

# Role of Vascular Smooth Muscle Cell Phenotypic Switching and Calcification in Aortic Aneurysm Formation Involvement of Vitamin K-Dependent Processes

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## Role of Vascular Smooth Muscle Cell Phenotypic Switching and Calcification in Aortic Aneurysm Formation

### Involvement of Vitamin K-Dependent Processes

Ploingarm Petsophonsakul, Malgorzata Furmanik, Rachael Forsythe, Marc Dweck, Geert Willem Schurink, Ehsan Natour, Chris Reutelingsperger, Michael Jacobs, Barend Mees, Leon Schurgers

**Abstract**—Aortic aneurysm is a vascular disease whereby the ECM (extracellular matrix) of a blood vessel degenerates, leading to dilation and eventually vessel wall rupture. Recently, it was shown that calcification of the vessel wall is involved in both the initiation and progression of aneurysms. Changes in aortic wall structure that lead to aneurysm formation and vascular calcification are actively mediated by vascular smooth muscle cells. Vascular smooth muscle cells in a healthy vessel wall are termed contractile as they maintain vascular tone and remain quiescent. However, in pathological conditions they can dedifferentiate into a synthetic phenotype, whereby they secrete extracellular vesicles, proliferate, and migrate to repair injury. This process is called phenotypic switching and is often the first step in vascular pathology. Additionally, healthy vascular smooth muscle cells synthesize VKDPs (vitamin K-dependent proteins), which are involved in inhibition of vascular calcification. The metabolism of these proteins is known to be disrupted in vascular pathologies. In this review, we summarize the current literature on vascular smooth muscle cell phenotypic switching and vascular calcification in relation to aneurysm. Moreover, we address the role of vitamin K and VKDPs that are involved in vascular calcification and aneurysm.

**Visual Overview**—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2019;39:1351-1368. DOI: 10.1161/ATVBAHA.119.312787.)

**Key Words:** aortic aneurysm ■ blood vessels ■ extracellular matrix ■ phenotypic switching ■ vascular calcification ■ vascular smooth muscle cell ■ vitamin K

Aortic aneurysm is a matrix degenerative disease defined by a dilated blood vessel. Aneurysms weaken the arterial vessel wall increasing risk of rupture, which results in massive, and often fatal, internal bleeding. Aortic aneurysms are the result of environmental and genetic risk factors, which lead to shear stress, inflammation, positive vascular remodeling, and ECM (extracellular matrix) degradation.<sup>1-4</sup> Aortic aneurysms resulted in >151 000 deaths globally in 2013 and are ranked in the top 15 causes of mortality in the United States.<sup>5</sup> However, there are no efficient interventions to prevent aneurysm progression.

The sequential pathophysiology of aneurysm formation is unclear, but it is believed that vascular smooth muscle cells (VSMCs) play a central role. Most VSMCs in the vessel wall display a contractile phenotype, which allows them to maintain vascular tone. However, VSMCs have the ability to differentiate into a synthetic phenotype. This process is termed phenotypic switching and is considered to be a key mechanism in arterial remodeling.<sup>6,7</sup> Synthetic VSMCs are characterized by decreased contractile protein expression and increased the

production of elastolytic enzymes (MMPs [matrix metalloproteinases]), which degrade the ECM and facilitate migration by detaching cells from the basement membrane and ECM.<sup>8</sup> Additionally, synthetic VSMCs can secrete extracellular vesicles that enhance local inflammation and promote vascular calcification.<sup>9,10</sup> Vascular calcification, an extreme form of arterial remodeling mediated by VSMCs, is characterized by deposition of calcium phosphate crystals in the vessel wall.<sup>11</sup> Calcification of the medial layer of the vessel wall is associated with arterial stiffening<sup>12</sup> and has been observed in aneurysm.<sup>13-16</sup> Vascular calcification is, in part, regulated by vitamin K-dependent mineralization-inhibiting proteins, such as MGP (matrix Gla protein).

Vitamin K is a fat-soluble vitamin, whose main function is to facilitate carboxylation of VKDPs (vitamin K-dependent proteins). High dietary intake of vitamin K has been shown to be associated with reduced coronary artery calcification and all-cause mortality,<sup>17,18</sup> but the potential influence of vitamin K on VSMC-mediated mechanisms of aortic aneurysm formation has not been analyzed.

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**Nonstandard Abbreviations and Acronyms**

<b>AAA</b>	abdominal aortic aneurysm
<b>BMP</b>	bone morphogenetic protein
<b>CHOP</b>	C/EBP homologous protein
<b>CNN</b>	SM-calponin
<b>COL3A</b>	type III collagen
<b>dp-ucMGP</b>	dephospho-uncarboxylated MGP
<b>ECM</b>	extracellular matrix
<b>ER</b>	endoplasmic reticulum
<b>Gas6</b>	growth arrest-specific gene 6
<b>GGCX</b>	$\gamma$ -glutamyl carboxylase
<b>HDL</b>	high-density lipoprotein
<b>KO</b>	vitamin K epoxide
<b>LDL</b>	low-density lipoprotein
<b>MAPK</b>	mitogen-activated protein kinase
<b>MGP</b>	matrix Gla protein
<b>MMP</b>	matrix metalloproteinase
<b>MYH11</b>	myosin heavy chain 11
<b>NADPH</b>	nicotinamide adenine dinucleotide phosphate
<b>NF-<math>\kappa</math>B</b>	nuclear factor- $\kappa$ B
<b>NOX</b>	NADPH oxidase
<b>OPN</b>	osteopontin
<b>PDGF</b>	platelet-derived growth factor
<b>PDI</b>	protein disulfide isomerase
<b>ROS</b>	reactive oxygen species
<b>SIRT1</b>	sirtuin 1
<b>SMMHC</b>	SM myosin heavy chain
<b>STAT3</b>	signal transducer and activator of transcription 3
<b>TAA</b>	thoracic aortic aneurysm
<b>TAAD</b>	thoracic aortic aneurysm and aortic dissection
<b>TGF-<math>\beta</math></b>	transforming growth factor $\beta$
<b>TIMP</b>	tissue inhibitor of MMPs
<b>TRX</b>	thioredoxin
<b>VKA</b>	vitamin K antagonist
<b>VKDPs</b>	vitamin K-dependent proteins
<b>VKOR</b>	vitamin K-oxidoreductase
<b>VKORC1</b>	vitamin K-oxidoreductase complex subunit 1
<b>VKORC1L1</b>	VKORC1-like 1
<b>VSMC</b>	vascular smooth muscle cell
<b>XBP1u</b>	unspliced X box protein 1

In this review, we summarize current knowledge on mechanisms regulating VSMC phenotypic switching and calcification in the context of aortic aneurysm formation. Additionally, we explore the role of vascular vitamin K in these processes to offer insight into therapeutic implications.

### Aneurysm Formation

The word aneurysm comes from the Greek word aneurysma, which means dilation. Clinically, an aneurysm is defined by a dilated blood vessel with an enlargement of 50% greater than the normal diameter.<sup>19</sup> The arterial wall subjected to an aneurysm is vulnerable to pressure. Increased blood pressure results in a continuing enlargement of the vessel wall, which may eventually lead to rupture and hemorrhage.

Aneurysms predominantly occur in the aorta and are clinically termed according to the location: (1) ascending aortic aneurysm, (2) aortic arch aneurysm, (3) descending thoracic aortic aneurysm (TAA), (4) thoracoabdominal aortic aneurysm, and (5) abdominal aortic aneurysm (AAA), the most common type. AAA is the 13th most commonly found cardiovascular disorder,<sup>20</sup> and most frequently seen in men, older than 60 years, and with one or more risk factors, including family history, high blood pressure, high cholesterol, obesity, and smoking.

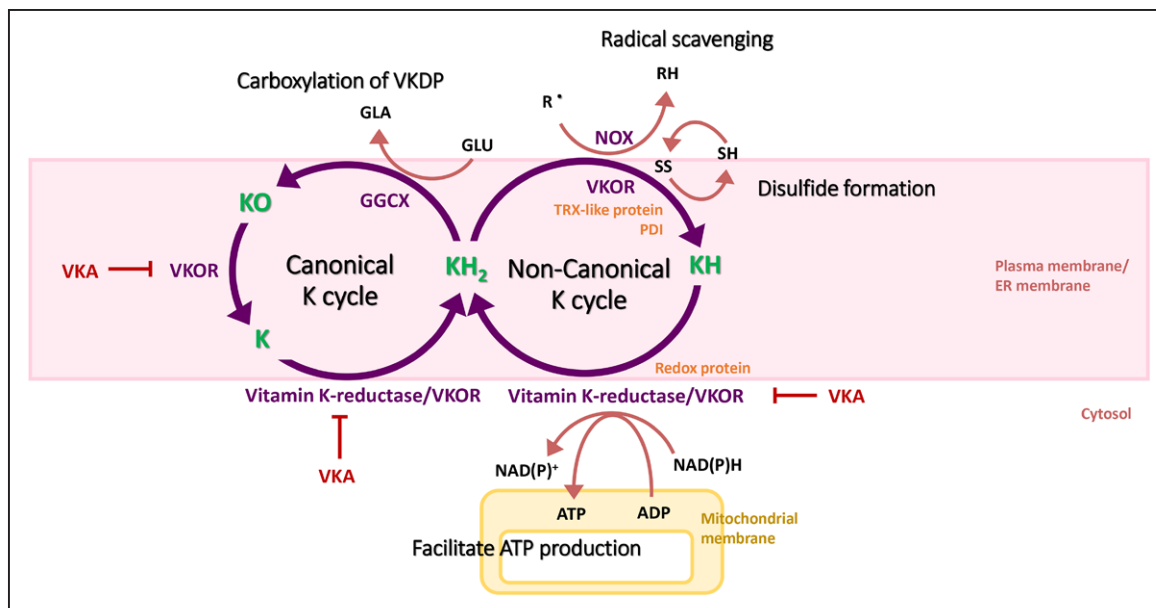
Aortic aneurysms are characterized by a disrupted vessel wall structure with degraded elastic laminae and disappearance of organized VSMC layers.<sup>21,22</sup> For a long time, biomechanical factors were thought to be the main cause of aneurysm formation and rupture. Currently, aneurysm pathophysiology is regarded as a complex biological process involving cellular-driven remodeling of the vessel wall.<sup>23</sup> Active and dynamic remodeling, rather than degeneration of the vessel wall, is the cause of AAA development.<sup>24</sup> In support of this, AAA is characterized histologically by inflammation, oxidative stress, VSMC apoptosis, and ECM degradation.<sup>4</sup> Moreover, a major difference between the healthy vessel wall and the aneurysm wall is the reduced number of VSMCs.<sup>25</sup>

Aortic aneurysms that develop above the diaphragm in the upper aortic segment are generally termed TAAs. TAAs are predominantly a result of genetic and connective tissue disorders, such as Marfan syndrome caused by mutations in fibrillin-1, which assists proper elastic fiber formation and elastin deposition.<sup>26</sup> Other TAA-related diseases are Ehlers-Danlos syndrome type IV and Loeys-Dietz syndrome, caused by mutations in COL3A (type III collagen) and TGF (transforming growth factor)- $\beta$  receptors (*TGF- $\beta$ 1* or *TGF- $\beta$ 2*) genes, respectively.<sup>27</sup> Moreover, TAA may develop in individuals harboring genetic predispositions without syndromic disorders, termed familial TAA and aortic dissection (TAAD).<sup>28</sup> Increased risk for TAAD is also found in patients with bicuspid aortic valve and mutations in VSMC contractile proteins.<sup>29–31</sup>

TAAs and AAAs share common features such as dilation and rupture of the aorta, proteolysis of ECM and depletion of VSMCs.<sup>22</sup> While the AAA is seen as an atherothrombotic origin presenting with intraluminal thrombus, oxidative stress, and adventitial inflammation, intraluminal thrombus, and immune response are not usually observed in the TAA.<sup>22</sup> However, infiltration of inflammatory cells in the aortic wall of TAA patients has been documented.<sup>32,33</sup> Pathological observation in a mouse model suggests that noninflammatory accumulation of SMC-like cells and elastin-poor ECM, which leads to vascular remodeling, is involved in the development of TAAs.<sup>34</sup> Mucoid degeneration, characterized by accumulation glycosaminoglycans and observed during aging of the human aorta, is also specific to TAA.<sup>22,35</sup>

### Vitamin K

Vitamin K is a fat-soluble vitamin. Naturally occurring vitamin K includes vitamin K1 (phylloquinone) and vitamin K2 (menaquinones). Phylloquinone is mainly found in leafy green vegetables, while menaquinones can be found in fermented



**Figure 1.** The canonical and noncanonical vitamin K cycle. In the canonical vitamin K cycle, vitamin K is a cofactor in the carboxylation of VKDPs (vitamin K-dependent proteins), which then become activated. The oxidation from vitamin K-hydroquinone (KH<sub>2</sub>) to vitamin K-epoxide (KO) is driven by GGCX (γ-glutamyl carboxylase). VKOR (vitamin K-oxidoreductase) and vitamin K-reductase enable the recycling process of vitamin K through conversion of KO to K and KH<sub>2</sub>, respectively. Imbalanced equilibrium of vitamin K forms may allow an alternative vitamin K cycle, whose reduction steps to semiquinone (KH) are also driven by VKOR. Through this noncanonical K cycle, VKOR and a redox protein (DTT or TRX [thioredoxin]) facilitate KH<sub>2</sub> conversion to KH, thereby exerting antioxidant properties such as preventing NADPH (nicotinamide adenine dinucleotide phosphate)-dependent lipid peroxidation and protecting the plasma membrane against reactive oxygen species (ROS) by regulating NOX (NADPH oxidase) activity. KH<sub>2</sub> is involved in protein disulfide-thiol interchange of NOX. Moreover, VKOR, together with PDI (protein disulfide isomerase) or TRX-like protein, contribute to disulfide formation and protein synthesis within the ER (endoplasmic reticulum) membrane. Vitamin K also improves mitochondrial oxygen consumption and is involved in ATP generation. In all cases, the presence of a VKA inhibits the recycling process of vitamin K and its subsequent functions. R\* indicates free radical species; RH, organic substrates; SH, disulfide-thiol; and SS, disulfide bond.

food.<sup>36</sup> Menaquinones have an unsaturated aliphatic side chain with a variable number of prenyl units. The number of prenyl units indicates the respective type of menaquinone. It has been shown that menaquinone-7 is absorbed most efficiently and has the best bioavailability.<sup>37,38</sup> During absorption, vitamin K is taken up by enterocytes in the small intestine where it is packaged into chylomicrons, which are taken up by the liver. Vitamin K<sub>2</sub>, specifically the long chain menaquinones, is redistributed into the circulation and available for extrahepatic tissues such as the vasculature.<sup>39,40</sup>

### Vitamin K Cycle

The main function of both vitamin K<sub>1</sub> and K<sub>2</sub> is acting as cofactors in carboxylation of VKDPs (Figure 1). Vitamin K serves as a cofactor for the enzyme GGCX (γ-glutamyl carboxylase), which catalyzes the conversion of glutamic acid in VKDP to γ-carboxyglutamic acid. This reaction is driven by oxidation of vitamin K-hydroquinone to vitamin K epoxide (KO). KO can be recycled by VKOR (vitamin K-oxidoreductase), which converts KO to vitamin K and back to vitamin K-hydroquinone.<sup>41,42</sup>

### VKOR Polymorphisms Versus Aneurysm Risk

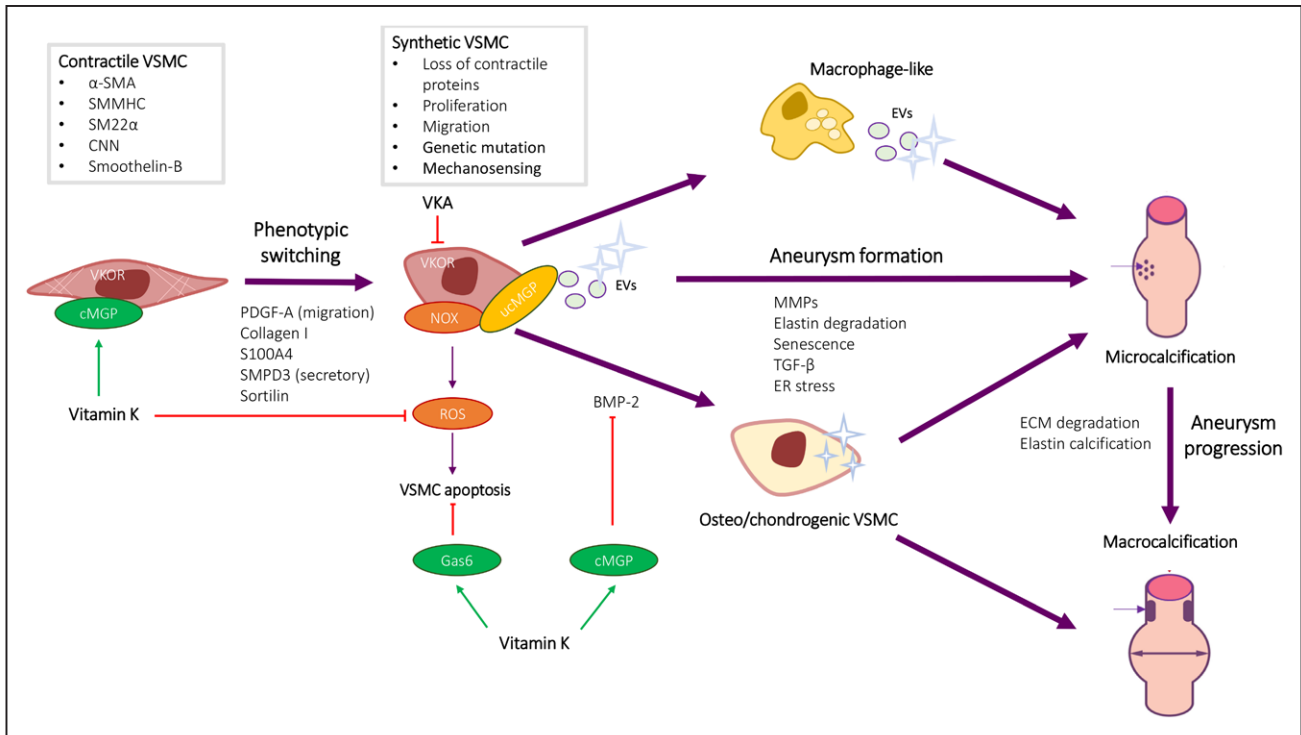
VKOR is expressed in various vascular cells and heart tissue. The first evidence of a role for vitamin K in aneurysm formation comes from a study showing increased expression of VKOR in vascular endothelial cells and ventricular aneurysm tissue of human heart.<sup>43</sup> Additionally, in a study of 253 cases

of aortic dissection, of which 11.5% had Marfan syndrome, and 416 controls, VKORC1 (VKOR complex subunit 1) polymorphisms were shown to be associated with an almost 2-fold higher risk of aortic dissection, independent of conventional vascular risk factors.<sup>44</sup> In the same study, a similar observation was made in patients with stroke and coronary heart disease, suggesting VKORC1 variation as a common genetic risk factor for all vascular diseases.<sup>44</sup> Indeed, the role of VKORC1 was highlighted as an emerging genetic marker of TAA and acute aortic dissection in hemostasis patients.<sup>45</sup> Identifying the genetic polymorphism such as VKORC1 in hemostasis patients may give more specificity in identifying patients at risk of TAA than the currently used protein markers such as plasma D-dimer.<sup>45</sup> Another study detecting the frequency of VKORC1 polymorphisms was performed in 189 patients with an aneurysm of the ascending aorta, excluding those with genetic disease, and 188 controls with matching age, sex, body mass index, and smoking status.<sup>46</sup> VKORC1 polymorphism rs9923231 was significantly associated with aneurysms of the ascending aorta, suggesting that carboxylation of VKDPs might be involved in TAA formation.<sup>46</sup>

