Aortic valve and arterial calcification in patients with familial hypercholesterolemia

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DOI: 10.33963/v.phj.98945

Received: June 19, 2023

Accepted: January 15, 2024

Early publication date: February 6, 2024

ABSTRACT

Heterozygous familial hypercholesterolemia (heFH) is an autosomal dominant lipid metabolism disorder. Its prevalence is 1:250–1:300 people in the population. Patients with heFH have an up to 13-fold increased risk of premature coronary artery disease (CAD). If left untreated, men and women with heFH typically develop early CAD before the ages of 55 and 60, respectively.

There is evidence that coronary artery calcification (CAC) and aortic valve calcification (AoVC) are more prevalent in FH patients than in the general population. It is documented that CAC and AoVC are predictors of increased risk of cardiovascular morbidity and mortality in heFH patients, like in the general population. However, the etiology and pathogenesis of vascular calcification in FH patients is not well understood. Risk factors for vascular calcification include age, increased levels of atherogenic lipoproteins, Lp(a), increased blood pressure, and inflammation. There are convincing data from clinical studies and animal atherosclerotic mouse models using low-density lipoprotein receptor (LDL-R) knockout mice that the vascular calcification processes in FH are associated with LDL-R mutations, probably partly due to a higher total cholesterol burden of FH subjects. Data from animal models as well as clinical studies indicate that the Wnt/beta-catenin pathway components and LDL receptor-related proteins 5 and 6 (LRP-5/6) might be involved in calcification processes in FH patients.

The purpose of the review is to describe the prevalence of coronary and aortic calcification and its risk factors in FH patients. The review covers data about the role of the Wnt/beta-catenin pathway and factors modulating calcification processes.

Key words: aortic valve calcification, coronary calcification, familial hypercholesterolemia

GENETIC BACKGROUND AND CLINICAL CONSEQUENCES OF FAMILIAL HYPERCHOLESTEROLEMIA

Familial hypercholesterolemia is caused by mutations in the low-density lipoprotein receptor (*LDLR*) gene, apolipoprotein *B100* gene, or the proprotein convertase subtilisin-kexin type 9 (*PCSK9*) gene. Defects in these genes lead to impaired clearance of LDL cholesterol (LDL-C) from the plasma. Low-density lipoprotein receptors are responsible for uptake of LDL particles and thus removal of cholesterol from plasma. Apolipoprotein B100 is the LDL-bound protein that binds to the LDL receptor. PCSK9 is responsible for the degradation of LDL receptors [1]. A single copy of the defective gene (heterozygous) leads to an increase of plasma LDL-C (5 to 13 mmol/l), whereas 2 copies of the same defective gene (homozygous) or 2 coexisting mutations (compound heterozygous) lead to very high concentrations (above 13 mmol/l) of LDL-C due to minimal or no LDL-C clearance. FH is associated with high circulating LDL-C levels from birth, which leads to the development of atherosclerosis early in life, premature atherosclerotic cardiovascular disease (ASCVD), and tendon xanthomas [1–3].

The goal of FH treatment is to reduce mortality and premature coronary artery disease (CAD) events by reducing plasma LDL

levels. Heterozygous familial hypercholesterolemia (heFH) patients are categorized as being at high risk of atherosclerotic cardiovascular disease, or as very-high-risk patients due to the presence of ASCVD or the presence of one of the major risk factors [3]. Pharmacotherapeutic management of heFH patients consists of high-dose statins usually in combination with ezetimibe as first-line therapy followed by a PCSK9 inhibitor to further lower LDL-C levels to achieve the recommended targets. Despite intensive drug therapy, most heFH patients are still at high cardiovascular risk, and, interestingly, patients with a monogenic form of hypercholesterolemia are at higher risk than those with a polygenic form [4]. This might be due to inadequate treatment, too late onset of treatment, or possibly due to not addressing all risk factors, such as calcification processes [5].

PREVALENCE OF CALCIFICATION IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

Vascular calcification is the pathological deposition of calcium phosphate mineral, usually hydroxyapatite, in vascular structures. Vascular calcification can be located in the intima (atherosclerotic intimal calcification), media — medial artery calcification (Monckeberg's sclerosis), and in cardiac valves. Calcification forms within the intimal and medial layers of the vessel wall and heart valves through active mechanisms similar to bone development. Coronary artery calcification can be characterized by early changes, such as microcalcification to well-developed calcified fibroatheromas [5, 6].

Calcification of aortic valves is a slowly progressive fibro-calcific remodeling of the valve leaflets leading to aortic valve stenosis. This process can be divided into two phases: the initiation phase, which shows similarities with atherosclerosis including endothelial damage due to increased mechanical stress and reduced shear stress, lipid deposition, inflammation, and oxidative stress, and the propagation phase, which is mainly characterized by fibrosis and calcification. Valve calcification is highly dependent on biomechanical and hemodynamic factors and has the characteristics of atherosclerotic and medial calcification, with a strong inflammatory component [7, 8]. Valvular interstitial cells (VICs), the most abundant valvular cells, play a major role in valve calcification and are responsible for differences between the pathobiology of aortic stenosis and atherosclerosis [9]. Mineralization of the heart valve includes nodule formation and stenosis, which causes the narrowing of the valve opening [10]. Increased oxidation of valvular lipids and subsequent chronic valvular inflammation lead to VIC differentiation into osteogenic phenotype. VICs can release osteopontin and osteocalcin, which are responsible for valvular calcification and pro-inflammatory cytokines, clotting factors, and proteins involved in the propagation of calcification [9]. Experimental data, comparing molecular profiles of calcification in aortic vascular smooth muscle cells and aortic VICs suggest that the pathogenesis of cardiovascular calcification is significantly more diverse than previously appreciated [11].

