

Editorial

Vitamin K Epoxide Reductase Complex and Vascular Calcification

Is This the Important Link Between Vitamin K and the Arterial Vessel Wall?

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Vitamin K is a cofactor in the γ -glutamyl carboxylation pathway, a posttranslational conversion of specific glutamate residues into γ -carboxyglutamic acid (Gla) residues, catalyzed by the endoplasmic reticulum enzyme γ -glutamyl carboxylase. The substrates of carboxylase are the so-called vitamin K–dependent proteins, which are involved in diverse physiological processes such as blood coagulation, bone and soft tissue mineralization, and cellular proliferation. The coagulation factors II (prothrombin), VII, IX, and X have procoagulant activity, whereas proteins C and S inhibit blood coagulation (see reviews by Dahlback¹ and Stafford²). Protein Z is involved in the fixation of thrombin by binding to a phospholipid surface. Matrix Gla protein (MGP) and osteocalcin (or bone Gla protein) are regulators of tissue mineralization, whereas Gas6 is involved in the regulation of cell growth. The mRNA sequences of 4 new putative vitamin K–dependent proteins were recently reported, but their functions remain elusive: 2 proline-rich Gla proteins, PRGP1 and PRGP2, and 2 transmembrane Gla proteins, TMG3 and TMG4. For each of these proteins, the presence of Gla residues is a prerequisite for Ca²⁺ binding and/or Ca²⁺-dependent interaction with negatively charged surfaces. Four key components are involved in the proper biosynthesis of the vitamin K–dependent proteins: the enzymes γ -glutamyl carboxylase and vitamin K epoxide reductase complex (VKORC1), vitamin K, and a precursor protein. Not the form in which it occurs in food (vitamin K quinone) but the reduced form, vitamin K hydroquinone (KH₂), is the active cofactor used by the enzyme γ -glutamyl carboxylase ([Figure](#)). KH₂ is converted into vitamin K epoxide (KO), and KO is subsequently reduced to vitamin K and vitamin KH₂ in 2 reactions by the action of VKORC1. This recycling mechanism is called the vitamin K cycle and explains why the daily requirement for vitamin K is low and why vitamin K deficiency is rarely seen in healthy subjects. Except for the dithiol-dependent pathway, vitamin K (and not KO) may also be reduced by DT diaphorase, an NAD(P)H-dependent dehydrogenase. Although DT diaphorase is highly expressed in the liver, the dithiol-dependent pathway is the most active one for KH₂ generation³ ([Figure](#)).

Vascular calcification occurs in 2 distinct forms: intimal calcification, which always occurs in the context of atherosclerosis, and medial calcification, which can occur in its absence. Intimal calcification begins as early as the second decade of life, soon after fatty streak formation, and increases with age and lesion progression.⁴ Whether intimal calcification stabilizes atherosclerotic plaques or promotes plaque rupture is still a matter of debate. Calcified lesions and fibrotic hypocellular lesions are much stiffer than cellular lesions.⁵ Furthermore, biomechanical data suggest that calcification reduces the "stresses" in a plaque and does not cause rupture.⁶ It has been suggested that when the entire fibrous cap covering a lesion is calcified, the underlying plaque is encapsulated and thus, protected from rupture. Plaques with a heavily calcified cap are 5 times stiffer than cellular lesions or the normal vessel wall and are very resistant to rupture.⁷ In contrast, coronary artery calcification has been linked to atherosclerotic plaque rupture, and coronary artery and aortic calcifications are closely associated with an increased risk for cardiovascular events.⁸ Calcium crystals have been shown to aggravate inflammation,⁹ and if this is the case in an atherosclerotic plaque, enhanced inflammation would be expected to indirectly cause plaque instability. These contradictory data have fueled the hypothesis that only extensive calcification renders the atherosclerotic plaque resistant to rupture, whereas early or intermediate stages of calcification may actually enhance plaque vulnerability to rupture.¹⁰ Tunica media calcification is an age-dependent process that occurs independently of atherosclerosis. Medial calcification is associated with type 2 diabetes and appears to be a strong independent predictor of cardiovascular and coronary heart disease (CHD) mortality.¹¹ Calcification of the arterial media contributes to stiffening of arterial tone, which may lead to an increase in cardiac work and systolic blood pressure, a highly significant predictor of myocardial infarction and vascular death.¹² Overall, vascular calcification maybe regarded as 1 of the major complications of cardiovascular disease and an independent risk factor for myocardial infarction and cardiac death.

The importance of MGP in the prevention of vascular calcification stems from several observations. Although the molecular mechanism of MGP action is unknown, data from various studies demonstrate that it plays a major role in the inhibition of soft tissue calcification. Most important, MGP-deficient mice showed 2 phenotypes: impaired bone growth and extensive medial calcification of the aorta, leading to death within 8 weeks after birth due to rupture of the thoracic or abdominal aorta.¹³ Furthermore, inhibition of vitamin K-dependent protein synthesis in extrahepatic tissues by a combination of warfarin and vitamin K1 resulted in calcifications of the elastic lamellae in arteries and heart valves of rats within 3 to 5 weeks via inhibition of the -glutamyl carboxylation of MGP.^{14,15}[R15-174274](#) In contrast, vitamin K2 (MK-4) prevented warfarin-induced vascular media calcification,¹⁵ suggesting differences in vitamin K1 and K2 tissue bioavailability and cofactor utilization in the reductase/carboxylase reaction. In humans, it has been demonstrated that oral anticoagulant (warfarin) treatment is associated with substantially increased heart valve calcification¹⁶ and that undercarboxylation of MGP is associated with intimal and medial vascular calcification.¹⁷

