Review article

J Atherosclerosis Prev Treat. 2021 Jan-Apr;12(1):7–13
doi.org/10.53590/japt.02.1017

Association of PCSK9 with human plasma Lipoproteins

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ABSTRACT

Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is a serine protease primarily expressed in the liver. The main function of circulating PCSK9 relates to its binding to the low-density lipoprotein receptor (LDL-R) in hepatocytes, increasing its endosomal and lysosomal degradation. This results in the inhibition of LDL-R recycling to the cell surface and therefore in the reduction of the hepatic LDL uptake, leading to the increase in plasma levels of LDL-cholesterol. Several studies have demonstrated that the plasma levels of PCSK9 are correlated with those of the ApoB-containing lipoproteins; LDL, Lp(a) and Triglyceride-Rich Lipoproteins (TRL). Furthermore, it has been shown that PCSK9 binds to the LDL and Lp(a) particles and significantly influences TRL metabolism. By contrast, controversial results exist concerning the association of PCSK9 with High Density Lipoprotein (HDL). In the present review we present existing data on the association of PCSK9 with human plasma lipoprotein particles and its possible pathophysiological role.

KEY WORDS: *ApoB-lipoproteins, High Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Lp(a), PCSK9, Triglyceride-Rich Lipoproteins*

INTRODUCTION

Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) is a serine protease primarily expressed in the liver and it is also detected in the central nervous system, the intestine and the kidney as well as in various cell types such as endothelium, smooth muscle cells and macrophages[1]. PCSK9 is also found in the cerebrospinal fluid [(2)] and at the sites of atherosclerotic plaques[3].

The PCSK9 circulating in plasma is mainly secreted by the liver[1]. The main function of PCSK9 relates to the binding to low-density lipoprotein receptor (LDL-R) in hepatocytes, increasing its endosomal and lysosomal degradation[4]. This leads to the inhibition of LDL-R recycling to the cell surface and therefore to the reduction of LDL hepatic uptake, leading to the elevation of LDL-cholesterol plasma levels, a major risk factor of cardiovascular diseases (CVD)[5]. Several studies have showed that PCSK9 is associated with various types of lipoproteins in human plasma primarily with LDL, VLDL and Lp(a), with controversial data concerning the association of PCSK9 with HDL. Aim of this review is to describe the association of PCSK9 with various lipoprotein particles in human plasma and to provide existing evidence for the possible pathophysiological role of this association.

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Submission: 04.03.2021, Acceptance: 21.04.2021

PCSK9 BIOSYNTHESIS, INTRACELLULAR METABOLISM, SECRETION AND PATHOPHYSIOLOGICAL ROLE

The human PCSK9 gene, is located on chromosome 1p32.3, which contains 12 exons and is 3,617 bps in length[6]. PCSK9 is synthesized as Pro-PCSK9, which consists of 692 amino acids and has a molecular weight of ~75kDa[7]. The Pro-PCSK9 is composed as a signal peptide (residues 1 to 30), a pro-domain extended between amino acids 31-152, a catalytic domain (residues 153 to 421) and a Cys-His-rich C-terminal domain (CHRD) that extends from 422 to 692 amino acids residues[8-10]. Following its synthesis, Pro-PCSK9 is directed to the Endoplasmic Reticulum (ER) by the signal peptide[11], where the pro-domain (~13kDa) is auto-catalytically cleaved off and binds non covalently to the catalytic domain of the remaining PCSK9 protein (~62kDa). This results in the formation of an intact heterodimer (62+13kDa) that represents the most active form of the PCSK9[6], which is then secreted into the bloodstream through the ER[12,13]. The 62kDa PCSK9 can also be proteolytically cleaved in the extracellular space, by protease furin[14]. This cleavage results in the production of a ~7kDa peptide and the furin-cleaved form (55kDa), of PCSK9 in plasma[14].

The plasma levels reported by different groups, depending on the methodology used, were 50-600 ng/ml with a mean value of 200ng/ml[15,16], 11-115ng/ml[15,17] and 0.1-9.3μg/ml with a mean value of 4μg/ml[15,18]. The above values refer to the total PCSK9 (intact and furin-cleaved) in plasma. The most commonly PCSK9 levels used as a reference are 0.3-0.8 μg/ml[19-21]. Currently there is no method to measure separately the levels of intact or furin-cleaved form of PCSK9.

