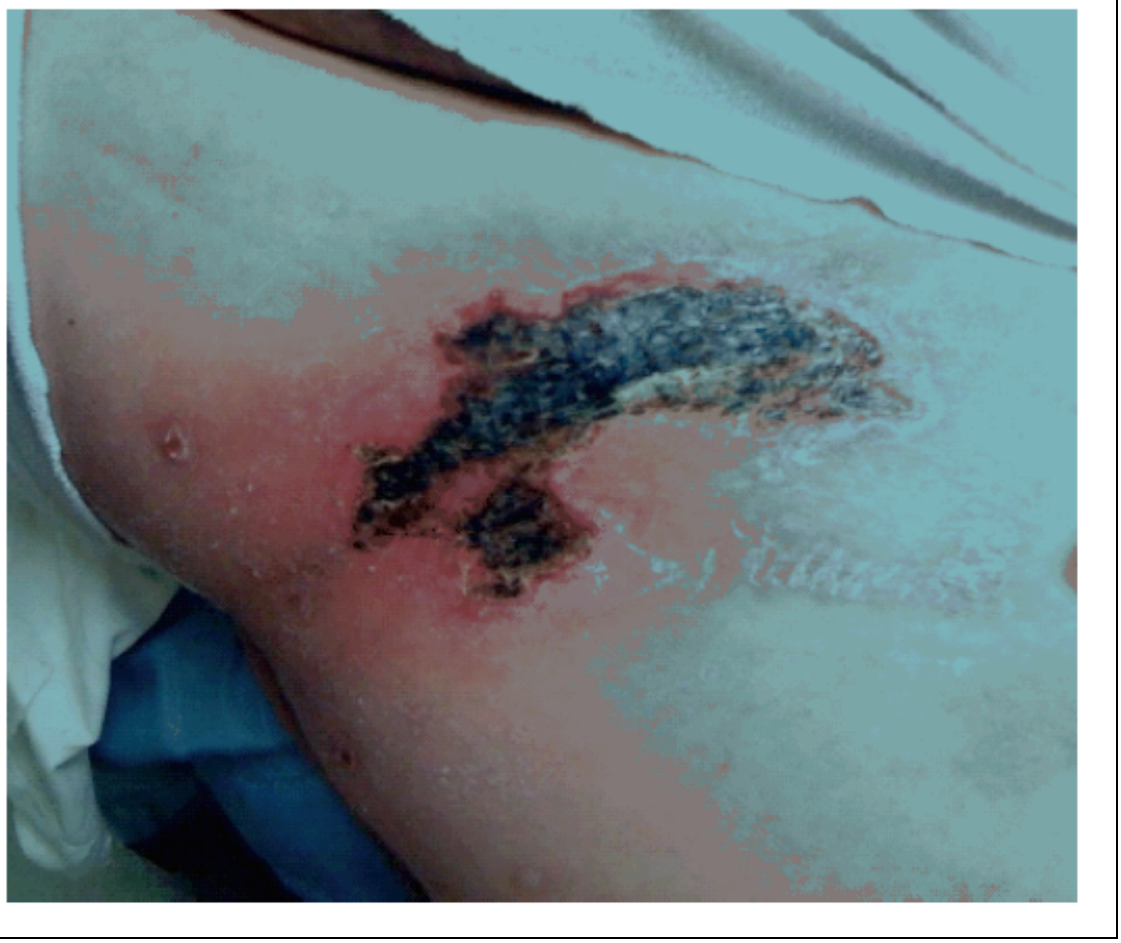

Fig. 5. Characteristic skin lesion of calcific uremic arteriolopathy (CUA). This middle-aged woman with type II diabetes and CKD5 suffered CUA 6 months following treatment with coumadin for deep vein thrombosis. Her serum PTH levels had actually ranged between 60 and 120 pg/ml – slightly below the NK-DOQI guidelines of 150–300 pg/ml. CUA is characterized by medial calcification of dermal *arterioles* and intimal fibroproliferative responses that lead to vessel occlusion and skin necrosis. Lesions such as this one significantly improve with sodium thiosulfate infusion (91). See text for additional details.