Given the proposed role for MGP and vitamin K in the prevention of calcium deposition, 3 factors influencing arterial calcification can be deduced: (1) the level of MGP expression, (2) vitamin K status, and (3) mutations/polymorphisms affecting the activities of either -glutamyl carboxylase or *VKORC1*. The report by Wang et al¹⁸ in this issue of *Circulation* demonstrates that a polymorphism in *VKORC1* is indeed associated with a higher risk of arterial vascular disease. It reports on the association of polymorphisms in the *VKORC1* gene with stroke (n=1811 patients), CHD (n=740 patients), and aortic dissection (n=253 patients) compared with matched

controls (n=1811, 740, and 416, respectively), who were recruited from 7 clinical centers located in 7 Chinese provinces (stroke) or Fuwai Hospital in Peking (CHD and aortic dissection). The results of this study indicate that the *CC* and *CT* genotypes of polymorphism +2255 are more prevalent in patients with stroke, CHD, and aortic dissection compared with controls and that the presence of a *C* allele of single nucleotide polymorphism +2255 is associated with an almost 2-fold increased risk of stroke, CHD, or aortic dissection. Although the authors suggest several possibilities, the biological mechanism behind the +2255 polymorphism in intron 1 of *VKORC1* and a 2-fold increased cardiovascular risk remains unclear.

First of all, the authors propose that the observed higher serum levels of undercarboxylated osteocalcin and PIVKA-II in subjects carrying the *TT* genotype of +2255 are the result of decreased promoter activity and lower mRNA expression levels. Undercarboxylated osteocalcin serum levels in subjects carrying the *TT* genotype, however, were only slightly higher compared with the reference range (1.236±0.569 ng/mL). Together with the observed variation in analysis within this group and the low numbers of total subjects tested (n=49), it is difficult to conclude that subjects carrying the *CC* genotype have a higher -glutamyl carboxylation activity compared with carriers of the *T* allele. Extending the analyses with more subjects would possibly enhance the statistical difference, thereby providing a stronger basis for the proposed hypothesis. Higher levels of *VKORC1* suggest better carboxylation, not only of osteocalcin and prothrombin but also of vascular MGP, which has been suggested to prevent or inhibit vascular calcification, as the authors state in their discussion. With regard to this point, it would be of interest to determine the serum levels of carboxylated and undercarboxylated MGP (however, these assays are not yet commercially available), as well as the state of vascular calcification within subjects carrying the *TT* and *CC* genotypes. On the other hand, increased carboxylation of vitamin K-dependent proteins within the arterial vessel wall suggests the presence of the more active coagulation factors prothrombin and factors VII and X in atherosclerotic lesions. These proteins have been shown to be present in all stages of atherosclerosis.^{19,20}[R20-174274](#) It has been known for many years that activated coagulation proteins are involved in atherosclerotic lesions, but the underlying mechanisms are unknown. The clotting end product fibrin, as well as the precursor protein fibrinogen, is abundant even in early lesions and may play a distinct role in different aspects of the pathophysiology of atherosclerosis.²¹ Factor VII has been localized at smooth muscle cells,¹⁹ and our recent data also demonstrate colocalization of factor X and thrombin in atherosclerotic lesions.²⁰ Together, these data suggest the presence of a fully operational coagulation cascade in the atherosclerotic vessel wall, of which at least some of the proteins are locally synthesized. The generation of specific proteases such as thrombin may have a crucial influence on the atherosclerotic process, as suggested in very recent experiments by Karanam et al,²² showing that prolonged inhibition of thrombin with the direct inhibitor melagatran diminished morphological plaque lesions as well as plaque ruptures in apolipoprotein (apo) E^{-/-} mice. These data are supported by the finding that mice with a defect in the intrinsic coagulation cascade that had been backcrossed with apoE^{-/-} mice had a reduced atherosclerotic burden compared with mice of the parent apoE^{-/-} background.²³ The association between the *CC* genotype and an increased risk of CHD could, at least in part, be explained by the contribution of increased -glutamyl carboxylation of blood coagulation proteins in either the liver or arterial vessel wall to the progression and thrombogenicity of atherosclerotic lesions.

In summary, the authors of this important report provide the first evidence of an association between the *CC* genotype of +2255 in *VKORC1* with a 2-fold increased risk for CHD. This haplotype may serve as a novel genetic marker for the risk of CHD. Their findings and those of

others support an important role for vitamin K–dependent proteins in biological processes involved in the onset of CHD, such as atherosclerosis. For many years, vitamin K has been considered the essential cofactor for γ -glutamyl carboxylation, and it was thought that vitamin K–dependent proteins were mainly involved in coagulation. Recent discovery of the *VKORC1* gene and the association of its polymorphisms with an increased risk of CHD are exciting developments in vitamin K research. Furthermore, the involvement of vitamin K–dependent proteins in diverse biological mechanisms besides coagulation may have significant clinical impact with respect to influences on the progression and thrombogenicity of atherosclerotic plaque. Although specific thrombin-inhibiting drugs are now being clinically validated for the prevention of (recurrent) atherothrombotic occlusion, these medications may have additional effects on the progression and thrombogenicity of atherosclerosis that may be of substantial therapeutic interest. Establishment of the presence and magnitude of these effects is of great importance especially in relation to the described requirements for a lower dose of warfarin in patients with a certain polymorphism in *VKORC1*.

The opinions expressed in this article are not necessary those of the editors or of the American Heart Association.



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