### Role of VSMCs in Aortic Aneurysm Formation

#### VSMC Phenotypic Plasticity

The vessel wall of large arteries consists of different cells, including endothelial cells, fibroblasts, and VSMCs. VSMCs reside in the tunica media and comprise the majority of cells in arteries.<sup>47</sup> The main function of VSMCs is to regulate vascular



**Figure 2.** Vascular smooth muscle cell (VSMC) plasticity in aneurysm formation. VSMC phenotypic switching between contractile and synthetic is essential for vascular remodeling and maintaining a healthy vasculature. In the presence of stimuli (such as PDGF [platelet-derived growth factor]), a contractile VSMC can acquire a synthetic phenotype which is characterized by downregulation of contractile proteins and increase in proliferation and migration. VSMC phenotypic switching precedes aneurysm formation and differentiation of VSMCs into osteo/chondrogenic and macrophage-like phenotypes, which promote further remodeling and calcification. VSMCs fail to maintain contractility in the presence of inactive vitamin K-dependent proteins (VKDPs) and secrete calcifying vesicles. Oxidative stress drives VSMC proliferation, VSMC apoptosis, and initiates microcalcification. This, in turn, causes destructive changes in the surrounding matrix and leads to weakening of the vessel wall. Macrocalcification develops over time and further aggravates the dilation of the vessel.  $\alpha$ -SMA indicates  $\alpha$ -smooth muscle actin; BMP, bone morphogenetic protein; cMGP, carboxylated matrix Gla protein; CNN, SM-calponin; ECM, extracellular matrix; EVs, extracellular vesicles; Gas6, growth arrest-specific gene 6; MMP, matrix metalloproteinase; NOX, nicotinamide adenine dinucleotide phosphate oxidases; ROS, reactive oxygen species; SMMHC, SM myosin heavy chain; TGF- $\beta$ , transforming growth factor  $\beta$ ; ucMGP, uncarboxylated matrix Gla protein; VKA, vitamin K antagonist; and VKOR, vitamin K-oxidoreductase.

tone and diameter through contraction and dilatation, thereby controlling blood pressure and blood flow distribution.<sup>48</sup> VSMCs are not terminally differentiated, which distinguishes them from skeletal muscle cells and cardiomyocytes. Indeed, VSMCs retain remarkably high plasticity, which allows them to modulate and switch phenotype on stress signals.<sup>7</sup> VSMC phenotypic switching is triggered by inflammation and injury.<sup>49</sup> Atherosclerosis, hypertension, and postangioplasty restenosis, as well as aneurysms, are all associated with VSMC phenotypic switching.<sup>50–52</sup>

VSMC phenotypic modulation can be characterized by changes in morphology, protein expression, proliferation, and migration (Figure 2). In vitro, contractile VSMCs are elongated and display spindle-shape morphology, while synthetic VSMCs are rhomboidal and display cobblestone morphology.<sup>53,54</sup> Synthetic VSMCs express lower levels of proteins involved in contraction, such as  $\alpha$ -SMA ( $\alpha$ -smooth muscle actin), SMMHC (SM myosin heavy chain), SM22 $\alpha$  (smooth muscle 22 $\alpha$ ), CNN (SM-calponin), and smoothelin-B.<sup>55</sup> Additionally, synthetic VSMCs are characterized by increased proliferation and migration.<sup>55,56</sup> VSMCs can also give rise to other cells in the vessel wall, such as osteo/chondrogenic-like and macrophage-like cells, which has been reviewed by others.<sup>57–59</sup>

The local environmental factors that modulate VSMC phenotype include growth factors, such as PDGF

(platelet-derived growth factor)<sup>56,60</sup> and TGF- $\beta$ ,<sup>56,61</sup> angiotensin II,<sup>62</sup> nitric oxide,<sup>56</sup> reactive oxygen species (ROS)<sup>63</sup> and the components of the ECM. Moreover, other factors, such as hyaluronan and heparin, are also known to influence VSMCs.<sup>64–66</sup>

### Phenotypic Switching of VSMCs Is Key in Aneurysm Formation

In this section, we discuss how mechanisms regulating VSMC phenotype are involved in aneurysm formation and how they could potentially be modulated by vitamin K.

#### Mutations in Genes Encoding Contractile Proteins and TGF- $\beta$

In line with loss of contractile function promoting aneurysm formation,<sup>67,68</sup> TAAs may arise from a variety of gene mutations encoding structural components of ECM, cytoskeletal/smooth muscle contraction proteins, and proteins associated with TGF- $\beta$  pathway, such as TGF- $\beta$  receptors and SMAD proteins.<sup>69</sup> For example, mutations in *MYH11* (myosin heavy chain 11) cause familial TAAD, aortic stiffness, alteration in aortic compliance, and arterial degeneration.<sup>70</sup> Mutations in  $\alpha$ -SMA, a target of TGF- $\beta$ -signaling, also cause TAAD,<sup>31</sup> as a result of impaired VSMC contractility, ability to maintain ECM,<sup>71</sup> differentiation, and proliferation.<sup>69</sup> Apart from genetic

mutations, alterations in vascular tone and interstitial pressure may induce interstitial edema, generate aortic wall stress, and initiate intraparietal dissection.<sup>72</sup> Pharmacological induction of VSMC contractility in a mouse model, which predisposed to aortic dissection, showed great protection against intramural delamination, an initiator of dissection.<sup>73</sup> These findings emphasize the importance of VSMC contractility in maintaining the structural integrity of the aorta.

TGF- $\beta$  is another factor implicated in regulating VSMC contractility.<sup>26</sup> Stimulation of VSMCs with TGF- $\beta$ 1 increases  $\alpha$ -SMA, SMMHC, and CNN mRNA expression<sup>61</sup> and reduces proliferation.<sup>74</sup> However, since reduced proliferation results in fewer VSMCs in the vessel wall, leading to AAA formation,<sup>75</sup> the exact effect of TGF- $\beta$ 1 signaling on VSMCs in aneurysm formation is likely dependent on the context and other mechanisms at play. Increased TGF- $\beta$  expression is observed in human AAA and TAA tissue<sup>75,76</sup> and in TAAs related to Marfan syndrome.<sup>26,77</sup> Increased TGF- $\beta$  activity has been shown to play a role in the pathology of TAAD in Marfan syndrome.<sup>78,79</sup> In line with that, a loss-of-function mutation in *TGF- $\beta$ 2* was sufficient to cause aortic root dilation in a mouse model of Marfan syndrome.<sup>80</sup> Paradoxically, these mice showed an upregulation of TGF- $\beta$  signaling and TGF- $\beta$ 1 expression.<sup>80</sup> Milewicz et al<sup>68</sup> suggested that overactivity of TGF- $\beta$  may be a secondary response to tissue injury in thoracic aortic diseases, rather than the primary cause. Indeed, aortic dilation and medial elastin damage in young Marfan mice developed in the absence of elevated VSMC TGF- $\beta$  signaling.<sup>81</sup> Furthermore, Loeys-Dietz syndrome mice (TGF- $\beta$  receptor knockin; *TGF- $\beta$ 2*<sup>G357W/+</sup>) showed increased TGF- $\beta$  signaling only in later stages of TAA development.<sup>82</sup> Conditional disruption of VSMC *TGF- $\beta$ 2* in postnatal mice impaired VSMC contractile apparatus induced a proliferative response and rapidly result in TAAD.<sup>83</sup> Moreover, TGF- $\beta$  neutralization in these mice exacerbated aortic disease, suggesting that basal TGF- $\beta$  is required to maintain structural integrity of VSMCs.<sup>83</sup> These studies highlight the fact that TGF- $\beta$  signaling contributes to aneurysm formation regardless of the presence of the fibrillin-1 mutation. Indeed, a growing body of evidence suggests emerging concepts such as mechanosensing and vascular tone regulation may better explain how aneurysms are formed in the context of TAAD.<sup>84</sup>

Interestingly, TGF- $\beta$  neutralization was shown to enhance angiotensin II-induced aortic rupture and aneurysm in mice, both at the thoracic and abdominal regions.<sup>85</sup> In a similar mouse model, TGF- $\beta$  was shown to protect against inflammatory aortic aneurysm progression and complications.<sup>34</sup> Whereas VSMC-extrinsic TGF- $\beta$  signaling protects against AAA, VSMC-intrinsic TGF- $\beta$  signaling protects the thoracic aorta in the angiotensin II-induced mice.<sup>86</sup> A recent study revealed that TGF- $\beta$ 1 suppressed a broad array of proinflammatory genes in cultured human VSMCs, partially through the STAT3 (signal transducer and activator of transcription 3) and NF- $\kappa$ B (nuclear factor- $\kappa$ B) pathway.<sup>87</sup> In parallel, TGF- $\beta$ 1 potently induced the expression of VSMC contractile genes.<sup>87</sup> Observation from this study suggests that the 2 events are independent, and suppression of the

proinflammatory genes is not the consequence of the induced contractile VSMC phenotype.<sup>87</sup>

The interaction of vitamin K and TGF- $\beta$  has not been studied in the context of aneurysm and VSMC biology. A study in osteoblast-like cells derived from osteosarcoma showed that vitamin K promoted TGF- $\beta$  mRNA expression.<sup>88</sup> However, a study in cancer cells showed no alteration in the expression of TGF- $\beta$ 1.<sup>89</sup> Conversely, TGF- $\beta$  was found to induce expression of VKDPs, such as MGP, in embryonic lung cell culture and Gas6 (growth arrest-specific gene 6) protein in VSMCs.<sup>90,91</sup>

Although mutational defects may not be easily amended, vitamin K may alleviate their downstream effects, pathologies, and vessel integrity in aortic aneurysm, such as elastin degradation, VSMC apoptosis, oxidative stress, and calcification, which we discuss in the following sections.

### Elastin Degradation

Phenotypic switching of VSMCs underlies the destructive changes that lead to aneurysm formation in animal models of TAA.<sup>67,92</sup> Investigating genetic diseases, in this case Marfan syndrome, provided a better understanding of this process.<sup>6</sup> The defective elastin lamellae impair VSMC attachment, which leads to switching of VSMCs towards a synthetic, proelastolytic phenotype.<sup>6,92</sup> The proelastolytic phenotype of VSMCs isolated from human AAA tissue is characterized by increased production of MMPs.<sup>93</sup> MMPs are a family of endopeptidases that exert proteolytic activity towards elastin and collagen. Increased MMPs, in particular, MMP-2 and MMP-9, degrade the ECM, thereby weakening the vascular wall and leading to AAA formation. TAA and AAA were found in ApoE mice with TIMP (tissue inhibitor of MMPs)-1 deficiency.<sup>94</sup> Moreover, VSMC migration is inhibited by inhibition of MMPs.<sup>95</sup> End-stage AAA usually features a destruction of elastin which is compensated by an increased synthesis of collagen.<sup>96,97</sup>

Although the effect of vitamin K on elastin degradation has not been studied in the context of aneurysm, a study in cancer cells showed that vitamin K2 inhibits production of MMPs by suppressing NF- $\kappa$ B and MAPK (mitogen-activated protein kinase) activity.<sup>98</sup> Vitamin K2 (45 mg/d) was shown to reduce serum MMP-3 in rheumatoid arthritis patients in a cross-sectional (n=158) and a longitudinal study (n=52).<sup>99</sup> However, vitamin K1 (10 mg/d) does not alter serum MMP-3 of rheumatoid arthritis patients as revealed by a randomized control trial study (n=64).<sup>100</sup>

### Apoptosis of VSMCs

AAAs have a reduced number of VSMCs,<sup>25</sup> leading to limited capacity to produce connective tissue and to repair elastin breaks. The reduced number of VSMCs is likely the consequence of apoptosis, because apoptotic VSMCs have been observed in the medial layer of AAAs.<sup>101</sup> VSMC apoptosis can result from proteolytic degradation of the ECM<sup>102</sup> and also from oxidative stress.<sup>103</sup> Other factors that may contribute to a proapoptotic milieu include inflammatory mediators, proliferative triggers, such as PDGF and cell stretch, hypoxia, and DNA damage.<sup>102</sup> Also, mechanical stress can contribute by enhancing endoplasmic reticulum (ER) stress-induced apoptosis.<sup>104</sup> In addition, VSMC senescence can ultimately progress into apoptosis.<sup>102</sup> Apoptosis is accompanied by the

generation of apoptotic bodies, which, if not cleared by phagocytosis, can stimulate calcification.<sup>105</sup> Calcium deposits, in turn, can aggravate inflammation and mechanical stress<sup>106,107</sup> indicating the fueling of an amplification loop spiraling down towards a mechanically compromised vessel wall.

Vitamin K may be associated with VSMC apoptosis through activation of Gas6, which is a VKDP containing  $\gamma$ -carboxyglutamic acid.<sup>108</sup> Gas6 inhibits VSMC apoptosis by binding to Axl, a tyrosine kinase receptor, thereby activating Akt.<sup>109</sup> Vitamin K2 was shown to inhibit rat VSMC calcification in culture through restoration of the Gas6/Axl/Akt antiapoptotic pathway.<sup>110</sup> Downregulation of the Gas6-Axl interaction is associated with inorganic phosphate (Pi)-induced human VSMC apoptosis and addition of human recombinant Gas6 inhibits Pi-induced apoptosis and calcification.<sup>111</sup> Additionally, Axl was found to be upregulated in cultured rat VSMCs after vascular injury and may mediate migration and proliferation of VSMCs.<sup>112</sup> Circulating Gas6 and soluble Axl in plasma has been measured in healthy controls (n=141) and patients with large (n=123) or small (n=122) AAA.<sup>113</sup> Gas6 concentration was found to positively correlate with AAA size, and the correlation was stronger with Gas6/Axl ratio.<sup>113</sup> While the authors of this study suggest that the higher Gas6 concentration in plasma may reflect the higher *Gas6* gene expression and Gas6/Axl plasma ratio might be a useful marker for AAA,<sup>113</sup> the mechanisms of action were not demonstrated. All of these studies point to the possibility that vitamin K could protect from aneurysm via Gas6/Axl and inhibition of apoptosis.