High LDL is associated with increased atherosclerosis and vascular calcification due to oxidative modification of LDL-C, lipid deposition, and inflammation [3–5].

Calcification commonly affects patients with diabetes mellitus, dyslipidemia, heart valve disease, and end-stage renal disease [12].

There is strong evidence that calcification processes are more prevalent in patients with FH than in the general population [13, 14]. Interestingly, some data indicate that vascular calcification processes in FH patients are associated with *LDLR* mutations [13–18].

In the study measuring the degree of aortic calcification in heFH patients compared to both homozygous familial hypercholesterolemia (hoFH) and controls, aortic calcification was observed in patients with heFH at a later time and was less extensive than in hoFH (34 vs. 14 years, respectively). In this study, the age at which the abdominal aorta calcium score reaches 1000 was 22, 28, and 62 years in hoFH, heFH, and controls, respectively. The authors suggested that aortic calcification may be partly independent of serum cholesterol levels in FH patients [16] (Table 1).

AoVC is associated with an elevated cardiovascular risk. The degree of AoVC is correlated with the severity of aortic stenosis, disease progression, heart failure, and the development of cardiovascular events [17–19].

In FH patients prevalence of AoVC is higher than in healthy controls. In the study by Ten Kate et al. [13], the prevalence of aortic valve calcification (%) and the AoVC-score (median, IQR) were markedly higher in 145 heFH patients than in controls: 41%, 51 (9–117) vs. 21%, 21 (3––49), respectively. In this study, patients' age, untreated maximal LDL-C equal to 7.1 ± 2.2 mmol/l, coronary artery calcification (CAC), and diastolic blood pressure were independently associated with AoVC. However, the strongest predictor of the AoVC-score was *LDLR*-negative mutational heFH [13]. Awan et al. examined 25 hoFH patients, at a mean age of 32 years and a mean baseline cholesterol level before treatment of 19.5 mmol/l. An elevated mean calcium score in aortic valves was found in patients under the age of 20 and correlated significantly with age [14]. In the study by Gałązka et al. [15] assessment of computed tomography (CT) calcium scores showed that patients with *LDLR* gene mutations have significantly increased AoVC scores in comparison to those without mutations: 13.8 ± 37.9 vs. 0.94 ± 3.1, respectively. Also, other authors showed that patients with FH due to mutations in the *LDLR* gene exhibited severe, premature aortic calcification in a gene-dosage, age-dependent fashion [20–22]. In the LDLR-deficient mouse model for aortic calcification, elevated cholesterol alone was not sufficient to produce severe aortic calcification. This observation suggests that the absence of the LDLR might be the major contributor to aortic calcification [23].

In heFH patients accelerated aortic calcification increases exponentially with age. According to Kindi M. et al. [20],

Table 1. Studies on coronary artery calcification (CAC) and aortic valve calcification in FH patients

Vascular calcification	Patients	Results	Ref No
Aortic valve calcification (AVC)	145 heFH patients and 131 non-FH controls, mean age 52 ± 8 years	Prevalence (%) of AVC was higher in heFH than in controls: 41% vs. 21% LDLR-negative mutational heFH was a strong predictor of AVCS (OR: 4.81; 95% CI, 2.22–10.4; *P* <0.001)	[8]
Aortic calcification (AoCa)	25 hoFH patients, mean age 32 years, mean baseline cholesterol 19 ± 5 mmol/l	Elevated calcium score was found by age 20 and correlated with age; 24% of patients underwent aortic valve surgery	[9]
Aortic valve calcification score (AVCS)	72 FH patients with LDLR mutation vs. 50 patients without mutation	AVCS was higher in patients with vs. without mutation: 13.8 ± 37.9 vs. 0.94 ± 3.1; *P* = 0.03 The *LDLR* mutation was a strong predictor of AVCS (OR, 7.83; 95% CI, 2.08–29.50; *P* = 0.002)	[10]
AoCa	heFH due to the French Canadian mutation (Delta15 kb del. null allele), Mean age 50 ± 15 years Initial cholesterol 10.45 ± 1.73 mmol/l Comparison to both hoFH and controls	A strong correlation between age and calcium score was found (r = 0.72; *P* =0.0016) Aortic calcification in heFH patients occurred later than in hoFH patients (34 vs. 14 years, respectively), and were less extensive, but much earlier than in controls, suggesting a gene-dosage effect of LDL-R mutations and aortic calcium deposition. Conclusion: aortic calcification may be partly independent of serum cholesterol levels in FH patients.	[11]
AoCa	16 heFH patients with the null LDLR DEL15Kb mutation Rescanned after 8.2 ± 0.8 years 38 controls	An abdominal AoCa score in heFH was 7916 ± 7060 Agatston U; and in controls: 1472 ± 2489; *P* <0.001 The rate of progression was 159 vs. 312 Agatston U/y in controls vs. those with heFH; heFH patients exhibited accelerated AoCa that increased exponentially with age	[15]
AoCa	LDLR-deficient mouse model	Aortic calcification was an age- and diet-dependent process. Data suggest that the absence of the LDLR is a major contributor to aortic calcification LDLR deficiency increases aortic calcification independently of cholesterol level	[18]
CAC	112 heFH patients (50% males), median age 45 years	The prevalence of CAC was 58% Patients without CAC showed lower total cholesterol burden (TCB) than patients with CAC (298 ± 110 vs. 417.9 ± 89 mmol-years/l, *P* <0.001) Multivariate analysis indicated that TCB was independently associated with CAC Conclusion: Asymptomatic heFH subjects exhibit early coronary atherosclerosis directly associated with TCB burden. CAC score may be useful to identify higher-risk heFH patients who can benefit from earlier and more aggressive treatment	[20]
CAC	Patients with genetically defined heFH (68 women and 78 men) 95 patients had prevalent CAC	In heFH patients age, family history of premature cardiovascular disease, sex, statin use, diet quality, smoking status, the LDLR genotype, and lipoprotein(a) levels were independently associated with CAC prevalence and severity	[21]
Coronary calcification Agatston score	50 heFH patients, diagnosed according to DLCN criteria and 70 controls	Significantly greater Agatston calcium score in heFH patients than in the controls (260 vs. 46; *P* = 0.002)	[22]

