Biologia Serbica, 2019, 41(2): 9-14

DOI 10.5281/zenodo.3532045

Review

The role of proprotein convertase subtilisin/kexin type 9 in atherosclerosis

Jelena VEKIĆ[1*], Dragana BOJANIN[2], Vesna SPASOJEVIĆ-KALIMANOVSKA[1]

[1] *Department of Medical Biochemistry, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia*
[2] *Department for Clinical Chemistry and Hematology, Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić", Belgrade, Serbia*

Received: 02 July 2019 / Accepted: 13 August 2019 / Published Online: 8 November 2019

Summary. Dyslipidemia is one of the predominant causes of atherosclerosis and cardiovascular disease (CVD) development. Accordingly, lifestyles approaches and therapeutic targeting of low-density lipoprotein (LDL)-cholesterol remain the main strategies for CVD prevention and treatment. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secretory serin-protease with important roles in lipoprotein metabolism. In particular, PCSK9 promotes degradation of hepatic LDL-receptors, leading to reduced clearance of LDL particles and increased plasma LDL-cholesterol levels. To date, a large body of evidence from experimental, genetic and clinical studies indicates that PCSK9 is implicated in the development of atherosclerosis. As this research is moving forward, additional roles of PCSK9 beyond cholesterol metabolism and atherosclerosis are being discovered. In the present paper, we will discuss our current knowledge of the role of PCSK9 in atherosclerosis and its associations with cardiometabolic risk factors, and provide a brief overview of the recent achievements in pharmacological inhibition of PCSK9-mediated LDL-receptor degradation toward LDL-cholesterol reduction and prevention of CVD development.

Keywords: atherosclerosis, cardiometabolic risk, dyslipidemia, LDL, PCSK9.

INTRODUCTION

For decades, atherosclerosis and cardiovascular diseases (CVD) remain the leading causes of worldwide mortality and, consequently, the focus of scientific research. The development of atherosclerosis involves interactions of multiple biochemical pathways, so the network of CVD risk factors is wide and complex. Nevertheless, the crucial role in atherogenesis is attributed to alterations in lipid homeostasis, while the accumulation of low-density lipoprotein (LDL) particles within the vessel wall is considered a hallmark of atherosclerosis. Furthermore, modern cardiovascular pharmacotherapy is grounded on the so-called "cholesterol hypothesis", advocating LDL-cholesterol targeting as the main strategy for CVD risk reduction (Vekic et al. 2019).

It is now firmly established that cholesterol levels *per se* does not account for extensive lipid deposition in vascular intima, but rather qualitative characteristics and the functional properties of lipoprotein particles, the carriers of cholesterol in plasma. Circulating LDL particles have different composition, density and size, and numerous experimental data have demonstrated that the smallest LDL subfractions have the highest atherogenic potential (Rizzo et al. 2009a). These small, dense LDL (sdLDL) particles are particularly prone to oxidative modifications, which is a critical step preceding their accumulation in the vascular intima (Rizzo et al. 2009b). In this context, the size and number of circulating LDL particles have emerged as novel lipid biomarkers, superior to LDL-cholesterol level in terms of CVD prediction (Allaire et al. 2017). A new chapter in the research of dyslipidemia was opened by the discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9) (Seidah et al. 2003). Shortly thereafter, a large body of research demonstrated that PCSK9 is an important factor that controls the atherogenicity of LDL

E-mail: jelena.vekic@pharmacy.bg.ac.rs

particles, and thus could be a potential therapeutic target (Shapiro et al. 2018). In this paper, we discuss the function and clinical significance of PCSK9 in atherosclerosis-related diseases, as well as strategies for its therapeutic modulation that have recently been approved.

BIOSYNTHESIS AND PLASMA HOMEOSTASIS OF PCSK9

PCSK9 is a secretory serin-protease that regulates the expression of LDL-receptors and plasma LDL-cholesterol levels. It is one of nine serine-proteases in the proprotein convertase superfamily (Horton et al. 2007). The liver is the chief organ responsible for PCSK9 synthesis and also its main target tissue (Seidah et al. 2003). PCSK9 is also produced in the intestine, pancreas, kidneys and brain, but to a much lesser extent than in the liver. Similarly to other secretory peptides, PCSK9 undergoes complex intracellular processing from preproPCSK9 to mature PCSK9, following autocatalytic cleavage of the prodomain in proPCSK9. Interestingly, the prodomain remains bound to the mature PCSK9, preventing its further catalytic activity (Norata et al. 2016). Although the mechanism by which PCSK9 accomplishes its roles in the metabolism of LDL particles is still not completely clear, it seems that it does not require the enzymatic activity of PCSK9 (Horton et al. 2007; Kosenko et al. 2013; Norata et al. 2016).

