

Clinical update

# Medial vascular calcification revisited: review and perspectives

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Vascular calcifications (VCs) are actively regulated biological processes associated with crystallization of hydroxyapatite in the extracellular matrix and in cells of the *media* (VCm) or *intima* (VCi) of the arterial wall. Both patterns of VC often coincide and occur in patients with type II diabetes, chronic kidney disease, and other less frequent disorders; VCs are also typical in senile degeneration. In this article, we review the current state of knowledge about the pathology, molecular biology, and nosology of VCm, expand on potential mechanisms responsible for poor prognosis, and expose some of the directions for future research in this area.

## Keywords

Vascular calcifications • Mönckeberg's media sclerosis • Vascular molecular biology and genetics • Vascular function

## Introduction

Vascular calcifications (VCs) are of similar composition to bone minerals. Vascular calcification of the *media* (VCm) are, in principal, deposits of hydroxyapatite with a high degree of crystallization.<sup>1</sup> Initially, VCs were thought to be the result of passive degenerative processes; however, recent studies illustrate that VC is an active process initiated and regulated via a variety of molecular signalling pathways.<sup>2</sup> While considerable progress elucidating the signalling pathways regulating VC formation has been achieved, the exact molecular basis of VC still remains elusive.<sup>3</sup>

With incoming new research data, the already large number of molecular mechanisms suggested to contribute to VC formation continues to grow. It appears that while deposition of hydroxyapatite represents the resulting commonality of VC, different initiating and propagating molecular mechanisms, as well as diverse crystalline compositions of calcium apatite crystals may be present in various forms of VC.<sup>4–6</sup> For example, it seems likely that VC processes associated with atheroma formation may be triggered by specific biochemical cascades that are altogether different from the cascades initiated by primary damage to elastic fibres which occurs in medial calcification; however, both ultimately result in ectopic VC. While experimental conditions

in a given model may replicate parts of the calcification process, they may not provide the whole picture in any of these specific conditions.

We believe that some of the emerging molecular complexity of VC may be accounted for by differences in experimental designs, tendencies to unite a host of different findings associated with different types of VC under a single umbrella hypothesis, undue focus on specific molecular cascades replicating parts of the calcification process in a given model, as well as disregard for specific aetiologies of VC types.

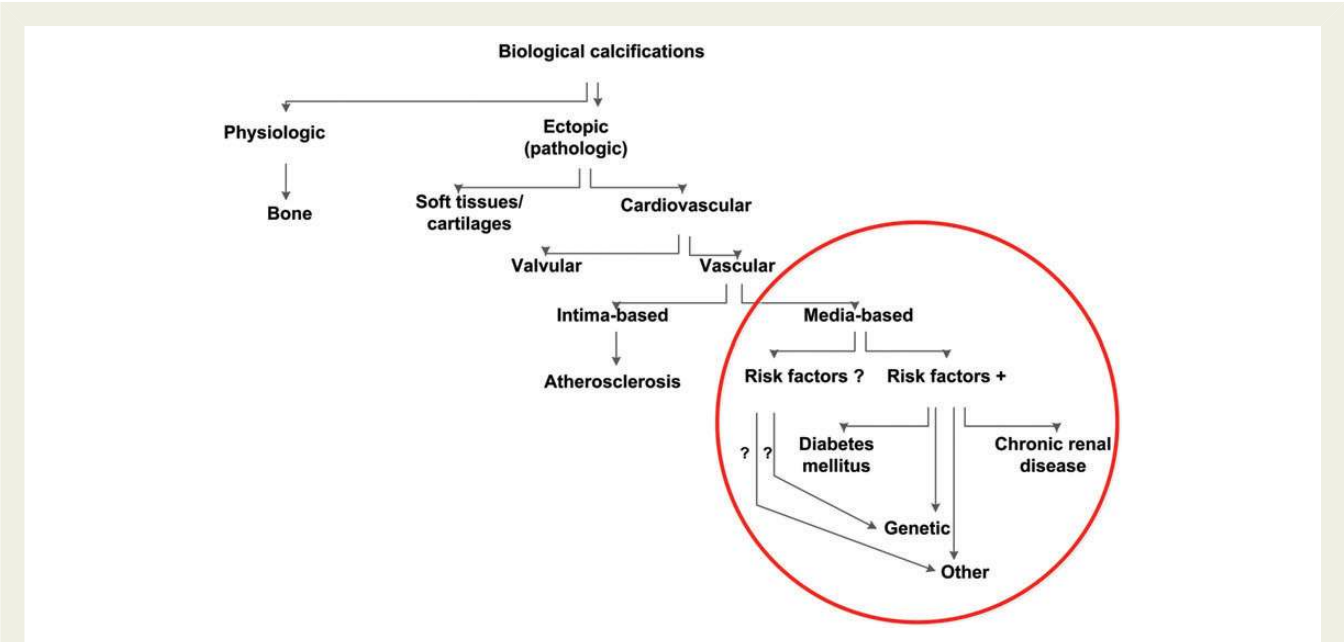
In this article, we review the current state of knowledge about the clinical pathology, molecular biology, and nosology of VCm and expose potential directions for future research in this exciting and clinically important area.

## Nomenclature

Establishing common nomenclature is not only the first step to sharing wisdom but also critical to focus research directions and interpret the results. While molecular pathogenesis-based nomenclature of VC is not available, provisory descriptive terminology should supply the need at present (Figure 1). While VCi is commonly associated with atherosclerotic plaques, VCm represents a group of

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**Figure 1** Simplified nomenclature of biological calcifications. Medial vascular calcifications comprise in humans a number of diseases; in the absence of knowledge of molecular pathogenesis of different types of medial vascular calcifications, the proposed nomenclature is based on the presence or absence of known risk factors. Mönckeberg’s media sclerosis represents the most common variety of medial calcifications; it is frequently associated with type II diabetes and chronic kidney disease, yet, in some cases none of these diseases and no other known risk factors for vascular calcifications are present. In the latter case genetic or other as yet not known risk factors are likely.

**Table 1** Main characteristics of media- and intima-related vascular calcifications

VCs types	Topography		Lesion calcify		Risk factors		Main molecular cascade	
	Systemic	Territorial	Early	Late	Traditional	Other	Inflammatory	Varied
Media	(X)	XX	XXX		X	XX	(X)	XXX
Intima	XXX			XXX	XXX		XXX	

Inflammatory cascades are largely linked to lipoproteins and cytokines, varied cascades are primarily related to systemic and local disturbances of calcium and phosphate metabolism, metabolism of glucose and possibly elastin degeneration.

distinct pathological conditions of differing aetiologies but a common final consequence (media calcification). Mönckeberg medial sclerosis (MMS) is the most common VCm variant; it is localized in the arteries of the extremities, and it is frequently associated with type 2 diabetes (T2D) and end-stage renal disease (ESRD). Some of the distinctive features between the calcifications of the intima (VCi) and VCm are reviewed in Table 1. However, both forms of VC often coincide and overlaps are frequent.