### ER Stress

Recently, aortic aneurysm formation has been shown to correlate with increased ER stress.<sup>114</sup> In line with this, transcription factor XBP1u (unspliced X box protein 1), which is expressed in the absence of ER stress, has been shown to maintain VSMC contractile phenotype. XBP1u deficiency causes VSMC dedifferentiation, enhances proinflammatory and proteolytic VSMC phenotype, thus aggravating TAA and AAA in vivo.<sup>115</sup> ER stress also promotes TAAD formation through CHOP (C/EBP homologous protein), an effector of the ER stress-induced unfolded protein response which regulates ER stress-induced apoptosis.<sup>116,117</sup> CHOP deletion prevents VSMC apoptosis and TAAD development, without affecting VSMC proliferation.<sup>117</sup> Moreover, ER stress inhibition was able to attenuate AAA in angiotensin II-induced ApoE<sup>-/-</sup> mice.<sup>118</sup>

The effect of vitamin K on ER stress has not been studied in the context of aneurysm. However, it is known that VKOR is associated with the ER disulphide forming pathway,<sup>119,120</sup> and ER transcription factors, such as XBP1, may affect the expression of *VKORC1* gene.<sup>121</sup>

### Oxidative Stress

Oxidative stress is a well-established factor promoting the development of aortic aneurysm, and it has been implicated in regulating features of VSMC phenotype. Both VSMCs and infiltrating inflammatory cells can contribute to the increase in ROS in the vessel wall.<sup>122</sup> Several sources of ROS exist (NOX [NADPH (nicotinamide adenine dinucleotide phosphate) oxidases]), lipoxygenases, cyclo-oxygenase, and nitric oxide synthase; however, NOX enzymes have been predominantly

implicated in AAA pathology thus far.<sup>123</sup> NOX enzymes form a family involved in the production of superoxide anion, and NOX1, NOX2, NOX4, and NOX5 are expressed in human VSMCs.<sup>124,125</sup> Genetic deletion of p47<sup>phox</sup>, a cytosolic subunit that associates with NOX1-4, reduced the incidence of AAA in an angiotensin II-induced mouse model of aneurysm.<sup>126</sup> Interestingly, systemic NOX2 deficiency reduced ROS in AAA lesion, however, it exacerbated angiotensin II-induced AAAs in mice by increasing vascular inflammation.<sup>127</sup> This was because of disruption of macrophage function, the main source of increased NOX2 expression in the AAAs.<sup>127</sup> NOX4-mediated oxidative stress has been shown to be implicated in VSMC apoptosis,<sup>124</sup> while increased NOX2 and NOX5 activity have been shown to induce VSMC proliferation.<sup>128-130</sup> Moreover, NOX2 and NOX5 are most prominently expressed NOX isoforms in the aorta of AAA patients.<sup>131</sup> NOX1-mediated ROS generation has been shown to decrease contractile protein expression in cerebral aneurysms.<sup>132</sup>

More generally, scavenging ROS attenuated the formation of AAA in mice and protected against aortic aneurysm development.<sup>133</sup> In another mouse model of AAA, aneurysm formation and rupture were decreased by vitamin E, a well-known antioxidant.<sup>134</sup> Flow loading and systemic antioxidant therapy lowered oxidative stress and early aortic dilation in a rat model of AAA.<sup>135</sup> Antioxidant treatment not only reduced macrophage infiltration into the abdominal aorta but also delayed AAA formation and reduced aortic rupture.<sup>134</sup> Additionally, in human subjects, increased circulating level of TRX (thioredoxin), a protein released from cells in response to oxidative stress, and release of TRX in the luminal part of AAA patients positively correlated with AAA size and expansion.<sup>136</sup> Correspondingly, proteomic analysis showed that TRX protein in the human AAA wall sample is negatively correlates with the growth rate, which is in line with hypothesis that upregulation of TRX in defense against ROS may slow down the aneurysm expansion rate.<sup>137</sup> A similar dependency of TAA on increased ROS has been observed, as in vitro and ex vivo studies on human TAA tissue revealed that oxidative stress mediates VSMC phenotypic switching towards synthetic phenotype through connective tissue growth factor.<sup>52</sup> These results were further validated in a mouse model of TAA, which showed similar pathology as observed in human tissue.<sup>52</sup> Taken together, these studies suggest that decreasing oxidative stress attenuates aneurysm formation; however, the underlying mechanisms have not been studied in detail.

Recent findings suggest a role for vitamin K as an antioxidant. Human VKORC1L1 (VKORC1-like 1), a paralogue enzyme of VKORC1 found in the ER, was found to regulate vitamin K-dependent intracellular antioxidant function in the cell membrane.<sup>138</sup> Both vitamin K1 and K2 were shown to mediate a VKORC1L1-dependent increase in cell viability. Genome-wide expression studies showed that VKORC1L1 is expressed throughout many tissues and may serve as a potential target for the regulation of intracellular redox homeostasis.<sup>138</sup>

Additionally, Mukai et al<sup>139</sup> showed that vitamin K-hydroquinone is a potent free radical scavenger. Kinetic studies showed vitamin K-hydroquinone has 10- to 100-fold higher antioxidant activity than other radical scavengers such

as  $\alpha$ -tocopherol and ubiquinone. In another study, menadione (a synthetic form of vitamin K without a side chain) and vitamin K1 and K2 inhibited NADPH-dependent microsomal lipid peroxidation in the presence of dithiothreitol, a non-physiological redox protein which drives VKOR.<sup>140</sup> This antioxidant effect was abolished when a vitamin K antagonist (VKA), namely warfarin, was added into the reaction. Vitamin K1 and K2 were shown to protect against oxidative stress in primary oligodendrocytes and immature cortical neurons.<sup>141</sup> Additionally, vitamin K1 and K2 prevented oxidative stress-induced cell death by inhibiting activation of 12-lipoxygenase in primary oligodendrocytes.<sup>142</sup> This vitamin K action was independent of the GG CX, though facilitated by the VKOR redox cycle.

Vitamin K has also been shown to directly modulate NOX activity. Bridge et al<sup>143</sup> have shown that reduced phyloquinone ( $K1H_2$ ) serves as a natural electron donor for NOX in the soybean plant and might protect the plasma membrane against ROS. In addition,  $K1H_2$  is also involved in protein disulfide-thiol interchange of NOX.<sup>143</sup> Electron transfer via hydroquinone was proposed as a mechanism sensing the redox changes across the plasma membrane that occur during aging and senescence.<sup>143–145</sup> Additionally, the VKOR recycling requires redox partners such as PDI (protein disulfide isomerase) and TRX-like protein to deliver reducing equivalence.<sup>146</sup> PDI is known to regulate the activity of NOX in various cell types and is essential for redox-mediated VSMC migration via PDGF, which is associated with NOX activation.<sup>147</sup> Intracellular PDI regulates expression and activity of the NOX, which contributes to ROS generation.<sup>148</sup> Further to that, VKOR significantly contributes to disulfide formation and redox homeostasis within the ER.<sup>120</sup> Reduction of VKOR activity by warfarin, in combination with other disulfide bond formation proteins, resulted in cell death.<sup>120</sup>

All these studies show that oxidative stress is an important factor promoting both AAA and TAA formation, and that vitamin K, as an antioxidant, has great potential in this field. However, as the antioxidant mechanisms of vitamin K have not been analyzed in VSMCs in the context of aneurysm, this warrants further study.

### Senescence of VSMCs

Many hallmarks of cellular senescence have been reported in aneurysms and aneurysm-derived VSMCs. Stress signals, such as uremic toxins and ROS, induce a cellular senescence response, which is characterized by a quiescent state and secretion of proinflammatory cytokines.<sup>149</sup> Senescent VSMC releases factors which drive differentiation of local VSMCs to become osteo/chondrogenic.<sup>150</sup> An in vitro study by Liao et al<sup>151</sup> indicated that VSMCs derived from human AAA exhibited a distinct phenotype resembling accelerated replicative senescence. These VSMCs displayed distortion in morphology and limited proliferation capacity in comparison to VSMCs derived from the inferior mesenteric artery. Additionally, a genome-wide association study revealed a common variant, rs10757278, found in human AAA.<sup>152</sup> Rs1075728 is adjacent to *CDKN2A/CDKN2B* (genes encode the cell cycle regulators), which is expressed in senescent VSMCs.<sup>59,153,154</sup>

Another senescence mechanism is related to lamin A. Persistent DNA damage and accumulation of prelamin A, the precursor to the component of nuclear membrane lamin A, is a key mediator that links premature senescence and accelerated vascular calcification.<sup>155</sup> Increased expression of lamin A was observed in the region of high wall stress and not in the nonaneurysmal vascular beds of AAA patients.<sup>156</sup> Furthermore, a physiological pulsatile stretch of VSMC in vitro caused a time-dependent elevation of prelamin A and lamin A expression.<sup>156</sup> Lamin A is also a direct activator of SIRT1 (sirtuin 1).<sup>157</sup> SIRT1 is a nicotinamide adenine dinucleotide-dependent protein deacetylase, highly expressed in the vasculature.<sup>158</sup> SIRT1 is known for its protective function in vascular aging and is involved in variety of cellular processes including the inhibition of apoptosis.<sup>159,160</sup> Inhibition of SIRT1 in VSMCs results in DNA damage, early senescence, and apoptosis.<sup>160</sup> In addition, reduction of SIRT1 in animal models induces medial degeneration, AAA formation, and aortic dissection providing a potential molecular basis for aneurysm formation in patients.<sup>160</sup> Concurrently, human AAA wall showed significantly lower SIRT1 immunoreactivity in comparison to nonaneurysmal aortic sections.<sup>161</sup> In addition, overexpression of VSMC-specific SIRT1 was able to suppress AAA formation in an animal model.<sup>161</sup> VSMCs cultured from end-stage human AAA expressed elevated levels of differentiation marker (microRNA 145), lower expression of SIRT1, and exhibited higher level of DNA damage compared with nonaneurysmal human saphenous vein.<sup>162</sup> These features did not correlate with patients' chronological age.<sup>162</sup>

Telomeres, which are DNA repeats at the end of chromosomes, have a protective role against age-associated diseases.<sup>163</sup> Telomere attrition (shortening) is one of the hallmarks of cellular aging and one of the causes of cellular senescence.<sup>164</sup> Shortening of telomeres has been documented in biopsies of AAA of aortic tissue.<sup>165</sup>

Senescence has been shown to accompany not only AAAs but also TAAs. VSMCs derived from bicuspid and tricuspid aortic valve-associated aneurysms of TAA patients displayed reduced proliferation and migration capacity. In addition, telomere length analyses showed that these VSMCs had significantly shorter telomeres compared with VSMCs derived from healthy tissue.<sup>166</sup> Aschacher et al<sup>167</sup> showed that telomere shortening was associated with reduced activity of telomerase, which in turn is involved in VSMC proliferation. It was concluded that VSMCs in TAAs undergo premature senescence. The cause-consequence relationship of VSMC senescence and TAA formation and the role of telomere shortening remains to be unraveled.<sup>167</sup>

Mitochondrial dysfunction is another hallmark of cellular senescence, and it has been implicated in vascular aging and cardiovascular diseases.<sup>168</sup> Mitochondrial respiratory chain dysfunction causes reduction of ATP synthesis and increased ROS generation.<sup>168,169</sup> The bioenergetic profiling of proliferating human VSMCs revealed that they rely on mitochondrial oxidative phosphorylation and have a high respiratory reserve capacity at rest, while aging cells have lower resting oxidative phosphorylation and reduced reserve capacity.<sup>170</sup> Bioinformatic analyzes suggest the importance of mitochondria and oxidative



phosphorylation in AAA.<sup>171</sup> Indeed, mitochondrial dysfunction was observed in synthetic VSMCs of human AAA tissue and of AAA mouse model.<sup>172</sup> A recent study demonstrated that VSMC mitochondrial respiration is reduced in TAAs of fibulin-4 mutant mice; this was coupled with increased ROS and dysregulated expression of genes involved in energy metabolism.<sup>173</sup> The reduction of mitochondrial respiration in VSMC was also observed in VSMCs of Loeys-Dietz syndrome mice and human fibroblasts of Marfan and Loeys-Dietz syndrome patients, suggesting that altered mitochondrial respiration may contribute to TAA formation.<sup>173</sup>

However, the direct effects of vitamin K on VSMC senescence have not been studied, with one exception. Vitamin K2 has been reported to be involved in ATP generation. A study in *Drosophila* mitochondria revealed that vitamin K2 acts as an electron carrier that can rescue deficiency of *PINK1* (PTEN-induced putative kinase 1),<sup>174</sup> a mitochondrial regulator of autophagy that determines VSMC fate.<sup>175,176</sup> In this study, vitamin K2 was shown to rescue mitochondrial dysfunction, improve mitochondrial oxygen consumption, and facilitate the production of ATP.<sup>174</sup>

Taken together, the studies of noncanonical roles of vitamin K at the background of our current understanding of aneurysm biology suggest that vitamin K antioxidative property seems to be the most promising effects on the pathogenesis of aneurysms (Figure 1). In the following parts, we discuss how the canonical function of vitamin K might play a role in the mechanisms of aneurysm formation through inhibition of VSMC phenotypic switching towards osteo/chondrogenic and VSMC calcification.

### VSMC Calcification in Aneurysm Formation

Calcification, the process of deposition of calcium phosphate crystals of the medial layer of the vessel wall, is associated with arterial stiffening and causes isolated systolic hypertension.<sup>177</sup> VSMCs are key players in vascular calcification.<sup>178</sup> Phenotypic switching of contractile VSMCs into osteo/chondrogenic VSMCs is accompanied by expression of bone-specific proteins that regulate ECM mineralization (Figure 2).<sup>179,180</sup> BMP-2 (bone morphogenetic protein 2), the main regulator of osteogenesis, binds the BMP receptor and activates SMAD signaling, which leads to expression and increased activity of osteo/chondrogenic transcription factors such as RUNX2 (Runt-related transcription factor 2), Osterix, and SOX9.<sup>59,181</sup> These transcription factors control expression of mineralization regulators, such as alkaline phosphatase, osteocalcin, OPN (osteopontin), osteoprotegerin, and bone sialoprotein.<sup>59</sup> In response to a variety of stress signals, VSMCs can cause and enhance calcification via several mechanisms, including increased apoptosis,<sup>105</sup> extracellular vesicle release,<sup>182</sup> and loss of natural calcification inhibitors, such as MGP.<sup>183</sup>

Calcification of bovine VSMCs suppressed the expression of elastic fiber protein, tropoelastin (elastin monomer), and fibrillin-1.<sup>184</sup> Conversely, the addition of recombinant tropoelastin was able to inhibit VSMC calcification.<sup>185</sup> Study in bovine VSMC culture shows that Vitamin K2 inhibits calcium deposition in a dose-dependent manner, and the addition of vitamin K to bisphosphonate treatment enhances the expression of tropoelastin.<sup>186</sup> Moreover, Vitamin K2 and bisphosphonate

inhibit bovine VSMC differentiation to osteo/chondrogenic phenotype.<sup>186</sup> Vitamin K alone increased MGP and decrease OPN expression of inorganic Pi-induced bovine VSMC calcification in culture.<sup>186</sup>

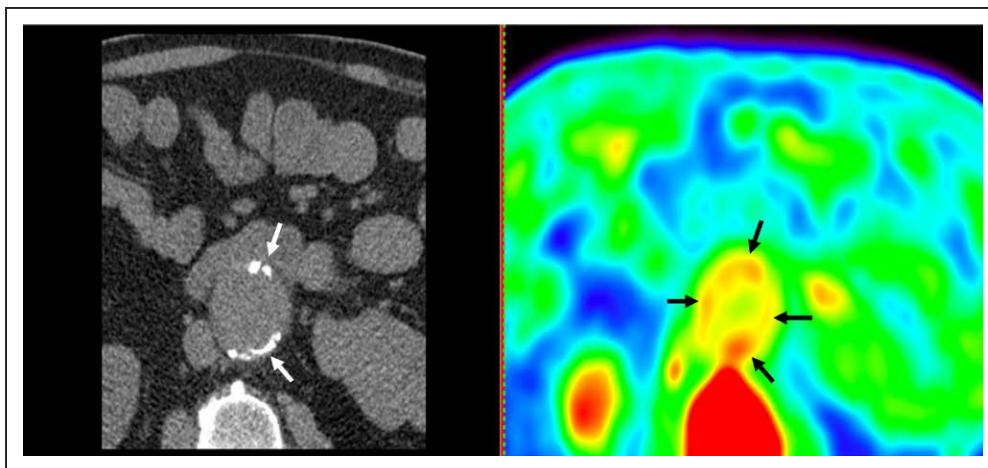
Apart from VSMCs, macrophages are now recognized as important contributors to the progression of calcification and proinflammatory macrophages release microcalcification-inducing extracellular vesicles.<sup>187,188</sup> Cholesterol loading converts VSMCs into a macrophage-like foam cells.<sup>189,190</sup> Interestingly, recent findings show that macrophage-like human VSMCs, which were induced by enzyme-modified nonoxidized LDL (low-density lipoprotein), have a genetic profile associated with calcification including upregulation of BMP and downregulation of MGP and had a higher propensity to calcify.<sup>191</sup> Taken together, these studies suggest that differentiation of VSMCs into macrophage-like cells may contribute to vascular calcification (Figure 2).

### Calcification of Aneurysms

Vascular calcification has been shown to be increased in symptomatic AAA and contribute to rupture risk in AAA, as it induces changes in mechanical properties of the aneurysm (Figure 2).<sup>192,193</sup> However, when categorized based on the size as microcalcification (<50  $\mu\text{m}$ ) and macrocalcification (>50  $\mu\text{m}$ ),<sup>194</sup> calcification has been suggested to promote different outcomes with respect to aneurysms.

Microcalcification, which precedes macrocalcification, is unequivocally considered detrimental and is present during a biologically active fast-dilating aneurysm.<sup>13</sup> Microcalcification colocalized with elastin degradation in the aorta of Marfan patients.<sup>15</sup> In Marfan mice, microcalcification was abundant in the ascending aorta and strongly correlated with the aortic root diameter.<sup>15</sup> In the same study, macrocalcification was found to be prominent in the aortic root of Marfan mice and correlated with aortic dilation to a lesser extent.<sup>15</sup> The data on macrocalcification are more complex. Recently, a prospective case-control and observational cohort study of 72 patients with asymptomatic AAA revealed that macrocalcification was not associated with aneurysm expansion or AAA events,<sup>13</sup> and it was suggested that macrocalcification of the aneurysm segment might stabilize the degenerated vascular wall.<sup>13</sup> However, research is conflicting and recently published data on the role of calcium scoring in aneurysmal aortic disease revealed macrocalcification of TAAs and AAAs was associated with significantly higher overall and cardiovascular mortality.<sup>16</sup> This was a retrospective, observational, single-center study performed on 319 patients (TAA=123 and AAA=196), who underwent computed tomography. Aortic aneurysm calcification scores were derived from computed tomography, and multivariate regression analysis was performed after correcting for potential confounders (age and blood pressure).<sup>16</sup> Although the study reflects clinical practice, renal function, a known cause for vascular calcification, was not analyzed.<sup>16</sup>

While macrocalcification may be less detrimental to the aneurysmal vessel wall than microcalcification, it does start as microcalcification. Therefore, detection of microcalcification could be useful to measure biological activity in the aneurysmal wall, thus identifying risk for AAA events.<sup>195</sup> Recently, it was shown that active mineralization in AAA, represented



**Figure 3.** Example of an aneurysm with areas of  $^{18}\text{F}$ -NaF uptake (positron emission tomography [PET]-computed tomography [CT], right) that do not correlate with calcification visible on CT (left). On the CT scan, calcification is visible at the anterior and posterior wall of the aorta (white arrows); however, there is almost circumferential  $^{18}\text{F}$ -NaF uptake (black arrows). This shows that there are areas where the PET-CT tracers detect active mineralization, where none is detected by CT scan. The signal intensity of  $^{18}\text{F}$ -NaF uptake visualized by PET-CT requires a careful quantitative analysis, which includes calculation of tissue-to-background ratios using background blood pool activity in the right atrium. Moreover, manual adjustment of regions of interest is important when analyzing  $^{18}\text{F}$ -NaF uptake in structures adjacent to the bone to ensure that any signal attributed to bone is discarded.

by a marked increase in  $^{18}\text{F}$ -NaF uptake on positron emission tomography-computed tomography (see Figure 3 as a visual example), correlates with presence and progression of AAA.<sup>13</sup>  $^{18}\text{F}$ -NaF specifically detects active calcification and puts forward microcalcification as a promising risk factor amendable for intervention in AAA.<sup>13,196</sup>

### Vitamin K-Dependent Calcification Inhibitors

For many decades, it was thought that calcification of the vasculature was a passive process. The discovery of inhibitors actively preventing vascular calcification revealed that this process is highly regulated by cells and proteins. Of specific interest are VKDPs, whose activity can be modulated by vitamin K or VKAs.<sup>42,197</sup> VKDPs can be found in hepatic and extrahepatic tissues (summarized in Table). In all cases, VKDPs only exhibit their function when being carboxylated with the aid of its unequivocal cofactor vitamin K.