universal, concomitants. The few studies that have been performed reveal that both BMP4 (89) and OPN (90) are expressed in tissue afflicted by CUA. Mortality is very high without treatment, approaching 100% within 12 to 18 months (91). However, intravenous sodium thiosulfate infusion – a reducing compound initially used for cyanide poisoning that improves tissue glutathionine levels, has anti-inflammatory properties, and solubilizes amorphous calcium phosphate (92) – has been successfully used to treat of the disorder (91;93; and unpublished personal experience). Since administration of vitamin K restores MGP γ-carboxylation and enhances the reversal of warfarin-induced calcification in rats (94), a combination of intravenous thiosulfate with oral vitamin K treatment may prove optimal for treating this deadly disease. Randomized, controlled trials will be required.

Conclusions and Future Directions

As evident from the above, the perspective is that the rising incidence of type II diabetes will synergize with increasing longevity to increase the prevalence of CAS, AMC, and AIC in Westernized societies. Since diabetes is responsible for approximately 40% of the dialysis burden of end-stage renal disease, the milieu of CKD5 that creates a “perfect storm” for progressive vascular calcium deposition (Table 1) will also increase in prevalence. Moreover, calcification is a major contributor to

bioprosthetic vascular tissue failure (24); this has not been discussed at all, but will become an increasingly important consideration as arterial tissue engineering is increasingly used to restore macrovascular functions (95). Other forms of disease, such as mitral valve calcification, mitral annulus calcification, and venous calcification of portal vein hypertension were not discussed – but do have clinical implications. Only in the last 2 decades have the mechanistic underpinnings of vascular calcium deposition been studied in any significant detail. Histopathology and molecular biology have now converged with nosology and etiology to orient research in the field. Non-invasive methodologies necessary for longitudinally following vascular calcium metabolism during disease initiation, progression, and potential regression are only beginning to be developed. Studies evaluating the mechanism and kinetics of mineral egress from calcified arteries and valves lag far, far behind those of vascular mineral deposition. Congenital deficiencies in Smad6 (96) and Notch1 (97) convey risk for aortic valve calcification; since both molecules negatively regulate osteogenic differentiation, they may also play critical roles in calcific vascular disease arising from metabolic insults or advanced age. Novel modulators of phosphate and magnesium homeostasis (*e.g.,* FGF23, klotho, EGF) (65) (98;99) have yet to be well-integrated into the working models of vascular mineralization. Criteria and potential mechanisms of bone, mineral and vascular disease in CKD5 have emerged; however, these insights do not strictly translate to patients with sufficient GFR (≥ 60 cc/minute) because of the profound perturbations of mineral metabolism that occur with end-stage renal disease. A comprehensive and sophisticated understanding of vascular mineralization – in all of its forms and complexities – will be needed in order to develop novel medical strategies for treating this burgeoning disorder of mineral metabolism. Patient-oriented research is necessary to determine the extent to which arterial pathophysiology can be meaningfully reversed by egress of vascular mineral deposits – and how differences in the regulation of vascular calcification vs. vascular ossification may impact arterial responses to calciotropic therapies. An interdisciplinary working group in mineral metabolism and vascular disease would certainly help orient and advance the field. As scientists, physicians, and educators with expertise in mineral metabolism, these challenges present unique opportunities for us to contribute – to help address an important, unmet clinical need in human health and healthcare.

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References

1. Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. *Arterioscler Thromb Vasc Biol*. 2004 Jul;24(7):1161-70.

2. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature*. 2001 Dec 13;414(6865):782-7.

3. Shao JS, Cai J, Towler DA. Molecular mechanisms of vascular calcification: lessons learned from the aorta. *Arterioscler Thromb Vasc Biol*. 2006 Jul;26(7):1423-30.

4. Murphy WA Jr, Nedden Dz D, Gostner P, Knapp R, Recheis W, Seidler H. The iceman: discovery and imaging. *Radiology*. 2003 Mar;226(3):614-29.

5. Virchow R. *Cellular Pathology as Based upon Physiological and Pathological Histology*. New York: Dover Publications; 1863:404-408.