LDL-C at baseline or during treatment seems to have little effect on the rate of progression of the AoCa score. However, Rajamannan et al. [22] pointed out in their publication that progression of calcification in treated FH patients was slower than in controls, which suggests the inhibiting effect of lipid-lowering therapy on calcification processes.

Interestingly, patients with FH caused by PCSK9 gain-of-function mutation exhibit an age- and gene-dosage-dependent increase in the incidence of AoVC [20]. Also, PCSK9 gain-of-function mutations in experimental animal models induced cardiovascular calcification [22–24].

Patients with heFH are characterized also by increased CAC, which is related to the total cholesterol burden and *LDLR* gene mutational status [25–27]. In a group of 112 heFH patients (50% males, median age 45 years), coronary artery calcification prevalence was 58% and, in multivariate analysis, was independently associated with the total cholesterol burden. Patients with CAC showed a significantly higher total cholesterol burden (TCB) than patients without CAC. Among patients aged <45 years, 39% exhibited CAC and a higher TCB compared with patients without CAC (352 ± 71 vs. 255 ± 88 mmol-years/l) due to higher total cholesterol levels at diagnosis (10.2 ± 2 vs. 8.7 ± 2 mmol/l) [25]. In a group of 146 heFH patients, aged 47.8 ± 14.1 years, included in the study by Drouin-Chartier et al. [26], 95 had prevalent CAC. CAC prevalence and severity in this study correlated independently with age, family history of premature cardiovascular disease, male sex, statin use and diet quality, smoking, receptor-negative phenotype, and lipoprotein(a) (Lp[a]) [26]. In the study by Medel et al. [27], a significantly higher Agatston calcium score, which is a measure of arterial calcification, was observed in comparison with the control group (260 vs. 46) and was associated, apart from age and HDL-C, with null allele *LDLR* gene mutations. Also, in the study by Ten Kate et al. [17], the CAC score was associated with *LDLR* mutational status (Table 1).

To conclude, data from clinical studies and animal atherosclerotic mouse models with the *LDLR* knockout mice suggest that vascular calcification processes in FH are associated, apart from the TCB, also with *LDLR* mutational status; however, the mechanisms of these processes remain to be elucidated.

ROLE OF CALCIFICATION IN PREDICTING CARDIOVASCULAR RISK

The CAC score has been widely used to predict the future risk of acute coronary events. The accuracy of coronary artery calcification score has been demonstrated in cardiovascular risk assessment in the general population,

Table 2. Trials on coronary artery calcification (CAC) and aortic valve calcification confirming their role as predictors of cardiovascular risk in FH patients

Vascular calcification	Patients	Result	Ref No
CAC	206 patients with molecularly proven FH mean age: 45 ± 14 years; 36.4% men; treated LDL-C: (150 ± 34 mg/dl) follow-up 3.7 years (IQR, 2.7–6.8 years)	CAC was the only marker independently associated with future cardiovascular events	[24]
CAC	SAFEHEART and REFERCHOL national registries on heFH 1624 patients (mean age: 48.5 ± 12.8 years; men: 45.7%), median follow-up of 2.7 years (IQR, 0.4–5.0) Atherosclerosis was present in 81 subjects	The presence of a CAC score of >100 was associated with a hazard ratio of 32.05 (95% CI, 10.08–101.94) of developing cardiovascular events as compared to a CAC score of 0. CAC score confirmed its use in improving cardiovascular risk stratification and cardiovascular events prediction in statin-treated heFH	[25]
Aortic root calcification	113 FH patients, age 52.1 ± 15.6 years, mean LDL-C 299 ± 94.6 mg/dl Follow-up period 1635 days	Independent predictor of major adverse cardiovascular events OR, 1.48; 95 CI, 1.11–1.87	[16]

and recent data in heFH patients confirm this observation [27–30]. In the study including 206 FH subjects receiving standard lipid-lowering therapy, CAC was independently associated with ASCVD events [28].