VSMCs synthesize several VKDP calcification inhibitors (Table). One of the most potent inhibitors for vascular calcification known in is MGP.<sup>183</sup> MGP regulates VSMC phenotypic switching through BMP signaling which transdifferentiates VSMC into osteo/chondrogenic phenotype.<sup>42,55,208</sup> This transdifferentiation, together with loss of VSMC markers;  $\alpha$ -SMA and SM22 $\alpha$ , was found in MGP-deficient mice.<sup>205,222</sup> MGP also acts locally to inhibit ECM mineralization in calcified vascular lesions.<sup>209–211</sup> MGP maintains contractility of VSMCs by binding to BMP-2 and thus inhibiting osteo/chondrogenic differentiation.<sup>208,223,224</sup> MGP also prevents calcification when it is loaded into extracellular vesicles that are secreted during the process of vascular remodeling.<sup>212–214</sup> Moreover, intrasection analysis of human coronary arteries (n=12) showed that MGP is associated with microcalcifications in early atherosclerotic lesions,<sup>225</sup> which is associated with increased plaque vulnerability.<sup>107,226,227</sup> A patient-based proteomic analysis revealed that MGP is increased in calcified AAA (n=6) and TAA (n=6) tissue sample in comparison to the adjacent normal aorta tissues.<sup>228</sup> It was suggested

that upregulation of the calcification inhibitor might be a feedback mechanism to prevent further calcium deposit.<sup>228</sup> However, circulating MGP has not been studied in the context of AAA and TAA.

### Phenotypic Switching of VSMCs in Aneurysm Promotes Calcification

VSMC phenotypic switching, as shown by loss of contractility markers and increases in MMPs, precedes AAA in mice.<sup>51</sup> Accordingly, synthetic VSMCs and upregulation of OPN was shown in the dilated region of human TAA.<sup>52</sup>

In the context of aneurysm formation, additional specific mechanisms promoting calcification are at play. MMP-mediated elastin degradation and misfolding of elastin were shown to be associated with calcium deposition on elastin fibers and calcification.<sup>210,229</sup> Additionally, elastic fiber degradation might cause detachment of VSMCs thereby altering their phenotype.<sup>92</sup> Elastin peptides, which are generated through elastin breaks, upregulate ALP and downregulate MGP expression of human VSMCs in vitro.<sup>15</sup> Absence of the calcification inhibitory protein MGP also promotes phenotypic switching of VSMCs.<sup>209</sup> Hence, VSMC phenotypic switching increases both cellular and ECM stiffness.<sup>26</sup> Elastin-derived peptides and TGF- $\beta$ 1 were shown to promote osteo/chondrogenic differentiation of VSMCs, which may lead to calcification.<sup>230</sup> Indeed, VSMCs of Marfan mice showed enhanced ALP activity and upregulation of osteogenic gene profile including ALP, bone  $\gamma$ -carboxyglutamic acid-containing protein, and RUNX2, in comparison to wild-type mice.<sup>15</sup>

It was proposed that osteo/chondrogenic differentiation of VSMCs is an attempt to prevent vessel wall degeneration by increased ECM production.<sup>230</sup> Differentiation of macrophages into osteoclast-like cells is also observed in the development of AAAs in both humans and mice.<sup>231,232</sup> It was shown that pharmacological inhibition of the activity of these osteoclasts (cells involved in resorbing calcification) using bisphosphonate was able to prevent AAA in a  $\text{CaCl}_2$ -induced

**Table. Hepatic and Extrahepatic VKDPs**

Vitamin K-Dependent Proteins	Function	Reference
<b>Hepatic</b>		
Coagulation factor VII, IX, and X	Procoagulant. Aid in the coagulation cascade.	Danziger <sup>198</sup>
Prothrombin (coagulation factor II)	Inhibits vascular calcification through binding of phosphatidylserine on EVs, thus activates coagulation. Loading of EVs with prothrombin reduces both their procalcific and procoagulant properties.	Kapustin et al <sup>199</sup>
Activated coagulation factor (FIIa, FVIIa, and FXa)	Activates cellular protease-activated receptors, thereby inducing cellular processes, such as inflammation, apoptosis, migration, fibrosis, and calcification.	Borisoff et al <sup>200</sup> and Schurgers and Spronk <sup>201</sup>
Protein C	Coagulation inhibitor. Assembles anticoagulant complex on cell surface.	Danziger, <sup>198</sup> Matsuzaka et al, <sup>202</sup> and Esmon et al <sup>203</sup>
Protein S	Coagulation inhibitor. Cofactor for activated protein C.	Danziger <sup>198</sup> and Matsuzaka et al <sup>202</sup>
Protein Z	Coagulation inhibitor. Cofactor for the inactivation of FXa.	Danziger <sup>198</sup> and Han et al <sup>204</sup>
<b>Extrahepatic</b>		
Osteocalcin	Bone tissue-specific protein, which is also expressed in osteo/chondrogenic VSMCs.	Willems et al, <sup>42</sup> Steitz et al, <sup>205</sup> Neve et al, <sup>206</sup> and Iyemere et al <sup>207</sup>
	Regulates mineral deposition.	
Matrix Gla protein	Regulates VSMC phenotypic switching through BMP signaling.	Willems et al, <sup>42</sup> Rensen et al, <sup>55</sup> Malhotra et al, <sup>208</sup> Luo et al, <sup>209</sup> Murshed et al, <sup>210</sup> Schurgers et al, <sup>211</sup> Kapustin et al, <sup>212</sup> Schurgers et al, <sup>213</sup> and Wallin et al <sup>214</sup>
	Locally, MGP inhibits ECM mineralization in vascular lesions.	
	Prevents calcification when loaded into EVs that are secreted during the process of vascular remodeling.	
Gla-rich protein (also called Uema)	Inhibits vascular calcification through direct binding at calcification sites, thus inhibiting crystal formation/maturation, and via loading into EVs and calcifying protein particles. Binds and inhibits BMP-2.	Viegas et al, <sup>215-217</sup> and Willems et al <sup>42,218</sup>
Growth arrest-specific gene 6	Involved in vascular homeostasis.	Clauser et al, <sup>90</sup> Nakano et al, <sup>108,219</sup> Melaragno et al, <sup>109</sup> Son et al, <sup>111</sup> Fridell et al, <sup>220</sup> and Laurance et al <sup>221</sup>
	In VSMCs, Gas6 inhibits apoptosis, induces migration, and promotes cell survival.	

These VKDPs have a high affinity for calcium ions because of their negatively charged Gla residues and are involved in inhibiting ectopic calcification. BMP indicates bone morphogenetic protein; ECM, extracellular matrix; EVs, extracellular vesicles; Gas6, growth arrest-specific gene 6; MGP, matrix Gla protein; VKDPs, vitamin K-dependent proteins; and VSMC, vascular smooth muscle cell.

mouse model of the aneurysm.<sup>231</sup> Furthermore, bisphosphonate attenuated AAA in angiotensin II–induced mouse model of aneurysm through reduction of vascular inflammation.<sup>233</sup> Conversely, inhibiting osteoclast activity with bisphosphonate failed to prevent aneurysm progression in an animal model of severe Marfan syndrome.<sup>234</sup> The contradictory outcome may lie on the different basic pathophysiology between TAA and AAA.<sup>233</sup>

### VKAs Promote and Vitamin K Rescues Calcification

The importance of vitamin K status in vascular calcification is supported by studies of VKAs,<sup>235</sup> which counteract the function of vitamin K by blocking VKOR and subsequently inhibiting vitamin K recycling.<sup>236</sup> VKAs, such as warfarin, are widely used as anticoagulants to prevent thromboembolic complications in cardiovascular diseases.<sup>237</sup>

In a study in mice, VKA-induced calcification was associated with increased apoptosis and reduced cellularity in the medial vascular layer in mice.<sup>238</sup> In rats, VKA treatment was shown to rapidly calcify the elastic lamellae of major arteries and aortic heart valves.<sup>239</sup> This was similar to what has been observed in MGP-deficient mice, suggesting that VKAs induce calcification by inhibiting MGP functionality.<sup>209</sup> It must

be noted that a daily high dose of VKA in combination with high-dose vitamin K1 was used to prevent bleeding and cause arterial calcification of rats (15 mg warfarin/100 g of body weight administered twice daily, while typical maintenance dose of VKA for human ranges between 2 and 10 mg/d).<sup>239</sup> This dosage is required to maintain a constant level of VKA in plasma without oscillation,<sup>239</sup> because of rapid clearance of warfarin from plasma in rats and considering the half-life of warfarin.<sup>240,241</sup> However, the authors showed no significant differences in blood chemistry between the VKA-treated and the control groups. Additionally, although observations in animal models are based on much higher doses of VKAs than human dosage, it appears that the international normalized ratio in these animals falls within the same target international normalized ratio range for patients.<sup>242</sup>

Ample evidence exists that patients using VKAs have higher levels of vascular calcification than VKA nonusers.<sup>243,244</sup> More than a decade ago, it was reported that VKAs are associated with increased valvular calcification in patients.<sup>243</sup> Since then, this has been verified in many subsequent studies. A large population-based cohort of 15 010 individuals enrolled in the Gutenberg health study, of which 278 patients received VKA treatment, demonstrated an increased arterial stiffness in

VKA-treated patients.<sup>245</sup> Interestingly, enhanced inflammation was also observed in VKA users in this study. However, the cross-sectional design of this study does not allow interpretation of cause and effect, and only a limited number of patients received direct-acting anticoagulants for oral anticoagulation therapy for comparison. Another study analyzed atherosclerotic plaques of patients with nonvalvular atrial fibrillation and showed higher coronary calcium score and significantly higher amount of spotty calcification (calcification of <3 mm) in VKA users compared with a matching demographics (age, sex, body mass index, family history, etc) control group (n=101 per group).<sup>246</sup> It must be noted that despite the propensity score matching, this study ignored the difference between VKA-treated patients and control with regards to some parameters, including HDL (high-density lipoprotein) and stroke risk score in patients with atrial fibrillation (CHADS<sub>2</sub>VASC<sub>2</sub>), which might contribute to spotty calcification. Serial coronary intravascular ultrasound examinations in a post hoc analysis of 8 prospective randomized clinical trials showed a significant progression of coronary artery calcification, independent of changes in atheroma volume in VKA users compared with the propensity matching control group (n=164 per group).<sup>247</sup> In a different study, VKA users had significantly more coronary artery calcification than VKA nonusers with matching gender and Framingham risk score (n=133 per group).<sup>242</sup> A small cross-sectional study on long-term VKA treatment showed an association of VKA users with extracoronary calcification.<sup>248</sup> Recently, small cross-sectional studies conducted in atrial fibrillation patients pointed towards increased prevalence of vascular calcification as compared to nonvitamin K antagonist oral anticoagulants or no treatment.<sup>249</sup> Data on vascular calcification from subgroup analyses of large controlled randomized clinical trials comparing warfarin with direct oral anticoagulants for various clinical indications might further clarify the role of vitamin K in aortic calcification or aneurysm formation. Such results would provide a valuable piece of the puzzle, given the limitations of smaller clinical studies of warfarin and of animal studies that used several log-fold excess of warfarin compared with that used clinically in humans.

In experimental animals, VKA-induced medial calcification was rescued by cotreatment with a high dose of vitamin K2.<sup>238</sup> Additionally, regression of arterial calcification was shown in an animal model with high vitamin K diet.<sup>250</sup> In an interventional randomized control trial of 53 long-term end-stage renal disease patients, who had a vitamin K deficiency, supplementation of vitamin K2 significantly decreased the levels of inactive MGP (dp-ucMGP [dephospho-uncarboxylated MGP]) in a dose- and time-dependent manner.<sup>251</sup> This indicates that circulating dp-ucMGP reflects bioavailability of vitamin K in the vasculature.<sup>213,251,252</sup> Indeed, low vitamin K status or intake is linked to vascular calcification in human subjects.<sup>253,254</sup> A study of 115 patients with suspected coronary artery disease showed reduction of carboxylated MGP correlates with coronary artery calcification progression.<sup>255</sup> Recent results from a prospective interventional study of 72 patients with aortic stenosis showed that vitamin K1 supplementation over a 12-month period significantly decelerated the progression of aortic valve calcification.<sup>252</sup> Hence, the level of dp-ucMGP can be used as a marker for vascular vitamin K

deficiency. As vascular calcification is suggested to increase risk of aneurysm rupture, treatment with vitamin K could hold promise to decrease this risk.

### VKAs in Aneurysm

The effect of VKAs on AAA progression has not been fully explored yet. They have been linked to continued AAA expansion of >5 mm in diameter and increased risk of persistent blood flow in aneurysm sac (endoleak) after endovascular aneurysm repair.<sup>256,257</sup> A few clinical cases have been reported in which the use of anticoagulants induced spontaneous rupture of bronchial artery aneurysms in patients who took heparin and VKAs and did not have an apparent aortic pathology.<sup>258</sup> Additionally, VKAs promote many effects similar to what has been observed in aneurysm pathology. In a mouse model of atherosclerosis, high-dose VKAs induce a vulnerable atherosclerotic plaque phenotype with elastin breaks.<sup>242</sup> High-dose warfarin treatment in rats results in activation of MMP-9, elastin degradation, vascular elastocalcinosis, and stiffness.<sup>259</sup> In addition, impaired vitamin K status in patients with end-stage renal disease is linked to VSMC phenotypic switching towards senescence-associated secretory phenotype, thus promoting premature vascular aging.<sup>260</sup> Taken together, VKAs affect VSMC properties, weaken the vascular media and might thus increase the susceptibility of aneurysms to progress and rupture.

### Conclusions

In summary, in this review, we presented what is known about the role of VSMC phenotypic switching in the pathophysiology of aneurysm formation. NOX enzymes in VSMCs are a major source of increased oxidative stress in aneurysms. Oxidative stress induces VSMC phenotypic switching, which results in ECM degradation and weakening of the arterial wall. VSMC phenotypic changes also lead to calcification, which has been recently proposed as a risk factor for aneurysm progression and rupture, as it results in segmental aortic stiffening generating distally increased aortic wall stress. Vitamin K and VKOR are involved in the regulation of NOX and ROS generation. Vitamin K is known to activate MGP by which it prevents vascular calcification. Moreover, vitamin K has the ability to scavenge free radicals, reduce oxidative stress, and decrease vascular calcification. Therefore, it is tempting to postulate that vitamin K deficiency plays a role in aneurysm formation. Vitamin K supplementation holds the potential to lower the risk of aortic aneurysms and improve cardiovascular outcome.

More studies are needed in patients to examine the effects of vitamin K supplementation on aneurysm progression and risk of rupture. Moreover, shedding light on the effect that vitamin K has on hallmarks of VSMC phenotype (such as proliferation, migration, and contractile protein expression) and the associated mechanisms (oxidative stress and ER stress) would help to fully understand the role of vitamin K in aneurysm formation.