6. Glass DA 2nd, Karsenty G. In vivo analysis of Wnt signaling in bone. *Endocrinology*. 2007 Jun;148(6):2630-4.

7. Eames BF, de la Fuente L, Helms JA. Molecular ontogeny of the skeleton. *Birth Defects Res C Embryo Today*. 2003 May;69(2):93-101.

8. Chung UI, Kawaguchi H, Takato T, Nakamura K. Distinct osteogenic mechanisms of bones of distinct origins. *J Orthop Sci*. 2004;9(4):410-4.

9. Cohen MM Jr. The new bone biology: pathologic, molecular, and clinical correlates. *Am J Med Genet A*. 2006 Dec 1;140(23):2646-706.

10. Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, Maeda T, Takano Y, Uchiyama M, Heaney S, Peters H, Tang Z, Maxson R, Maas R. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. *Nat Genet*. 2000 Apr;24(4):391-5.

11. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell*. 1997 May 30;89(5):755-64.

12. Wang Y, Wan C, Deng L, Liu X, Cao X, Gilbert SR, Bouxsein ML, Faugere MC, Guldberg RE, Gerstenfeld LC, Haase VH, Johnson RS, Schipani E, Clemens TL. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. *J Clin Invest*. 2007 Jun;117(6):1616-26.

13. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002 Jan 11;108(1):17-29.

14. Hassan MQ, Tare RS, Lee SH, Mandeville M, Morasso MI, Javed A, van Wijnen AJ, Stein JL, Stein GS, Lian JB. BMP2 commitment to the osteogenic lineage involves activation of Runx2 by DLX3 and a homeodomain transcriptional network. *J Biol Chem*. 2006 Dec 29;281(52):40515-26.

15. Tyson KL, Reynolds JL, McNair R, Zhang Q, Weissberg PL, Shanahan CM. Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. *Arterioscler Thromb Vasc Biol*. 2003 Mar 1;23(3):489-94.

16. Shanahan CM. Inflammation ushers in calcification: a cycle of damage and protection? *Circulation*. 2007 Dec 11;116(24):2782-5.

17. Rajamannan NM, Bonow RO, Rahimtoola SH. Calcific aortic stenosis: an update. *Nat Clin Pract Cardiovasc Med*. 2007 May;4(5):254-62.

18. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. *Circ Res*. 2006 Nov 10;99(10):1044-59.

19. Moe SM, Chen NX. Pathophysiology of vascular calcification in chronic kidney disease. *Circ Res*. 2004 Sep 17;95(6):560-7.

20. O'Brien KD. Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more). *Arterioscler Thromb Vasc Biol*. 2006 Aug;26(8):1721-8.

21. Rosenhek R, Binder T, Porenta G, Lang I, Christ G, Schemper M, Maurer G, Baumgartner H. Predictors of outcome in severe, asymptomatic aortic stenosis. *N Engl J Med*. 2000 Aug 31;343(9):611-7.

22. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of 'degenerative' valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation*. 1994 Aug;90(2):844-53.

23. O'Brien KD, Kuusisto J, Reichenbach DD, Ferguson M, Giachelli C, Alpers CE, Otto CM. Osteopontin is expressed in human aortic valvular lesions. *Circulation*. 1995 Oct 15;92(8):2163-8.

24. Srivatsa SS, Harrity PJ, Maercklein PB, Kleppe L, Veinot J, Edwards WD, Johnson CM, Fitzpatrick LA. Increased cellular expression of matrix proteins that regulate mineralization is associated with calcification of native human and porcine xenograft bioprosthetic heart valves. *J Clin Invest*. 1997 Mar 1;99(5):996-1009.

25. Rajamannan NM, Subramaniam M, Rickard D, Stock SR, Donovan J, Springett M, Orszulak T, Fullerton DA, Tajik AJ, Bonow RO, Spelsberg T. Human aortic valve calcification is associated with an osteoblast phenotype. *Circulation*. 2003 May 6;107(17):2181-4.

26. Caira FC, Stock SR, Gleason TG, McGee EC, Huang J, Bonow RO, Spelsberg TC, McCarthy PM, Rahimtoola SH, Rajamannan NM. Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. *J Am Coll Cardiol*. 2006 Apr 18;47(8):1707-12.