Interestingly, Mszar et al. [31, 32] noticed, using data from coronary CT performed on more than 1000 heFH patients, that coronary atherosclerotic disease burden in middle-aged heFH patients is heterogeneous. The authors found that nearly half of the heFH patients were free of clinical ASCVD and demonstrated no detectable CAC despite significantly elevated LDL-C levels. These data indicate that determining calcification scores in heFH patients would be helpful as prognostic factors to identify those with higher risk who can benefit from earlier and more aggressive treatment [31]. The severity of CAC was recently identified as the most discriminant risk factor associated with the incidence of cardiovascular events in patients with heFH [29–33]. Also, the aortic root calcification score was an independent risk factor for cardiac events in FH [21]. A large study on the heFH population conducted by Gallo et al. [30] included 1624 patients with molecular diagnosis of heFH, at the mean age of 45 years. They were followed for a median of 2.7 years (interquartile range [IQR], 0.4–5.0 years). In this study, CAC scores of 0.1–100, and >100 were observed in 38.7%, 32.2%, and 29% of study participants, respectively. Patients with clinical events exhibited higher CAC scores (387 [IQR, 146–879] vs. 8 [IQR, 0–109]) and a higher frequency of CAC of >100 (82.72% vs. 26.18%). Cardiovascular endpoints in this study were acute coronary syndromes, stroke, aortic valve replacement (which is also an important endpoint in heFH patients), peripheral artery disease, cardiovascular death, and elective myocardial revascularization (Table 2).

SUGGESTED MECHANISMS OF ARTERIAL CALCIFICATION AND THE WNT/ /BETA-CATENIN PATHWAY

The mechanisms of vascular calcification are not fully elucidated. In the available literature, the data on mechanism of CAC and AoVC in FH are sparse and derived mainly from experimental models. Coronary artery calcification and aortic valve calcification was thought to be a passive and degenerative disorder; however, it has been shown to be an active process, very similar to bone mineralization, and many of the key regulators of bone mineralization are active in cardiovascular calcification [5].

Studies have found that vascular calcification is thought to be mediated by osteoblast-like cells. Calcifying vascular cells originate from local smooth muscle cells and circulating hematopoietic stem cells, especially in intimal calcification. There is evidence, that also endothelial cells can function as an additional source of osteogenic progenitors in vascular calcification [34, 35]. The process of transition of endothelial-to-mesenchymal cells possessing osteogenic potential and leading to vascular calcification requires cooperation between distinct stimuli, including inflammatory cytokines and transforming growth factor beta family members.

During calcification vascular smooth muscle cells (VSMC) switch into cells similar to bone-forming osteoblasts with upregulation of osteogenic markers and extracellular matrix remodeling. In the calcifying condition, e. g. with a high level of serum phosphate, VSMCs begin to express osteogenic markers including RUNX2 (Runt-related Transcription Factor 2), SP7 (Osterix), osteopontin (OPN, SPP1), osteocalcin (OCN, BGLAB1), alkaline phosphatase (ALPL), SOX9 (SRY-box transcription factor 9), collagen types I and X. Calcifying VSMCs form small discrete regions of calcification. A similarity was found between bone formation and artery calcification. However, as Patel et al. [36] showed by comparing mouse osteoblast with controls and calcifying VSMCs, early osteoblast markers in calcifying VSMCs (Runx2, Sp7) were 6-fold upregulated but still 3-fold lower in comparison to the osteoblast. Because the *RUNX2* gene is directly targeted by the canonical WNT signaling pathway, it was postulated that mineral deposition, like bone formation, is regulated by the WNT signaling pathway. The WNT pathway activates the genes connected with bone formation during development. The WNT signaling pathway regulates many cellular functions during development and in adults (e.g., cell fate determination, differentiation, proliferation, and morphogenesis). The WNT signaling pathway is stimulated by binding of extracellular WNT ligands to Frizzled receptors. WNT ligands

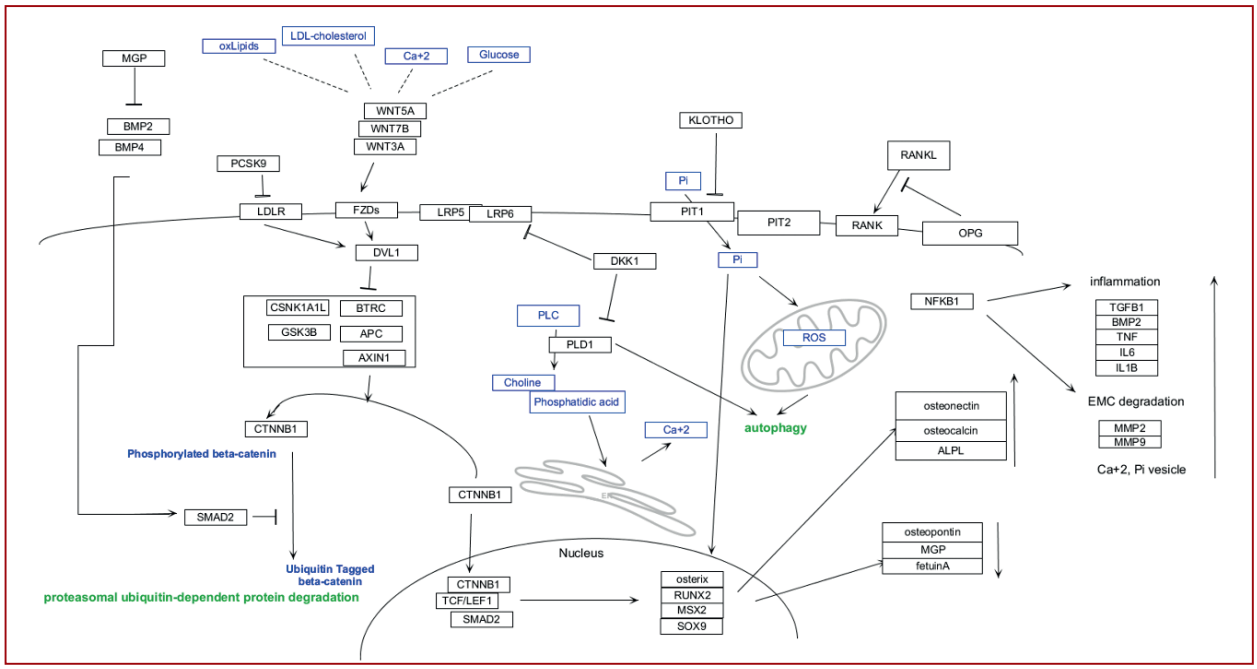

Figure 1. Proteins involved in vascular calcification processes in vascular smooth muscle cells (VSCM). Prepared using Pathvisio 3.2.1