A major pathophysiological role of PCSK9 is the regulation of the LDL-R on the surface of hepatocytes, thus PCSK9 significantly affects the plasma levels of LDL-cholesterol[22]. This role is exerted by both forms of PCSK9 (62kDa and 55kDa), but it is more pronounced for the 62kDa PCSK9[14]. More specifically, once secreted into the circulation, PCSK9 interacts with the Endothelial Growth Factor-A (EGF-A) domain of the LDL-R. This complex is internalized in endosomes via clathrin-coated pits and the cytosolic adaptor protein, a phosphotyrosine binding protein named as autosomal recessive hypercholesterolemia (ARH)[23]. The binding affinity of PCSK9 with LDL-R is increased in endosomes due to the existing acidic pH, thus the PCSK9 interaction with the receptor is further potentiated. This increase in the binding affinity may be due to the creation of additional binding sites on the receptor, occurring via intramolecular interactions among receptor domains[24,25]. Importantly, the strong association of PCSK9 with the LDL-R in the endosome prevents the conformational changes occurring on the receptor in the absence of PCSK9, which allow LDL-R to dissociate from its lipoprotein ligand and recycle back to the cell surface[5,24]. Consequently, the binding of PCSK9 to the LDL-R leads the receptor to the lysosome for degradation. It should be noted that the domain of the LDL-R which binds to PCSK9 is differentiated from the domain that recognizes and binds to LDL. Indeed, elimination of the LDL binding domain on the LDL-R, has no significant effect on the PCSK9 binding to the receptor. This suggests that the association of the PCSK9 with LDL-R occurs independently on the LDL binding. Since a proportion of circulating PSCK9 is associated with LDL particles (this is described below in this review), it remains to be established whether PCSK9 binds to the LDL-R as a free form or as a complex with LDL particles or in both forms[5].

Besides the binding and degradation of the LDL-R, PCSK9 also binds and leads to degradation of other receptors and membrane proteins, including the VLDL receptor[26], the Apolipoprotein E Receptor 2 (ApoER2)[27], the differentiation clusters 36 and 81 (CD36 and CD81, respectively)[28,29], the beta-secretase 1 (BACE 1)[30] and the epithelial (NA+) channel (ENaC)[31].

ASSOCIATION OF PCSK9 WITH LIPOPROTEINS

Association of PCSK9 with LDL

Clinical studies have demonstrated that plasma PCSK9 levels are positively correlated with LDL levels[32-35] (Table 1), a correlation that could be attributed to the main PCSK9 function which is the induction of the LDL-R degradation. However, it has been suggested that the above correlation could also be attributed to the association of PCSK9 with LDL particles in the circulation. Indeed, studies have demonstrated that approximately 30% to 40% of plasma PCSK9 is associated with LDL through a protein-protein interaction with its ApoB-100 content[36] (figure 1) and there is evidence that this association occurs within the secretory pathways of hepatocytes[37]. Moreover, the PCSK9 interaction with ApoB containing lipoproteins leads to the suppression of the ApoB degradation through the autophagosome/lysosome pathway[37]. In parallel, studies have demonstrated that the interaction of PCSK9 with ApoB-100 plays an important role in ApoB-100 metabolism, in an LDL-R independent manner[37]. In support of the above results, another study demonstrated that the removal of ApoB-100 containing lipoproteins from plasma by apheresis, reduced the levels of PCSK9 in plasma by 50%. Using immunoblot analysis, this study also showed that the intact form of PCSK9 (62kDa) is bound to LDL whereas

TABLE 1. *In vivo* studies demonstrating the correlation of PCSK9 with Lipoproteins