PCSK9 circulates in the plasma in two different forms. The first is in the form of an intact heterodimer, which has high affinity for the LDL-receptor and stimulates its degradation, so is considered to be an active form of PCSK9. The second, less abundant form results from hydrolytic cleavage of PCSK9 by furin. Such PCSK9 molecules have lower affinity for the LDL-receptor and therefore are denoted as the less active or inactive form (Norata et al. 2016). It should be mentioned that nearly 30% of circulating PCSK9 is associated with LDL particles (Kosenko et al. 2013). Apart from LDL, PCSK9 can be linked to lipoprotein (a), but not to other lipoproteins containing apolipoprotein B, i.e. triglyceride-rich lipoproteins and their remnants (Tavori et al. 2016). However, the physiological importance of PCSK9 complexes with lipoproteins is still undefined. Some authors have suggested that LDL particles actually protect PCSK9 from degradation by furin; and it has been shown that PCSK9-LDL complexes have higher affinity for LDL-receptors, compared to free PCSK9 (Tavori et al. 2013). This observation suggests that PCSK9-LDL complexes might be a functional form of this protein. Of note, there is also evidence that PCSK9 bound to LDL particles exhibit delayed plasma clearance, but are unable to mediate LDL-receptor degradation (Tavori et al. 2013). Considering the above, LDL particles seem to be important regulators of PCSK9, but the exact physiological role of LDL-bound PCSK9 will need to be established in the future.

The synthesis of PCSK9 is regulated by intracellular cholesterol levels, via sterol regulatory element-binding protein-2 (SREBP-2) (Jeong et al. 2007). This transcription factor modulates expression of several genes involved in lipoprotein metabolism, including LDL-receptors (Horton et al. 2002). As a result, the depletion of intracellular cholesterol content up-regulates the synthesis of both PCSK9 and LDL-receptors. Data from clinical and epidemiological studies have demonstrated that plasma concentrations of PCSK9 have high inter-individual variation (Lakoski et al. 2009). Indeed, PCSK9 levels are largely affected by hormonal and nutritive status (Fig. 1).

The concentrations of PCSK9 are significantly reduced during prolonged fasting. Also, it has been established that PCSK9 has a diurnal rhythm that corresponds to that of cholesterol synthesis (Persson et al. 2010). For that reason, plasma PCSK9 measurement should be performed preferably in the morning, after an overnight fast. Dietary patterns are also able to modulate circulating PCSK9 level. In particular, high calorie intake increases PCSK9 (Cariou et al. 2013), whereas a Mediterranean diet decreases concentrations of PCSK9 in the plasma (Richard et al. 2012). Investigations in pediatric populations showed that levels of PCSK9 increase with age in girls, while in boys a decrease of PCSK9 levels with age was reported (Baass et al. 2009). In adults, PCSK9 levels are higher in women than in men (Lakoski et al. 2009), which could be attributable to the effects of estrogen on PCSK9 synthesis (Fig. 1).

PCSK9 AND ATHEROSCLEROSIS

The interaction between apolipoproteins on the surface of the lipoprotein particles and membrane receptors of different cells is critical for lipid transfer into the cells,

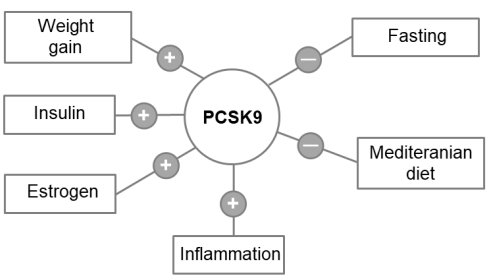

Fig. 1. Factors that modulate PCSK9 concentrations in plasma.

but also for the effective removal of potentially atherogenic lipoproteins from circulation. As previously mentioned, the most likely confirmed role of PCSK9 is the regulation of LDL receptor expression and, consequently, clearance of LDL particles. Since its discovery, numerous gain-of-function and loss-of-function mutations in the PCSK9 gene have been described and associated with increased and decreased LDL-cholesterol levels, respectively (Shapiro et al. 2018). Nowadays, gain-of-function mutations in the PCSK9 gene are accepted as the third cause of familiar hypercholesterolemia (Lakoski et al. 2009), in addition to mutations of the genes encoding LDL-receptors and apolipoprotein B.