27. Mohler ER 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation*. 2001 Mar 20;103(11):1522-8.

28. Cowell SJ, Newby DE, Prescott RJ, Bloomfield P, Reid J, Northridge DB, Boon NA; Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE) Investigators. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med*. 2005 Jun 9;352(23):2389-97.

29. Moura LM, Ramos SF, Zamorano JL, Barros IM, Azevedo LF, Rocha-Gonçalves F, Rajamannan NM. Rosuvastatin affecting aortic valve endothelium to slow the progression of aortic stenosis. *J Am Coll Cardiol*. 2007 Feb 6;49(5):554-61.

30. Weiss RM, Ohashi M, Miller JD, Young SG, Heistad DD. Calcific aortic valve stenosis in old hypercholesterolemic mice. *Circulation*. 2006 Nov 7;114(19):2065-9.

31. Aikawa E, Nahrendorf M, Figueiredo JL, Swirski FK, Shtatland T, Kohler RH, Jaffer FA, Aikawa M, Weissleder R. Osteogenesis associates with inflammation in early-stage atherosclerosis evaluated by molecular imaging in vivo. *Circulation*. 2007 Dec 11;116(24):2841-50.

32. Katz R, Wong ND, Kronmal R, Takasu J, Shavelle DM, Probstfield JL, Bertoni AG, Budoff MJ, O'Brien KD. Features of the metabolic syndrome and diabetes mellitus as predictors of aortic valve calcification in the Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2006 May 2;113(17):2113-9.

33. 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 Aug;16(8):978-83.

34. 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 Sep;18(9):1731-40.

35. Nelson RG, Gohdes DM, Everhart JE, Hartner JA, Zwemer FL, Pettitt DJ, Knowler WC. Lower-extremity amputations in NIDDM. 12-yr follow-up study in Pima Indians. *Diabetes Care*. 1988 Jan;11(1):8-16.

36. Clarke P, Gray A, Legood R, Briggs A, Holman R. The impact of diabetes-related complications on healthcare costs: results from the United Kingdom Prospective Diabetes Study (UKPDS Study No. 65). *Diabet Med*. 2003 Jun;20(6):442-50.

37. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet*. 2005 Nov 12;366(9498):1719-24.

38. Hoerger TJ, Downs KE, Lakshmanan MC, Lindrooth RC, Plouffe L Jr, Wendling B, West SL, Ohsfeldt RL. Healthcare use among U.S. women aged 45 and older: total costs and costs for selected postmenopausal health risks. *J Womens Health Gend Based Med*. 1999 Oct;8(8):1077-89.

39. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. *Bull World Health Organ*. 2003;81(9):646-56.

40. Al-Aly Z, Shao JS, Lai CF, Huang E, Cai J, Behrmann A, Cheng SL, Towler DA. Aortic Msx2-Wnt calcification cascade is regulated by TNF-alpha-dependent signals in diabetic Ldlr-/- mice. *Arterioscler Thromb Vasc Biol*. 2007 Dec;27(12):2589-96.

41. Shao JS, Cheng L, Charlton-Kachigian N, Loewy AP, Towler DA. Teriparatide (human parathyroid hormone (1-34)) inhibits osteogenic vascular calcification in diabetic low density lipoprotein receptor-deficient mice. *J Biol Chem*. 2003 Dec 12;278(50):50195-202.

42. Towler DA, Bidder M, Latifi T, Coleman T, Semenkovich CF. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor-deficient mice. *J Biol Chem*. 1998 Nov 13;273(46):30427-34.

43. Shao JS, Cheng SL, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, Towler DA. Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *J Clin Invest*. 2005 May;115(5):1210-20.

44. Zhang L, Zalewski A, Liu Y, Mazurek T, Cowan S, Martin JL, Hofmann SM, Vlassara H, Shi Y. Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation*. 2003 Jul 29;108(4):472-8.

45. Moreno PR, Fuster V. New aspects in the pathogenesis of diabetic atherothrombosis. *J Am Coll Cardiol*. 2004 Dec 21;44(12):2293-300.

46. Langheinrich AC, Michniewicz A, Sedding DG, Walker G, Beighley PE, Rau WS, Bohle RM, Ritman EL. Correlation of vasa vasorum neovascularization and plaque progression in aortas of apolipoprotein E(-/-)/low-density lipoprotein(-/-) double knockout mice. *Arterioscler Thromb Vasc Biol*. 2006 Feb;26(2):347-52.