Abbreviations: APC, APC regulator of the WNT signaling pathway; AXIN1, axin 1; BMP2, bone morphogenetic protein 2; BMP4, bone morphogenetic protein 4; BTRC, beta-transducin repeat containing E3 ubiquitin protein ligase; CSNK1A1L, casein kinase 1 alpha 1 like; CTNNB1, catenin beta 1; DKK1, dickkopf WNT signaling pathway inhibitor 1; DVL1, dishevelled segment polarity protein 1; FZDs, frizzled class receptors; GSK3B, glycogen synthase kinase 3 beta; IL1B, interleukin 1b; IL6, interleukin 6; LEF1, lymphoid enhancer binding factor 1; LRP5, LDL receptor related protein 5; LRP6, LDL receptor related protein 6; LDLR, low density lipoprotein receptor; MMP2, matrix metallopeptidase 2; MMP9, matrix metallopeptidase 9; MSX2, msh homeobox 2; NFKB1, nuclear factor kappa B subunit 1; OPG, (TNFRSF11B) TNF receptor superfamily member 11b; OSTERIX (Sp7), Sp7 transcription factor; PCSK9, proprotein convertase subtilisin/kexin type 9; PLD1, phospholipase D1; PIT1, (SLC20A1) solute carrier family 20 member 1; PIT2, (SLC20A2) solute carrier family 20 member 2; RANK, (TNFRSF11A) TNF receptor superfamily member 11a; RANKL, (TNFSF11) TNF superfamily member 11; RUNX2, RUNX family transcription factor 2; SMAD2, SMAD family member 2; SOX9, SRY-box transcription factor 9; TCF, (HNF4A) hepatocyte nuclear factor 4 alpha; TGFB1, transforming growth factor beta 1; TNF, tumor necrosis factor; WNT3A, Wnt family member 3A; WNT5A, Wnt family member 5A; WNT7B, Wnt family member 7B

(family of 19 proteins) activate intracellular signaling by binding to Frizzled receptors associated with co-receptors LRP5/LRP6 (low-density lipoprotein receptor family) and recruit cytoplasmic phosphoprotein Disheveled (DVL). DVL serves as a branch point for the canonical and non-canonical WNT signaling pathways. Propagation of canonical WNT signaling through receptor activation inhibits proteasomal beta-catenin utilization. Release of beta-catenin from the inhibitory complex (Axin, Glycogen Synthase Kinase 3 (GSK-3), Casein Kinase 1 (CK1), adenomatous polyposis coli protein (APC), and the E3-ubiquitin ligase β-TrCP (BTRC) leads to beta-catenin translocation to nucleus and induces expression of WNT target genes. Some studies have begun testing specific WNT ligands to determine if any of them contributes most significantly toward calcification. Multiple WNT ligands conveying canonical and noncanonical actions, including WNT7b, WNT5a, and WNT2 are related to vascular smooth muscle calcification [37, 38].

The most studied WNT ligand in vascular calcification is WNT3a. WNT3a, through activation of its downstream mediator beta-catenin, can induce RUNX2 and osteocalcin expression and promote calcification in VSMCs [39].

Sheng et al. [40] showed that cholesterol is enriched around the WNT-activated Frizzled receptors and LRP 5/6 co-receptors and plays an essential role in DVL-mediated formation and maintenance of the canonical WNT signaling complex. Cholesterol specifically facilitates the membrane recruitment of the PDZ (Postsynaptic density protein [PSD95]/Drosophila disc large tumor suppressor [Dlg1]/Zonula occludens-1 protein) domain of DVL and its interaction with other proteins [40]. VSMCs from high-fat-fed rats showed higher mRNA expressions of Wnt3a, beta-catenin, and Tcf4 as well as in VSMCs cultured with hyperlipidemic serum [38]. Cholesterol depletion from myoblast membranes induces activation of the WNT signaling pathway and myogenic differentiation [42].

DKK1 slowed vascular calcification by promoting PLD1 degradation via regulating autophagosome formation and maturation. Phospholipase D (PLD) exists widely in biological tissues and can hydrolyze PLC (phosphatidylcholine) to phosphatidic acid (PA) and free choline. PA acts as the second messenger in cells to regulate intracellular calcium [43] (Figure 1).

There is evidence that lack of LDLR may modify osteoblast function, resulting in increased deposition in calcifying vascular tissue [18, 22, 23]. Data increasingly indicate that the WNT/beta-catenin pathway and LDL receptor-related protein 5/6 (LRP5/6) co-receptors are

also involved in osteoblasts differentiation, initiating and promoting calcification processes [18, 44, 45]. Beta-catenin plays a key role in the commitment of early progenitors to osteoblast precursors by mediating WNT signal transduction. Low-density lipoprotein cholesterol, a strong stimulator of atherosclerosis and inflammation, signals LRP5 to initiate WNT signaling in the vasculature and valve interstitial cells. These cells then develop bone formation signaling and cause ectopic bone matrix synthesis and calcification over time [45]. The role of involvement of WNT /beta-catenin pathway components and LRP5/6 in calcification processes in FH patients was confirmed by data on knockout and transgenic animal models [18, 22, 35, 44–48].

