

# VASCULAR CALCIFICATION IN CHRONIC KIDNEY DISEASE: AN UPDATE

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## ABSTRACT

Vascular calcification involves passive degeneration and an active process of arterial mineralisation, resembling osteogenesis. In chronic kidney disease, several proteins that physiologically control bone mineralisation, are also involved in the molecular and cellular mechanisms of the pathogenesis of vascular calcification. In fact, arterial cells grown in culture are induced to become osteogenic by inflammatory and atherogenic stimuli, such as high phosphate concentration. Mechanisms linking them must be considered in clinical decisions. Further understanding of processes causing vascular calcification may be considered for new therapeutic options for vascular disease in renal patients.

**Keywords:** Vascular calcifications, secondary hyperparathyroidism, phosphate, calcium.

## INTRODUCTION

Patients with chronic kidney disease (CKD) develop vascular calcification (VC) much faster than the general population.<sup>1</sup> In particular, it has been widely demonstrated how CKD represents an independent risk factor of cardiovascular mortality and all-cause mortality. Vascular calcifications are not only the result of the mere passive process of crystal deposition, but also an actively regulated process that develops in response to physiological and pathological conditions.<sup>2</sup> Several risk factors play a key role in this rapid vascular ageing. They are divided into “classic” risk factors such as age, gender, dialysis vintage, inflammatory status, calcium-phosphate disorders, and diabetes,<sup>3</sup> and new “non-classic” risk factors such as bone-related proteins: fetuin-A (2-Heremans-Schmid glycoprotein, AHSG), matrix-carboxyglutamic acid protein (MGP), pyrophosphate, osteoprotegerin (OPG), and bone morphogenetic

protein-2 (BMP-2). (Table 1) In addition, CKD promotes atherosclerosis.<sup>6</sup> In fact; the reduction of renal function promotes the development of an inflammatory status (increased levels of C-reactive protein) and lipid abnormalities that contribute to

INHIBITORS	PROMOTERS
Fetuin-A (2-Heremans-Schmid glycoprotein, AHSG)	OPG (Osteoprotegerin)
MGP (Matrix-GLA-Protein)	BMP 2/4 (Bone Morphogenetic Protein 2/4)
Pyrophosphate	

**Table 1. Inhibitors and promoters of vascular calcification.**

endothelial dysfunction and vascular calcification. The prevalence and progression of vascular calcification increases dramatically once patients are on dialysis,<sup>4</sup> and the vascular phenotype of even young dialysis patients can be compared with that of octogenarians.<sup>5</sup> Vascular calcification starts developing in the early stages of CKD (stage III 25%, stage IV 35%) and is present in over 50% of patients at the time of dialysis.

## PATHOPHYSIOLOGY

Ectopic vascular calcifications follow a very similar developing process to physiological bone formation. At sites of calcification, there is an up-regulated expression of mineralisation proteins, normally confined to bone and cartilage; this event induces osteo-chondrocyte-like changes in vascular smooth muscle cells (VSMCs).<sup>7</sup> These proteins include a number of transcription factors, such as Runx2 (Cbfa-1), Osterix, Msx2, and Sox9.<sup>8,9</sup> To create a microenvironment that is permissive for calcification, specialised membrane-bound bodies called matrix vesicles, serve as nucleation sites for hydroxyapatite.<sup>9,10</sup> VSMC-derived vesicles do not calcify until calcification inhibitors, such as Fetuin-A and MGP, are maintained in normal ranges. When calcification inhibitor levels are low, VSMCs produce mineralisation-competent vesicles that contain preformed hydroxyapatite.<sup>10,11</sup>

## HIGH PHOSPHATE AND VASCULAR CALCIFICATION

Phosphate (P) homeostasis in normal subjects is regulated by intestinal absorption, renal excretion, and bone resorption. However, in subjects with CKD, P renal excretion is reduced. Nevertheless, P levels are maintained among normal limits by reducing P tubular resorption through increasing parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). In the same setting, reduction of intestinal phosphorous absorption happens due to the reduction of plasma levels of calcitriol.<sup>12</sup> At stages IV and V of CKD, dietary intake of P tends to exceed renal excretion capacity, resulting in hyperphosphataemia. Abnormalities in mineral metabolism have been claimed to be a causal factor for the development of vascular calcification in CKD patients.

Several studies have shown that high phosphorus levels stimulate the development of VC in an *in vitro* model of VSMCs.<sup>13</sup> *In vitro* studies have demonstrated that high phosphate concentration is responsible for

VC formation through a specific activation of the core-binding factor alpha-1 (Cbfa-1), an osteoblast-specific gene that regulates the expression of several bone morphogenetic proteins.<sup>14</sup> In CKD, the expression of these proteins was also stimulated by uraemic patients' serum with normal serum phosphate, suggesting that uraemic milieu also has a role in CV pathogenesis.<sup>15</sup> Interestingly, calcified arteries from CKD patients showed an increased expression of both Cbfa-1 and osteopontin.<sup>16</sup> These data suggest that VC is an active process due, not only to calcium-phosphate salt deposition in artery wall, but also to a genomic regulation driven by uraemic environment and elevations in serum phosphate levels.