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## References

- Vorp DA, Raghavan ML, Webster MW. Mechanical wall stress in abdominal aortic aneurysm: influence of diameter and asymmetry. *J Vasc Surg.* 1998;27:632–639.
- Dua MM, Dalman RL. Hemodynamic influences on abdominal aortic aneurysm disease: Application of biomechanics to aneurysm pathophysiology. *Vascul Pharmacol.* 2010;53:11–21. doi: 10.1016/j.vph.2010.03.004
- Zamaneh Kassiri RB, Kassiri Z. Extracellular matrix remodelling and abdominal aortic aneurysm. *J Clin Exp Cardiol.* 2013;4:1–8.
- Kuivaniemi H, Ryer EJ, Elmore JR, Tromp G. Understanding the pathogenesis of abdominal aortic aneurysms. *Expert Rev Cardiovasc Ther.* 2015;13:975–987. doi: 10.1586/14779072.2015.1074861
- GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2015;385:117–171. doi: 10.1016/S0140-6736(14)61682-2
- vanVarik BJ, Rennensperger CP, Kroon AA, deLeeuw PW, Schurgers LJ. Mechanisms of arterial remodeling: lessons from genetic diseases. *Front Genet.* 2012;3:290. doi: 10.3389/fgene.2012.00290
- Alexander MR, Owens GK. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. *Annu Rev Physiol.* 2012;74:13–40. doi: 10.1146/annurev-physiol-012110-142315
- Willis AI, Pierre-Paul D, Sumpio BE, Gahtan V. Vascular smooth muscle cell migration: current research and clinical implications. *Vasc Endovascular Surg.* 2004;38:11–23. doi: 10.1177/153857440403800102
- Kapustin AN, Shanahan CM. Emerging roles for vascular smooth muscle cell exosomes in calcification and coagulation. *J Physiol.* 2016;594:2905–2914. doi: 10.1113/JP271340
- Schurgers LJ, Akbulut AC, Kaczor DM, Halder M, Koenen RR, Kramann R. Initiation and propagation of vascular calcification is regulated by a concert of platelet- and smooth muscle cell-derived extracellular vesicles. *Front Cardiovasc Med.* 2018;5:36. doi: 10.3389/fcvm.2018.00036
- Duer MJ, Frisci D, Proudfoot D, Reid DG, Schoppet M, Shanahan CM, Skepper JN, Wise ER. Mineral surface in calcified plaque is like that of bone: further evidence for regulated mineralization. *Arterioscler Thromb Vasc Biol.* 2008;28:2030–2034. doi: 10.1161/ATVBAHA.108.172387
- Mackey RH, Venkitchalam L, Sutton-Tyrrell K. Calcifications, arterial stiffness and atherosclerosis. *Adv Cardiol.* 2007;44:234–244. doi: 10.1159/000096744
- Forsythe RO, Dweck MR, McBride OMB, et al. 18F-sodium fluoride uptake in abdominal aortic aneurysms: the SoFIA3 study. *J Am Coll Cardiol.* 2018;71:513–523. doi: 10.1016/j.jacc.2017.11.053
- Nakayama A, Morita H, Hayashi N, Nomura Y, Hoshina K, Shigematsu K, Ohtsu H, Miyata T, Komuro I. Inverse correlation between calcium accumulation and the expansion rate of abdominal aortic aneurysms. *Circ J.* 2016;80:332–339. doi: 10.1253/circj.CJ-15-1065
- Wanga S, Hibender S, Ridwan Y, van Roomen C, Vos M, van der Made I, van Vliet N, Franken R, van Riel LA, Groenink M, Zwinderman AH, Mulder BJ, de Vries CJ, Essers J, de Waard V. Aortic microcalcification is associated with elastin fragmentation in Marfan syndrome. *J Pathol.* 2017;243:294–306. doi: 10.1002/path.4949
- Chowdhury MM, Zieliński LP, Sun JJ, Lambracos S, Boyle JR, Harrison SC, Rudd JHF, Coughlin PA. Editor's choice – calcification of thoracic and abdominal aneurysms is associated with mortality and morbidity. *Eur J Vasc Endovasc Surg.* 2018;55:101–108.
- Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, Hofman A, Witteman JC. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr.* 2004;134:3100–3105. doi: 10.1093/jn/134.11.3100
- Beulens JW, Bots ML, Atsma F, Bartelink ML, Prokop M, Geleijnse JM, Witteman JC, Grobbee DE, van der Schouw YT. High dietary menaquinone intake is associated with reduced coronary calcification. *Atherosclerosis.* 2009;203:489–493. doi: 10.1016/j.atherosclerosis.2008.07.010
- Johnston KW, Rutherford RB, Tilson MD, Shah DM, Hollier L, Stanley JC. Suggested standards for reporting on arterial aneurysms. Subcommittee on Reporting Standards for Arterial Aneurysms, Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. *J Vasc Surg.* 1991;13:452–458.
- Choke E, Cockerill G, Wilson WR, Sayed S, Dawson J, Loftus I, Thompson MM. A review of biological factors implicated in abdominal aortic aneurysm rupture. *Eur J Vasc Endovasc Surg.* 2005;30:227–244. doi: 10.1016/j.ejvs.2005.03.009
- Henderson EL, Geng YJ, Sukhova GK, Whittemore AD, Knox J, Libby P. Death of smooth muscle cells and expression of mediators of apoptosis by T lymphocytes in human abdominal aortic aneurysms. *Circulation.* 1999;99:96–104.
- Michel JB, Jondeau G, Milewicz DM. From genetics to response to injury: vascular smooth muscle cells in aneurysms and dissections of the ascending aorta. *Cardiovasc Res.* 2018;114:578–589. doi: 10.1093/cvr/cvy006
- Choke E, Thompson MM, Dawson J, Wilson WR, Sayed S, Loftus IM, Cockerill GW. Abdominal aortic aneurysm rupture is associated with increased medial neovascularization and overexpression of proangiogenic cytokines. *Arterioscler Thromb Vasc Biol.* 2006;26:2077–2082. doi: 10.1161/01.ATV.0000234944.22509.f9
- Raaz U, Zöllner AM, Schellinger IN, et al. Segmental aortic stiffening contributes to experimental abdominal aortic aneurysm development. *Circulation.* 2015;131:1783–1795. doi: 10.1161/CIRCULATIONAHA.114.012377
- López-Candales A, Holmes DR, Liao S, Scott MJ, Wickline SA, Thompson RW. Decreased vascular smooth muscle cell density in medial degeneration of human abdominal aortic aneurysms. *Am J Pathol.* 1997;150:993–1007.
- Crosas-Mollet E, Meirelles T, López-Luque J, et al. Vascular smooth muscle cell phenotypic changes in patients with Marfan syndrome. *Arterioscler Thromb Vasc Biol.* 2015;35:960–972. doi: 10.1161/ATVBAHA.114.304412
- Elefteriades JA, Pomianowski P. Practical genetics of thoracic aortic aneurysm. *Prog Cardiovasc Dis.* 2013;56:57–67. doi: 10.1016/j.pcad.2013.06.002
- Pannu H, Avidan N, Tran-Fadulu V, Milewicz DM. Genetic basis of thoracic aortic aneurysms and dissections: potential relevance to abdominal aortic aneurysms. *Ann N Y Acad Sci.* 2006;1085:242–255. doi: 10.1196/annals.1383.024
- Ando M, Okita Y, Morota T, Takamoto S. Thoracic aortic aneurysm associated with congenital bicuspid aortic valve. *Cardiovasc Surg.* 1998;6:629–634.
- Freeze SL, Landis BJ, Ware SM, Helm BM. Bicuspid aortic valve: a review with recommendations for genetic counseling. *J Genet Couns.* 2016;25:1171–1178. doi: 10.1007/s10897-016-0002-6
- Guo DC, Pannu H, Tran-Fadulu V, et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. *Nat Genet.* 2007;39:1488–1493. doi: 10.1038/ng.2007.6
- He R, Guo DC, Sun W, Papke CL, Duraisamy S, Estrera AL, Safi HJ, Ahn C, Buja LM, Arnett FC, Zhang J, Geng YJ, Milewicz DM. Characterization of the inflammatory cells in ascending thoracic aortic aneurysms and sporadic aneurysms. *J Thorac Cardiovasc Surg.* 2008;136:922.e1–929.e1. doi: 10.1016/j.jtcvs.2007.12.063
- Tang PC, Yakimov AO, Teesdale MA, Coody MA, Dardik A, Elefteriades JA, Tellides G. Transmural inflammation by interferon-gamma-producing T cells correlates with outward vascular remodeling and intimal expansion of ascending thoracic aortic aneurysms. *FASEB J.* 2005;19:1528–1530. doi: 10.1096/fj.05-3671fje
- Wang Y, Ait-Oufella H, Herbin O, Bonnín P, Ramkhalawon B, Taleb S, Huang J, Offenstadt G, Combadière C, Rénia L, Johnson JL, Tharaux PL, Tedgui A, Mallat Z. TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. *J Clin Invest.* 2010;120:422–432. doi: 10.1172/JCI38136
- Schlattmann TJ, Becker AE. Histologic changes in the normal aging aorta: implications for dissecting aortic aneurysm. *Am J Cardiol.* 1977;39:13–20.
- Schurgers LJ, Geleijnse JM, Grobbee DE, Pols HAP, Hofman A, Witteman JCM, Vermeer C. Nutritional intake of vitamins K1 (Phylloquinone) and K2 (Menaquinone) in The Netherlands. *J Nutr Environ Med.* 1999;9:115–122.
- Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food. *Pathophysiol Haemost Thromb.* 2000;30:298–307.
- Schurgers LJ, Teunissen KJ, Hamulyák K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. *Blood.* 2007;109:3279–3283. doi: 10.1182/blood-2006-08-040709

39. Schurgers LJ, Vermeer C. Differential lipoprotein transport pathways of K-vitamins in healthy subjects. *Biochim Biophys Acta*. 2002;1570:27–32.
40. Shearer MJ, Newman P. Metabolism and cell biology of vitamin K. *Thromb Haemost*. 2008;100:530–547.
41. van Gorp RH, Schurgers LJ. New insights into the pros and cons of the clinical use of Vitamin K Antagonists (VKAs) versus Direct Oral Anticoagulants (DOACs). *Nutrients*. 2015;7:9538–9557. doi: 10.3390/nu7115479
42. Willems BA, Vermeer C, Reutelingsperger CP, Schurgers LJ. The realm of vitamin K dependent proteins: shifting from coagulation toward calcification. *Mol Nutr Food Res*. 2014;58:1620–1635. doi: 10.1002/mnfr.201300743
43. Wang Y, Zhen Y, Shi Y, Chen J, Zhang C, Wang X, Yang X, Zheng Y, Liu Y, Hui R. Vitamin k epoxide reductase: a protein involved in angiogenesis. *Mol Cancer Res*. 2005;3:317–323. doi: 10.1158/1541-7786.MCR-04-0221
44. Wang Y, Zhang W, Zhang Y, et al. VKORC1 haplotypes are associated with arterial vascular diseases (stroke, coronary heart disease, and aortic dissection). *Circulation*. 2006;113:1615–1621. doi: 10.1161/CIRCULATIONAHA.105.580167
45. Parolari A, Tremoli E, Songia P, Pillozzi A, Di Bartolomeo R, Alamanni F, Mestres CA, Pacini D. Biological features of thoracic aortic diseases. Where are we now, where are we heading to: established and emerging biomarkers and molecular pathways. *Eur J Cardiothorac Surg*. 2013;44:9–23. doi: 10.1093/ejcts/ezs647
46. Andreas M, Panzenboeck A, Shabaniyan S, Kocher A, Mannhalter C, Petzl A, Hueblauer J, Wolzt M, Ehrlich M, Lang I. The VKORC1 polymorphism rs9923231 is associated with aneurysms of the ascending aorta in an Austrian population. *Thromb Res*. 2017;152:41–43. doi: 10.1016/j.thromres.2017.02.009
47. Anwar MA, Shalhoub J, Lim CS, Gohel MS, Davies AH. The effect of pressure-induced mechanical stretch on vascular wall differential gene expression. *J Vasc Res*. 2012;49:463–478. doi: 10.1159/000339151
48. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev*. 2004;84:767–801. doi: 10.1152/physrev.00041.2003
49. Owens GK. Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev*. 1995;75:487–517. doi: 10.1152/physrev.1995.75.3.487
50. Rzuicido EM, Martin KA, Powell RJ. Regulation of vascular smooth muscle cell differentiation. *J Vasc Surg*. 2007;45(suppl A):A25–A32. doi: 10.1016/j.jvs.2007.03.001
51. Ailawadi G, Moehle CW, Pei H, Walton SP, Yang Z, Kron IL, Lau CL, Owens GK. Smooth muscle phenotypic modulation is an early event in aortic aneurysms. *J Thorac Cardiovasc Surg*. 2009;138:1392–1399. doi: 10.1016/j.jtcvs.2009.07.075
52. Branchetti E, Poggio P, Sainger R, Shang E, Grau JB, Jackson BM, Lai EK, Parmacek MS, Gorman RC, Gorman JH, Bavaria JE, Ferrari G. Oxidative stress modulates vascular smooth muscle cell phenotype via CTGF in thoracic aortic aneurysm. *Cardiovasc Res*. 2013;100:316–324. doi: 10.1093/cvr/cvt205
53. Hao H, Gabbiani G, Bochaton-Piallat ML. Arterial smooth muscle cell heterogeneity: implications for atherosclerosis and restenosis development. *Arterioscler Thromb Vasc Biol*. 2003;23:1510–1520. doi: 10.1161/01.ATV.0000090130.85752.ED
54. Chamley-Campbell J, Campbell GR, Ross R. The smooth muscle cell in culture. *Physiol Rev*. 1979;59:1–61. doi: 10.1152/physrev.1979.59.1.1
55. Rensen SS, Doevendans PA, van Eys GJ. Regulation and characteristics of vascular smooth muscle cell phenotypic diversity. *Neth Heart J*. 2007;15:100–108.
56. Hao H, Ropraz P, Verin V, Camenzind E, Geinoz A, Pepper MS, Gabbiani G, Bochaton-Piallat ML. Heterogeneity of smooth muscle cell populations cultured from pig coronary artery. *Arterioscler Thromb Vasc Biol*. 2002;22:1093–1099.
57. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. *Circ Res*. 2016;118:692–702. doi: 10.1161/CIRCRESAHA.115.306361
58. Allahverdian S, Chaabane C, Boukais K, Francis GA, Bochaton-Piallat ML. Smooth muscle cell fate and plasticity in atherosclerosis. *Cardiovasc Res*. 2018;114:540–550. doi: 10.1093/cvr/cvy022
59. Durham AL, Speer MY, Scatena M, Giachelli CM, Shanahan CM. Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. *Cardiovasc Res*. 2018;114:590–600. doi: 10.1093/cvr/cvy010
60. Hellström M, Kalén M, Lindahl P, Abramsson A, Betsholtz C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development*. 1999;126:3047–3055.
61. Hautmann MB, Madsen CS, Owens GK. A transforming growth factor beta (TGFbeta) control element drives TGFbeta-induced stimulation of smooth muscle alpha-actin gene expression in concert with two CarG elements. *J Biol Chem*. 1997;272:10948–10956. doi: 10.1074/jbc.272.16.10948
62. Bascands JL, Girolami JP, Trolly M, Escargueil-Blanc I, Nazzari D, Salvayre R, Blaes N. Angiotensin II induces phenotype-dependent apoptosis in vascular smooth muscle cells. *Hypertension*. 2001;38:1294–1299.
63. Clempus RE, Griendling KK. Reactive oxygen species signaling in vascular smooth muscle cells. *Cardiovasc Res*. 2006;71:216–225. doi: 10.1016/j.cardiores.2006.02.033
64. Nataatmadja M, West J, West M. Overexpression of transforming growth factor-beta is associated with increased hyaluronan content and impairment of repair in Marfan syndrome aortic aneurysm. *Circulation*. 2006;114(1 suppl):I371–I377. doi: 10.1161/CIRCULATIONAHA.105.000927
65. Reilly CF, Kindy MS, Brown KE, Rosenberg RD, Sonenshein GE. Heparin prevents vascular smooth muscle cell progression through the G1 phase of the cell cycle. *J Biol Chem*. 1989;264:6990–6995.
66. Beamish JA, Geyer LC, Haq-Siddiqi NA, Kottke-Marchant K, Marchant RE. The effects of heparin releasing hydrogels on vascular smooth muscle cell phenotype. *Biomaterials*. 2009;30:6286–6294. doi: 10.1016/j.biomaterials.2009.08.004
67. Mao N, Gu T, Shi E, Zhang G, Yu L, Wang C. Phenotypic switching of vascular smooth muscle cells in animal model of rat thoracic aortic aneurysm. *Interact Cardiovasc Thorac Surg*. 2015;21:62–70. doi: 10.1093/icvts/ivv074
68. Milewicz DM, Prakash SK, Ramirez F. Therapeutics targeting drivers of thoracic aortic aneurysms and acute aortic dissections: insights from predisposing genes and mouse Models. *Annu Rev Med*. 2017;68:51–67. doi: 10.1146/annurev-med-100415-022956
69. Lindsay ME, Dietz HC. The genetic basis of aortic aneurysm. *Cold Spring Harb Perspect Med*. 2014;4:a015909. doi: 10.1101/cshperspect.a015909
70. Zhu L, Vranckx R, Khau Van Kien P, Lalande A, Boisset N, Mathieu F, Wegman M, Glancy L, Gasc JM, Brunotte F, Bruneval P, Wolf JE, Michel JB, Jeunemaitre X. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. *Nat Genet*. 2006;38:343–349. doi: 10.1038/ng1721
71. Kim HW, Stansfield BK. Genetic and epigenetic regulation of aortic aneurysms. *Biomed Res Int*. 2017;2017:7268521. doi: 10.1155/2017/7268521
72. Mallat Z, Tedgui A, Henrion D. Role of microvascular tone and extracellular matrix contraction in the regulation of interstitial fluid: implications for aortic dissection. *Arterioscler Thromb Vasc Biol*. 2016;36:1742–1747. doi: 10.1161/ATVBAHA.116.307909
73. Ferruzzi J, Murtada SI, Li G, Jiao Y, Uman S, Ting MY, Tellides G, Humphrey JD. Pharmacologically improved contractility protects against aortic dissection in mice with disrupted transforming growth factor-β signaling despite compromised extracellular matrix properties. *Arterioscler Thromb Vasc Biol*. 2016;36:919–927. doi: 10.1161/ATVBAHA.116.307436
74. Chen PY, Qin L, Li G, Tellides G, Simons M. Smooth muscle FGF/TGFβ cross talk regulates atherosclerosis progression. *EMBO Mol Med*. 2016;8:712–728. doi: 10.15252/emmm.201506181
75. Fukui D, Miyagawa S, Soeda J, Tanaka K, Urayama H, Kawasaki S. Overexpression of transforming growth factor beta1 in smooth muscle cells of human abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg*. 2003;25:540–545. doi: 10.1053/ejvs.2002.1857
76. Nataatmadja M, West J, Prabowo S, West M. Angiotensin II receptor antagonism reduces transforming growth factor beta and smad signaling in thoracic aortic aneurysm. *Ochsner J*. 2013;13:42–48.
77. Doyle JJ, Gerber EE, Dietz HC. Matrix-dependent perturbation of TGFβ signaling and disease. *FEBS Lett*. 2012;586:2003–2015. doi: 10.1016/j.febslet.2012.05.027
78. Lindsay ME, Dietz HC. Lessons on the pathogenesis of aneurysm from heritable conditions. *Nature*. 2011;473:308–316. doi: 10.1038/nature10145
79. Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science*. 2006;312:117–121. doi: 10.1126/science.1124287
80. Lindsay ME, Schepers D, Bolar NA, et al. Loss-of-function mutations in TGFB2 cause a syndromic presentation of thoracic aortic aneurysm. *Nat Genet*. 2012;44:922–927. doi: 10.1038/ng.2349
81. Wei H, Hu JH, Angelov SN, Fox K, Yan J, Enstrom R, Smith A, Dichek DA. Aortopathy in a mouse model of marfan syndrome is not mediated by altered transforming growth factor β signaling. *J Am Heart Assoc*. 2017;6:e004968.