47. Tintut Y, Alfonso Z, Saini T, Radcliff K, Watson K, Boström K, Demer LL. Multilineage potential of cells from the artery wall. *Circulation*. 2003 Nov 18;108(20):2505-10.

48. Heistad DD, Marcus ML, Larsen GE, Armstrong ML. Role of vasa vasorum in nourishment of the aortic wall. *Am J Physiol*. 1981 May;240(5):H781-7.

49. Carrington AL, Abbott CA, Griffiths J, Jackson N, Johnson SR, Kulkarni J, Van Ross ER, Boulton AJ. Peripheral vascular and nerve function associated with lower limb amputation in people with and without diabetes. *Clin Sci (Lond)*. 2001 Sep;101(3):261-6.

50. Forst T, Pfützner A, Kann P, Lobmann R, Schäfer H, Beyer J. Association between diabetic-autonomic-C-fibre-neuropathy and medial wall calcification and the significance in the outcome of trophic foot lesions. *Exp Clin Endocrinol Diabetes*. 1995;103(2):94-8.

51. Maiellaro K, Taylor WR. The role of the adventitia in vascular inflammation. *Cardiovasc Res*. 2007 Sep 1;75(4):640-8.

52. Alexandraki K, Piperi C, Kalofoutis C, Singh J, Alaveras A, Kalofoutis A. Inflammatory process in type 2 diabetes: The role of cytokines. *Ann N Y Acad Sci*. 2006 Nov;1084:89-117.

53. Shao JS, Aly ZA, Lai CF, Cheng SL, Cai J, Huang E, Behrmann A, Towler DA. Vascular Bmp Msx2 Wnt signaling and oxidative stress in arterial calcification. *Ann N Y Acad Sci*. 2007 Nov;1117:40-50.

54. Lai CF, Seshadri V, Huang K, Shao JS, Cai J, Vattikuti R, Schumacher A, Loewy AP, Denhardt DT, Rittling SR, Towler DA. An osteopontin-NADPH oxidase signaling cascade promotes pro-matrix metalloproteinase 9 activation in aortic mesenchymal cells. *Circ Res*. 2006 Jun 23;98(12):1479-89.

55. Bidder M, Shao JS, Charlton-Kachigian N, Loewy AP, Semenkovich CF, Towler DA. Osteopontin transcription in aortic vascular smooth muscle cells is controlled by glucose-regulated upstream stimulatory factor and activator protein-1 activities. *J Biol Chem*. 2002 Nov 15;277(46):44485-96.

56. Steitz SA, Speer MY, McKee MD, Liaw L, Almeida M, Yang H, Giachelli CM. Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. *Am J Pathol*. 2002 Dec;161(6):2035-46.

57. Scatena M, Liaw L, Giachelli CM. Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. *Arterioscler Thromb Vasc Biol*. 2007 Nov;27(11):2302-9.

58. Bruemmer D, Collins AR, Noh G, Wang W, Territo M, Arias-Magallona S, Fishbein MC, Blaschke F, Kintscher U, Graf K, Law RE, Hsueh WA. Angiotensin II-accelerated atherosclerosis and aneurysm formation is attenuated in osteopontin-deficient mice. *J Clin Invest*. 2003 Nov;112(9):1318-31.

59. Collins AR, Schnee J, Wang W, Kim S, Fishbein MC, Bruemmer D, Law RE, Nicholas S, Ross RS, Hsueh WA. Osteopontin modulates angiotensin II-induced fibrosis in the intact murine heart. *J Am Coll Cardiol*. 2004 May 5;43(9):1698-705.

60. Lenga Y, Koh A, Perera AS, McCulloch CA, Sodek J, Zohar R. Osteopontin expression is required for myofibroblast differentiation. *Circ Res*. 2007 Dec 13; [Epub ahead of print]

61. Eicke BM, Bauer J, Mink S, Kuhl V, Hlawatsch A, Küstner E, Victor A. Sympathetic vasomotor response of the radial artery in patients with diabetic foot syndrome. *Diabetes Care*. 2003 Sep;26(9):2616-21.