Prevalence and relevance

The prevalence of VCm of MMS type—based on ankle-brachial index (ABI) > 1.3—has been estimated to occur in ~0.5% of adults, is more prevalent in men than women (3 : 2) and the highest prevalence of VCm has been observed in patients with T2D.<sup>7</sup> The prevalence of VCm in patients with newly diagnosed T2D was 17%,<sup>8</sup> and among patients with established T2D receiving oral anti-diabetics, the

prevalence of VCm was as high as 41.5%.<sup>9</sup> In patients with ESRD, the prevalence of VCm was 27%. Compared with ESRD patients with VCi, the patients with VCm were younger, had less conventional risk factors for atherosclerosis, greater prevalence of calcium–phosphate disorder, and a length of dependence on haemodialysis.<sup>10</sup> In patients awaiting renal transplantation, moderate-to-severe calcifications were seen in 39.5% of individuals;<sup>11</sup> in all cited studies plain X-ray radiography of the pelvis and/or thigh was used for detection and differentiation between VCi and VCm. Vascular calcification of the media have been less frequently reported in association with other disorders (Table 2)<sup>8–36</sup>. While VCm are common to all of these conditions phenotypes may vary. For example, in calciphylaxis VCm is associated with extensive ischaemic necrotic skin and skeletal muscle lesions due to microthrombi, thrombotic occlusions, endo-vascular fibrosis, and massive ‘metastatic’ calcifications of small arteries (30–600 µm; average 100 µm);<sup>33–35</sup> all features absent in the other varieties of VCm.

**Table 2** Diseases associated with media calcifications

Condition	Markers and findings	Comments	Reference
Atherosclerosis	Lipoprotein and glucose metabolism analysis, genetic testing; duplex carotis ABI	Frequent coincidence between intima and media calcifications	8–11
Diabetes mellitus II	Fasting glucose profile; HbA1c quantification; CT		7–9
Chronic renal disease	Creatinine, glomerular filtration rate, urea, phosphate, calcium		10–12
Ageing	Pyrophosphate?		13–15
Hyperparathyroidism	Phosphate, Calcium analysis; quantification of parathormon (PTH)		16,17
Vitamin D disorders	Phosphate, calcium, 25(OH)D <sub>2</sub> , 25(OH)D <sub>3</sub> , 1,25(OH) <sub>2</sub> D <sub>2</sub> , 1,25(OH) <sub>2</sub> D <sub>3</sub>		18
Vitamin K deficiency	Coagulation studies, F VII, IX, and X, osteocalcin		19
Vitamin K–antagonist anticoagulants	Coagulation studies		20
Osteoporosis	Bone mineral density analysis	Deficiency of OPG, dietary essential fatty acids, and hyperlipidaemia	21
Kawasaki disease	Persistent fever; elevated TNF- $\alpha$ , IL-1, IL-6, B/T lymphocytes	Infants and children <5 years of age	22
ACDC	Genetic testing for NTSE mutations; calcification in joint capsules; decreased ABI; decreased limb perfusion	Autosomal recessive pattern of inheritance	23
GACI	Genetic testing for ENPP1 mutations. Calcification can be detected <i>in utero</i> , in newborn or in early childhood	Clinical overlap with PXE, autosomal recessive pattern of inheritance	24
IBGC	Genetic testing for SLC20A2, PDGF-B mutations. Cerebral vascular calcifications and progressive neurological defects	Autosomal dominant pattern of inheritance	25
PXE	Skin biopsy; genetic testing for ABCC6 mutations	Adolescence; clinical overlap with GACI. Autosomal recessive pattern of inheritance	26–28
Rheumatoid arthritis	Anti-CCP antibodies, ERS, CRP	Treated with steroids, NSAIDs, DMARDs; coronary artery calcification	29
Singleton–Merten syndrome	Aortic calcification, dental dysplasia and skeletal changes resembling severe anaemia		30,31
$\beta$ -Thalassemia	Complete blood count, Hb tests, Hb-electrophoresis	Patients also exhibit angiod streaks and skin lesions similar to PXE patients	32
Calciophylaxis	Coagulation studies, blood count	Arteriopathy, associated thrombosis, ischaemic dermal damage; uraemia and non-uraemic causes	33–35
?	Laboratory screening for traditional and non-traditional risk factors	No known risk factors, no associated diseases	36

ACDC, arterial calcification due to deficiency of CD73; GACI, generalized arterial calcification of infants; IBGC, idiopathic basal ganglia calcification; PXE, *Pseudoxanthoma elasticum*.

Vascular calcifications are considered an independent marker of cardiovascular risk.<sup>8–11,37</sup> Nevertheless, as highlighted by issues concerning biomechanical stability of calcified coronary atherosclerotic plaques<sup>38,39</sup> generalizations regarding detrimental effects of this form of VC may be fallacious and to decide on these matters requires careful re-evaluation of different pathologies.

## Pathology

Traditionally, two major forms of ectopic (pathologic) calcifications were distinguished; *dystrophic* refers to VC occurring in damaged tissues while *metastatic* was associated with systemic disorders of calcium and phosphate metabolism; these descriptions reflect the

differences between vascular ossifications (active process) and petrifications (passive process) described by Virchow.<sup>40</sup> While Virchow also differentiated between VCI and VCM, the latter were systematically studied in the arteries of the extremities by Mönckeberg.<sup>41</sup> Subsequently, VCM confirmed by histology were observed in large elastic type arteries (ascending aorta), medium-sized visceral and kidney arteries, and small transitional arteries (coronary, temporal, uterine, ovarian, parathyroid, mammary gland, and other) with diameter of at least 0.5 mm.<sup>42–45</sup> In calciophylaxis predominantly arteriolar VCM are present.<sup>33–35</sup> A systemic distribution of VCM appears uncommon,<sup>46</sup> yet the true incidence has not been established. Interestingly, on autopsy, patients with ESRD had a high degree of VCI but no evidence of VCM in coronary arteries.<sup>47</sup>