PCSK9/ ApoB	Positive correlation (r=0.226, p=0.006)	Guardiola M, Plana N, Ibarretxe D, Cabré A, González M, Ribalta J, et al Clin Sci (Lond). 2015 Jun;128(12):877-82
PCSK9/ VLDL	Positive correlation (r=0.210, p=0.001)	
PCSK9/ IDL	Positive correlation (r=0.206, p=0.001)	
PCSK9/ Small LDL	Positive correlation (r=0.224, p<0.001)	
PCSK9/ Medium LDL	Positive correlation (r=0.235, p<0.001)	
PCSK9/ Very Small LDL	Positive correlation (r=0.220, p<0.001)	
PCSK9/ HDL-Cholesterol	Positive correlation (r=0.149, p=0.012)	Xu RX, Li S, Zhang Y, Li XL, Guo YL, Zhu CG, et al. Lipids Health Dis. 2014 Dec 11;13:188.
PCSK9/ non HDL-Cholesterol	Positive correlation (r=0.221, p=0.000)	
PCSK9/ Apo AI	Positive correlation (r=0.184, p=0.003	
PCSK9/ LDL-Cholesterol	Positive correlation (r=0.219, p=0.000)	
PCSK9/ ApoB	Positive correlation (r=0.260, p=0.000)	
PCSK9/ Small LDL	Positive correlation (r=0.166, p=0.005)	
PCSK9/ Intermediate LDL	Positive correlation (r=0.145, p=0.015)	
PCSK9/ Small HDL	Positive correlation (r=0.124, p=0.048)	
PCSK9/ Intermediate HDL	Positive correlation (r=0.157, p=0.013)	
PCSK9/ LDL-Cholesterol	Positive correlation (r=0.516, p<0.001)	Kwakernaak AJ, Lambert G, Dullaart RP Clin Biochem. 2014 May;47(7-8):679-82.
PCSK9/ non HDL-Cholesterol	Positive correlation (r=0.547, p<0.001)	
PCSK9/ HDL-Cholesterol	Not correlated (r=0.063, p=0.66)	
PCSK9/ VLDL	Positive correlation (r=0.333, p=0.022)	
PCSK9/ LDL	Positive correlation (r=0.373, p=0.010)	
PCSK9/ IDL	Positive correlation (r=0.532, p=<0.001)	
PCSK9/ Small LDL	Positive correlation (r=0.329, p=0.024)	
PCSK9/ LDL-Cholesterol	Positive correlation (p<0.001)	Ferri N, Ruscica M, Coggi D, Bonomi A, Amato M, Frigerio B, et al, Atherosclerosis. 2020 Sep;309:39-46
PCSK9/ HDL-Cholesterol	Positive correlation (p<0.001)	

most of the furin-cleaved form is in the ApoB-100-free fraction of plasma[38].

The interaction between PCSK9 and LDL has also been studied, *in vitro*[39]. In this regard, LDL isolated by flotation ultracentrifugation, by which the endogenous PCSK9 was completely detached, was incubated *in vitro* with fluorescently labeled recombinant PCSK9. The association between LDL and PCSK9 was studied using immunoprecipitation and western blot analysis[39]. The results showed that the isolated LDL binds to the PCSK9[39]. Regarding the PCSK9 domains involved in its binding with LDL[39], it has been demonstrated that the N-terminal sequence of the PCSK9 prodomain containing the 31-52 amino acids is required for the binding of PCSK9 with LDL[40] (figure 1). Interestingly, deletion of this amino acid sequence, prevents the LDL binding to PCSK9, however it increases the PCSK9 binding affinity to the LDL-R[40].

Since LDL is consisted of a heterogeneous particle population, it was investigated whether there are differences in the association of PCSK9 with various LDL subfractions. In this regard, it has been shown that the PCSK9 levels are positively and independently correlated with small and intermediate LDL subfractions in plasma of patients with stable CAD[33]. Another study demonstrated that PCSK9 levels are positively correlated with medium, small and very small LDL subfractions, in plasma of patients with high cardiovascular risk[34]. A gender analysis performed in this study showed that this correlation, is observed in male subjects but not in females, suggesting that the differential association of PCSK9 with LDL subfractions may represent a new underlying mechanism for gender disparity in the development of CAD[33]. In conclusion, the PCSK9 levels in plasma are positively correlated with LDL, preferentially with the intermediate, and small LDL particles. The intact form of PCSK9 rather than its the furin-cleaved form is primarily associated LDL through the binding of its N-terminal region of the prodomain to ApoB-100.

