Review Article

Vascular Calcification: Current Genetics Underlying This Complex Phenomenon

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Abstract

Objective: Vascular calcification is the consequence of the complex interaction between genetic, environmental, and vascular factors, which ultimately lead to the deposition of calcium in the *tunica intima* (atherosclerotic calcification) or *tunica media* (Mönckenberg's sclerosis). Vascular calcification is also closely related to other pathologies, such as diabetes mellitus, dyslipidemia, and chronic kidney disease. It has been concluded that the degree of vascular calcification may vary from person to person, even if the associated pathologies and environmental factors are the same. Therefore, this suggests an important genetic contribution to the development of vascular calcification. This review aimed to find the most recent evidence about vascular calcification pathophysiology regarding the genetic aspects and molecular pathways.

Data Sources: We conducted an exhaustive search in Scopus, EBSCO, and PubMed with the keywords "genetics and vascular calcification", "molecular pathways, genetic and vascular calcification" and included the main articles from January 1995 up to August 2016. We focused on the most recent evidence about vascular calcification pathophysiology regarding the genetic aspects and molecular pathways.

Study Selection: The most valuable published original and review articles related to our objective were selected.

Results: Vascular calcification is a multifactorial disease; thus, its pathophysiology cannot be explained by a single specific factor, rather than by the result of the association of several genetic variants, molecular pathway interactions, and environmental factors that promote its development.

Conclusion: Although several molecular aspects of this mechanism have been elucidated, there is still a need for a better understanding of the factors that predispose to this disease.

Key words: Atherosclerosis; Cardiovascular Risk; Genetics; Polymorphisms; Vascular Calcification

Introduction

Calcium phosphate, in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), is the main inorganic component of the bony tissue. Its crystals grow from vesicles secreted by osteoblasts and function as major nests of bony tissue formation.[1] Vascular smooth muscle cells can similarly secrete these vesicles, promoting ectopic formation of bony tissue and creating what it is generally known as vascular calcification.[2]

Vascular calcification is the result of a deregulated mineral metabolism, which ultimately leads to hydroxyapatite deposits on the vascular wall. It has been reported that vascular calcification prevalence increases with age. Moreover, when pathologies such as diabetes mellitus, dyslipidemia, hypertension, and chronic kidney disease (CKD) are present, the mechanisms that inhibit the deposition hydroxyapatite crystals at the vascular level are prone to be affected.[3,4] Therefore, in the last 20 years, the perception of vascular calcification as a pathology evoked by a passive consequence of aging has changed to the concept of an active phenomenon,

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in which hemodynamical, pathological, anatomical, genetic, and epigenetic factors interact to facilitate calcium deposition at the vascular level.[5]

Vascular calcification is an inherent phenomenon, as ancient as the human species. Historically, ancient civilizations also suffered from this pathology. In fact, computed tomography (CT) scans of mummies from the Egyptian empire show high prevalence of calcium phosphate deposits affecting coronary, carotid, iliac, femoral, and aorta arteries.[6]

From the histological standpoint, there are two types of vascular calcification. The first is the calcification of the *tunica intima*, also known as atherosclerotic calcification, while the second is the calcification of the *tunica media* or Mönckenberg's sclerosis.[3] Although studies have found that atherosclerotic calcification has a higher association with a chronic vascular inflammatory process than the Mönckenberg's sclerosis, this has not been completely proven in genetic and image trials aiming to define their underlying mechanisms. As a consequence, it is established that it is difficult to discriminate between these two phenomena. Further, both of these entities might share some of the same pathophysiological pathways.[7]

The presence of vascular calcification catalyzes the deposition of new ectopic calcium in large amounts,[3] increasing the risks for other cardiovascular diseases and adverse cardiovascular effects.[8]

Moreover, the linkage between vascular calcification and genetics has been confirmed by a multiethnic study, which aimed to understand the genetic basis of atherosclerosis. In this study, an association was found between familial coronary disease and the prevalence of coronary artery calcification (CAC). These results were consistent in all ethnic groups. In addition, this study revealed a high hereditable component for both coronary artery and abdominal aorta calcification.[9]

In this article, we have taken into account the different aspects in which the genetic and molecular pathways are presumably involved with vascular calcification. Consequently, this review is directed to physicians and its aim is to elucidate the different associations and mechanisms that predispose to vascular calcification with a genetics scope. In addition, a brief discussion of its clinical approach in the diagnosis and treatment has been incorporated into this work.