Vascular calcification of the media lesions, whether in elastic, transitional or muscular-type arteries, appear indiscernible when examined microscopically. The four stages of lesion progression distinguish the extent and severity of MMS VC type. In stage 1, calcifications appear on haematoxylin–eosin (H&E) staining as irregular blue or violet deposits embedded within the media. On a high-resolution light microscopy using H&E, Elastica-van-Gieson, von Kossa, or Alizarin- staining deposits consisting of fine granulations, which increase in size and become confluent, are revealed. Both intra- and extracellular deposits are present. Intracellular deposits are located in vascular smooth muscle cells (VSMCs); extracellular deposits are largely associated with damaged and fractured elastic fibres embedded within the extracellular matrix. In muscular and transitional arteries, with H&E-staining granular calcifications develop alongside the internal elastic membrane (IEM) and nearby VSMC. In our experience, the involvement of the IEM is common. These bands of calcium-rich deposits may thicken, becoming solid plates extending deep into the inner layer of the media. With further progression of the disease calcifications may distort the junctions of the innermost and outermost layers of the media, spanning up to three quadrants of the cross section (stage 2) or it may involve the entire circumference (stage 3). In stages 2 and 3, large conglomerates of calcifications may form solid plates or sheaths, progressively distorting the architecture of the media; intrusions upon the intima are then common.<sup>48</sup> In the absence of atherosclerotic lesions, the intima shows a subendothelial hyperplasia. Figure 2A–D demonstrates the characteristic features of stages 1–3 on histological specimen. In stage 4 of VCm foci of bone formation within the arterial media may be found, calcifications may undergo osseous metaplasia giving rise to true bony trabeculae. These structures delineate true medullary spaces harbouring haematopoietic cells interspersed with adipocytes<sup>49</sup> (Table 3).

In the arterial wall, calcification deposits associated with VCm may be perceived as foreign bodies and induce granuloma formation within the media; these structures often contain multinucleated giant cells. Other inflammatory components such as foam cells, lymphocytes, and mast cells may also be present. In advanced stages, large calcifications may induce secondary changes in the intima such as subendothelial hyperplasia characterized by an increase in cellularity (e.g. myofibroblasts, fibroblasts, fibrocytes) and ulcerations characterized by infiltrations of the intima or even protrusions into the lumen. It should be noted that VCm lesions do not spontaneously regress and the clinical complications may vary according to the site and the amount of calcification. Vascular calcification of the media lesions may become sites of thrombosis or they may detach and cause peripheral embolism.<sup>49</sup> Although VCm are not necessarily indicative of peripheral artery disease (PAD), the co-incidence is common. Anatomically, VCm lesions may also involve the microcirculation, e.g. arterioles (10–500 µm in diameter). However, to our knowledge, systematic studies concerning the extent of VCm lesions towards the microcirculation have not been performed. In VCm, the affected arteries display linear calcifications of the media and IEM; VCi are patchy clusters located in the intima. While calcifications are present in all VCm lesions, they do not represent consistent finding in atheroma (Stary stages IV–VII).<sup>50–52</sup>

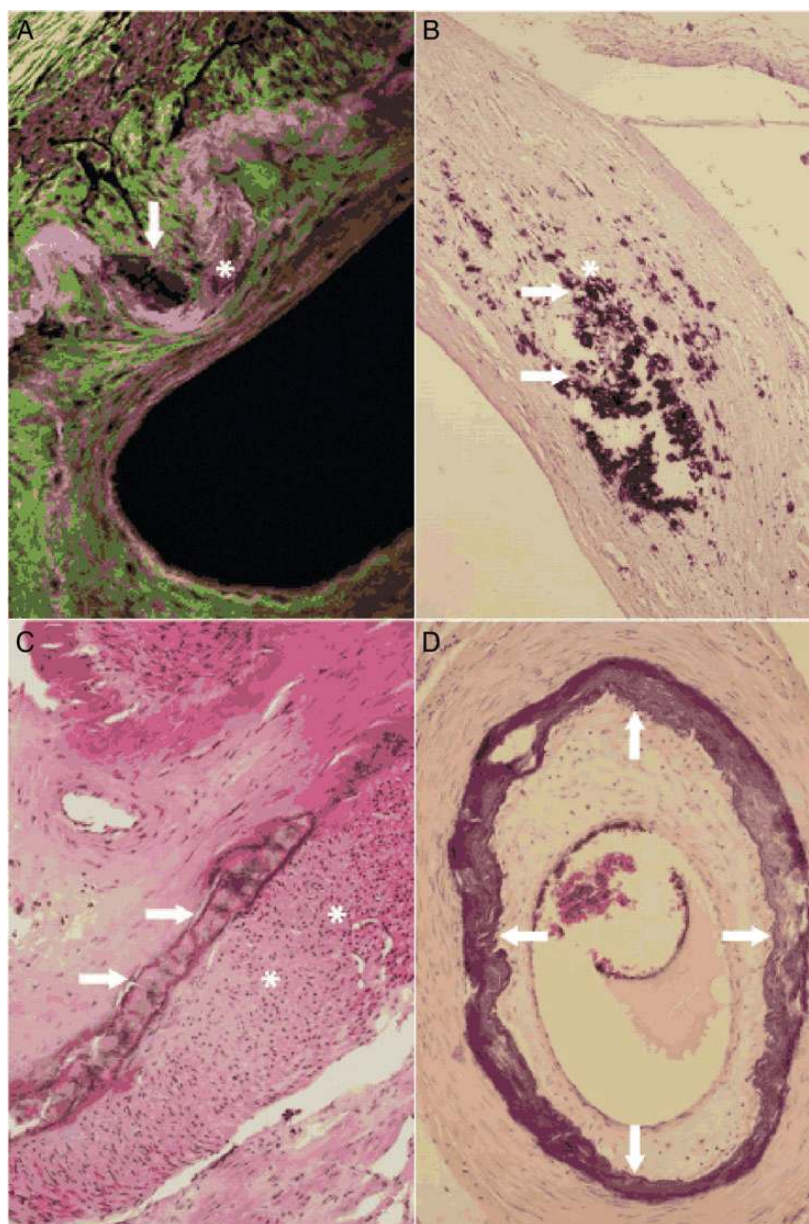
## Biochemistry and molecular biology

The discovery of the expression of the osteoblast-related bone morphogenetic protein (BMP2) in calcified human atherosclerotic lesions<sup>2</sup> has pioneered the hypothesis that VC is an active biological process reminiscent of osteogenesis. This notion has been reinforced with the observation of bone-forming events, including the production of matrix vesicles and apoptotic bodies in VC.<sup>53</sup> In addition to this pathogenic view, results from several groups supported an additional hypothesis, that calcification is the consequence of the loss of local and/or circulating calcification inhibitors, such as vitamin-K dependent matrix-Gla protein (MGP),<sup>54</sup> inorganic pyrophosphate (PPi),<sup>55</sup> fetuin A,<sup>56</sup> and osteoprotegerin (OPG).<sup>57</sup> While both pathogenic models are not mutually exclusive<sup>58</sup> determining whether the expression of bone-related genes is the cause or the consequence of the calcification process<sup>59</sup> is critical, as therapies will differ depending on which mechanism is the initiating process.<sup>3,5</sup>

In recent years, these two concepts have been expanded and edited based on new evidence comprising a range of data concerning molecular genetic cascades and cellular transformations (for expert review, see<sup>3,5,12,60,61</sup>). Rather than recapitulating these complex biological scenarios, here we wish to focus on specific aspects particularly relevant to VCm.