62. Takahashi T, Nishizawa Y, Emoto M, Kawagishi T, Matsumoto N, Ishimura E, Inaba M, Okuno Y, Shimada H, Morii H. Sympathetic function test of vasoconstrictor changes in foot arteries in diabetic patients. *Diabetes Care*. 1998 Sep;21(9):1495-1501.

63. Lavery LA, Higgins KR, Lanctot DR, Constantinides GP, Zamorano RG, Armstrong DG, Athanasiou KA, Agrawal CM. Home monitoring of foot skin temperatures to prevent ulceration. *Diabetes Care*. 2004 Nov;27(11):2642-7.

64. Schreyer SA, Vick C, Lystig TC, Mystkowski P, LeBoeuf RC. LDL receptor but not apolipoprotein E deficiency increases diet-induced obesity and diabetes in mice. *Am J Physiol Endocrinol Metab*. 2002 Jan;282(1):E207-14.

65. Razzaque MS, St-Arnaud R, Taguchi T, Lanske B. FGF-23, vitamin D and calcification: the unholy triad. *Nephrol Dial Transplant*. 2005 Oct;20(10):2032-5.

66. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev*. 1998 May 1;12(9):1260-8.

67. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature*. 1997 Mar 6; 386(6620):78-81.

68. Johnson K, Polewski M, van Etten D, Terkeltaub R. Chondrogenesis mediated by PPi depletion promotes spontaneous aortic calcification in NPP1-/- mice. *Arterioscler Thromb Vasc Biol*. 2005 Apr;25(4):686-91.

69. Price PA, Faus SA, Williamson MK. Warfarin-induced artery calcification is accelerated by growth and vitamin D. *Arterioscler Thromb Vasc Biol*. 2000 Feb;20(2):317-27.

70. Price PA, Chan WS, Jolson DM, Williamson MK. The elastic lamellae of devitalized arteries calcify when incubated in serum: evidence for a serum calcification factor. *Arterioscler Thromb Vasc Biol*. 2006 May;26(5):1079-85.

71. Boström K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest*. 1993 Apr;91(4):1800-9.

72. Dwyer JR, Sever N, Carlson M, Nelson SF, Beachy PA, Parhami F. Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. *J Biol Chem*. 2007 Mar 23;282(12):8959-68.

73. Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, Demer LL. Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. *Arterioscler Thromb Vasc Biol*. 1997 Apr;17(4):680-7.

74. Tintut Y, Patel J, Parhami F, Demer LL. Tumor necrosis factor-alpha promotes in vitro calcification of vascular cells via the cAMP pathway. *Circulation*. 2000 Nov 21;102(21):2636-42.

75. Burke AP, Kolodgie FD, Farb A, Weber D, Virmani R. Morphological predictors of arterial remodeling in coronary atherosclerosis. *Circulation*. 2002 Jan 22;105(3):297-303.

76. Ward MR, Pasterkamp G, Yeung AC, Borst C. Arterial remodeling. Mechanisms and clinical implications. *Circulation*. 2000 Sep 5;102(10):1186-91.

77. Aikawa E, Nahrendorf M, Sosnovik D, Lok VM, Jaffer FA, Aikawa M, Weissleder R. Multimodality molecular imaging identifies proteolytic and osteogenic activities in early aortic valve disease. *Circulation*. 2007 Jan 23;115(3):377-86.

78. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *J Am Soc Nephrol*. 2004 Nov;15(11):2857-67.

79. Reynolds JL, Skepper JN, McNair R, Kasama T, Gupta K, Weissberg PL, Jahnen-Dechent W, Shanahan CM. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. *J Am Soc Nephrol*. 2005 Oct;16(10):2920-30.

80. Steitz SA, Speer MY, Curinga G, Yang HY, Haynes P, Aebersold R, Schinke T, Karsenty G, Giachelli CM. Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. *Circ Res*. 2001 Dec 7;89(12):1147-54.

81. Hruska KA, Mathew S, Saab G. Bone morphogenetic proteins in vascular calcification. *Circ Res*. 2005 Jul 22;97(2):105-14.