Genetics

The etiology of vascular calcification is multifactorial. It has been well defined that genetics plays a pivotal role in the pathogenesis of this disease. Indeed, studies have associated up to 50% of the components of this pathological entity to heredity.[7] In the human genome, multiple loci have been associated with the presence of CAC and atherosclerosis.[3]

Studies have reported a risk allele of a single-nucleotide polymorphism (SNP) in chromosome 9p21.3, in which the cyclin-dependent kinase inhibitor (*CDKN2A* and *CDKN2B*) genes are located. The genetic variants located in both genes (*CDKN2A* and *CDKN2B*) increase the risk for both CAC and premature atherosclerosis, suggesting that the presence of polymorphisms in this gene sequence affects the normal modulation of vascular smooth muscle cell proliferation.[10,11] As a consequence, this causes vascular calcification, which will be explained below. In addition, other SNPs associated with CAC are located in region 6p24, within the phosphate and actin regulator 1 gene.[3,11,12] A genomic hypertension study also associates regions 6p21.3 and 10q21.3 to CAC.[7,13]

The current state of knowledge cannot completely explain the mechanism by which the different genomic variants may affect the genetic expression, posttranscriptional modifications (e.g., alternative splicing), or peptide sequence, which ultimately modulate vascular calcification. According to the genetic and physiological influences, other genes have been suggested as possible causative agents. In addition, other polymorphisms with possible associations with vascular calcification have been found. These genes have been grouped according to their mechanism of action in different categories [Table 1]. Furthermore, polymorphism studies have reported conflicting data on the aforementioned genes; in other words, both positive evidence and negative evidence regarding their association with vascular calcification have been published. Therefore, no conclusions should be drawn on this matter at the moment.[7]

Pathophysiology

Broadly speaking, two major pathophysiological pathways explain the origins of ectopic calcification, namely the unregulated induction of osteogenesis and second, the loss of mineralization inhibition factors.

Unregulated induction of osteogenesis

Smooth muscle cell differentiation to bony tissue can be induced through different pathways. As discussed

Table 1: Genes associated with vascular calcification grouped according to their mechanism of action

Category	Gene
Influence on endothelial function and leukocyte adhesion	*CCR2*
	ACE
	ELAM-1
	EPHX2
Oxidative stress modulation	*GPx-1*
Extracellular matrix remodeling	*MMP3*
Bone metabolism	*BMP*
Vitamin K metabolism	*MGP*
	CALU
	VKORC1

CCR2: Chemokine receptor type 2; *ACE*: Angiotensin-converting enzyme; *ELAM-1*: Endothelial leukocyte adhesion molecule 1, or E-selectin; *EPHX2*: Epoxide hydrolase 2; *GPx-1*: Glutathione peroxidase 1; *MMP3*: Matrix metalloproteinase 3; *BMP*: Bone morphogenetic protein; *MGP*: Matrix Gla Protein; *CALU*: Calumenin; *VKORC1*: Vitamin K epoxide reductase complex subunit 1.

below, oxidative stress, endothelial dysfunction, and proinflammatory factors play a major role in this differentiation.

Onset of vascular osteogenesis

Calcifying cells, a subfamily of vascular smooth muscle cells, establish an important role in the aforementioned process.[14] In *in vitro* studies, calcifying nodules have been observed within these cells, sharing important characteristics with bony tissue, such as increased activity of alkaline phosphatase (ALP) and expression of osteopontin (OPN), osteonectin, and osteocalcin.[15] In addition, calcifying cells represent up to 20% of the vascular smooth muscle cell population, and can potentially generate a mesenchymal lineage of osteoblasts, promoting osteogenesis.[2]

Alternatively, pericytes are another kind of vascular osteogenic inductor – a group of contractile cells that surround the endothelial cells in the capillaries. These cells have a high potential to become mesenchymal progenitors and differentiate into osteoblasts and chondrocytes.[16] In addition, pericytes adopt a behavior similar to calcifying cells by generating *in vitro* calcified nodules,[17] presenting molecules such as type I collagen, OPN, matrix Gla protein (MGP),[18] and osteocalcin within the vasculature. When an atherosclerotic lesion is present, these factors act as osteoprogenitors in the vascular wall. Moreover, pericytes are part of the microvasculature endothelium, and so, they are deeply involved in angiogenesis,[19] which is closely related to vascular calcification as explained below.