Study of chronic kidney disease (CKD)-related hyperphosphataemia refocused attention to the importance of inorganic phosphate (Pi)/PPi homeostasis. Patients with CKD-induced hyperphosphataemia develop rapid and extensive VCm, due in part to mineral dysregulation stemming from the primary renal disorder. Under these morbid conditions, vasculotoxicity of excess Pi, calcium, and other ions form nanocrystals that nucleate in soft tissues, including the vascular wall.<sup>62</sup> In the presence of physiological cytoplasmic concentrations of Pi (~1 mM Pi), calcification is only observed in specific genetic conditions (see below) or after long periods of exposure, either *in vitro*<sup>58,63</sup> or *in vivo* as in ageing.<sup>64,65</sup> However, in the presence of pathological hyperphosphataemic (> 2 mM Pi) conditions calcium phosphate deposition is accelerated and easily observed. This strongly suggests that deposition is prevented by the presence and activity of calcification inhibitors, and that calcification is therefore accelerated when the limited capability of calcification inhibitors is overcome.<sup>58,66</sup>

In contrast to bone, calcium phosphate in the arteries is not predominantly deposited on type I collagen but rather on the amorphous elastin that comprises the elastic lamellae.<sup>67</sup> This process is augmented when elastin is degraded by elastase and other proteases.<sup>68</sup> While the elastin fibres can calcify both *in vitro* and *in vivo* as in elastocalcinosi,<sup>65,69</sup> the role of the VSMCs and other cellular determinants (e.g. vascular progenitor cells, pericytes, calcifying vascular cells) is more complicated. There are at least three key aspects to be considered and elucidated regarding the role of these cells in the pathogenesis of VC: (i) the transformation into an osteochondrogenic phenotype, (ii) the generation of nucleating structures for calcium phosphate deposition, and (iii) the endocytosis and toxicity of calcium phosphate nanocrystals. The phenotypic plasticity of the mesenchymal-derived VSMC allows them to *trans-differentiate in vitro* and *in vivo*, gaining osteochondrogenic-like characteristics and expression of bone-related genes – e.g. Cbfa1/Runx2, Msx2, BMP2, alkaline phosphatase,



**Figure 2** Microscopical images of medial calcifications. Small calcifications (arrow) alongside the internal elastic membrane (asterisk) characteristic of Mönckeberg medial sclerosis stage 1 are shown (femoral artery, fluorescence microscopy, 400 ×) (A). Larger amounts of medial calcifications (arrow) in vicinity of vascular smooth muscle cells (asterisk) in Mönckeberg medial sclerosis stage 1 lesion are demonstrated (femoral artery, haematoxylin–eosin stain, 200 ×) (B). Calcifications becoming confluent and forming solid plates (arrows) and subendothelial hyperplasia (asterisks) in the intima are seen; the findings correspond to Mönckeberg medial sclerosis stage 2 (femoral artery, haematoxylin–eosin stain, 40 ×) (C). Mönckeberg medial sclerosis stage 3 is characterized by calcifications distorting the media spanning the entire circumference (arrows) (tibial anterior artery, haematoxylin–eosin stain, 40 ×) (D).

osteopontin (OPN).<sup>69</sup> The identity of these cells and impetus for their *trans*-differentiation has yet to be elucidated.<sup>70</sup> Recent findings demonstrating that persistent DNA damage signalling associated with cellular senescence can activate osteogenic pathways in VSMCs are of significance, as ageing is the most dominant risk factor for the progression of VCm.<sup>71,72</sup> In addition, recent evidence

also supports the view that osteogene expression can be a consequence of the nucleation of calcium phosphate crystals, as bone-related gene expression can be completely prevented with calcification inhibitors such as PPI or phosphonoformic acid, even in the presence of high concentrations of calcium or phosphate.<sup>58,73</sup> Furthermore, VC is associated with a decrease in the concentration of

**Table 3** Stages of medial calcifications and their histological aspects

Stage	Histological aspects of vascular calcifications; MMS type
I	Granular calcifications alongside the internal elastic membrane Calcification nearby vascular smooth muscle cells
II	Calcifications increasing in size and becoming confluent Solid plates distorting the media spanning up to the incomplete circumference Association of subendothelial hyperplasia in the intima
III	Calcifications distorting the media spanning the entire circumference Association of subendothelial hyperplasia in the intima
IV	Calcifications and foci of bone formation (osseous metaplasia)

MMS, Mönckeberg' media sclerosis.

extracellular PPI and an increase of Pi.<sup>74</sup> In order to activate osteogenic gene expression, nanoparticles of 30–500 nm need to be endocytosed and accumulated in the lysosomes, where the crystals are then dissolved. Increased calcium concentration in the cytosol can additionally be a cause of apoptosis and necrosis.<sup>73,75</sup>

Vascular smooth muscle cells also participate in the nucleation of calcium phosphate crystals through the formation of extracellular vesicles. Classification of these vesicles is a controversial area of research; recent attempts have been made to standardize nomenclature and classification.<sup>76</sup> Matrix vesicles (30–300 nm in diameter) and fragmented apoptotic bodies (50–5000 nm in diameter) are the most studied vesicular structures in calcification.<sup>12</sup> Matrix vesicles seem to be released by macrophages and VSMCs in calcified atheroma<sup>77</sup> and by VSMCs in medial calcification.<sup>78</sup>

Apoptotic bodies released by VSMCs can act as nucleating agents for the deposition of crystals in the aortic wall.<sup>53</sup> Normal vesicles contain inhibitors of calcification, especially in the presence of serum, and do not initiate calcification while *in vitro*<sup>79</sup> and *ex vivo*.<sup>77</sup> data suggest that calcification due to the production of apoptotic bodies and matrix vesicles is dependent on the extracellular concentrations of calcium and phosphate.<sup>77,78</sup> Apoptosis of VSMCs is also associated with calcification and medial degeneration;<sup>80</sup> cell death can be caused by lysosome-dependent dissolution of internalized calcium phosphate crystals <1 µm.<sup>75</sup> Cell death is not restricted to apoptosis, as necrotic cell debris also has been shown to induce calcification in a mesenchymal *in vitro* model.<sup>80</sup>