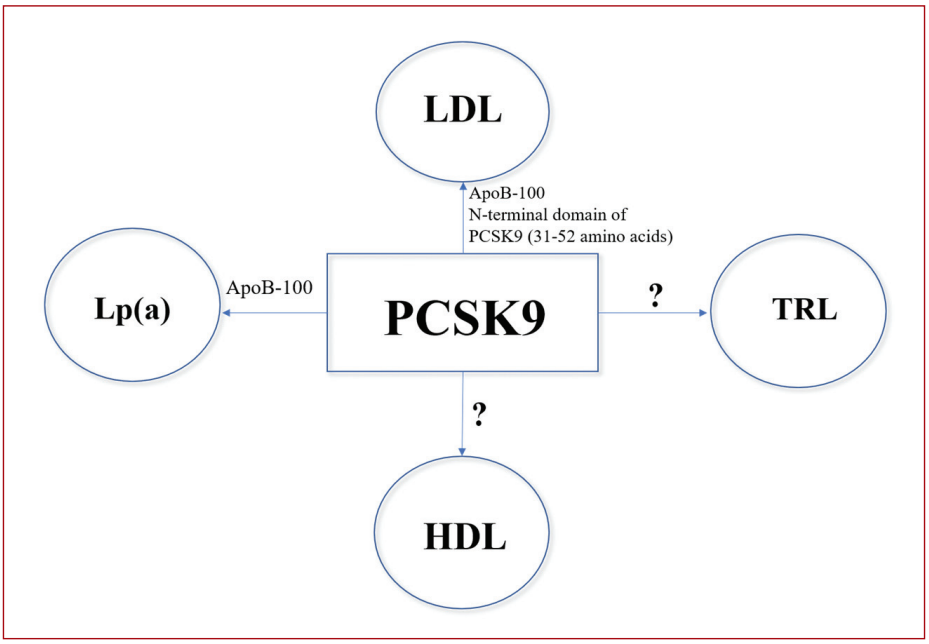

FIGURE 1. Association of PCSK9 with human lipoprotein particles and the domains which play a crucial role in this association.

Association of PCSK9 with Triglyceride-Rich Lipoproteins (TRL)

Triglyceride-rich lipoproteins (TRL) include chylomicrons (CM), very low density lipoproteins (VLDL) and their remnants[41]. Studies have demonstrated a positive correlation between circulating PCSK9 and plasma triglyceride levels[32-34]. This correlation was the first indication that PCSK9 may play a role in TRL metabolism[42]. Previously published data have demonstrated that the reduction in plasma PCSK9 levels is associated with a decrease in TG levels, possibly through an enhancement of the hepatic catabolism of intermediate density lipoprotein (IDL) via the LDL-R pathway[43]. Consistent with the above results, is the finding that the plasma PCSK9 levels are positively correlated with IDL and VLDL levels[32,34] (Table 1). It has been demonstrated that PCSK9 does not bind to isolated VLDL from human plasma[39], whereas a study of isolated VLDL from mice plasma using Fast Protein Liquid Chromatography (FPLC) interacts with PCSK9[37]. Another mechanism by which PCSK9 may affect VLDL levels is through its binding to the VLDL receptor, probably through the EGF-A domain in a similar manner as it binds to the LDL-R[44]. Moreover, increased cellular and secreted ApoB-48 and ApoB-100 levels have been shown after treatment of human enterocytes with recombinant PCSK9, indicating that PCSK9 may induce the production of CM in the intestinal cells[41]. In addition, incubation of this enterocyte cell line with a human Gain of Function D374Y-PCSK9 had as a consequence an enhanced cholesterol uptake in these cells (due to increased expression of cholesterol transporters NPC1L1 and CD36) and an increased CM secretion (due to increased lipid and ApoB-48 biogenesis) as compared with normal PCSK9[41]. In conclusion, PCSK9 levels are positively correlated with plasma levels of triglyceride rich lipoproteins, including CM, VLDL and IDL. PCSK9 may influence CM production as well as VLDL and IDL degradation, however the binding of PCSK9 with VLDL particles needs further investigation.

Association of PCSK9 with Lp(a)

Lp(a) consists of an LDL-like particle on which a unique apolipoprotein (a) is attached, through a single disulfide bond formed with the LDL ApoB-100 moiety[45]. High Lp(a) levels in plasma, are positively correlated with PCSK9 levels, in patients not receiving any lipid lowering drug[11]. Moreover, several studies have demonstrated that in addition to LDL, PCSK9 may bind to Lp(a), since it also contains ApoB-100[11,38] (figure 1). To establish the association of PCSK9 with Lp(a), investigators isolated LDL and Lp(a) from subjects with elevated levels of Lp(a), using iodixanol-based ultracentrifugation. The possible association of PCSK9 with Lp(a) was investigated by native gel electrophoresis and immunoblotting. Using these techniques authors showed that the Lp(a) band contained ApoB, Apo(a) and also PCSK9, strengthening the notion that PCSK9 associates with Lp(a)[38]. The PCSK9 bound on Lp(a) consisted of both the intact and the furin-cleaved form[11]. Finally, it has been suggested that PCSK9 could preferentially be associated with Lp(a)

as compared with LDL, due to longer Lp(a) half-life in human plasma[11,46].