Two separate routes of LDL-receptor degradation by PCSK9 have been described. In the extracellular pathway, the catalytic domain of PCSK9 binds to the epidermal growth factor precursor homology domain A (EGF-A) of the LDL-receptor. It was recently revealed that the formation of this PCSK9-LDL-receptor complex is facilitated by heparin-sulphate proteoglycans at the surface of hepatocytes (Gustafsen et al. 2017). The complex PCSK9-LDL-receptor enters the cell by endocytosis, but PCSK9 prevents recycling of the receptor to the cell membrane. Instead, the entire complex is subject to degradation in lysosomes (Lagace 2014). PCSK9-mediated degradation LDL-receptors can be also achieved via an intracellular pathway, where newly-synthesized PCSK9 within the cell binds nascent LDL-receptors and directs them for lysosomal degradation (Strøm et al. 2014).

Numerous studies suggest a link between PCSK9 and traditional cardiometabolic risk factors (Fig. 2). As we recently reviewed, PCSK9 is positively associated with body mass index, and PCSK9 levels are higher in obese individuals (Vekic et al. 2019). However, plasma PCSK9 levels seem to be unaffected by weight loss (Filippatos et al. 2017). Data from experimental models convincingly demonstrated that insulin enhances PCSK9 synthesis (Fig. 1). Namely, reduced plasma level and PCSK9 mRNA were found in hepatocytes of mice with liver-specific knockout of the insulin receptor, as well as in streptozotocin-induced insulin deficient mice and ob/ob mice treated with antisense oligonucleotides against the insulin receptor (Miao et al. 2015). In line with previous clinical studies, significant correlations were found between PCSK9 and serum glucose concentrations, as well as with HOMA-IR, an index of insulin resistance, in both adult and pediatric populations (Baass et al. 2009; Lakoski et al. 2009). Recently, a positive correlation between PCSK9 and glycated hemoglobin (HbA_{1c}) was shown in patients with type 2 diabetes mellitus (Levenson et al. 2017), indicating that PCSK9 might be one of the factors responsible for development of diabetic dyslipidemia.

As expected, PCSK9 plasma levels show a strong, positive correlation with pro-atherogenic serum lipid parameters,

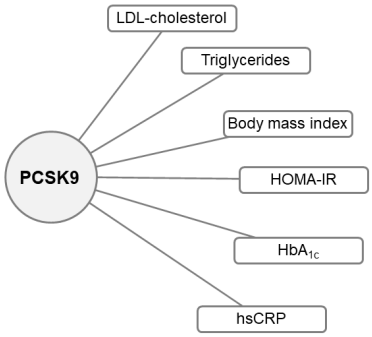

Fig. 2. Cardiometabolic risk factors associated with increased PCSK9 levels.

such as LDL-cholesterol and triglycerides levels (Baass et al. 2009; Lakoski et al. 2009). Although high-density lipoproteins (HDL) are considered to be essentially atheroprotective particles, the available data suggest that plasma PCSK9 concentrations are positively associated with HDL-cholesterol levels (Victor et al. 2004). Furthermore, a positive correlation between PCSK9 and sdLDL particles was found in patients with CVD (Zhang et al. 2015; Nozue et al. 2016), while such an association does not exist in healthy individuals (Kwakernaak et al. 2014). We recently analysed the relationship between plasma PCSK9 levels and lipoprotein subclasses in pediatric patients with type 1 diabetes mellitus, who were treated by intensive insulin therapy and had no diabetic complications (Bojanin et al. 2019). Our data showed that plasma PCSK9 levels significantly increased with worsening metabolic control, supporting the role of PCSK9 in the development of diabetic dyslipidemia. In addition, we found that PCSK9 was positively associated with HbA_{1c}, triglycerides, total and LDL-cholesterol levels in patients with deteriorated metabolic control, whereas such associations were not seen in patients with well-controlled diabetes. The same research revealed that PCSK9 levels are inversely associated with sdLDL particles in patients with good glucoregulation, which was explained by the authors to be due to the effects of insulin therapy (Zhang et al. 2016). Having in mind previously discussed findings that plasma PCSK9 bound to LDL particles has reduced affinity toward LDL-receptors (Tavori et al. 2013), we hypothesised that sdLDL particles may serve as a feedback mechanism that regulates PCSK9 activity in well-controlled type 1 diabetes (Bojanin et al. 2019).