82. Gallo EM, Loch DC, Habashi JP, et al. Angiotensin II-dependent TGF- $\beta$  signaling contributes to Loeys-Dietz syndrome vascular pathogenesis. *J Clin Invest*. 2014;124:448–460. doi: 10.1172/JCI69666
83. Li W, Li Q, Jiao Y, Qin L, Ali R, Zhou J, Ferruzzi J, Kim RW, Geirsson A, Dietz HC, Offermanns S, Humphrey JD, Tellides G. Tgfr2 disruption in postnatal smooth muscle impairs aortic wall homeostasis. *J Clin Invest*. 2014;124:755–767. doi: 10.1172/JCI69942
84. Mallat Z, Ait-Oufella H, Tedgui A. The pathogenic transforming growth factor- $\beta$  overdrive hypothesis in aortic aneurysms and dissections: a mirage? *Circ Res*. 2017;120:1718–1720. doi: 10.1161/CIRCRESAHA.116.310371
85. Chen X, Rateri DL, Howatt DA, Balakrishnan A, Moorleghen JJ, Cassis LA, Daugherty A. TGF- $\beta$  neutralization enhances angii-induced aortic rupture and aneurysm in both thoracic and abdominal regions. *PLoS One*. 2016;11:e0153811. doi: 10.1371/journal.pone.0153811
86. Angelov SN, Hu JH, Wei H, Airhart N, Shi M, Dichek DA. TGF- $\beta$  (Transforming Growth Factor- $\beta$ ) signaling protects the thoracic and abdominal aorta from angiotensin II-induced pathology by distinct mechanisms. *Arterioscler Thromb Vasc Biol*. 2017;37:2102–2113. doi: 10.1161/ATVBAHA.117.309401
87. Gao P, Wu W, Ye J, Lu YW, Adam AP, Singer HA, Long X. Transforming growth factor  $\beta$ 1 suppresses proinflammatory gene program independent of its regulation on vascular smooth muscle differentiation and autophagy. *Cell Signal*. 2018;50:160–170. doi: 10.1016/j.cellsig.2018.07.002
88. Zhang Y, Yin J, Ding H, Zhang C, Gao YS. Vitamin K2 ameliorates damage of blood vessels by glucocorticoid: a potential mechanism for its protective effects in glucocorticoid-induced osteonecrosis of the femoral head in a rat model. *Int J Biol Sci*. 2016;12:776–785. doi: 10.7150/ijbs.15248
89. Wang Z, Wang M, Finn F, Carr BI. The growth inhibitory effects of vitamins K and their actions on gene expression. *Hepatology*. 1995;22:876–882.
90. Clauser S, Meilhac O, Bièche I, Raynal P, Bruneval P, Michel JB, Borgel D. Increased secretion of Gas6 by smooth muscle cells in human atherosclerotic carotid plaques. *Thromb Haemost*. 2012;107:140–149. doi: 10.1160/TH11-05-0368
91. Zhao J, Warburton D. Matrix Gla protein gene expression is induced by transforming growth factor-beta in embryonic lung culture. *Am J Physiol*. 1997;273(1 pt 1):L282–L287. doi: 10.1152/ajplung.1997.273.1.L282
92. Bunton TE, Biery NJ, Myers L, Gayraud B, Ramirez F, Dietz HC. Phenotypic alteration of vascular smooth muscle cells precedes elastolysis in a mouse model of Marfan syndrome. *Circ Res*. 2001;88:37–43.
93. Airhart ND, Arif B, Curci J. Vascular smooth muscle cells from abdominal aortic aneurysms have uniquely high elastolytic potential associated with activation of MMP-2. *Journal of Surgical Research*. 2013;179:282.
94. Silence J, Collen D, Lijnen HR. Reduced atherosclerotic plaque but enhanced aneurysm formation in mice with inactivation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) gene. *Circ Res*. 2002;90:897–903.
95. Bendeck MP, Irvin C, Reidy MA. Inhibition of matrix metalloproteinase activity inhibits smooth muscle cell migration but not neointimal thickening after arterial injury. *Circ Res*. 1996;78:38–43.
96. Menashi S, Campa JS, Greenhalgh RM, Powell JT. Collagen in abdominal aortic aneurysm: typing, content, and degradation. *J Vasc Surg*. 1987;6:578–582.
97. Tanios F, Gee MW, Pelisek J, Kehl S, Biehler J, Grabher-Meier V, Wall WA, Eckstein HH, Reeps C. Interaction of biomechanics with extracellular matrix components in abdominal aortic aneurysm wall. *Eur J Vasc Endovasc Surg*. 2015;50:167–174. doi: 10.1016/j.ejvs.2015.03.021
98. Ide Y, Zhang H, Hamajima H, Kawaguchi Y, Eguchi Y, Mizuta T, Yamamoto K, Fujimoto K, Ozaki I. Inhibition of matrix metalloproteinase expression by menatrenone, a vitamin K2 analogue. *Oncol Rep*. 2009;22:599–604.
99. Ebina K, Shi K, Hirao M, Kaneshiro S, Morimoto T, Koizumi K, Yoshikawa H, Hashimoto J. Vitamin K2 administration is associated with decreased disease activity in patients with rheumatoid arthritis. *Mod Rheumatol*. 2013;23:1001–1007. doi: 10.1007/s10165-012-0789-4
100. Shishavan NG, Gargari BP, Kolahi S, Hajjalilo M, Jafarabadi MA, Javadzadeh Y. Effects of vitamin K on matrix metalloproteinase-3 and rheumatoid factor in women with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *J Am Coll Nutr*. 2016;35:392–398. doi: 10.1080/07315724.2015.1026004
101. Rowe VL, Stevens SL, Reddick TT, Freeman MB, Donnell R, Carroll RC, Goldman MH. Vascular smooth muscle cell apoptosis in aneurysmal, occlusive, and normal human aortas. *J Vasc Surg*. 2000;31:567–576.
102. Thompson RW, Liao S, Curci JA. Vascular smooth muscle cell apoptosis in abdominal aortic aneurysms. *Coron Artery Dis*. 1997;8:623–631.
103. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases *in vitro*. Implications for atherosclerotic plaque stability. *J Clin Invest*. 1996;98:2572–2579. doi: 10.1172/JCI119076
104. Jia LX, Zhang WM, Li TT, Liu Y, Piao CM, Ma YC, Lu Y, Wang Y, Liu TT, Qi YF, Du J. ER stress dependent microparticles derived from smooth muscle cells promote endothelial dysfunction during thoracic aortic aneurysm and dissection. *Clin Sci (Lond)*. 2017;131:1287–1299. doi: 10.1042/CS20170252
105. Proudfoot D, Skepper JN, Hegyi L, Bennett MR, Shanahan CM, Weissberg PL. Apoptosis regulates human vascular calcification *in vitro*: evidence for initiation of vascular calcification by apoptotic bodies. *Circ Res*. 2000;87:1055–1062.
106. Nadra I, Mason JC, Philippidis P, Florey O, Smythe CD, McCarthy GM, Landis RC, Haskard DO. Proinflammatory activation of macrophages by basic calcium phosphate crystals via protein kinase C and MAP kinase pathways: a vicious cycle of inflammation and arterial calcification? *Circ Res*. 2005;96:1248–1256. doi: 10.1161/01.RES.0000171451.88616.c2
107. Vengrenyuk Y, Carlier S, Xanthos S, Cardoso L, Ganatos P, Virmani R, Einav S, Gilchrist L, Weinbaum S. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. *Proc Natl Acad Sci USA*. 2006;103:14678–14683.
108. Nakano T, Higashino K, Kikuchi N, Kishino J, Nomura K, Fujita H, Ohara O, Arita H. Vascular smooth muscle cell-derived, Gla-containing growth-potentiating factor for Ca(2+)-mobilizing growth factors. *J Biol Chem*. 1995;270:5702–5705. doi: 10.1074/jbc.270.11.5702
109. Melaragno MG, Cavet ME, Yan C, Tai LK, Jin ZG, Haendeler J, Berk BC. Gas6 inhibits apoptosis in vascular smooth muscle: role of Axl kinase and Akt. *J Mol Cell Cardiol*. 2004;37:881–887. doi: 10.1016/j.yjmcc.2004.06.018
110. Qiu C, Zheng H, Tao H, Yu W, Jiang X, Li A, Jin H, Lv A, Li H. Vitamin K2 inhibits rat vascular smooth muscle cell calcification by restoring the Gas6/Axl/Akt anti-apoptotic pathway. *Mol Cell Biochem*. 2017;433:149–159. doi: 10.1007/s11010-017-3023-z
111. Son BK, Kozaki K, Iijima K, Eto M, Kojima T, Ota H, Senda Y, Maemura K, Nakano T, Akishita M, Ouchi Y. Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. *Circ Res*. 2006;98:1024–1031. doi: 10.1161/01.RES.0000218859.90970.8d
112. Melaragno MG, Wuthrich DA, Poppa V, Gill D, Lindner V, Berk BC, Corson MA. Increased expression of Axl tyrosine kinase after vascular injury and regulation by G protein-coupled receptor agonists in rats. *Circ Res*. 1998;83:697–704.
113. Ekman C, Site DF, Gottsäter A, Lindblad B, Dahlbäck B. Plasma concentrations of growth arrest specific protein 6 and the soluble form of its tyrosine kinase receptor Axl as markers of large abdominal aortic aneurysms. *Clin Biochem*. 2010;43:110–114. doi: 10.1016/j.clinbiochem.2009.07.025
114. Shanahan CM, Furmanik M. Endoplasmic reticulum stress in arterial smooth muscle cells: a novel regulator of vascular disease. *Curr Cardiol Rev*. 2017;13:94–105. doi: 10.2174/1573403X12666161014094738
115. Zhao G, Fu Y, Cai Z, Yu F, Gong Z, Dai R, Hu Y, Zeng L, Xu Q, Kong W. Unspliced XBP1 confers VSMC homeostasis and prevents aortic aneurysm formation via FoxO4 interaction. *Circ Res*. 2017;121:1331–1345. doi: 10.1161/CIRCRESAHA.117.311450
116. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep*. 2006;7:880–885. doi: 10.1038/sj.embor.7400779
117. Jia LX, Zhang WM, Zhang HJ, Li TT, Wang YL, Qin YW, Gu H, Du J. Mechanical stretch-induced endoplasmic reticulum stress, apoptosis and inflammation contribute to thoracic aortic aneurysm and dissection. *J Pathol*. 2015;236:373–383. doi: 10.1002/path.4534
118. Qin Y, Wang Y, Liu O, Jia L, Fang W, Du J, Wei Y. Tauroursodeoxycholic acid attenuates angiotensin II induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice by inhibiting endoplasmic reticulum stress. *Eur J Vasc Endovasc Surg*. 2017;53:337–345. doi: 10.1016/j.ejvs.2016.10.026
119. Wajih N, Hutson SM, Wallin R. Disulfide-dependent protein folding is linked to operation of the vitamin K cycle in the endoplasmic reticulum. A protein disulfide isomerase-VKORC1 redox enzyme complex appears to be responsible for vitamin K1 2,3-epoxide reduction. *J Biol Chem*. 2007;282:2626–2635. doi: 10.1074/jbc.M608954200

120. Rutkevich LA, Williams DB. Vitamin K epoxide reductase contributes to protein disulfide formation and redox homeostasis within the endoplasmic reticulum. *Mol Biol Cell*. 2012;23:2017–2027. doi: 10.1091/mbc.E12-02-0102
121. Askari B, Khaleqsefat E, Khalafkhani D, Khalaj-Kondori M, Khademvatan K, Soraya H. Study on a novel polymorphism in the VKORC1 promoter region using bioinformatic tools and warfarin dosing data. *Thromb Res*. 2017;158:76–78. doi: 10.1016/j.thromres.2017.08.012
122. Miller FJ Jr, Sharp WJ, Fang X, Oberley LW, Oberley TD, Weintraub NL. Oxidative stress in human abdominal aortic aneurysms: a potential mediator of aneurysmal remodeling. *Arterioscler Thromb Vasc Biol*. 2002;22:560–565.
123. Siu KL, Li Q, Zhang Y, Guo J, Youn JY, Du J, Cai H. NOX isoforms in the development of abdominal aortic aneurysm. *Redox Biol*. 2017;11:118–125. doi: 10.1016/j.redox.2016.11.002
124. Konior A, Schramm A, Czesnikiewicz-Guzik M, Guzik TJ. NADPH oxidases in vascular pathology. *Antioxid Redox Signal*. 2014;20:2794–2814. doi: 10.1089/ars.2013.5607
125. McCormick ML, Gavrila D, Weintraub NL. Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol*. 2007;27:461–469. doi: 10.1161/01.ATV.0000257552.94483.14
126. Thomas M, Gavrila D, McCormick ML, Miller FJ Jr, Daugherty A, Cassis LA, Dellsperger KC, Weintraub NL. Deletion of p47phox attenuates angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice. *Circulation*. 2006;114:404–413. doi: 10.1161/CIRCULATIONAHA.105.607168
127. Kigawa Y, Miyazaki T, Lei XF, Nakamachi T, Oguchi T, Kim-Kaneyama JR, Taniyama M, Tsunawaki S, Shioda S, Miyazaki A. NADPH oxidase deficiency exacerbates angiotensin II-induced abdominal aortic aneurysms in mice. *Arterioscler Thromb Vasc Biol*. 2014;34:2413–2420. doi: 10.1161/ATVBAHA.114.303086
128. Lu WW, Jia LX, Ni XQ, Zhao L, Chang JR, Zhang JS, Hou YL, Zhu Y, Guan YF, Yu YR, Du J, Tang CS, Qi YF. Intermedin1-53 attenuates abdominal aortic aneurysm by inhibiting oxidative stress. *Arterioscler Thromb Vasc Biol*. 2016;36:2176–2190. doi: 10.1161/ATVBAHA.116.307825
129. Guzik TJ, Chen W, Gongora MC, Guzik B, Lob HE, Mangalat D, Hoch N, Dikalov S, Rudzinski P, Kapelak B, Sadowski J, Harrison DG. Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. *J Am Coll Cardiol*. 2008;52:1803–1809. doi: 10.1016/j.jacc.2008.07.063
130. Jay DB, Papaharalambus CA, Seidel-Rogol B, Dikalova AE, Lassègue B, Griendling KK. Nox5 mediates PDGF-induced proliferation in human aortic smooth muscle cells. *Free Radic Biol Med*. 2008;45:329–335. doi: 10.1016/j.freeradbiomed.2008.04.024
131. Guzik B, Sagan A, Ludew D, Mrowiecki W, Chwała M, Bujak-Gizycka B, Filip G, Grudzien G, Kapelak B, Zmudka K, Mrowiecki T, Sadowski J, Korbut R, Guzik TJ. Mechanisms of oxidative stress in human aortic aneurysms—association with clinical risk factors for atherosclerosis and disease severity. *Int J Cardiol*. 2013;168:2389–2396. doi: 10.1016/j.ijcard.2013.01.278
132. Starke RM, Thompson JW, Ali MS, Pascale CL, Martínez Lege A, Ding D, Chalouhi N, Hasan DM, Jabbar P, Owens GK, Toborek M, Hare JM, Dumont AS. Cigarette smoke initiates oxidative stress-induced cellular phenotypic modulation leading to cerebral aneurysm pathogenesis. *Arterioscler Thromb Vasc Biol*. 2018;38:610–621. doi: 10.1161/ATVBAHA.117.310478
133. Xiong W, Mactaggart J, Knispel R, Worth J, Zhu Z, Li Y, Sun Y, Baxter BT, Johanning J. Inhibition of reactive oxygen species attenuates aneurysm formation in a murine model. *Atherosclerosis*. 2009;202:128–134. doi: 10.1016/j.atherosclerosis.2008.03.029
134. Gavrila D, Li WG, McCormick ML, Thomas M, Daugherty A, Cassis LA, Miller FJ Jr, Oberley LW, Dellsperger KC, Weintraub NL. Vitamin E inhibits abdominal aortic aneurysm formation in angiotensin II-infused apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2005;25:1671–1677. doi: 10.1161/01.ATV.0000172631.50972.0f
135. Nakahashi TK, Hoshina K, Tsao PS, Sho E, Sho M, Karwowski JK, Yeh C, Yang RB, Topper JN, Dalman RL. Flow loading induces macrophage antioxidative gene expression in experimental aneurysms. *Arterioscler Thromb Vasc Biol*. 2002;22:2017–2022.
136. Martinez-Pinna R, Lindholt JS, Blanco-Colio LM, Dejouengel T, Madrigal-Matute J, Ramos-Mozo P, Vega de Ceniga M, Michel JB, Egido J, Meilhac O, Martin-Ventura JL. Increased levels of thioredoxin in patients with abdominal aortic aneurysms (AAAs). A potential link of oxidative stress with AAA evolution. *Atherosclerosis*. 2010;212:333–338. doi: 10.1016/j.atherosclerosis.2010.05.031
137. Behr Andersen C, Lindholt JS, Urbonavicius S, Halekoh U, Jensen PS, Stubbe J, Rasmussen LM, Beck HC. Abdominal aortic aneurysms growth is associated with high concentrations of plasma proteins in the intraluminal thrombus and diseased arterial tissue. *Arterioscler Thromb Vasc Biol*. 2018;38:2254–2267. doi: 10.1161/ATVBAHA.117.310126
138. Westhofen P, Watzka M, Marinova M, Hass M, Kirfel G, Müller J, Bevens CG, Müller CR, Oldenburg J. Human vitamin K 2,3-epoxide reductase complex subunit 1-like 1 (VKORC1L1) mediates vitamin K-dependent intracellular antioxidant function. *J Biol Chem*. 2011;286:15085–15094. doi: 10.1074/jbc.M110.210971
139. Mukai K, Morimoto H, Kikuchi S, Nagaoka S. Kinetic study of free-radical-scavenging action of biological hydroquinones (reduced forms of ubiquinone, vitamin K and tocopherol quinone) in solution. *Biochim Biophys Acta*. 1993;1157:313–317.
140. Vervoort LM, Ronden JE, Thijssen HH. The potent antioxidant activity of the vitamin K cycle in microsomal lipid peroxidation. *Biochem Pharmacol*. 1997;54:871–876.
141. Li J, Lin JC, Wang H, Peterson JW, Furie BC, Furie B, Booth SL, Volpe JJ, Rosenberg PA. Novel role of vitamin k in preventing oxidative injury to developing oligodendrocytes and neurons. *J Neurosci*. 2003;23:5816–5826.
142. Li J, Wang H, Rosenberg PA. Vitamin K prevents oxidative cell death by inhibiting activation of 12-lipoxygenase in developing oligodendrocytes. *J Neurosci Res*. 2009;87:1997–2005. doi: 10.1002/jnr.22029
143. Bridge A, Barr R, Morrè DJ. The plasma membrane NADH oxidase of soybean has vitamin K(1) hydroquinone oxidase activity. *Biochim Biophys Acta*. 2000;1463:448–458.
144. Kishi T, Morrè DM, Morrè DJ. The plasma membrane NADH oxidase of HeLa cells has hydroquinone oxidase activity. *Biochim Biophys Acta*. 1999;1412:66–77.
145. Morrè DM, Lenaz G, Morrè DJ. Surface oxidase and oxidative stress propagation in aging. *J Exp Biol*. 2000;203(pt 10):1513–1521.
146. Schulman S, Wang B, Li W, Rapoport TA. Vitamin K epoxide reductase prefers ER membrane-anchored thioredoxin-like redox partners. *Proc Natl Acad Sci USA*. 2010;107:15027–15032.
147. Pescatore LA, Bonatto D, Forti FL, Sadok A, Kovacic H, Laurindo FR. Protein disulfide isomerase is required for platelet-derived growth factor-induced vascular smooth muscle cell migration, Nox1 NADPH oxidase expression, and RhoGTPase activation. *J Biol Chem*. 2012;287:29290–29300. doi: 10.1074/jbc.M112.394551
148. Trevelin SC, Lopes LR. Protein disulfide isomerase and Nox: new partners in redox signaling. *Curr Pharm Des*. 2015;21:5915–5963.
149. Katsuumi G, Shimizu I, Yoshida Y, Minamino T. Vascular senescence in cardiovascular and metabolic diseases. *Front Cardiovasc Med*. 2018;5:18. doi: 10.3389/fcvm.2018.00018
150. Shanahan CM. Mechanisms of vascular calcification in CKD—evidence for premature ageing? *Nat Rev Nephrol*. 2013;9:661–670. doi: 10.1038/nrneph.2013.176
151. Liao S, Curci JA, Kelley BJ, Sicard GA, Thompson RW. Accelerated replicative senescence of medial smooth muscle cells derived from abdominal aortic aneurysms compared to the adjacent inferior mesenteric artery. *J Surg Res*. 2000;92:85–95. doi: 10.1006/jrsr.2000.5878
152. Helgadottir A, Thorleifsson G, Magnusson KP, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. *Nat Genet*. 2008;40:217–224. doi: 10.1038/ng.72
153. Bennett MR, Clarke MC. Killing the old: cell senescence in atherosclerosis. *Nat Rev Cardiol*. 2017;14:132. doi: 10.1038/nrcardio.2016.217
154. Michel JB, Martin-Ventura JL, Egido J, Sakalihasan N, Treska V, Lindholt J, Allaire E, Thorsteinsdottir U, Cockerill G, Swedenborg J; FAD EU consortium. Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. *Cardiovasc Res*. 2011;90:18–27. doi: 10.1093/cvr/cvq337
155. Liu Y, Drozdov I, Shroff R, Beltran LE, Shanahan CM. Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. *Circ Res*. 2013;112:e99–109. doi: 10.1161/CIRCRESAHA.111.300543
156. Malkawi A, Pirianov G, Torsney E, Chetter I, Sakalihasan N, Loftus IM, Nordon I, Huggins C, Charolidi N, Thompson M, Xu XY, Cockerill GW. Increased expression of lamin A/C correlate with regions of high wall stress in abdominal aortic aneurysms. *Aorta (Stamford)*. 2015;3:152–166. doi: 10.12945/j.aorta.2015.14.069
157. Liu B, Ghosh S, Yang X, Zheng H, Liu X, Wang Z, Jin G, Zheng B, Kennedy BK, Suh Y, Kaerberlein M, Tryggvason K, Zhou Z. Resveratrol rescues SIRT1-dependent adult stem cell decline and alleviates progeroid