Is PCSK9 associated with High Density Lipoprotein?

Some clinical studies have demonstrated that the PCSK9 levels in plasma are positively correlated with HDL levels[33,35], whereas others failed to show such correlation[32] (Table 1). In addition, there are contrasting results as concern the association of PCSK9 with HDL particles. Previous data have shown that PCSK9 is associated with apoB-containing lipoproteins such as LDL and Lp(a) but not with HDL[39]. In contrast, a more recent study suggests that the main carrier of PCSK9 in plasma are the HDL particles[47]. By using plasma from healthy volunteers, this study demonstrated that approximately 20% of total plasma PCSK9 was immunoprecipitated with a specific anti-ApoB antibody which binds to all apoB-containing lipoproteins, including Lp(a)[47]. Importantly, removal of HDL from plasma by immunoprecipitation, using a specific anti-ApoA1 antibody, resulted in an approximately 90% reduction of total PCSK9 plasma levels[47]. The association of PCSK9 with HDL in this study was further supported by the finding that immunoblotting of total plasma, HDL-depleted plasma and HDL particles prepared from plasma. Results showed that HDL particles contain both intact and furin-cleaved PCSK9 forms. In this regard it should be emphasized that immunoisolation of HDL, rather than isolation by sequential ultracentrifugation of plasma, may reduce the loss of HDL-associated PCSK9, since studies have shown that the high g forces required for HDL isolation by ultracentrifugation, may result in dissociation of PCSK9 from HDL particles[39]. Based on the above controversial results on whether PCSK9 is associated with HDL, it is concluded that the possible association of PCSK9 with HDL particle needs further investigation.

Acknowledgment

The present study was supported by a research grant from the Hellenic Atherosclerosis Society which was received by Tsouka N. Aikaterini.

Conflict of interest

The authors have no conflicts of interest related to this publication.

ΠΕΡΙΛΗΨΗ

Η αλληλεπίδραση της PCSK9 με τις λιποπρωτεΐνες του ανθρώπινου πλάσματος

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Η PCSK9 είναι μια σερινοπρωτέαση που εκφράζεται πρωταρχικώς στο ήπαρ. Βασική λειτουργία της κυκλοφορούσας PCSK9 είναι η σύνδεση της στον υποδοχέα της χαμηλής πυκνότητας λιποπρωτεΐνης (LDL-R) που βρίσκεται στην επιφάνεια των ηπατοκυττάρων, αυξάνοντας την ενδοσωμική και λυσοσωμική αποικοδόμηση του. Η λειτουργία αυτή έχει ως συνέπεια την αναστολή της ανακύκλωσης του LDL-R στην επιφάνεια του ηπατοκυττάρου, και επομένως την μείωση της πρόσληψης της LDL από τα ηπατοκύτταρα, αυξάνοντας έτσι τα επίπεδα της LDL χοληστερόλης στο πλάσμα. Πολλές μελέτες υποστηρίζουν ότι τα επίπεδα της PCSK9 στο πλάσμα συσχετίζονται με τα επίπεδα των λιποπρωτεϊνών πλούσιων σε ApoB απολιποπρωτεΐνη , όπως είναι η LDL, η Lp(a) και οι λιποπρωτεΐνες πλούσιες σε τριγλυκερίδια. Επιπροσθέτως, έχει αποδειχθεί η αλληλεπίδραση της PCSK9 με την LDL και την Lp(a), ενώ επηρεάζει σημαντικά τον μεταβολισμό των λιποπρωτεϊνών πλούσιων σε τριγλυκερίδια. Εν αντιθέσει, υπάρχουν αντικρουόμενα δεδομένα αναφορικά με την αλληλεπίδραση της PCSK9 με την υψηλής πυκνότητας λιποπρωτεΐνη (HDL). Στο παρόν άρθρο, παρουσιάζουμε την υπάρχουσα βιβλιογραφία αναφορικά με την αλληλεπίδραση της PCSK9 με τα λιποπρωτεϊνικά σωματίδια στο πλάσμα καθώς και τον παθοφυσιολογικό ρόλο αυτής της αλληλεπίδρασης.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: *PCSK9, ApoB λιποπρωτεΐνες, λιποπρωτεΐνες πλούσιες σε τριγλυκερίδια, υψηλής πυκνότητας λιποπρωτεΐνη (HDL), χαμηλής πυκνότητας λιποπρωτεΐνη (LDL)*

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