82. London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul MC. Arterial calcifications and bone histomorphometry in end-stage renal disease. *J Am Soc Nephrol*. 2004 Jul;15(7):1943-51.

83. Raggi P, James G, Burke SK, Bommer J, Chasan-Taber S, Holzer H, Braun J, Chertow GM. Decrease in thoracic vertebral bone attenuation with calcium-based phosphate binders in

hemodialysis. *J Bone Miner Res*. 2005 May;20(5):764-72.

84. Hashiba H, Aizawa S, Tamura K, Shigematsu T, Kogo H. Inhibitory effects of etidronate on the progression of vascular calcification in hemodialysis patients. *Ther Apher Dial*. 2004 Jun;8(3):241-7.

85. Rodan G, Reszka A, Golub E, Rizzoli R. Bone safety of long-term bisphosphonate treatment. *Curr Med Res Opin*. 2004 Aug;20(8):1291-300.

86. Janigan DT, Hirsch DJ, Klassen GA, MacDonald AS. Calcified subcutaneous arterioles with infarcts of the subcutis and skin ("calciphylaxis") in chronic renal failure. *Am J Kidney Dis*. 2000 Apr;35(4):588-97.

87. Coates T, Kirkland GS, Dymock RB, Murphy BF, Brealey JK, Mathew TH, Disney AP. Cutaneous necrosis from calcific uremic arteriolopathy. *Am J Kidney Dis*. 1998 Sep;32(3):384-91.

88. Wilmer WA, Magro CM. Calciphylaxis: emerging concepts in prevention, diagnosis, and treatment. *Semin Dial*. 2002 May-Jun;15(3):172-86.

89. Griethe W, Schmitt R, Jurgensen JS, Bachmann S, Eckardt KU, Schindler R. Bone morphogenic protein-4 expression in vascular lesions of calciphylaxis. *J Nephrol*. 2003 Sep-Oct;16(5):728-32.

90. Ahmed S, O'Neill KD, Hood AF, Evan AP, Moe SM. Calciphylaxis is associated with hyperphosphatemia and increased osteopontin expression by vascular smooth muscle cells. *Am J Kidney Dis*. 2001 Jun;37(6):1267-76.

91. Araya CE, Fennell RS, Neiberger RE, Dharnidharka VR. Sodium thiosulfate treatment for calcific uremic arteriolopathy in children and young adults. *Clin J Am Soc Nephrol*. 2006 Nov;1(6):1161-6.

92. Meissner M, Kaufmann R, Gille, J. Sodium thiosulphate: a new way of treatment for calciphylaxis? *Dermatology*. 2007;214(4):278-82.

93. Rogers NM, Teubner DJ, Coates PT. Calcific uremic arteriolopathy: advances in pathogenesis and treatment. *Semin Dial*. 2007 Mar-Apr;20(2):150-7.

94. Schurgers LJ, Spronk HM, Soute BA, Schiffers PM, DeMey JG, Vermeer C. Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. *Blood*. 2007 Apr 1;109(7):2823-31.

95. Badylak SF. Regenerative medicine approach to heart valve replacement. *Circulation*. 2005 May 31;111(21):2715-6.

96. Galvin KM, Donovan MJ, Lynch CA, Meyer RI, Paul RJ, Lorenz JN, Fairchild-Huntress V, Dixon KL, Dunmore JH, Gimbrone MA Jr, Falb D, Huszar D. A role for smad6 in development and homeostasis of the cardiovascular system. *Nat Genet*. 2000 Feb;24(2):171-4.

97. Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, King IN, Grossfeld PD, Srivastava D. Mutations in NOTCH1 cause aortic valve disease. *Nature*. 2005 Sep 8;437(7056):270-4.

98. Ichikawa S, Imel EA, Kreiter ML, Yu X, Mackenzie DS, Sorenson AH, Goetz R, Mohammadi M, White KE, Econs MJ. A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest*. 2007 Sep;117(9):2684-91.

99. Groenestege WM, Thébault S, van der Wijst J, van den Berg D, Janssen R, Tejpar S, van den Heuvel LP, van Cutsem E, Hoenderop JG, Knoers NV, Bindels RJ. Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest*. 2007 Aug;117(8):2260-7.