Recently an additional cellular phenomenon called autophagy<sup>81</sup> has been described that counteracts the pro-calcific effect of apoptosis. In addition, recent findings suggest that the initiating events and the early pathogenic steps in the various ectopic calcification disorders may be different. Calcifying atherosclerosis, for example, occurs in the absence of hyperphosphataemia, and the strong inflammatory component is sufficient to create the conditions for nucleating calcium phosphates.<sup>82</sup> This localized inflammatory response has not been observed in VCm, in particular macrophage accumulation does not occur at sites of medial calcification. During CKD (a major cause of VCm), a generalized, non-localized pro-inflammatory

status is observed, with overexpression of TNFα, which could activate the osteochondrogenic programme in VSMCs.<sup>12</sup>

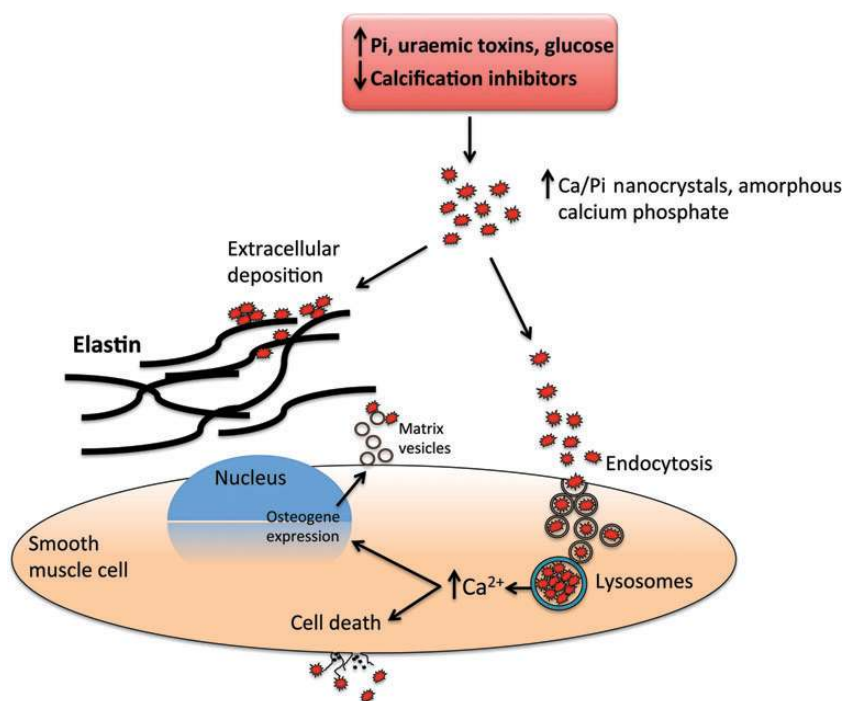
Many other general cellular mechanisms have been related to VC, such as microRNA control of gene expression,<sup>83</sup> endoplasmic reticulum stress,<sup>84</sup> and activation of the inflammasome.<sup>85</sup> Clearly, VC represents a highly complex biological process. Elucidation of the main regulatory mechanisms and differentiating them from secondary phenomena is one of the urgent pending questions that must be addressed. Specifically related to the involvement of VSMCs in VC, we must identify which cell types are involved in the osteochondrogenic *trans*-differentiation and under what conditions; why do some cells respond apoptotically to endocytosis of nanocrystals, while other cells simply modify gene expression? And what are the specific conditions that determine apoptosis, necrosis, and other types of cell death as well as autophagy in the vessel wall?

The success of on-going and future studies will enhance our understanding of these specific questions, add to our knowledge of VC pathogenesis and identify therapeutic targets. Figure 3 shows potential molecular pathogenic pathways associated with VCm. The event responsible for triggering precipitation of hydroxyapatite from the metastable supersaturated solution of calcium and phosphate within the environment of the media—i.e. VSMCs and matrix—may result from either the presence of toxins, genetic predisposition, and/or acquired deficiency in calcification inhibitors.

## Molecular genetics

There are several genetically defined vascular diseases associated with primary vascular media calcification and understanding the role of these genes in this pathogenic process will help to uncover the shared mechanism regulating VCm. One monogenetic autosomal recessive disease that seems most closely to resemble the classical description of MMS is arterial calcification due to deficiency of CD73 (ACDC), a rare vascular disease hallmarked by tortuous arteries with accompanied medial calcifications and medial hyperplasia.<sup>23,86</sup> In retrospect, it appears that ACDC patients were described as having atypical VCm, however, genetic confirmation on these patients cannot be pursued.<sup>87–89</sup> Arterial calcification due to deficiency of CD73 patients develop massive VC in the medial layer of muscular-type arteries primarily located in the lower-extremities.<sup>86</sup> These patients have no indication of classic atherosclerosis, impaired kidney function, or diabetes. Arterial calcification due to deficiency of CD73 is caused by loss-of-function mutations in the gene encoding for CD73 protein. This cell membrane-bound extracellular enzyme generates adenosine and Pi from AMP. Loss of CD73 activity results in an increase in activity of tissue non-specific alkaline phosphatase (TNAP), a key protein in bone formation that is rescued *in vitro* via exogenous adenosine administration. Increase in TNAP activity can lead to the hydrolyzation of extracellular PPI, a potent steric inhibitor of hydroxyapatite formation, thus increasing the concentration of Pi and tipping the scales towards a pro-calcific microenvironment. The discovery of ACDC opens up a new field of research as to the role of adenosine signalling on this disease phenotype.

Generalized arterial calcification in infants (GACI, also referred to as idiopathic infantile arterial calcification) is another rare vascular disease with systemic progressive VC that develop *in utero* and



**Figure 3** Suggested pathogenesis of medial calcifications. Shown are simplified molecular pathways potentially associated with initiation and propagation of medial calcifications. Nanocrystals of calcium phosphates could be nucleated and either deposited on elastine fibres and extracellular vesicles or endocysted and directed to lysosomes where they are then dissolved at low pH and  $\text{Ca}^{2+}$  are released to the cytosol. Cell death or osteochondrotic transdifferentiation could follow the transient increase in calcium concentration.

during the early postnatal period.<sup>24</sup> Histologically, it is characterized by medial calcifications that lead to neointimal formation and vessel occlusion. This systemic vascular proliferative disease results in multi-organ failure due to non-atherosclerosis-related myocardial infarction as early as 3 months of age. Generalized arterial calcification in infant is an autosomal recessive disease caused by mutations in the gene ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1). This cell membrane-bound extracellular enzyme generates AMP and PPi from ATP and is therefore directly upstream of CD73, the causal gene for ACDC, in the extracellular purine metabolic pathway. Pyrophosphate is a potent physiological inhibitor of TNAP and the lack of PPi and subsequent increase in TNAP activity is believed to be the main driver for this pathological malignant form of primary medial calcification.

