JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 7, 2020

EDITORIAL COMMENT

PCSK9 and Calcific Aortic Valve Stenosis

Moving Beyond Lipids*

Jane A. Leopold, MD

Ectopic calcification in the cardiovascular system is a highly prevalent comorbidity that heralds the presence of occult coronary or valvular heart disease. Calcific aortic valve stenosis (CAVS), in particular, is common in the general population and is associated with increasing age and risk factors for atherosclerosis. The incidence of CAVS has been reported to be approximately 4.9 per 1,000 people annually, with a heterogeneous geographic distribution, which suggests that genetic and environmental factors may play a role in the pathobiology and progression of CAVS (1). CAVS, like all cardiovascular calcification, remains a vexing clinical problem because it confers an increased risk of major adverse cardiac events in patients; currently, there are no pharmacotherapeutics that can prevent or inhibit its progression. This has led to a resurgence of interest in elucidating and understanding the determinants of CAVS to offer therapies beyond percutaneous or surgical valve replacement.

Although plasma lipoproteins and other lipids are involved in the pathogenesis of CAVS, accumulating evidence indicates that PCSK9 may also contribute to calcification of the aortic valve. Studies have reported that increased plasma levels of PCSK9 correlate with the presence of CAVS, and conversely, that the PCSK9 R46L loss-of-function variant may lower the risk of CAVS (2). Similarly, patients treated with PCSK9 inhibitors in the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) study had a lower hazard of new or worsening aortic stenosis or aortic valve replacement (3). Although the aforementioned studies are suggestive of a PCSK9–CAVS association, preclinical studies performed with PCSK9 knockout mice provide more definitive support for a causal relationship. Analysis of aortic valves harvested from mice and exposure of PCSK9 knockout valve interstitial cells (VICs) to calcification medium found that indexes of calcification were lower in tissues and cells isolated from knockout mice compared to wild-type mice with functional PCSK9 (2). Although limited, these converging lines of data indicate that PCSK9 is relevant in CAVS.

In this issue of *JACC: Basic to Translational Science*, Perrot et al. (4) provided compelling data to advance our understanding of the role of PCSK9 in the pathogenesis of CAVS. They provided a meta-analysis of 10 genetic association studies that included 12,059 patients with CAVS and 541,081 control subjects to reveal that the odds of CAVS were lower in carriers of the PCSK9 R46L loss-of-function variant. Within 2 of the larger population-based studies, the weighted genetic risk score for CAVS was associated with lower low-density lipoprotein cholesterol (LDL-C), but not lipoprotein(a) [Lp(a)] levels. Next, to demonstrate that PCSK9 is important to the pathobiology of CAVS, they showed that PCSK9 expression was increased in calcified human aortic valves compared with normal valves; that a pro-calcifying stimulus increased PCSK9 expression and secretion from isolated human

*Editorials published in the *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

From the Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts. This work was supported by the National Institutes of Health (grant HL125215) and by the American Heart Association (grant 19AIML34980000). Dr. Leopold has reported that she has no relationships relevant to the contents of this paper to disclose.

The author attests they are in compliance with human studies committees and animal welfare regulations of the author's institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC:* Basic to Translational Science author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2020.06.004

VICs; and that increased PCSK9 expression occurred concomitant with an increase in cellular calcium content, a marker for calcification. When VICs were treated with a neutralizing antibody to PCSK9, calcification was abrogated. Taken together, these findings further implicated PCSK9 in the pathobiology of CAVS.

The findings from the meta-analysis achieved importance because it provided confirmation of earlier studies that reported that harboring a PCSK9 loss-of-function variant was protective for CAVS. Data from the loss-of-function variant meta-analysis was also intriguing because it served as a surrogate for the potential benefits of pharmacologic PCSK9 inhibition in limiting aortic valve calcification. When considered in concert with the preliminary analysis from the FOURIER study, it becomes evident that a clinical trial of PCSK9 inhibition in CAVS is warranted. What remains less certain, however, is the mechanism of action by which inhibition of PCSK9 abrogates CAVS. This is necessary to understand because it has implications for the use of PCSK9 inhibitors as therapeutics for cardiovascular calcification. To date, the explanation for the beneficial effects of decreased PCSK9 functional activity in CAVS has centered on the corresponding decrease in lipid levels, specifically LDL-C and Lp(a) levels. In the current study, only LDL-C, and not Lp(a), correlated with the PCSK9 loss-of-function variant, which suggested that the protective effects of the PCSK9 loss-of-function variant was likely conferred by lower LDL-C levels in the cohort studied. Although others have implicated Lp(a) as a risk determinant for CAVS, it is plausible that characteristics unique to the population studied or geographic heterogeneity may account for the absence of correlation between PCSK9 variants, Lp(a), and CAVS risk in the current study.