## FETUIN - A

Human fetuin-A (AHSG, alpha2-Heremans Schmid glycoprotein, alpha2HS-glycoprotein, alpha2-HSG) is an extracellular calcium-regulatory protein acting as a potent inhibitor of calcium phosphate precipitation. It is a member of a family of four structurally-related plasma proteins containing cystatin-like protein domains. The cystatin family harbours type 1 (mainly intracellular proteins), type 2 (mainly extracellular proteins), and type 3 cystatins (plasma proteins). Cystatin domain 1 in fetuin-A is strongly negatively charged with a high affinity for calcium-rich minerals.<sup>17</sup>

Fetuin-A is one of the non-collagenous, most abundant proteins in bone, accounting for 25% non-collagenous proteins. Serum fetuin-A has an anti-inflammatory property; the demonstration that this protein specifically prevents neutrophils from activation by hydroxyapatite crystals, supports this issue.<sup>18</sup> Furthermore, anti-apoptotic activity of fetuin-A has been observed in smooth muscle cells.<sup>19</sup> Beside these findings, fetuin-A showed an important role in the mineralisation process.

Fetuin-A is responsible for mineral accumulation in bone from plasma, thanks to its high affinity for bone minerals, especially for nascent apatite mineral. For this reason, it is an inhibitor of *de novo* apatite formation from supersaturated mineral solutions, but it does not dissolve preformed minerals.<sup>20</sup> Specifically, fetuin-A binds calcium phosphate and calcium carbonate with high affinity. Haemodialysis patients with low serum AHSG levels have a major risk of CV and all-cause mortality.<sup>21</sup> This observation by Ketteler et al.<sup>21</sup> suggests that AHSG may be involved in preventing the accelerated extraskelatal calcification observed in CKD. A recent study in a

population of 115 haemodialysis patients supports this hypothesis, as VC was associated not only with increasing age and a history of cardiovascular events, but also with abnormal values of inflammatory markers, such as reduction in AHSG and albumin and an increase in C-reactive protein and fibrinogen.<sup>22</sup>

## MATRIX GLA PROTEIN

Extracellular matrix GLA protein (MGP) is a member of the vitamin-K-dependent protein family, and it is a calcification inhibitor found in vascular and other soft tissue.<sup>23,24</sup> MGP promotes VSMC differentiation, antagonises BMP (BMP2 and BMP4) signalling and prevents osteochondrogenic lineage reprogramming of VSMCs. In mice, targeted deletion of the MGP gene results in rapid and complete arterial calcification, resulting in death by 6 weeks.<sup>25</sup> MGP is synthesised in the uncarboxylated form (ucMGP) and performs its action after vitamin K-dependent carboxylation. Without sufficient vitamin K, it remains decarboxylated and does not inhibit calcification.<sup>26,27</sup> The degree of  $\gamma$ -carboxylation required for MGP to inhibit calcification in humans is not known. Decarboxylated MGP form seems to be in high concentrations in calcified vessels, while carboxylated MGP form is more abundant in healthy vascular tissue.<sup>28</sup> This demonstrates that lack of functional MGP increases risk for vascular calcification. In addition to being carboxylated, MGP needs a post-translational phosphorylation, which is also thought to contribute to its functionality.<sup>29</sup> The phosphorylated ucMGP accumulates in a detectable amount in plasma.<sup>30</sup>

The role of MGP in vascular calcification has been elucidated in animal models, whereas in humans, data are conflicting. It has been suggested that the amount of ucMGP in the circulation is increased among patient populations characterised by pathologic soft-tissue calcification.<sup>29-31</sup> The studies that examined the association between plasma ucMGP and vascular calcification are limited to case-control comparisons or specific disease populations.<sup>29-31</sup> To evaluate the utility of ucMGP as a predictive marker of coronary artery calcification (CAC), it is necessary to examine a population free of clinical events.

In a randomised controlled trial with vitamin K supplementation, Shea et al.<sup>32</sup> found that older community-dwelling adults who adhered to phylloquinone (vitamin K1) supplementation showed less CAC progression over 3 years. The impact of MGP on regulation of calcification in humans appears to

have a genetic component. An association between polymorphisms of the MGP gene and myocardial infarction has been described in low-risk individuals.<sup>33</sup> Furthermore, their distribution has proved to differ significantly in CKD/haemodialysis patients as compared to healthy controls, and particular alleles are associated with an increase in cardiovascular events in haemodialysis patients.<sup>34</sup> Potentially, the identification of polymorphisms of the MGP gene, and their association with cardiovascular morbidity, is a critical step towards the understanding of the pathogenetic mechanisms of VC in CKD and dialysis patients.