Familial idiopathic basal ganglia calcification (IBGC, also commonly known as Fahr's disease) is an additional rare disease with a wide spectrum of neuropathological symptoms that have recently been attributed to the medial calcification of small blood vessel that supply the area of the basal ganglia.<sup>25</sup> Genetic evaluations of a subset of IBGC families identified variants in the gene sodium-dependent phosphate transporter 2 (SLC20A2 or PiT-2). PiT-2 is a  $\text{Na}^+/\text{Pi}$  co-transporter that plays an important role in Pi homeostasis and may be functionally linked to ENPP1 and CD73 in extracellular purine metabolism. A comprehensive review of genetic diseases leading to VC is available.<sup>61</sup>

Overall these three distinct genetic vascular diseases, all with medial calcification as their common primary pathology, highlight

the importance of extracellular purine metabolism and downstream adenosine signalling, and phosphate homeostasis in the development of VCm. The clinical presentation of the garden variety of VCm as described by Mönckeberg is clearly distinct from the devastating pathologies seen in patients suffering from ACDC, GACI, and IBGC; however, it is therefore important to genetically evaluate VCm variants to understand their molecular pathogenesis and to develop specific treatment strategies targeting VCm. For murine models of VC, see Supplementary material online, Appendix.

## Functional implications

Arterial compliance (C) and distensibility (C/A) are given by the slope of the non-linear relation between the transmural pressure (p) and the luminal cross-sectional area (A), an expression of the elastodynamic coupling between the blood flow dynamics and vessel wall mechanics. The speed of the pressure wave, which is inversely proportional to the square root of the wall distensibility, can be also computed using the Moens–Korteweg equation;<sup>90</sup> arterial calcification adversely affects blood flow dynamics and vessel wall mechanics.

Arterial medial calcifications have several major consequences according to the diseased arterial compartment. In the *macrocirculation* stiffening of the arterial wall is associated with increase in pulse wave velocity, increase in pulse pressure and pulse wave deformation (premature wave reflection, diastolic decay steepening).<sup>10,91,92</sup> Attenuation of the Windkessel effect results in increased pulsatility

potentially affecting organ perfusion, whereas augmented afterload results in left ventricular hypertrophy,<sup>93</sup> diastolic dysfunction,<sup>94</sup> and heart failure with preserved ejection fraction.<sup>95</sup> In advanced stages of VCm of MMS type, loss of elasticity of arterial walls is associated with increasingly deteriorating peripheral tissue perfusion finally resulting in arterial flow stasis and diffuse thrombus formation that is similar in appearance to deep vein thrombosis. Pathophysiological consequences of an increased pulsatility are less well understood. In the *microcirculation*, the loss in elasticity and increase of arterial blood pressure primarily interferes with autoregulation secondary to calcifications surrounding and within VSMC. Metabolic and endothelium-related responses all require a normal adjoining VSMC.

Arterial wall stiffening represents a strong independent predictor of future cardiovascular events and all-cause mortality.<sup>96</sup> While the impact of the loss of Windkessel function on LV function is well understood, the impact of the loss of elasticity in patients with VCm of MMS type appears less well understood. In addition to the decreasing propulsion of the antegrade blood flow with ultimate stasis and thrombus formation in the end-stage disease, we suggest three additional pathogenic principles potentially responsible for the poor prognosis of MMS patients. First, the ring-like calcifications present in advanced VCm are likely to interfere with positive arterial remodelling in the presence of atherosclerosis as described by Glagov et al.<sup>97</sup> and with arterial remodelling in a broader sense of arterial adaptation to haemodynamic alterations, noxes, and senescence.<sup>98</sup> For example, in cases of coincidence with atherosclerosis VCm would prevent compensatory remodelling and markedly accelerate progression of the disease. Second, stage 4 VCm with secondary invasion of the intima shall increase the risk of thrombo-embolic events. Third, VCm arteriolar lesions may

alter mechanotransduction, disturb autoregulation, and impair peripheral tissue perfusion. Thus, to determine the full functional impact of VCm on vascular function not only are measurements of pressure wave velocity<sup>99</sup> required, but also concurrent and reliable measurements of vascular dimensions over time,<sup>100</sup> as well as assessments of microcirculatory and endothelial functions.<sup>101,102</sup>

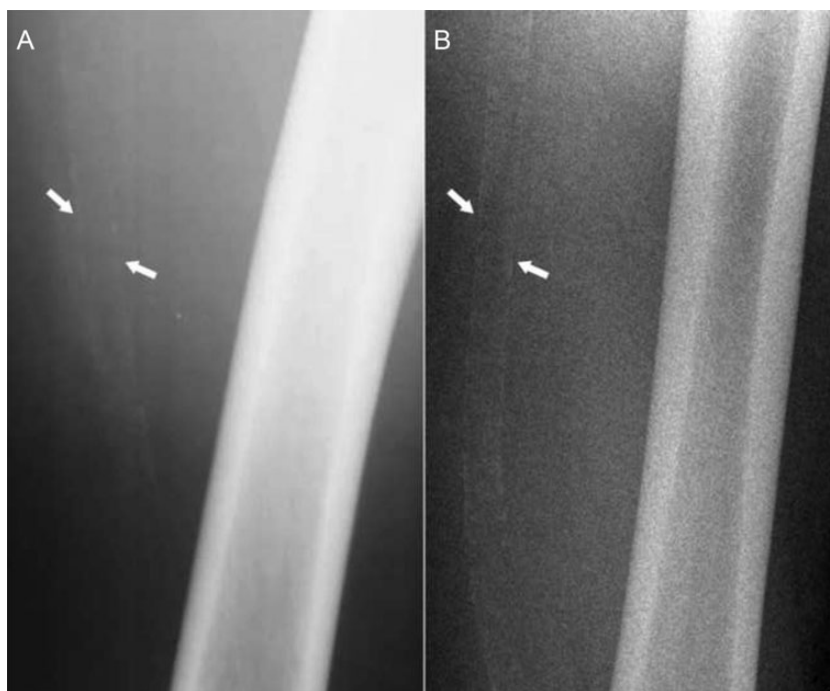
## Diagnostic evaluations

Vascular calcification of the media of the MMS type is indicated by measuring the ABI; MMS is diagnosed with an ABI  $\geq 1.1$ . It has been proposed that ankle-brachial index readings of 1.1–1.3, 1.3–1.5, and  $>1.5$  denote an early, intermediate, and late MMS, respectively.<sup>103</sup> In patients with suggested MMS (ABI  $\geq 1.1$ ), the toe-brachial index (TBI) has been proposed to improve the specificity of segmental blood pressure measurements.<sup>104</sup> However, questions have been raised about the validity of this approach, and the role of TBI in diagnostics of MMS remains uncertain.<sup>105</sup> Nevertheless, despite limitations ABI remains the most important screening tool to suggest the presence of both, PAD and MMS.<sup>106</sup>