Although not posited by the study directly, it is difficult to believe that lower LDL-C levels correlated with the PCSK9 loss-of-function variant were the sole explanation for protection from CAVS for several reasons. First, interventional studies of the effect statins on CAVS, such as the double-blind, placebo-controlled SALTIRE (Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression) study, demonstrated a >50% reduction in LDL-C yet found no difference in the progression of valvular calcification in the statin-treated arm (5). Second, although it is critically important to lower and maintain guideline-directed LDL-C levels, at the cellular and molecular level, it is the oxidized form of LDL (ox-LDL) that increases the calcification potential of human VICs. Cells exposed to ox-LDL increase expression of the phosphate transporter Pit1 and the pro-calcific mediator bone morphogenetic protein-2, both of which were shown to promote transition of VICs to osteoblast-like calcifying cells (6). The idea that an oxidant-rich milieu promotes calcification may also explain, in part, the link between Lp(a) and CAVS because Lp(a) is the major lipoprotein carrier of oxidized phospholipids. Clinical studies showed that the top tertile of Lp(a) was associated with the highest aortic valve calcification activity by ^{15}F-NaF positron emission tomography imaging (7). Thus, to understand the role of PCSK9 in mediating CAVS, it is necessary to look at the biological activity of PCSK9 beyond its effects on regulating lipid levels.

It is now becoming apparent that PCSK9 has several pleiotropic effects that are likely to play an important role in mediating CAVS. Microarray profiling revealed that PCSK9 is involved in the expression of genes related to inflammation, stress responses, cell cycle functions, and xenobiotic metabolism. PCSK9 modulates inflammation, a critical contributor to calcification, by activating macrophages directly to release proinflammatory cytokines, including tumor necrosis factor-α, interleukin-6, interleukin-1β, and C-reactive protein. PCSK9 also activates T cells, which infiltrate calcifying aortic valves, undergo clonal expansion, and facilitate a local adaptive immune response. PCSK9 has been implicated in other pathways germane to calcification, such as autophagy and apoptosis, the latter through a mechanism that involves regulation of mitochondrial DNA damage and mitochondrial oxidant stress. PCSK9 may affect the local redox environment via activation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, reactive oxygen species generation, and upregulation of the lectin-type oxidized LDL receptor 1 (LOX-1), which is the primary scavenger receptor for ox-LDL, a known contributor to cardiovascular calcification. In other cell types, such as vascular smooth muscle cells, a decrease in PCSK9 expression and activity maintains the differentiated contractile phenotype, which is lost when these cells are exposed to procalcific mediators (8).

Thus, although the relationship among PCSK9, lipid levels, and atherosclerosis has been studied extensively, far less is known about PCSK9 and CAVS. The study by Perrot et al (4) moves the field forward by directly implicating PCSK9 in VIC

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 5, NO. 7, 2020
JULY 2020:662–4

calcification and by providing convincing evidence that decreased PCSK9 activity is protective from CAVS. Collectively, these findings open the door to future studies that will define the mechanism(s) by which PCSK9, beyond its effects on lipid levels, promotes CAVS.

ADDRESS FOR CORRESPONDENCE: Dr. Jane A. Leopold, Division of Cardiovascular Medicine, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, NRB 0630K, Boston, Massachusetts 02115. E-mail: jleopold@bwh.harvard.edu.

REFERENCES

1. Lindman BR, Clavel MA, Mathieu P, et al. Calcific aortic stenosis. Nat Rev Dis Primers 2016;2:16006.

2. Poggio P, Songia P, Cavallotti L, et al. PCSK9 involvement in aortic valve calcification. J Am Coll Cardiol 2018;72:3225–7.

3. Bergmark BA, O'Donoghue ML, Murphy SA, et al. An exploratory analysis of proprotein convertase subtilisin/kexin type 9 inhibition and aortic stenosis in the FOURIER trial. JAMA Cardiol 2020;5:709–13.

4. Perrot N, Vincenza V, Moschetta D, et al. Genetic and in vitro inhibition of *PCSK9* and calcific aortic valve stenosis. J Am Coll Cardiol Basic Trans Science 2020;5:649–61.

5. Cowell SJ, Newby DE, Prescott RJ, et al. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. N Engl J Med 2005;352:2389–97.

6. Nadlonek NA, Lee JH, Weyant MJ, Meng X, Fullerton DA. Ox-LDL induces PiT-1 expression in human aortic valve interstitial cells. J Surg Res 2013;184:6–9.

7. Zheng KH, Tsimikas S, Pawade T, et al. Lipoprotein(a) and oxidized phospholipids promote valve calcification in patients with aortic stenosis. J Am Coll Cardiol 2019;73:2150–62.

8. Ding Z, Pothineni NVK, Goel A, Luscher TF, Mehta JL. PCSK9 and inflammation: role of shear stress, pro-inflammatory cytokines, and LOX-1. Cardiovasc Res 2020;116:908–15.

KEYWORDS calcific aortic valve stenosis, lipids, PCSK9