It is now firmly established that low-grade inflammation is an independent risk factor for the development of CVD. The concentrations of PCSK9 are positively associated with high-sensitive C-reactive protein (hsCRP) levels, indicating a possible role of low-grade inflammation in PCSK9 regulation (Gencer et al. 2016). Indeed, it has been demonstrated that

pro-inflammatory cytokines TNF-α and resistin are able to up-regulate PCSK9 synthesis in hepatocytes (Melone et al. 2012). New insight into the association between PCSK9 and inflammation was provided by a recent study documenting that PCSK9 promotes pro-inflammatory cytokines secretion in macrophages, by activating NF-κB pathway (Tang et al. 2017). These data suggest that PCSK9 may accelerate development of atherosclerosis through mechanisms independent of LDL-receptor degradation. In support of this hypothesis, it was confirmed that plasma PCSK9 levels are associated with carotid intima media thickness (cIMT), a non-invasive marker of subclinical atherosclerosis, independently of LDL cholesterol concentrations (Xie et al. 2016). Also, PCSK9 concentrations are an independent predictor of coronary artery calcium (CAC) scores (Zhao et al. 2018), another verified indicator of subclinical atherosclerosis.

In summary, PCSK9 levels are significantly increased in patients with high cardiovascular risk, such as obesity, metabolic syndrome and diabetes, as well as in patients with CVD. However, in spite of numerous epidemiological data linking PCSK9 and atherosclerosis, the measurement of PCSK9 is currently employed only for research purposes. Obviously, PCSK9 might represent a biomarker of familial hypercholesterolemia, in addition to conventional lipid and genetic markers. According to the available data, PCSK9 determination could be useful in identifying patients who require more intensive therapy to lower LDL-cholesterol levels. Statins, which act as inhibitors of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, are recommended as first-line therapy for the prevention and treatment of CVD. Yet, broad spectrums of patients with high CVD risk fail to achieve recommended LDL-cholesterol levels due to statin intolerance or resistance. Furthermore, statins are able to increase plasma PCSK9 levels, which may account for their limited efficacy in lowering LDL-cholesterol. Until now, various approaches to inhibit PCSK9 and prevent LDL-receptors degradation have been tested. Among them, monoclonal antibodies against PCSK9 emerged as the most promising strategy to neutralize circulating PCSK9. Data from several clinical studies convincingly demonstrated a dramatic reduction of LDL-cholesterol levels, with satisfactory safety and tolerability (Sabatine et al. 2017). In addition to LDL-cholesterol levels, PCSK9 inhibitors showed beneficial effects toward other pro-atherogenic lipid parameters, such as triglycerides and lipoprotein (a). Therefore, the latest cholesterol-lowering guidelines recommend the use of PCSK9 inhibitors in addition to intensive statin therapy in patients with very-high CVD risk (Grundy et al. 2019). There is no doubt that the introduction of PCSK9 inhibitors has launched a new era in personalized medicine. However, the cost-effectiveness of this therapy remains to be justified in the near future (Korman et al. 2018). More recently, it is becoming clear that the impact of PCSK9 on atherosclerosis development extends beyond the effects on LDL-cholesterol levels. However, the clinical consequences of PCSK9's pleiotropic effects require further investigation. Several preclinical studies investigating potential novel applications of PCSK9 inhibitors in cancer therapy are currently underway. These studies will hopefully yield routes for new therapeutic strategies aimed to reduce the proliferation of cancer cells by blocking intracellular cholesterol accumulation.

Acknowledgments

This work was supported by a grant from the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. 175035).

REFERENCES

Allaire J, Vors C, Couture P, Lamarche B. 2017. LDL particle number and size and cardiovascular risk: anything new under the sun? Current Opinion in Lipidology. 28(3):261–266.

Baass A, Dubuc G, Tremblay M, Delvin EE, O'Loughlin J, Levy E, Davignon J, Lambert M. 2009. Plasma PCSK9 is associated with age, sex, and multiple metabolic markers in a population-based sample of children and adolescents. Clinical Chemistry. 55(9):1637–1645.

Bojanin D, Vekic J, Milenkovic T, Vukovic R, Zeljkovic A, Stefanovic A, Janac J, Ivanisevic J, Mitrovic K, Spasojevic-Kalimanovska V. 2019. Association between proprotein convertase subtilisin/kexin 9 (PCSK9) and lipoprotein subclasses in children with type 1 diabetes mellitus: effects of glycemic control. Atherosclerosis. 280:14–20.