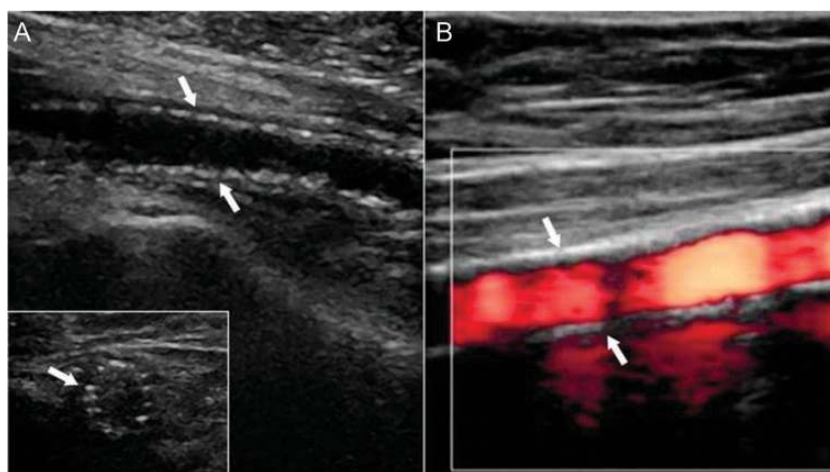
In clinical settings, MMS is frequently identified accidentally from conventional X-ray radiographs of pelvis or lower extremities; MMS lesions are visualized as uniform linear radiopaque 'railroad tracks' as opposed to the discrete irregular radio-opaque lesions seen in atherosclerosis.<sup>107</sup> With progressing disease granulations become coarser and less regular. Figure 4A and B shows a typical radiographic appearance of MMS lesions on native and angiographic X-ray films. Figure 5A and B shows the progression of MMS on 16-year follow-up in a male patient.



**Figure 4** X-ray images of left femoral bifurcation. Shown are native X-ray image (A) and selective needle angiogram (B) of the femoral bifurcation of a 56-year-old male patient. The typical 'railroad tracks' pattern (arrows) of medial calcification (A) and smooth endothelial interface (arrows) (B) of the femoral superficial and profunda arteries typical for Mönckeberg's type of medial calcification can be appreciated.



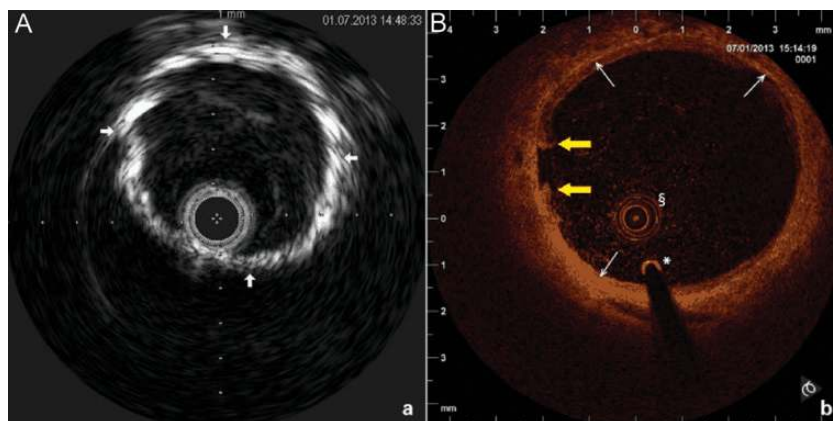
**Figure 5** Native X-ray images of a left thigh. Shown are native X-ray images of the left thigh of a male patient acquired at baseline at the age of 46 years (A) and on 16-year follow-up at the age of 62 years (B). The typical 'railroad trucks' pattern of medial calcification of the superficial femoral artery is seen on both images (arrows), however, on the image at baseline the pattern appears finely granulated while on the follow-up image the pattern appears coarse and more prominent signifying the progression of the disease.



**Figure 6** Ultrasound images of medial vascular calcifications. Shown are typical B-mode (A) and colour-mode (B) ultrasound images of a superficial femoral artery. B-mode images show the typical 'string-of-beads' pattern of media calcifications (arrows) with intact endothelial interface in both longitudinal sector and cross section (insert) images (courtesy C Garn). On colour-mode ultrasound image, the dense pattern of media calcifications (arrows), smooth endothelial interface, and homogeneous blood flow pattern (red colour) is shown.

Vascular ultrasound-based imaging techniques allow relatively inexpensive and non-invasive widely available means to detect VC and to differentiation between MMS- and the atherosclerosis-related lesions. In B-mode ultrasound images, MMS lesions are seen as

distinct echogenic granules located in the abluminal layers of the arterial walls. Depending on the stage of the disease lesions can appear as a 'string of beads' (Figure 6A) or they may be larger and confluent. Employing the colour flow duplex ultrasound in addition flow



**Figure 7** Typical images of medial vascular calcifications type Mönckeberg acquired by invasive imaging techniques. Intravascular ultrasound (A) and optical coherence tomography (B) cross-sectional images of a proximal superficial femoral artery are shown. On the intravascular ultrasound image dense tunica media calcification (arrows), the absence of acoustic shadowing and freedom from intimal atherosclerotic disease is demonstrated (Eagle Eye catheter, Volcano, 20 MHz, lateral resolution 200–250  $\mu$ , axial resolution 80–100  $\mu$ ). On the optical coherence tomography image, the circumferential layer of medial calcifications (arrows) is documented. The intraluminal prolapsing fibrotic plaque is also seen (yellow arrows);  $\delta$ OCT catheter, \*0.014 inch guidewire (Akquise, St Jude Medical, C7 Dragonfly catheter, lateral resolution 20  $\mu$ , axial resolution 10  $\mu$ ).

patterns can be visualized (Figure 6B). In patients undergoing endovascular interventions, MMS lesions may be visualized employing intravascular ultrasound (IVUS) or optical coherence tomography (OCT). On IVUS MMS lesions are seen as highly echogenic zones located within the media. Owing to the presence of fibrotic tissue typically no acoustic 'shadowing' is seen (Figure 7A). In contrast, calcifications of the intima, as seen in atherosclerosis, acoustic 'shadowing' is frequent. Compared with IVUS OCT provides higher resolution and substantially a better visualization of the innermost layers of arterial walls. Mönckeberg medial sclerosis lesions are distinctly visualized within the tunica media (Figure 7B). Computed tomography represents another excellent tool to detect VC;<sup>108</sup> however, distinction between intima- and media- type VC is unreliable and improved technology is required.<sup>109</sup>

While imaging studies employing conventional X-ray or ultrasound allow unequivocal definition of VCm, standardized laboratory tests to detect VC are not available at present. To assess high-risk patients or those with newly diagnosed with VCm, screening for cardiovascular risk factors for atherosclerosis including diabetes mellitus<sup>110,111</sup> and screening for risk factors for CKD-mineral and bone disorder (CKD-MBD)<sup>112</sup> should be performed. Laboratory evaluations of biomarkers specific for VC such as Pi, fibroblast growth factor 23 (FGF23), OPN, OPG, MGP, fetuin-A, alkaline phosphatase, and interleukin-6 (IL-6) have produced negative<sup>113</sup> or equivocal results<sup>114–118</sup> and have not yet been standardized for routine clinical evaluations.