LRP5/6 are members of the LDLR family and are composed of structurally related cell surface receptors. They act, in consort with Frizzled receptors, as coreceptors to mediate the Wnt/beta-catenin signaling pathway [49–51]. LRP5 and LRP6 are single-pass transmembrane proteins and are indispensable for Wnt signal transduction [49]. LRP5 is an LDLR co-receptor involved in activation of skeletal bone formation, but it is also implicated in cholesterol metabolism [18, 45, 50, 51]. It is expressed in low concentration in many tissues including the aortic valve. There is increasing data that LRP5 apart from the canonical WNT signaling pathway is active in cardiovascular calcification. Both LRP5 and LRP6 are involved in cardiac disease [52]. LRP5, apart from inducing osteogenic differentiation in heart valves was demonstrated to play a protective role in the injured heart following MI in mice. On the other hand, LRP6 inhibition limited myocardial fibrosis, improved cardiac repair in myocardial infarction, decreased blood pressure in hypertensive animals, and reduced adipogenesis and lipogenesis to prevent elevated serum LDL, triglycerides, and glucose levels [53, 54].

LRP5 is expressed in low levels also in the aortic valve, and upregulation of LRP5 in hypercholesterolemic rabbit aortic valve confirms that the LRP5/Wnt pathway is implicated in aortic stenosis progression. Rajamannan et al. [55] found that hypercholesterolemic AoV calcification is mediated in part by the LPR5/beta-catenin pathway and is attenuated by atorvastatin. In their study, the cholesterol diet induced complex bone formations in the calcified AoVs, with an increase in the number of LRP5 receptors, osteopontin, and p42/44 expression.

A recent review by Bundy et al. [44] discussed the potential role of the canonical WNT signaling pathway in vascular calcification and the WNT ligands that specifically aid in VSMC transdifferentiation.

FACTORS INFLUENCING CALCIFICATION PROCESSES

Many local and systemic factors influence the calcification process, including hyperlipidemia, hypertension, systemic inflammatory diseases, kidney disease, and diabetes [53, 54]. Also, medical therapies, such as statins and warfarin, exhibit pro-calcific effects on the vessel wall [5, 57].

Serum lipids play an important role in vascular calcification. Several clinical studies documented associations between LDL and coronary artery and aortic valve calcification [57–62]. High LDL-C levels were also associated with increased calcification progression in patients with known aortic calcification. In the CARDIA study, LDL-C, male sex, and the body mass index were significant risk factors for CAC and calcification progression [60]. The role of LDL-C was confirmed by the increased calcification of valves and arteries in patients with heterozygous LDLR null familial hypercholesterolemia [16, 17, 20, 58]. On the other hand, HDL (high-density lipoprotein) appears to have beneficial effects on vascular calcification through effects on bone preosteoclasts [57].

Higher apo B levels, apolipoprotein present in LDL and other liver-delivered lipoproteins, were associated with CAC prevalence, incidence, and progression. Apo B discordance, relative to LDL-C or non-HDL-C, was inconsistently associated with CAC prevalence and progression [62].

Other factors that might be responsible for the development of coronary and aortic valve calcification in patients FH include Lp(a) and circulating PCSK9 level [63–67]. Lp(a) is an independent risk factor for aortic valve stenosis and AoVC in the general population as well as in FH patients [63–67]. In the study including 129 heFH patients, AoVC was present in 38.2% of patients. In this study Lp(a) level was significantly correlated with the presence and severity of AoVC, but not with CAC [66]. Lp(a) is a major carrier of phospholipids and their oxidized forms, which are co-expressed with Lp(a) within the stenotic leaflets and promote valvular calcification. Interestingly, Kopytek et al. [68] recently showed that in severe aortic stenosis, oxidized phospholipids are associated with Lp(a) in relation to hypofibrinolysis, which is also linked to the severity of aortic stenosis.

Also, PCSK9, a protein regulating LDLR activity, is involved in premature artery calcification. Alonso et al. [69] selected 161 molecularly defined FH patients, measured CAC with cardiac CT using the Agatson score and correlated these measurements to PCSK9 levels, plasma Lp(a) levels, and apo(a) levels. They found that both plasma PCSK9 levels and Lp(a) were independently predictive of elevated coronary artery calcium scores. According to their findings, circulating PCSK9 levels were significantly lower in patients without coronary artery calcification while patients with the highest CAC scores had the highest levels of PSCK9 and Lp(a) [66]. The study by Poggio P et al.[70], indicates that PCSK9 is also involved in aortic valve calcification. On the contrary, Acena A. et al [71] described the independent role of low PCSK9 levels in progression of aortic stenosis in patients with ischemic heart disease. Interestingly, recent data suggest an association between PCSK9 serum levels and recurrent cardiovascular events in FH patients [72] (Table 3).