## PYROPHOSPHATE

Isopentenyl Pyrophosphate (IPP), a well-known inhibitor of hydroxyapatite formation in urine produced by VSMCs, chondrocytes and osteoblasts, is an important inhibitor of vascular calcifications. Its reactive chemical nature suggests that it is a compound used to bind or deliver oxygen and phosphate at tissue level for rapid employment. Several intracellular enzymatic reactions are responsible for its production.<sup>35</sup> Plasma IPP is normally cleared by the kidney,<sup>36</sup> however, serum IPP levels in haemodialysis patients are reduced.<sup>37</sup> Furthermore, the calcification-inhibitory action of IPP *in vivo* is well-documented. However, its simple chemical composition and heterogeneous metabolism, as well as the local nature of its action, hinder the development of a preparation for the clinical setting.

## OSTEOPROTEGERIN

Osteoprotegerin (OPG) belongs to the tumour necrosis factor receptor superfamily. It acts as a soluble decoy receptor for the receptor activator of nuclear factor-kappa B Ligand (RANKL), lying on osteoclast membrane, and inhibits its interaction with membrane-bound receptor RANK. Through this mechanism, OPG is able to inhibit osteoclasts differentiation. RANKL/OPG/RANK axis is not only involved in regulation of bone-remodelling,<sup>38,39</sup> but recent findings supported its role in carcinogenesis as well as central thermoregulation.<sup>40,41</sup>

This system has also been linked to the development of atherosclerosis and plaque destabilisation.<sup>42,43</sup> In observational studies, elevated circulating OPG levels have been associated with prevalence and severity of coronary artery disease, cerebrovascular disease, and peripheral vascular disease. Elevated OPG levels have also been associated with the degree

of coronary calcification in the general population as a marker of coronary atherosclerosis.<sup>44</sup> OPG is produced by osteoblasts but also by many different tissues and cell types, including the lung, kidney, intestine and endothelial cells. Its biological effects are still not completely understood, but it seems to be involved in apoptosis.<sup>45</sup>

In renal patients, increased levels have been associated with abnormal aortic calcifications in patients on dialysis<sup>46</sup> and with coronary artery calcifications in both dialysis and transplantation patients.<sup>47</sup> There is an association of serum OPG levels with all-cause and cardiovascular mortality, both in patients on dialysis<sup>48</sup> and in transplantation patients.<sup>49</sup>

## BONE MORPHOGENIC PROTEINS

Bone morphogenic proteins (BMP) are secreted polypeptides, a subgroup of the transforming growth factor-beta (TGF- $\beta$ ) superfamily of growth factors. BMPs were first identified in demineralised and pulverised bone powder capable of inducing ectopic endochondral bone formation in muscle.<sup>26</sup> Over the years, more than 15 distinct BMP family members have been identified. In 1993, Bostrom, Demer and colleagues first demonstrated the expression of BMP2 in calcified human atherosclerotic plaques, and the capacity of BMPs to direct osteogenic programming of vascular mesenchymal progenitors of the pericyte lineage. When BMP2, BMP4, and BMP6, were detected in calcified areas of atherosclerotic lesions,<sup>50-52</sup> it was therefore presumed that they

enhanced vascular calcification, even more so when it became evident that vascular calcification is largely driven by osteogenesis in the vascular media.<sup>53,54</sup> However, BMP signalling is not only driving ectopic calcification but is also essential for cardiovascular development, with critical roles in the establishment of endothelial cells during vasculogenesis, the recruitment and differentiation of VSMC precursor cells, and vascular patterning.<sup>55,56</sup> BMP activity is important for the regulation of phenotypic plasticity, proliferation, and differentiation in VSMC.<sup>52</sup> BMP2 in particular has an inhibitory effect on VSMC proliferation and differentiation, whereas BMP7 promotes the VSMC phenotype transformation.<sup>54</sup> Furthermore, BMP inhibition, potentially in later steps, appears to be a key actor in maintaining VSMC differentiation. Many are the causes that promote increased levels of BMP. Among these, endothelial activation in response to pathogenic stimuli, such as inflammatory cytokines and shear stress, appear to play a key role in regulating serum levels of BMP.<sup>57,58</sup>

## CONCLUSIONS

The astonishing mortality rate due to cardiovascular events in CKD has led to a great effort to identify causes and new potential strategies to improve survival in CKD. It seems that bone mineral abnormalities play a major role in inducing and sustaining cardiovascular damage in CKD. Improving understanding of cellular and molecular mechanisms of vascular calcification in CKD will give major tools to the clinicians to evaluate and choose treatments.

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