- features in laminopathy-based progeria. *Cell Metab.* 2012;16:738–750. doi: 10.1016/j.cmet.2012.11.007
158. Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, Dequiedt F, Haendeler J, Mione M, Dejana E, Alt FW, Zeiher AM, Dimmeler S. SIRT1 controls endothelial angiogenic functions during vascular growth. *Genes Dev.* 2007;21:2644–2658. doi: 10.1101/gad.435107
  159. Kida Y, Goligorsky MS. Sirtuins, cell senescence, and vascular aging. *Can J Cardiol.* 2016;32:634–641. doi: 10.1016/j.cjca.2015.11.022
  160. Gorenne I, Kumar S, Gray K, Figg N, Yu H, Mercer J, Bennett M. Vascular smooth muscle cell sirtuin 1 protects against DNA damage and inhibits atherosclerosis. *Circulation.* 2013;127:386–396. doi: 10.1161/CIRCULATIONAHA.112.124404
  161. Chen HZ, Wang F, Gao P, et al. Age-associated Sirtuin 1 reduction in vascular smooth muscle links vascular senescence and inflammation to abdominal aortic aneurysm. *Circ Res.* 2016;119:1076–1088. doi: 10.1161/CIRCRESAHA.116.308895
  162. Riches K, Clark E, Helliwell RJ, Angelini TG, Hemmings KE, Bailey MA, Bridge KL, Scott DJA, Porter KE. Progressive development of aberrant smooth muscle cell phenotype in abdominal aortic aneurysm disease. *J Vasc Res.* 2018;55:35–46. doi: 10.1159/000484088
  163. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell.* 2013;153:1194–1217. doi: 10.1016/j.cell.2013.05.039
  164. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of lifespan by introduction of telomerase into normal human cells. *Science.* 1998;279:349–352.
  165. Wilson WR, Herbert KE, Mistry Y, Stevens SE, Patel HR, Hastings RA, Thompson MM, Williams B. Blood leucocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease. *Eur Heart J.* 2008;29:2689–2694. doi: 10.1093/eurheartj/ehn386
  166. Blunder S, Messner B, Aschacher T, Zeller I, Türkcan A, Wiedemann D, Andreas M, Blüschke G, Laufer G, Schachner T, Bernhard D. Characteristics of TAV- and BAV-associated thoracic aortic aneurysms—smooth muscle cell biology, expression profiling, and histological analyses. *Atherosclerosis.* 2012;220:355–361. doi: 10.1016/j.atherosclerosis.2011.11.035
  167. Aschacher T, Salameh O, Enzmann F, Messner B, Bergmann M. Telomere biology and thoracic aortic aneurysm. *Int J Mol Sci.* 2017;19:E3.
  168. Yu E, Mercer J, Bennett M. Mitochondria in vascular disease. *Cardiovasc Res.* 2012;95:173–182. doi: 10.1093/cvr/cvs111
  169. Yu E, Foote K, Bennett M. Mitochondrial function in thoracic aortic aneurysms. *Cardiovasc Res.* 2018;114:1696–1698. doi: 10.1093/cvr/cvy180
  170. Yang M, Chadwick AE, Dart C, Kamishima T, Quayle JM. Bioenergetic profile of human coronary artery smooth muscle cells and effect of metabolic intervention. Singh PK, ed. *PLoS One.* 2017;12:e0177951.
  171. Yuan K, Liang W, Zhang J. A comprehensive analysis of differentially expressed genes and pathways in abdominal aortic aneurysm. *Mol Med Rep.* 2015;12:2707–2714. doi: 10.3892/mmr.2015.3709
  172. Gabrielson M, Vorkapic E, Folkesson M, Welander M, Matussek A, Dimberg J, Länne T, Skogberg J, Wägåsäter D. Altered PPAR $\gamma$  coactivator-1  $\alpha$  expression in abdominal aortic aneurysm: possible effects on mitochondrial biogenesis. *J Vasc Res.* 2016;53:17–26. doi: 10.1159/000446653
  173. van der Pluijm I, Burger J, van Heijningen PM, et al. Decreased mitochondrial respiration in aneurysmal aortas of Fibulin-4 mutant mice is linked to PGC1A regulation. *Cardiovasc Res.* 2018;114:1776–1793. doi: 10.1093/cvr/cvy150
  174. Vos M, Esposito G, Edirisinghe JN, Vilain S, Haddad DM, Slabbaert JR, Van Meensel S, Schaap O, De Strooper B, Meganathan R, Morais VA, Verstreken P. Vitamin K2 is a mitochondrial electron carrier that rescues pink1 deficiency. *Science.* 2012;336:1306–1310. doi: 10.1126/science.1218632
  175. Swiader A, Nahapetyan H, Faccini J, D'Angelo R, Mucher E, Elbaz M, Boya P, Vindis C. Mitophagy acts as a safeguard mechanism against human vascular smooth muscle cell apoptosis induced by atherogenic lipids. *Oncotarget.* 2016;7:28821–28835. doi: 10.18632/oncotarget.8936
  176. Grootaert MOJ, Moulis M, Roth L, Martinet W, Vindis C, Bennett MR, De Meyer GRY. Vascular smooth muscle cell death, autophagy and senescence in atherosclerosis. *Cardiovasc Res.* 2018;114:622–634. doi: 10.1093/cvr/cvy007
  177. Kalra SS, Shanahan CM. Vascular calcification and hypertension: cause and effect. *Ann Med.* 2012;44(suppl 1):S85–S92. doi: 10.3109/07853890.2012.660498
  178. Shanahan CM, Crouthamel MH, Kapustin A, Giachelli CM. Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Towler DA, ed. *Circ Res.* 2011;109:697–711.
  179. Neven E, Persy V, Dauwe S, De Schutter T, De Broe ME, D'Haese PC. Chondrocyte rather than osteoblast conversion of vascular cells underlies medial calcification in uremic rats. *Arterioscler Thromb Vasc Biol.* 2010;30:1741–1750. doi: 10.1161/ATVBAHA.110.204834
  180. Jono S, McKee MD, Murray CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res.* 2000;87:E10–E17.
  181. Paloian NJ, Giachelli CM. A current understanding of vascular calcification in CKD. *Am J Physiol Renal Physiol.* 2014;307:F891–F900. doi: 10.1152/ajprenal.00163.2014
  182. Kapustin AN, Chatrou ML, Drozdov I, et al. Vascular smooth muscle cell calcification is mediated by regulated exosome secretion. *Circ Res.* 2015;116:1312–1323. doi: 10.1161/CIRCRESAHA.116.305012
  183. Schurgers LJ, Uitto J, Reutelingsperger CP. Vitamin K-dependent carboxylation of matrix Gla-protein: a crucial switch to control ectopic mineralization. *Trends Mol Med.* 2013;19:217–226. doi: 10.1016/j.molmed.2012.12.008
  184. Sugitani H, Wachi H, Mecham RP, Seyama Y. Accelerated calcification represses the expression of elastic fiber components and lysyl oxidase in cultured bovine aortic smooth muscle cells. *J Atheroscler Thromb.* 2002;9:292–298.
  185. Wachi H, Sugitani H, Murata H, Nakazawa J, Mecham RP, Seyama Y. Tropoelastin inhibits vascular calcification via 67-kDa elastin binding protein in cultured bovine aortic smooth muscle cells. *J Atheroscler Thromb.* 2004;11:159–166.
  186. Saito E, Wachi H, Sato F, Sugitani H, Seyama Y. Treatment with vitamin K(2) combined with bisphosphonates synergistically inhibits calcification in cultured smooth muscle cells. *J Atheroscler Thromb.* 2007;14:317–324.
  187. Rogers MA, Aikawa M, Aikawa E. Macrophage heterogeneity complicates reversal of calcification in cardiovascular tissues. *Circ Res.* 2017;121:5–7. doi: 10.1161/CIRCRESAHA.117.311219
  188. New SE, Goettsch C, Aikawa M, Marchini JF, Shibusaki M, Yabusaki K, Libby P, Shanahan CM, Croce K, Aikawa E. Macrophage-derived matrix vesicles: an alternative novel mechanism for microcalcification in atherosclerotic plaques. *Circ Res.* 2013;113:72–77. doi: 10.1161/CIRCRESAHA.113.301036
  189. Allahverdian S, Chehroudi AC, McManus BM, Abraham T, Francis GA. Contribution of intimal smooth muscle cells to cholesterol accumulation and macrophage-like cells in human atherosclerosis. *Circulation.* 2014;129:1551–1559. doi: 10.1161/CIRCULATIONAHA.113.005015
  190. Vengrenyuk Y, Nishi H, Long X, Ouimet M, Savji N, Martinez FO, Cassella CP, Moore KJ, Ramsey SA, Miano JM, Fisher EA. Cholesterol loading reprograms the microRNA-143/145-myocardin axis to convert aortic smooth muscle cells to a dysfunctional macrophage-like phenotype. *Arterioscler Thromb Vasc Biol.* 2015;35:535–546. doi: 10.1161/ATVBAHA.114.304029
  191. Chellan B, Rojas E, Zhang C, Hofmann Bowman MA. Enzyme-modified non-oxidized LDL (ELDL) induces human coronary artery smooth muscle cell transformation to a migratory and osteoblast-like phenotype. *Sci Rep.* 2018;8:11954. doi: 10.1038/s41598-018-30073-w
  192. Buijs RV, Willems TP, Tio RA, Boersma HH, Tielliu IF, Slart RH, Zeebregts CJ. Calcification as a risk factor for rupture of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg.* 2013;46:542–548. doi: 10.1016/j.ejvs.2013.09.006
  193. O'Leary SA, Mulvihill JJ, Barrett HE, Kavanagh EG, Walsh MT, McLaughlin TM, Doyle BJ. Determining the influence of calcification on the failure properties of abdominal aortic aneurysm (AAA) tissue. *J Mech Behav Biomed Mater.* 2015;42:154–167. doi: 10.1016/j.jmbm.2014.11.005
  194. Kelly-Arnold A, Maldonado N, Laudier D, Aikawa E, Cardoso L, Weinbaum S. Revised microcalcification hypothesis for fibrous cap rupture in human coronary arteries. *Proc Natl Acad Sci USA.* 2013;110:10741–10746.
  195. Forsythe RO, Newby DE, Robson JM. Monitoring the biological activity of abdominal aortic aneurysms Beyond Ultrasound. *Heart.* 2016;102:817–824. doi: 10.1136/heartjnl-2015-308779
  196. Reeps C, Essler M, Pelisek J, Seidl S, Eckstein HH, Krause BJ. Increased 18F-fluorodeoxyglucose uptake in abdominal aortic aneurysms in positron emission/computed tomography is associated with inflammation, aortic wall instability, and acute symptoms. *J Vasc Surg.* 2008;48:417–423; discussion 424. doi: 10.1016/j.jvs.2008.03.059
  197. Chatrou ML, Winckers K, Hackeng TM, Reutelingsperger CP, Schurgers LJ. Vascular calcification: the price to pay for anticoagulation therapy with vitamin K-antagonists. *Blood Rev.* 2012;26:155–166. doi: 10.1016/j.blre.2012.03.002

198. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. *Clin J Am Soc Nephrol.* 2008;3:1504–1510. doi: 10.2215/CJN.00770208
199. Kapustin AN, Schoppet M, Schurgers LJ, Reynolds JL, McNair R, Heiss A, Jahnke-Dechent W, Hackeng TM, Schlieper G, Harrison P, Shanahan CM. Prothrombin loading of vascular smooth muscle cell-derived exosomes regulates coagulation and calcification. *Arterioscler Thromb Vasc Biol.* 2017;37:e22–e32. doi: 10.1161/ATVBAHA.116.308886
200. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. Schwartz RS, ed. *N Engl J Med.* 2011;364:1746–1760.
201. Schurgers LJ, Spronk HM. Differential cellular effects of old and new oral anticoagulants: consequences to the genesis and progression of atherosclerosis. *Thromb Haemost.* 2014;112:909–917. doi: 10.1160/TH14-03-0268
202. Matsuzaka T, Tanaka H, Fukuda M, Aoki M, Tsuji Y, Kondoh H. Relationship between vitamin K dependent coagulation factors and anticoagulants (protein C and protein S) in neonatal vitamin K deficiency. *Arch Dis Child.* 1993;68(3 Spec No):297–302. doi: 10.1136/adc.68.3\_spec\_no.297
203. Esmon CT, Vigano-D'Angelo S, D'Angelo A, Comp PC. Anticoagulation proteins C and S. *Adv Exp Med Biol.* 1987;214:47–54.
204. Han X, Fiehler R, Broze GJ Jr. Isolation of a protein Z-dependent plasma protease inhibitor. *Proc Natl Acad Sci USA.* 1998;95:9250–9255.
205. Steitz SA, Speer MY, Curinga G, Yang HY, Haynes P, Aebersold R, Schinke T, Karsenty G, Giachelli CM. Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. *Circ Res.* 2001;89:1147–1154.
206. Neve A, Corrado A, Cantatore FP. Osteocalcin: skeletal and extra-skeletal effects. *J Cell Physiol.* 2013;228:1149–1153. doi: 10.1002/jcp.24278
207. Iyemere VP, Proudfoot D, Weissberg PL, Shanahan CM. Vascular smooth muscle cell phenotypic plasticity and the regulation of vascular calcification. *J Intern Med.* 2006;260:192–210.
208. Malhotra R, Burke MF, Martyn T, Shakartzi HR, Thayer TE, O'Rourke C, Li P, Derwall M, Spagnoli E, Kolodziej SA, Hoeft K, Mayeur C, Jiramongkolchai P, Kumar R, Buys ES, et al. Inhibition of bone morphogenetic protein signal transduction prevents the medial vascular calcification associated with matrix gla protein deficiency. Aikawa E, ed. *PLoS One.* 2015;10:e0117098.
209. Luo G, Ducey P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature.* 1997;386:78–81. doi: 10.1038/386078a0
210. Murshed M, Schinke T, McKee MD, Karsenty G. Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. *J Cell Biol.* 2004;165:625–630. doi: 10.1083/jcb.200402046
211. Schurgers LJ, Teunissen KJ, Knapen MH, Kwaijtaal M, van Diest R, Appels A, Reutelingsperger CP, Cleutjens JP, Vermeer C. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. *Arterioscler Thromb Vasc Biol.* 2005;25:1629–1633. doi: 10.1161/01.ATV.0000173313.46222.43
212. Kapustin AN, Davies JD, Reynolds JL, McNair R, Jones GT, Sidibe A, Schurgers LJ, Skepper JN, Proudfoot D, Mayr M, Shanahan CM. Calcium regulates key components of vascular smooth muscle cell-derived matrix vesicles to enhance mineralization. *Circ Res.* 2011;109:e1–e12. doi: 10.1161/CIRCRESAHA.110.238808
213. Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. *Thromb Haemost.* 2008;100:593–603.
214. Wallin R, Cain D, Hutson SM, Sane DC, Loeser R. Modulation of the binding of matrix Gla protein (MGP) to bone morphogenetic protein-2 (BMP-2). *Thromb Haemost.* 2000;84:1039–1044.
215. Viegas CS, Cavaco S, Neves PL, Ferreira A, João A, Williamson MK, Price PA, Cancela ML, Simes DC. Gla-rich protein is a novel vitamin K-dependent protein present in serum that accumulates at sites of pathological calcifications. *Am J Pathol.* 2009;175:2288–2298. doi: 10.2353/ajpath.2009.090474
216. Viegas CS, Rafael MS, Enriquez JL, Teixeira A, Vitorino R, Luís IM, Costa RM, Santos S, Cavaco S, Neves J, Macedo AL, Willems BA, Vermeer C, Simes DC. Gla-rich protein acts as a calcification inhibitor in the human cardiovascular system. *Arterioscler Thromb Vasc Biol.* 2015;35:399–408. doi: 10.1161/ATVBAHA.114.304823
217. Viegas CSB, Santos L, Macedo AL, Matos AA, Silva AP, Neves PL, Staes A, Gevaert K, Morais R, Vermeer C, Schurgers L, Simes DC. Chronic kidney disease circulating calciprotein particles and extracellular vesicles promote vascular calcification: a role for GRP (Gla-Rich Protein). *Arterioscler Thromb Vasc Biol.* 2018;38:575–587. doi: 10.1161/ATVBAHA.117.310578
218. Willems BA, Furmanik M, Caron MMJ, Chatrou MLL, Kusters DHM, Welting TJM, Stock M, Rafael MS, Viegas CSB, Simes DC, Vermeer C, Reutelingsperger CPM, Schurgers LJ. Ucma/GRP inhibits phosphate-induced vascular smooth muscle cell calcification via SMAD-dependent BMP signalling. *Sci Rep.* 2018;8:4961. doi: 10.1038/s41598-018-23353-y
219. Nakano T, Kawamoto K, Higashino K, Arita H. Prevention of growth arrest-induced cell death of vascular smooth muscle cells by a product of growth arrest-specific gene, gas6. *FEBS Lett.* 1996;387:78–80.
220. Fridell YW, Villa J Jr, Attar EC, Liu ET. GAS6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. *J Biol Chem.* 1998;273:7123–7126. doi: 10.1074/jbc.273.12.7123
221. Laurance S, Lemarié CA, Blostein MD. Growth arrest-specific gene 6 (gas6) and vascular hemostasis. *Adv Nutr.* 2012;3:196–203. doi: 10.3945/an.111.001826
222. Speer MY, Li X, Hiremath PG, Giachelli CM. Runx2/Cbfa1, but not loss of myocardin, is required for smooth muscle cell lineage reprogramming toward osteochondrogenesis. *J Cell Biochem.* 2010;110:935–947. doi: 10.1002/jcb.22607
223. Zeboudj AF, Imura M, Boström K. Matrix GLA protein, a regulatory protein for bone morphogenetic protein-2. *J Biol Chem.* 2002;277:4388–4394. doi: 10.1074/jbc.M109683200
224. Sweatt A, Sane DC, Hutson SM, Wallin R. Matrix Gla protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. *J Thromb Haemost.* 2003;1:178–185.
225. Chatrou MLL, Cleutjens JP, van der Vusse GJ, Roijers RB, Mutsaers PHA, Schurgers LJ. Intra-section analysis of human coronary arteries reveals a potential role for micro-calcifications in macrophage recruitment in the early stage of atherosclerosis. Gadeau A-P, ed. *PLoS One.* 2015;10:e0142335.
226. Joshi NV, Newby DE, Dweck MR. Identifying high risk plaques prior to heart attack using PET-CT. *Future Cardiol.* 2014;10:307–310. doi: 10.2217/fca.14.22
227. Ehara S, Kobayashi Y, Yoshiyama M, Shimada K, Shimada Y, Fukuda D, Nakamura Y, Yamashita H, Yamagishi H, Takeuchi K, Naruko T, Haze K, Becker AE, Yoshikawa J, Ueda M. Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction: an intravascular ultrasound study. *Circulation.* 2004;110:3424–3429. doi: 10.1161/01.CIR.0000148131.41425.E9
228. Matsumoto K, Maniwa T, Tanaka T, Satoh K, Okunishi H, Oda T. Proteomic analysis of calcified abdominal and thoracic aortic aneurysms. *Int J Mol Med.* 2012;30:417–429. doi: 10.3892/ijmm.2012.985
229. Basalyga DM, Simionescu DT, Xiong W, Baxter BT, Starcher BC, Vyavahare NR. Elastin degradation and calcification in an abdominal aorta injury model: role of matrix metalloproteinases. *Circulation.* 2004;110:3480–3487. doi: 10.1161/01.CIR.0000148367.08413.E9
230. Simionescu A, Philips K, Vyavahare N. Elastin-derived peptides and TGF-beta1 induce osteogenic responses in smooth muscle cells. *Biochem Biophys Res Commun.* 2005;334:524–532. doi: 10.1016/j.bbrc.2005.06.119
231. Takei Y, Tanaka T, Kent KC, Yamanouchi D. Osteoclastogenic differentiation of macrophages in the development of abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol.* 2016;36:1962–1971. doi: 10.1161/ATVBAHA.116.307715
232. Tanaka T, Kelly M, Takei Y, Yamanouchi D. RANKL-mediated osteoclastogenic differentiation of macrophages in the abdominal aorta of angiotensin II-infused apolipoprotein E knockout mice. *J Vasc Surg.* 2018;68(6S):48S.e1–59S.e1. doi: 10.1016/j.jvs.2017.11.091
233. Tsai SH, Huang PH, Peng YJ, Chang WC, Tsai HY, Leu HB, Chen JW, Lin SJ. Zoledronate attenuates angiotensin II-induced abdominal aortic aneurysm through inactivation of Rho/ROCK-dependent JNK and NF-κB pathway. *Cardiovasc Res.* 2013;100:501–510. doi: 10.1093/cvr/cvt230
234. Nistala H, Lee-Arteaga S, Carta L, Cook JR, Smaldone S, Siciliano G, Rifkin AN, Dietz HC, Rifkin DB, Ramirez F. Differential effects of alendronate and losartan therapy on osteopenia and aortic aneurysm in mice with severe Marfan syndrome. *Hum Mol Genet.* 2010;19:4790–4798. doi: 10.1093/hmg/ddq409
235. Moreau C, Bajolle F, Siguret V, Lasne D, Golmard JL, Elie C, Beaune P, Cheurfi R, Bonnet D, Loriot MA. Vitamin K antagonists in children with heart disease: height and VKORC1 genotype are the main determinants

- of the warfarin dose requirement. *Blood*. 2012;119:861–867. doi: 10.1182/blood-2011-07-365502
236. Stafford DW. The vitamin K cycle. *J Thromb Haemost*. 2005;3:1873–1878. doi: 10.1111/j.1538-7836.2005.01419.x
  237. Beinema M, Brouwers JR, Schalekamp T, Wilffert B. Pharmacogenetic differences between warfarin, acenocoumarol and phenprocoumon. *Thromb Haemost*. 2008;100:1052–1057.
  238. Krüger T, Oelenberg S, Kaesler N, Schurgers LJ, van de Sandt AM, Boor P, Schlieper G, Brandenburg VM, Fekete BC, Veulemans V, Ketteler M, Vermeer C, Jahnhen-Dechent W, Floege J, Westenfeld R. Warfarin induces cardiovascular damage in mice. *Arterioscler Thromb Vasc Biol*. 2013;33:2618–2624. doi: 10.1161/ATVBAHA.113.302244
  239. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. *Arterioscler Thromb Vasc Biol*. 1998;18:1400–1407.
  240. Kekki M, Julkunen RJ, Wahlström B. Distribution pharmacokinetics of warfarin in the rat, a non-linear multicompartment model. *Naunyn Schmiedeberg's Arch Pharmacol*. 1977;297:61–73.
  241. Shepherd AM, Hewick DS, Moreland TA, Stevenson IH. Age as a determinant of sensitivity to warfarin. *Br J Clin Pharmacol*. 1977;4:315–320. doi: 10.1111/j.1365-2125.1977.tb00719.x
  242. Schurgers LJ, Joosen IA, Laufer EM, Chatrou MLL, Herfs M, Winkens MHM, Westenfeld R, Veulemans V, Krueger T, Shanahan CM, Jahnhen-Dechent W, Biessen E, Narula J, Vermeer C, Hofstra L, et al. Vitamin K-antagonists accelerate atherosclerotic calcification and induce a vulnerable plaque phenotype. Aikawa E, ed. *PLoS One*. 2012;7:e43229.
  243. Schurgers LJ, Aebert H, Vermeer C, Bültmann B, Janzen J. Oral anti-coagulant treatment: friend or foe in cardiovascular disease? *Blood*. 2004;104:3231–3232. doi: 10.1182/blood-2004-04-1277
  244. Weijs B, Blaauw Y, Rennenberg RJ, Schurgers LJ, Timmermans CC, Pison L, Nieuwlaar R, Hofstra L, Kroon AA, Wildberger J, Crijns HJ. Patients using vitamin K antagonists show increased levels of coronary calcification: an observational study in low-risk atrial fibrillation patients. *Eur Heart J*. 2011;32:2555–2562. doi: 10.1093/eurheartj/ehr226
  245. Eggebrecht L, Prochaska JH, Schulz A, Arnold N, Jünger C, Göbel S, Laubert-Reh D, Binder H, Beutel ME, Pfeiffer N, Blankenberg S, Lackner KJ, Spronk HM, Ten Cate H, Münzel T, et al. Intake of vitamin K antagonists and worsening of cardiac and vascular disease: results from the Population-Based Gutenberg Health Study. *J Am Heart Assoc*. 2018;7:e008650.
  246. Plank F, Beyer C, Friedrich G, Stühlinger M, Hintringer F, Dichtl W, Wildauer M, Feuchtnner G. Influence of vitamin K antagonists and direct oral anticoagulation on coronary artery disease: a CTA analysis. *Int J Cardiol*. 2018;260:11–15. doi: 10.1016/j.ijcard.2018.03.019
  247. Andrews J, Psaltis PJ, Bayturan O, Shao M, Stegman B, Elshazly M, Kapadia SR, Tuzcu EM, Nissen SE, Nicholls SJ, Puri R. Warfarin use is associated with progressive coronary arterial calcification: insights from serial intravascular ultrasound. *JACC Cardiovasc Imaging*. 2018;11:1315–1323. doi: 10.1016/j.jcmg.2017.04.010
  248. Rennenberg RJ, van Varik BJ, Schurgers LJ, Hamulyak K, Ten Cate H, Leiner T, Vermeer C, de Leeuw PW, Kroon AA. Chronic coumarin treatment is associated with increased extracoronary arterial calcification in humans. *Blood*. 2010;115:5121–5123. doi: 10.1182/blood-2010-01-264598
  249. Peeters F, Dudink E, Kimenai D, Weijs B, Altintas S, Heckman L, Muhl C, Schurgers L, Wildberger J, Meex S, Kietselaer B, Crijns H. Vitamin K antagonists, non-vitamin K antagonist oral anticoagulants, and vascular calcification in patients with atrial fibrillation. *TH Open*. 2018;2:e391–e398.
  250. Schurgers LJ, Spronk HM, Soute BA, Schiffers PM, DeMey JG, Vermeer C. Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. *Blood*. 2006;109:2823–31.
  251. Westenfeld R, Krueger T, Schlieper G, Cranenburg EC, Magdeleyns EJ, Heidenreich S, Holzmann S, Vermeer C, Jahnhen-Dechent W, Ketteler M, Floege J, Schurgers LJ. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. *Am J Kidney Dis*. 2012;59:186–195. doi: 10.1053/j.ajkd.2011.10.041
  252. Brandenburg VM, Reinartz S, Kaesler N, Krüger T, Dirrachs T, Kramann R, Peeters F, Floege J, Keszei A, Marx N, Schurgers LJ, Koos R. Slower progress of aortic valve calcification with vitamin K supplementation: results from a prospective interventional proof-of-concept study. *Circulation*. 2017;135:2081–2083. doi: 10.1161/CIRCULATIONAHA.116.027011
  253. Shea MK, Booth SL, Miller ME, Burke GL, Chen H, Cushman M, Tracy RP, Kritchevsky SB. Association between circulating vitamin K1 and coronary calcium progression in community-dwelling adults: the Multi-Ethnic Study of Atherosclerosis. *Am J Clin Nutr*. 2013;98:197–208. doi: 10.3945/ajcn.112.056101
  254. Cranenburg EC, Schurgers LJ, Uiterwijk HH, Beulens JW, Dalmeijer GW, Westerhuis R, Magdeleyns EJ, Herfs M, Vermeer C, Laverman GD. Vitamin K intake and status are low in hemodialysis patients. *Kidney Int*. 2012;82:605–610. doi: 10.1038/ki.2012.191
  255. Jono S, Ikari Y, Vermeer C, Dissel P, Hasegawa K, Shioi A, Taniwaki H, Kizu A, Nishizawa Y, Saito S. Matrix Gla protein is associated with coronary artery calcification as assessed by electron-beam computed tomography. *Thromb Haemost*. 2004;91:790–794. doi: 10.1160/TH03-08-0572
  256. Bobadilla JL, Hoch JR, Levenson GE, Tefera G. The effect of warfarin therapy on endoleak development after endovascular aneurysm repair (EVAR) of the abdominal aorta. *J Vasc Surg*. 2010;52:267–271. doi: 10.1016/j.jvs.2010.02.290
  257. Seike Y, Tanaka H, Fukuda T, Itonaga T, Morita Y, Oda T, Inoue Y, Sasaki H, Minatoya K, Kobayashi J. Influence of warfarin therapy on the occurrence of postoperative endoleaks and aneurysm sac enlargement after endovascular abdominal aortic aneurysm repair. *Interact Cardiovasc Thorac Surg*. 2017;24:615–618. doi: 10.1093/icvts/ivw383
  258. Wang Z, Xu C, Ding X, Chen J, Xin H. Spontaneous rupture of a mediastinal bronchial artery aneurysm induced by anticoagulant agent. *Thorac Cardiovasc Surg Rep*. 2016;5:18–20. doi: 10.1055/s-0036-1578813
  259. Bouvet C, Moreau S, Blanchette J, de Blois D, Moreau P. Sequential activation of matrix metalloproteinase 9 and transforming growth factor beta in arterial elastocalcinosis. *Arterioscler Thromb Vasc Biol*. 2008;28:856–862. doi: 10.1161/ATVBAHA.107.153056
  260. Stenvinkel P, Luttrupp K, McGuinness D, Witasp A, Qureshi AR, Wernerson A, Nordfors L, Schalling M, Ripsveden J, Wennberg L, Söderberg M, Bárány P, Olauson H, Shiels PG. CDKN2A/p16INK4a expression is associated with vascular progeria in chronic kidney disease. *Aging (Albany NY)*. 2017;9:494–507. doi: 10.18632/aging.101173

## Highlights

- Vascular smooth muscle cell phenotypic switching and extracellular vesicle release contribute to microcalcification driven aortic aneurysm formation.
- Calcification is involved in both abdominal aortic aneurysm and thoracic aortic aneurysm formation. Early detection of microcalcification may help to hold aortic aneurysm progression.
- Vitamin K-dependent processes are involved in the inhibition of calcification, and vitamin K might be a potential treatment option for an aortic aneurysm.