Inflammation is a key feature of arterial and aortic valve calcification. Clinical, animal, and *in vitro* studies implicate

Table 3. Factors influencing vascular calcification processes

Factor	Effect on calcification	Mechanism of action
LDL-C	Stimulatory	Oxidized low-density lipoprotein stimulates vascular calcification by driving osteoblastic differentiation of vascular smooth muscle cells and inhibiting osteoclast differentiation
VLDL-C	Stimulatory	
HDL-C	Inhibitory	High-density lipoprotein exerts beneficial effects on vascular calcification through effects on bone preosteoclasts
Lp(a)	Potentially casual	Lp(a) is a vehicle for oxidized phospholipids and oxysterols, which potentiate inflammation and atherosclerosis
Treatment with statin	Increase plaque calcification	Statins and high-intensity exercise promote calcification without increasing risk
Treatment with PCSK9-I	Increase plaque calcification	

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein a; PCSK9-I, proprotein convertase subtilisin-kexin type 9 inhibitor; VLDL-C, very low-density lipoprotein cholesterol

hyperlipidemia-induced inflammation in the genesis and progression of vascular calcification.

Diabetes mellitus is also one of the important factors involved in vascular calcification [12]. FH and DM significantly increase the risk and progression of arterial and aortic valve calcification, by inducing oxidative stress and inflammatory state. In FH patients, deficiency or dysfunction of LDL receptors results in increased plasma residence time of LDL in the circulation and, in consequence, increased exposure of vascular tissue to LDL particles and increased oxidative modifications of LDL, inflammatory state, and pathological conditions leading to calcification. In DM increased accumulation of advanced glycoxidation end products (AGEs) in aortic valves leads to enhanced valvular oxidative stress, inflammation, and expression of coagulation factors and, in consequence, calcification markers. Interestingly, AGE levels are better predictors for vascular calcification than HbA1c [57, 73].

Several studies confirmed the involvement of inflammatory cytokines, such as TNF alfa, Il-6, Il-1beta, CRP, and PTX3 in calcification processes [74–77]. Moreover, observations made in human aortic valves of diabetic patients with aortic stenosis demonstrated that the number of pro-inflammatory proteins impacts stenosis progression [78]. These data indicate the importance of inflammation status for the severity of aortic calcification. Data from genetic studies further confirm the role of inflammation in calcification: polymorphisms of inflammation factor genes (Interleukin-6 receptor gene, C-reactive protein gene) were associated with an attenuated systemic inflammatory state and less severe aortic stenosis or, conversely, might predispose to larger aortic valve calcification. Therefore, they potentially can be novel genetic risk markers of disease progression [79, 80]. Interestingly, Plunde and Back [81] described the important role of omega-6 fatty acid arachidonic acid that gives rise to the mediators prone to elicit a pro-inflammatory response and enhance calcification-related mechanisms in aortic valves.

Recent data, published by Sánchez-Duffhues et al. [77] indicate, that the pro-inflammatory cytokines, TNF alfa, and Il-1 beta, induce endothelial cell transformation to osteogenic differentiation. The authors suggest that the bone morphogenetic protein type II receptor (BMPR2) BMPR2-JNK signaling axis is a key pathway regulating inflammation-induced endothelial cell transformation and contributing to calcification.

GlycA, a novel composite biomarker of systemic inflammation, reflects posttranslational glycosylation of acute phase reactants and is measured by nuclear magnetic resonance spectroscopy. GlycA has been associated with a greater prevalence of coronary artery calcium, cardiovascular disease (CVD) events, and mortality. In cross-sectional analysis, in the Multi-Ethnic Study of Atherosclerosis, GlycA was positively associated with prevalent aortic valve calcification as well as descending thoracic aorta and other extra-coronary calcification [82].

Biomarkers that play a role in the pathophysiology of cardiac calcification can be measured in plasma samples for early detection and disease prevention. These include fetuin-A (AHSG), matrix Gla protein (MGP), osteoprotegerin (OPG), Klotho, fibroblast growth factor 23 (FGF23), nucleotide pyrophosphatase/phosphodiesterase-1 (NPP1) [25]. A loss of local and circulating calcification inhibitory proteins, such as MGP, fetuin-A, and OPG, also contribute significantly to the formation of vascular calcification [83, 84].

DETECTION OF CORONARY AND AORTIC VALVE CALCIFICATION

Coronary artery calcium imaging, according to the American Society for Preventive Cardiology, is included on the list of imaging tests helpful with the diagnosis and prognosis of CVD [85]. CT angiography without contrast infusion is used for assessment of CAC and atherosclerotic plaque characterization. It is a noninvasive procedure and patient radiation exposure is 1mSv [83]. Improvements in technology, especially high-speed multislice CT scans, allow objective measurement of both the density and extent of coronary calcification, usually calculated by using the Agatston method et al. [86]. For calcification assessment, the Agatston coronary calcium score, as well as the aortic valve calcification score, is calculated. Another noninvasive imaging biomarker of active coronary atherosclerotic mineralization recently suggested by Moss et al. [87] is positron emission tomography (PET) computed tomography using 18-F fluorodeoxyglucose (FDG-PET/CT) and 18F-sodium fluoride-tracers [86]. This analysis is useful for early detection of early calcification [5, 6, 87]. ^{18}NaF- PET/CT is also useful in aortic valve sclerosis detection. This imaging

system using PET detects smaller calcium deposits that are below CT resolution (200–500 µm) and intravascular ultrasound (200 µm lateral resolution). ^{18}F-NaF PET/CT imaging, which has higher sensitivity for calcium minerals, is used in human and animal studies to identify high-risk vulnerable lesions. Abdelbaky et al. [88] have found unequivocal evidence that early aortic valve inflammation may predispose to valve sclerosis using this technique.