Cariou B, Langhi C, Le Bras M, Bortolotti M, Lê KA, Theytaz F, Le May C, Guyomarc'h-Delasalle B, Zaïr Y, Kreis R, et al. 2013. Plasma PCSK9 concentrations during an oral fat load and after short term high-fat, high-fat high-protein and high-fructose diets. Nutrition and Metabolism. 10(1):4.

El Khoury P, Elbitar S, Ghaleb Y, Khalil YA, Varret M, Boileau C, Abifadel M. 2017. PCSK9 mutations in familial hypercholesterolemia: from a groundbreaking discovery to anti-PCSK9 therapies. Current Atherosclerosis Reports. 19(12):49.

Filippatos TD, Liberopoulos E, Georgoula M, Tellis CC, Tselepis AD, Elisaf M. 2017. Effects of increased body weight and short-term weight loss on serum PCSK9 levels - a prospective pilot study. Archives of Medical Science - Atherosclerotic Diseases. 2:e46–e51.

Gencer B, Montecucco F, Nanchen D, Carbone F, Klingenberg R, Vuilleumier N, Aghlmandi S, Heg D, Räber L, Auer R, et al. 2016. Prognostic value of PCSK9 levels in patients with acute coronary syndromes. European Heart Journal. 37(6):546–553.

Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, et al. 2019. 2018 AHA/ACC/AACVPR/

AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: executive summary. Circulation. 139:e1046–e1081.

Gustafsen C, Olsen D, Vilstrup J, Lund S, Reinhardt A, Wellner N, Larsen T, Andersen CBF, Weyer K, Li JP, et al. 2017. Heparan sulfate proteoglycans present PCSK9 to the LDL receptor. Nature Communications. 8(1):503.

Horton J, Goldstein J, Brown M. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. Journal of Clinical Investigation. 109(9):1125–1131.

Horton JD, Cohen JC, Hobbs HH. 2007. Molecular biology of PCSK9: its role in LDL metabolism. Trends in Biochemical Sciences. 32(2):71–77.

Jeong H, Lee H, Kim K, Kim Y, Yoon D, Park S. 2007. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. Journal of Lipid Research. 49(2):399–409.

Korman MJ, Retterstøl K, Kristiansen IS, Wisløff T. 2018. Are PCSK9 inhibitors cost effective? Pharmacoeconomics. 36(9):1031–1041.

Kosenko T, Golder M, Leblond G, Weng W, Lagace TA. 2013. Low density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated low density lipoprotein receptor degradation. Journal of Biological Chemistry. 288(12):8279–8288.

Kwakernaak AJ, Lambert G, Dullaart RP. 2014. Plasma proprotein convertase subtilisin-kexin type 9 is predominantly related to intermediate density lipoproteins. Clinical Biochemistry. 47(7-8):679–682.

Lagace T. 2014. PCSK9 and LDLR degradation: regulatory mechanisms in circulation and in cells. Current Opinion in Lipidology. 25(5):387–393.

Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. 2009. Genetic and metabolic determinants of plasma PCSK9 levels. Journal of Clinical Endocrinology and Metabolism. 94(7):2537–2543.

Levenson AE, Shah AS, Khoury PR, Kimball TR, Urbina EM, de Ferranti SD, Maahs DM, Dolan LM, Wadwa RP, Biddinger SB. 2017. Obesity and type 2 diabetes are associated with elevated PCSK9 levels in young women. Pediatric Diabetes. 18(8):755–760.

Melone M, Wilsie L, Palyha O, Strack A, Rashid S. 2012. Discovery of a new role of human resistin in hepatocyte low-density lipoprotein receptor suppression mediated in part by proprotein convertase subtilisin/kexin type 9. Journal of the American College of Cardiology. 59(19):1697–1705.

Miao J, Manthena PV, Haas ME, Ling AV, Shin DJ, Graham MJ, Crooke RM, Liu J, Biddinger SB. 2015. Role of insulin in the regulation of proprotein convertase subtilisin/kexin type 9. Arteriosclerosis, Thrombosis, and Vascular Biology. 35(7):1589–1596.

Norata GD, Tavori H, Pirillo A, Fazio S, Catapano AL. 2016. Biology of proprotein convertase subtilisin kexin 9: beyond low-density lipoprotein cholesterol lowering. Cardiovascular Research. 112(1):429–442.