The formation of new small intravascular blood vessels (angiogenesis) is common around vascular calcium deposits. In fact, the larger the calcified lesion is, the higher the level of angiogenesis will be found. However, the new vessels are prone to bleed within the plaque, which can ultimately lead to plaque disruption and ischemic events.[20] Concerning this process, we can thus say that the greater the number of blood vessels, the higher the input of circulating factors that will potentially initiate calcification. Pericytes and calcifying cells can differentiate into osteoblasts too, which, in addition to their physiologic osteogenic role, can produce vascular endothelial growth factor. In turn, this promotes angiogenesis and creates the particular pathways essential for vascular calcification.[5]

Oxidative stress and cytokines

Bone morphogenetic protein (BMP) molecules are known for their ectopic bone formation and are recognized as important mediators of vascular calcification. BMP2 and 4 favor mineralization while BMP7 has demonstrated its ability to retard this process.[21,22] BMP2 and 4 act primarily through the interaction with their receptors, which phosphorylate regulatory Smads (intracellular proteins that transduce extracellular signals and carry ligands to the nucleus where they activate a pathway of genetic transcription)[23-25] and modulate genetic expression, thus favoring vascular calcification.[26] On the other hand, BMP7 regulates expression of smooth muscle α-actin, preventing the osteoblastic differentiation.[21] By the same token, it is suggested that polymorphisms in the *BMP7* gene are associated with a higher vascular calcification risk.[27]

While BMPs have shown to be important mediators of calcifying processes, their effects are insufficient by themselves to create a calcified environment. To achieve calcification, a subsequent activation of other osteogenic transcription factors is needed. The main example of such molecules is the core-binding factor-α1 (Cbfa1).

Cbfa1 is a key element in the physiological osteoblastic differentiation. However, its function is not limited to bony tissue maturation. As a matter of fact, its expression in the vascular wall serves as a definite marker in the ectopic calcium deposits, generating the very first step in vascular calcification.[5] Cbfa1 further activates osterix, which will influence the vascular calcification phenotype. Cbfa1 also controls the expression of several other proteins that will promote ectopic osteoblastic differentiation, such as osteocalcin, OPN, and type I collagen.[28,29]

As for these molecular pathways, high expression of BMP2 is observed when endothelial cells are exposed to free radicals,[30] indicating that BMP2 expression is mediated by oxidative stress. For this reason, reactive oxygen species (ROS) can further incite other markers of vascular calcification, such as ALP activity.[31-33]

BMP2 induces cyclooxygenase 2 activity and, as a result, it stimulates both prostaglandin production and nicotinamide adenine dinucleotide phosphate-oxidase oxidase subunit nox1 expression, which increases ROS formation. The role of BMP2 over the ROS regulation consequently provides an important link between vascular calcification and the inflammatory process, which is associated with endothelial dysfunction.[32] Hence, both proinflammatory microenvironment and vascular calcification coexist in atherosclerotic lesions.[5]

Inorganic phosphate can also activate Cbfa1. In fact, vascular cellular culture studies have been carried out with the intention to describe the effect of Cbfa1 over osteoblastic differentiation, and they have reported an induced mineralization and OPN expression after inorganic phosphate was added.[34-36] This can be clinically related to the high presence of calcified plaques in patients with CKD,[34,35] who suffer from high levels of phosphate for two main reasons: poor glomerular filtration rate and, as a result, secondary hyperparathyroidism (a common complication of CKD). Accordingly, inorganic phosphate promotes osteoblastic phenotype through a type IIc sodium-dependent phosphate transporter that regulates calcium metabolism activating Cbfa1.[35,36] Alternatively, parathyroid hormone (PTH) is commonly elevated in patients with CKD and promotes the expression of protein kinase A, which can phosphorylate and activate Cbfa1.[37,38]

