PCSK9 involvement in aortic valve calcification

Paolo Poggio[*] PhD, Paola Songia[*] PhD, Laura Cavallotti[*] MD, PhD, Silvia S. Barbieri[*] PhD, Ilaria Zanotti[†] PhD, Benoît J. Arsenault[‡,§] PhD, Vincenza Valerio[*] BS, Nicola Ferri[?] PhD, Romain Capoulade[#] PhD, and Marina Camera[*,**] PhD

* Centro Cardiologico Monzino IRCCS, Milan, Italy.

[†] University of Parma, Department of Food and Drug, Parma, Italy.

[‡] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Québec Canada.

[§] Département de médecine, Faculté de médecine, Université Laval, Québec, Canada.

[?] University of Padua, Department of Pharmacological Sciences, Padua, Italy.

[#] L'institut du thorax, INSERM, CNRS, CHU Nantes, UNIV Nantes, Nantes, France.

[**] Università degli Studi di Milano, Department of Pharmacological and Biomolecular Sciences, Milan, Italy.

Address for correspondence

Marina Camera, PhD

Associate Professor of Pharmacology

Dept. of Pharmacological and Biomolecular Sciences

Università degli Studi di Milano

Via Balzaretti 9, 20133 Milan, Italy

and

Head of Cell and Molecular Biology in Cardiovascular Diseases Unit

Centro Cardiologico Monzino IRCCS

Via Carlo Parea 4, 20138 Milan, Italy

Telephone: +39 02.5800.2255/6

Fax: +39 02.5800.2342

Email: Marina.Camera@unimi.it

Twitter handle: @marinacamera_

OR

Paolo Poggio, PhD

Head of the Unit for the Study of Aortic, Valvular, and Coronary Pathologies

Centro Cardiologico Monzino IRCCS

Via Carlo Parea 4, 20138 Milan, Italy

Telephone: +39 02.5800.2853

Fax: +39 02.5800.2750

Email: Paolo.Poggio@ccfm.it

Twitter handle: @pao_poggio

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Conflicts of interest

None of the authors have any conflict of interest.

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PCSK9 inhibition therapy could be efficient in patients with CAVS and then it represents a novel pharmacological treatment for these patients.

MAIN TEXT

High levels of proprotein convertase subtilisin/kexin 9 (PCSK9) (>305 ng/mL) have been recently associated, along with insulin resistance and lipoprotein-associated phospholipase A2 activity, with aortic bioprosthesis calcification and able to predict hemodynamic valve deterioration (1). Interestingly, high levels of PCSK9 have been previously shown to correlate also with the presence of calcific aortic valve stenosis (CAVS) (2) and an early study showed that carriers of the PCSK9 R46L loss-of-function genetic variant might have a low CAVS risk (3).

In the present study, we took advantage from the PCSK9 knockout mouse model in order to explore the link between PCSK9 and aortic valve calcification (AVC). In addition, in human specimens, we evaluated if there was an association between calcified aortic valves and PCSK9 valve content.

First, whole aortic valve tissue extracts from 12-month-old *Pcsk9*$^{-/-}$ mice and wild-type (WT) fed with normal diet (n=5/group) were used to assess AVC by calcium colorimetric assay kit (Biovision, Mountain View, CA). AVC was five times lower in *Pcsk9*$^{-/-}$ than in WT mice (2.72±2.9 *vs.* 14.99±7.9 ng Ca^{2+}/μg proteins, respectively; p=0.008) (**Figure – Aortic Valve**). Second, valve interstitial cells (VIC) isolated from both mouse groups were used to evaluate the *in vitro* calcification potential related to PCSK9-deficiency in basal condition and upon a calcification-promoting medium (*i.e.* 10mM β-glycerophosphate and 50μg/mL ascorbic acid [βGAA]). Results showed that, after seven days of culture, *Pcsk9*$^{-/-}$ VICs (untreated) calcified to a lesser extent than WT VICs (4.37±1.6 *vs.* 8.04±1.7 ng Ca^{2+}/μg proteins, respectively; p=0.0003) (**Figure - VICs**). Interestingly, βGAA treatment, although inducing calcification in both groups, exerted a significantly lower calcification rate in *Pcsk9*$^{-/-}$ VICs compared to WT VICs (41.85±12.4 *vs.* 55.89±12.1 ng Ca^{2+}/μg proteins, respectively; p=0.01).

Since these *in vitro* data suggested a VIC-related PCSK9 effect in AVC, we then assessed, as a proof of concept, the presence of PCSK9 in human aortic valves with and without calcification (n=5/group). Automated capillary electrophoresis Western blotting (ProteinSimple, San Jose, CA)

and immunohistochemistry (Abcam, Cambridge, UK) analyses showed that PCSK9 was indeed highly expressed in calcified aortic valves compared to non-calcified ones (+4.9±0.5 log2 fold change; p=0.004).

Interestingly, quantitative PCR (data not shown) and Enzyme-Linked Immunosorbent Assay (R&D, Minneapolis, MN) revealed that human VICs isolated from calcified aortic valves expressed and secreted PCSK9 (0.54±0.07 and 2.51±0.33 ng/mL, respectively), which positively correlated with the VIC calcification potential assessed after seven days of *in vitro* culture (ρ=0.96; p=0.009). On the other hand, PCSK9 mRNA and protein levels were not detectable in valve endothelial cells.

Overall, we showed that i) old *Pcsk9*$^{-/-}$ mice have low AVC; ii) *Pcsk9*$^{-/-}$ VICs are partially protected from *in vitro* calcification; iii) PCSK9 is highly expressed in human calcified aortic valves; and iv) human AVC might be due to VIC-related PCSK9 expression. Taken together, these data strongly support a direct effect of PCSK9 on CAVS development and progression, while remain to be established if PCSK9 may facilitate calcification by acting intracellularly or extracellularly.

In the last decades, all efforts aimed at finding medical therapy or therapeutic agents that could prevent or stop the progression of CAVS have failed due to the paucity of underlying mechanisms (4). Furthermore, it is currently debated whether the mechanisms for bioprosthesis deterioration, due to leaflet calcification, are similar to the ones involved in native aortic valve calcification. Our results suggest that VIC-associated PCSK9 mediated mechanism(s) could be relevant only to the native aortic valve calcification, whereas circulating PCSK9 might influence both types of calcification.

Hence, PCSK9 inhibition therapy, in addition to its positive effects in coronary artery disease patients, could also be efficient in patients with CAVS and then represents a novel pharmacological treatment for these patients. This hypothesis will be confirmed in the currently ongoing and future clinical trials where the therapeutic approaches to inhibit PCSK9 may carry the added value to control AVC.

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FIGURE LEGEND

Figure. PCSK9 and Calcification.

Total calcium content in aortic valve leaflets and in valve interstitial cells from *Pcsk9*$^{-/-}$ and wild-type mice. The **central line** illustrates the median, while **box limits** indicate the 25th and 75th percentiles.