## Clinical management

Because of the typical absence of stenotic lesions until the late twentieth century, VCm has been considered rather a benign condition. Today, VCm is recognized as a key negative predictor of

cardiovascular morbidity and mortality,<sup>8–11,96</sup> requiring consequent prevention and, where possible, vigorous treatment. In all the patients with documented VCm regardless of potential cause and underlined pathogenesis secondary prevention and optimum treatment of associated diseases, if any, is required, in particular atherosclerosis, T2D, and CKD-MBD.

Based on current knowledge, disturbances in phosphate metabolism have been identified as a driving force for VCm and the number of potential triggers has been identified.<sup>5,12,23–25</sup> Consequently, in patients with a documented disorder of phosphate homeostasis such as those with CKD-MBD for prevention and treatment of VCm administration of phosphate binders, low-dose vitamin D, activators of calcium-sensing receptor (calcimimetics), magnesium, analogues of PPI (bisphosphonate), sodium thiosulfate, and more recently aldosterone antagonists has been proposed.<sup>119–122</sup> Nevertheless, the utility of these treatment strategies has not yet been fully established and remains subject of clinical trials and investigations. In patients with T2D, the importance of inflammatory signalling<sup>123</sup> and in particular receptors for advanced glycation end products<sup>124</sup> direct the attention to the control of glucose metabolism; however, to our knowledge, no studies are available to document the impact of the quality of metabolic control on VCm.

Vascular calcification has been a well-documented co-morbidity in patients with osteoporosis, particularly in post-menopausal women, and those with CKD-MBD.<sup>125</sup> Several common pathogenetic pathways including vitamin K deficiency<sup>126–128</sup> have been proposed, yet the common links between these disease phenotypes remain tentative. In patients with vitamin K disorders depending on the cause discontinuation of vitamin K antagonists such as oral anticoagulants responsible for inhibition of carboxylation required for activation of a number of vitamin-K-dependent proteins including the potent inhibitor of VC MGP, coagulation and anticoagulation factors FII, VII, IX,

and X, and protein C and protein S, respectively,<sup>19,129</sup> or supplementation of the active form of vitamin K, mostly vitamins K2, such as menaquinone-4<sup>130</sup> are indicated.

In patients with calciphylaxis, combined medical and surgical treatment including lowering calcium and phosphate concentration, increasing frequency of haemodialysis, i.v. administration of thiosulfate, hyperbaric oxygen, wound care, and debridement of necrotic tissue are indicated depending on clinical manifestation.<sup>131</sup>

The ageing process of arteries is characterized by a gradual increase in stiffness<sup>132</sup> and calcification,<sup>13</sup> potentially associated with numerous pathogenetic principles including generation of reactive oxygen species, systemic inflammation, endothelial dysfunction,<sup>133</sup> disturbances in phosphate metabolism.<sup>74</sup> Yet to date, no proven therapeutic principles exist to slow the ageing process of the arteries. In patients with VCm-type MMS without known risk factors, no treatment is available at present.

## Perspectives

To improve our knowledge of pathogenesis and treatment of the various types of VCm, several steps are proposed.

Rules for the development of the standard nomenclature of VCs with and without the presence of extravascular tissue calcifications in human disease and in animal models should be established.

Morphological studies should be carried out to determine the systemic macro- and microcirculatory incidence of various types of VCm including MMS in human disease in different vascular beds.

Functional studies should be performed to determine the impact of various types of VCm including MMS on vascular health; specifically, the effect of various types of VCm on arterial remodelling, blood flow propulsion, and peripheral tissue perfusion should be determined.

Imaging and biomarker technology allowing sensitive detection of different stages of medial and intimal calcifications should be improved and implemented.

At the molecular and cellular level, several key issues should also be addressed. It is important, for example, to precisely define and refine the existing laboratory models of VCm, especially *in vitro*, and limit inappropriate generalizations from these studies. To coordinate the rapidly growing body of data, a dedicated database for mineral metabolism and VC research should be initiated, and this is the best accomplished by adopting an interdisciplinary approach.<sup>134</sup> This will lead to the distinction between primary mechanisms of VCm and consequences, complications or accessory findings, in order to design appropriate therapies.

For example, it remains unclear whether the expression of bone-related genes is a cause or consequence of VCm in man; therefore, it is important that research focuses on the very early stages of VCm. In CKD where patients develop an extreme form of VCm, a mineral bone disorder seems to trigger the accumulation of calcium (but not Pi) in the aortic wall,<sup>135</sup> and the same findings have been obtained with an electron microscopy and even in the presence of a Pi-rich diet.<sup>136</sup> Calcium accumulation is accompanied by a concomitant increase in plasma concentrations of Pi, FGF23, and PTH among other agents.<sup>135</sup> Therefore, in the very early stages increased calcium content must originate from calcium uptake or accumulation in extracellular structures of the media; these important issues also need to be studied.

To identify critical pathways and mechanisms regulating VCm, we believe that it is also important to utilize genetic approaches. In families exhibiting inborn errors leading to VCm next generation sequencing technologies should be applied to identify novel genes regulating this disease process. Further, cohorts of patients exhibiting VCm, such as primary MMS and MMS associated with chronic diseases mainly T2D and CKD-MBD, should be screened to identify SNPs that co-segregate with disease patients.

It is also important to understand the nature of the immediate consequences, once calcifications have been first established. Are extracellular and intracellular deposits of calcium phosphates in the vascular wall equally harmful, and will they differentially respond to the emerging therapies?

There is also the important issue of VCm reversal. Animal studies have shown that a very low Pi diet is able to modulate (reverse) the accumulation of both, calcium and Pi in the aorta of uraemic rats with established VCm.<sup>137</sup> The Pi plasma concentration is therefore a key determinant of VCm in CKD and in the general population and further work on the cell biological effects of P on VSMCs is key to designing therapies.

Other areas that the scientific community should target are defining the factors that drive VCm in different disease states such as ageing,<sup>138</sup> diabetes,<sup>60</sup> and CKD<sup>5,12</sup> as these may also be valid targets to slow the development of VCm, or even reverse it.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

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