Recent evidence supports the essential role played by different proteins in the calcification of atherosclerotic lesions, such

as OPN, OPG, receptor activator of nuclear factor-κB ligand (RANKL), and ALP. It has been indicated that serum OPG levels are associated with vascular calcification in humans. In addition, the determination of serum OPG has been suggested as a prognostic marker of cardiovascular disease. Chang *et al*. reported that a high concentration of glucose promotes the expression of OPG, and inhibits the expression of RANKL in vascular smooth muscle cells, a molecular pathway that could partially explain the mechanisms underlying diabetic vascular calcification.[39-41]

Medications implicated in calcification

Warfarin, a Vitamin K antagonist, is commonly used as an oral anticoagulant to treat atherothrombotic diseases. Its use promotes vascular calcification by ultimately stopping gamma-carboxylation of MGP, an important inhibitory factor of calcification.[5] By its mechanism of action, warfarin inhibits the expression of Vitamin K epoxide reductase complex subunit 1 gene, which is one of the candidate genes. This gene is responsible for converting Vitamin K epoxide into Vitamin K hydroquinone, an essential substrate for MGP carboxylation.[7] When carboxylated MGP is absent, a procalcification environment is promoted.

Another molecule involved in vascular calcification is calcitriol (1,25-dihydroxyvitamin D), commonly used in secondary hyperparathyroidism. Especially in patients with CKD, high levels of Vitamin D can cause hypercalcemia, which then leads to a higher risk of soft-tissue calcification.[42] Calcitriol increases ALP activity,[43] and is physiologically inactivated by a chain reaction that starts with a hydroxylation reaction by 24-hydroxilase,[44] an enzyme codified by cytochrome p450, family 24, subfamily A, polypeptide 1 (*CYP24A1*).[7] Studies showed that *CYP24A1*-deficient mice indeed present excessively high levels of ectopic calcification.[45] Hence, this result confirms that calcitriol is an enhancer of vascular calcification.

Other osteogenic induction factors

As previously mentioned, ALP activity is an important factor in the vascular calcification orchestra, because it serves as an early marker for the presence of calcium deposits. Since it is essential in the formation of hydroxyapatite during ossification, when this enzyme is active in other tissues, it indicates the initiation of ectopic ossification. ALP further modulates calcification by diminishing inorganic pyrophosphate levels, an important inhibitory factor of calcification.[46,47]

Leptin, another vascular calcification mediator, physiologically modulates bone mass, binding to and activating the ventromedial hypothalamic nucleus as well as β-adrenergic receptors in osteoblasts.[48,49] In *in vitro* models, it has been demonstrated that leptin plays a crucial role in vascular calcification. This occurs through mechanisms[50] that promote osteoblast differentiation[51] and increase oxidative stress in endothelial cells, which then induce the expression of BMP2, a well-known marker for vascular calcification.[52,53]

Loss of mineralization inhibition factor

As previously noted, the second pathophysiologic pathway present in vascular calcification refers to the loss or malfunction of the factors that, under normal circumstances, would inhibit calcification.

Pyrophosphate pathway

Under physiological conditions, calcium phosphate deposits in the vascular wall can trigger unfavorable reactions. Pyrophosphate is in charge of blocking the generation of these crystals, naturally inhibiting processes that lead to vascular calcification. Hence, it is suggested that vascular calcification is a consequence of pyrophosphate regulation alterations.[3]

Associations between ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) and idiopathic infantile arterial calcification have been well established. This gene codes for NPP1, an enzyme responsible for processing extracellular ATP (adenosine triphosphate), which then generates pyrophosphate. Thus, the loss of function of *ENPP1* is associated with autosomal recessive inheritance. This, in turn, results in a substantial decrease of serum pyrophosphate,[54-56] promoting vascular calcification.