Interestingly, another method, recently described — circulating microvesicles (cMV) — appeared to indicate vascular atherosclerotic plaques and calcification. Circulating microvesicles are released when cells are activated. Chiva-Blanch et al. [89] found that endothelial-, granulocyte-, neutrophil- and platelet-derived cMV discriminate and map coronary atherosclerotic plaque and calcification in asymptomatic patients with FH. In their study, the Agatston coronary calcium score correlated with granulocyte-, platelet-, and endothelial-derived cMV. Circulating microvesicles could be useful biomarkers to better characterize and individualize cardiovascular risk prediction in FH patients.

TREATMENT OF VASCULAR CALCIFICATION

There is no treatment to decrease vascular calcification. Statin therapy might increase, decrease, or cause no changes in coronary calcification [22, 61, 90, 91]. Statins and high-intensity exercise promote calcification without increasing risk [61]. Statins and other LDL-lowering therapies markedly diminish the progression of atherosclerosis in FH patients; however, statins do not seem to reduce the rate of progression of coronary calcification despite a beneficial effect on the progression of atherosclerosis [92, 93]. Statins may also increase calcification associated with plaque quiescence/healing. There is a possibility that statins increase calcification and CAC scores by reducing plaque volume and increasing the density of plaque calcium leading to plaque stabilization [94]. Statins may also limit the progression of vascular calcification by reducing inflammation in atheroma associated with the decrease in LDL-C [95]. Summing up, strategies to prevent aortic valve and aorta calcification with statins have not been met with clinical success, and novel approaches are required.

The SEAS study examining the role of combined simvastatin and ezetimibe therapy in aortic stenosis involved 1873 patients with mild-to-moderate asymptomatic aortic stenosis. The authors [96] found that during a median follow-up of 52.2 months, simvastatin and ezetimibe reduced the incidence of ischemic cardiovascular events but not events related to aortic valve stenosis. However, secondary analysis from the SEAS trial showed that these drug combinations reduced the need for aortic valve replacement in patients with mild aortic stenosis and high LDL-C levels (>4.0 mmol/l), but not in patients with moderate aortic stenosis [97].

Plunde and Back suggested the need for clinical trials of high-dose EPA supplements as a treatment for aortic valve calcification, considering that fatty acids serve as substrates for many lipid mediators involved in the resolution of inflammation. Aortic valves incorporate omega-3 polyunsaturated fatty acid, and high omega-3 fatty acids are associated with slower progression of aortic valve stenosis [81].

Therapy with inhibitors of PCSK9: monoclonal antibodies alirocumab and evolocumab in statin-treated patients resulted in substantial reductions in atherogenic lipoprotein cholesterol-carrying particles and a decrease of CAC rate progression in comparison to statin monotherapy [89–91].

As previously mentioned, Lp(a) causal in ischemic heart disease is also associated with increased risk of calcification. Current treatment options for high Lp(a) include PCSK9 inhibitors that reduce its levels by 25%–30% on average [98, 99]. The new drugs lowering Lp(a) include antibodies against Lp(a), and therapies with ASO (antisense oligonucleotides) and siRNA (small interfering RNA), which currently are under clinical trials [100]. Therapies with ASO and siRNA olpasiran and pelacarsen reduce circulating Lp(a) levels by 85%–90%. Cholesteryl ester transfer protein (CETP) inhibitors also significantly reduce atherogenic lipoproteins, apolipoprotein B, small LDL particles, and Lp (a), and increase HDL-C. Obicetrapib, a next-generation, oral, once-daily, low-dose CETP inhibitor was characterized in clinical trials by an excellent safety and tolerability profile and markedly lowered atherogenic lipoproteins. Moreover, the REVEAL trial demonstrated that adding CETP inhibitor anacetrapib to intensive statin therapy reduced the risk of major coronary events, and this effect increased with longer follow-up duration. A recently published article by Bortnick AE showed in a multiethnic population that HDL-C, HDL-P, large HDL-P, and apoC3-lacking HDL-C were inversely associated with long-term incidence and progression of AVC. These data raise the possibility, that drugs increasing HDL-C, such as CETP inhibitors, could be a potential therapy for AoC [101].

Therapies using PCSK9 inhibitors, antisense oligonucleotides targeting apo(a) and thus lowering Lp(a) or cholesteryl transfer protein inhibitors and their role in reducing the risk of aortic stenosis progression have been described in detail in a recent review [102–105].

The findings on the role of inflammatory factors in arterial and AoVC raise the question about inhibitors of the IL-6 pathway, such as the IL-6 receptor antagonists: tocilizumab and sarilumab (106). Experimental data indicate that aortic calcification can be inhibited by an IL-1β mAb in LDLR-deficient mice [107, 108].

Apart from inflammation markers also the beta-catenin-dependent pathway is a potential target in the prophylaxis and treatment of vascular complications.

CONCLUSION AND FUTURE RESEARCH

There are still many controversies and unresolved questions concerning arterial calcification – although CAC severity is a strong predictor of cardiovascular morbidity and mortality, is it a healing process of vulnerable plaque? Or does it

increase the risk of plaque rupture? What is the role of statins and lipid-lowering drugs in calcification processes and in preventing the development of calcification? What is the role of LDLR defect and the WNT/beta-catenin pathway? So far there have been no convincing data on whether and how to treat vascular calcification and how the treatment will affect cardiovascular risk.

There is a need to estimate the role of CAC determination value in clinical practice in FH patients also in Poland, as there are specialized centers able to diagnose genetic background and treat FH patients with PCSK9 inhibitors [109, 110].

Article information

Conflict of interest: None declared.

Funding: UJ CM N41/DBS/000982

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