Nozue T, Hattori H, Ogawa K, Kujiraoka T, Iwasaki T, Hirano T, Michishita I. 2016. Correlation between serum levels of proprotein convertase subtilisin/kexin type 9 (PCSK9) and atherogenic lipoproteins in patients with coronary artery disease. Lipids in Health and Disease. 15(1):165.

Persson L, Cao G, Ståhle L, Sjöberg BG, Troutt JS, Konrad RJ, Gälman C, Wallén H, Eriksson M, Hafström I, et al. 2010. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. Arteriosclerosis, Thrombosis, and Vascular Biology. 30(12):2666–2672.

Richard C, Couture P, Desroches S, Benjannet S, Seidah NG, Lichtenstein AH, Lamarche B. 2012. Effect of the Mediterranean diet with and without weight loss on surrogate markers of cholesterol homeostasis in men with the metabolic syndrome. British Journal of Nutrition. 107(5):705–711.

Rizzo M, Berneis K, Zeljkovic A, Vekic J. 2009a. Should we routinely measure low-density and high-density lipoprotein subclasses? Clinical Laboratory. 55(11-12):421–429.

Rizzo M, Kotur-Stevuljevic J, Berneis K, Spinas G, Rini GB, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V, Vekic J. 2009b. Atherogenic dyslipidemia and oxidative stress: a new look. Translational Research.153(5):217–223.

Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, et al; FOURIER Steering Committee and Investigators. 2017. Evolocumab and clinical outcomes in patients with cardiovascular disease. New England Journal of Medicine. 376(18):1713–1722.

Seidah N, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin S, Stifani S, Basak A, Prat A, Chretien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proceedings of the National Academy of Sciences of the United States of America.100(3):928–933.

Shapiro MD, Tavori H, Fazio S. 2018. PCSK9: from basic science discoveries to clinical trials. Circulation Research. 122(10):1420–1438.

Strøm T, Tveten K, Leren T. 2014. PCSK9 acts as a chaperone for the LDL receptor in the endoplasmic reticulum. Biochemical Journal. 457(1):99–105.

Tang ZH, Peng J, Ren Z, Yang J, Li TT, Li TH, Wang Z, Wei DH, Liu LS, Zheng XL, et al. 2017. New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF-κB pathway. Atherosclerosis. 262:113–122.

Tavori H, Giunzioni I, Linton M, Fazio S. 2013. Loss of plasma proprotein convertase subtilisin/kexin 9 (PCSK9) after lipoprotein apheresis. Circulation Research. 113(12):1290–1295.

Tavori H, Christian D, Minnier J, Plubell D, Shapiro MD, Yeang C, Giunzioni I, Croyal M, Duell PB, Lambert G, et al. 2016. PCSK9 association with lipoprotein(a). Circulation Research. 119(1):29–35.

Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. 2019. Obesity and dyslipidemia. Metabolism: Clinical and Experimental. 92:71–81.

Victor R, Haley R, Willett D, Peshock R, Vaeth P, Leonard D, Basit M, Cooper RS, Iannacchione VG, Visscher WA, et al. 2004. The Dallas Heart Study: a population-based probability

sample for the multidisciplinary study of ethnic differences in cardiovascular health. American Journal of Cardiology. 93(12):1473–1480.

Xie W, Liu J, Wang W, Wang M, Qi Y, Zhao F, Sun J, Liu J, Li Y, Zhao D. 2016. Association between plasma PCSK9 levels and 10-year progression of carotid atherosclerosis beyond LDL-C: a cohort study. International Journal of Cardiology. 215:293–298.

Zhang Y, Xu RX, Li S, Zhu CG, Guo YL, Sun J, Li JJ. 2015. Association of plasma small dense LDL cholesterol with PCSK9 levels in patients with angiographically proven coronary artery disease. Nutrition, Metabolism and Cardiovascular Diseases. 25(4):426–433.

Zhang Y, Jenkins AJ, Basu A, Stoner JA, Lopes-Virella MF, Klein RL; DCCT/EDIC Research Group, Lyons TJ. 2016. Associations between intensive diabetes therapy and NMR-determined lipoprotein subclass profiles in type 1 diabetes. Journal of Lipid Research. 57(2):310–317.

Zhao X, Zhang HW, Li S, Zhang Y, Xu RX, Zhu CG, Wu NQ, Guo YL, Qing P, Li XL, et al. 2018. Association between plasma proprotein convertase subtisilin/kexin type 9 concentration and coronary artery calcification. Annals of Clinical Biochemistry. 55(1):158–164.