Another enzyme related to the pyrophosphate pathway is cluster of differentiation 73, a membrane surface protein coded by ecto-5'-nucleotidase gene (*NT5E*). This enzyme catalyzes adenosine monophosphate conversion into inorganic phosphate, which makes it a calcification accelerator. Mutations in this gene are less severe and phenotypically not as restrictive as those in *ENPP1*. Nonetheless, it confirms the role of ATP derivative pyrophosphate pathway in vascular calcification from a genetic perspective.[57]

Similarly, an additional mutation related to vascular calcification is present in ATP-binding cassette, subfamily C, member 6 (*ABCC6*),[58] a gene that codes for a transmembrane ATP-dependent protein. Knockout mice models for this gene show the development of ectopic calcification in skin, retina, medium-sized arteries, aorta, and vena cava. Therefore, *ABCC6* gene is considered responsible for elastic pseudoxanthoma,[59] a disease with the aforementioned characteristics, in addition to dystrophic calcinosis, which refers to calcium deposits in necrotic and inflamed spots.[60] A molecular link between elastic pseudoxanthoma and vascular calcification is established on the clinical and histological similarities between the two, suggesting that they both arise through mutations in *ABCC6,* which affect the pyrophosphate pathway[61] and reduce MGP gamma-carboxylation.[62]

ENPP1 and *NT5E* interact with receptor of advanced glycation end products (RAGE),[63] a receptor expressed on endothelial cells, macrophages, dendritic cells, and smooth muscle cells. When activated, the receptor initiates a signal cascade well evidenced to participate actively in vascular disease in general.[64-67] However, members of

the S100 proteins family, RAGE ligands, also known as calgranulins, have been identified to interact specifically in the processes that lead to vascular calcification. A direct association has been made between the expression of the S100A12 protein, or calgranulin C, and vascular calcification in transgenic mice S100A12tg/ApoE deficient.[68,69] Vascular smooth muscle cells in those mice showed a rise in osteoblast genetic expression, as well as more calcium deposits.

Moreover, S100A8 (calgranulin A) and S100A9 (calgranulin B) both relate to ROS production.[70] In addition, free radicals generate other reactions in the vasculature, including activation of RUNX-2: a regulator of osteoblast genetic expression.[71] Besides, it is possible that S100 proteins may indirectly promote calcification through apoptotic mediators, creating necrotic bodies as potential calcification nests.[72]

Matrix Gla protein

Its mechanism of action is complex; it modulates calcification by inhibiting the interaction between BMPs and their receptors.[73-75] The variants in its serum levels regulate mineral deposition and osteogenic differentiation; while intermediate levels inhibit the process, both low and high levels may favor calcification.[74]

To become functional, MGP needs to undergo γ-carboxylation, and when it is carboxylated, MGP may bind to calcium. This causes conformational changes in the molecule, which will ultimately inhibit BMP interaction with its receptors, thus functioning as a physiologic inhibitor of calcification.[76,77] Note that when carboxylated, MGP inhibits calcification; however, this protein promotes calcification in its noncarboxylated form.

Other inhibitory factors

There is a great amount of other agents that inhibit vascular calcification; we will only mention a few of them. Similar to MGP, OPN and OPG inhibit the process by acting directly on the vascular wall. Alternatively, fetuin-A or α2-Heremans-Schmid glycoprotein, a circulating inhibitory factor, may stop the formation of hydroxyapatite crystals.[78] Finally, adiponectin, an adipocyte derivative hormone, modulates osteoblastic differentiation through its receptors pathway 1/p38.[79]

Vascular Calcification Assessment

There are a number of noninvasive imaging tools that clinicians use to identify and assess vascular calcification. Nonetheless, all of them provide important information that could improve prediction of cardiovascular risk beyond the traditional risk factors, especially in patients with CKD, in which vascular calcification is an important component of the disease.[80]

Through a plain radiography, it is possible to recognize patterns in the artery wall, which might differentiate *tunica intima* from *tunica media* calcification. Patchy and irregular radiopaque lesions on the artery course usually characterize intimal calcification. On the other hand, *tunica media* calcification typically presents as linear tram-track radiopaque lesion along the artery border (angiogram-like).[81-83] In addition, carotid artery calcifications can be spotted on the panoramic radiograph below the mandibular angle and adjacent to the cervical vertebrae at the level of the intervertebral junction of C3 and C4.[84]

Ultrasonography is a rapid, available, and inexpensive method to investigate the vascular system, due to its ability to both detect calcifications and measure the vascular thickness, blood flow, and its characteristics. Literature supports, as a prognostic tool, the measurement of the carotid intima-media thickness in the prediction of cardiovascular events.[85] There are also reports in which renal and carotid artery calcification was easily detected with ultrasound.[84-86]

The coronary artery calcium detection by CT scan is probably one of the most frequent and reliable methods to measure vascular calcification. Coronary artery calcium score is determined through the method of Agatston *et al*.,[87,88] an important predictor for the incidence of coronary artery disease correlated with the histological findings.[89,90] It is even considered that the degree of coronary artery calcium can be an isolated risk factor for acute coronary syndrome. Determining the coronary artery calcium score may detect coronary artery disease in asymptomatic patients and this allows therapeutic decision-making in the group of patients with subclinical coronary artery disease.[91,92] With the intent to clarify the coronary artery calcium score, a scale has been proposed [Table 2].[93] An example of the usefulness of this coronary artery calcium score was reported in a study, in which asymptomatic patients for coronary artery disease with the diagnosis of diabetes mellitus were analyzed. During follow-up, the incidence of myocardial infarction was 0% in the group with a coronary artery calcium score from 0 to 10, 18.4% in patients with a score from 11 to 100, 22.9% in patients with 101–400, 48.3% in the group with a score from 401 to 1000, and 71.4% in patients with over 1000 ($P < 0.0001$).[94]

Current and Emerging Therapies for Vascular Calcification

The best treatment for vascular calcification is the management of the risk factors that predispose to cardiovascular disease

Table 2: Coronary artery calcium score classification according to the severity of the atherosclerotic involvement

Coronary artery calcium score	Plaque burden
0	No evidence of coronary artery disease
0–10	Minimal
11–100	Mild
101–400	Moderate
401–1000	Severe
>1000	Very severe

and the disorder of bone and mineral metabolism. Because hydroxyapatite is insoluble and stable under physiological conditions, the treatment for vascular calcification focuses on preventing its progression.[95] Furthermore, several medications have been used for the treatment of vascular calcification in humans and animal models, which take a particular relevance in CKD patients.[96]

Many therapies used in cardiovascular disease, osteoporosis, and CKD could be promising for treating vascular calcification. Some examples are as follows: (1) the endothelin receptor antagonists, which act by reducing blood pressure, (2) statins, which inhibit cholesterol production, (3) bisphosphonates, which inhibit osteoclast proliferation and survival, (4) denosumab and osteoprotegerin, which binds RANKL thus inhibiting binding to RANK, (5) teriparatide, which stimulates bone formation, (6) phosphate binder, which binds to dietary phosphate thus preventing its absorption, (7) Vitamin D receptor agonists, which treat secondary hyperparathyroidism and Vitamin D deficiency associated with CKD, (8) calcimimetics, which bind to calcium-sensing receptors treating secondary hyperparathyroidism, (9) Vitamin K, which is a cofactor in various metabolic pathways and frequently deficient in CKD patients, and (10) sodium thiosulfate, which acts as a vasodilator, antioxidant, and a calcium chelator thus preventing calcification.[96]

Conclusions

From the histological point of view, vascular calcification represents a complicated, multifactor disease that can be classified as *tunica intima* calcium deposition (atherosclerotic calcification) or *tunica media* calcification (Mönckenberg's sclerosis). The presence of this pathology increases the risks of adverse cardiovascular events and represents a serious public health problem. The pathophysiology of vascular calcification cannot be explained by a single specific factor; rather it is the result of the association of several genetic variants, molecular pathways interactions, and environmental factors that promote vascular calcification by either increasing osteogenesis or inhibiting its regulatory pathways. Although several molecular aspects of this disease have been elucidated, there is still a need for a better understanding of the factors that predispose to this disease.

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Conflicts of interest

There are no conflicts of interest.

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