

PCSK9 positively correlates with plasma sdLDL in community-dwelling population but not in diabetic participants after confounder adjustment

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Abstract

This study aimed to investigate the relationship between plasma proprotein convertase subtilisin kexin 9 (PCSK9) and small dense low-density lipoprotein (sdLDL) in diabetic and non-diabetic participants in a community-dwelling cohort.

The plasma levels of PCSK9 and sdLDL were detected in 1766 participants (median age: 61.40 years; 733 males vs 1033 females; 383 diabetic vs 1383 non-diabetic patients) from the Pingguoyuan community of Beijing, China.

Results showed that Pearson correlation analysis revealed a positive correlation between PCSK9 and sdLDL ($r=0.263$, $P<.001$). Multiple linear regression analysis showed a significant positive correlation between plasma PCSK9 and sdLDL in the whole population study. sdLDL was used as the dependent variable, and the potential cofounders were adjusted. However, any independent relationship was not observed between circulating PCSK9 and sdLDL in the diabetic subpopulation ($r=0.269$, $P<.05$, $\beta = 9.591$, $P>.05$).

Thus, there is a positive correlation between plasma PCSK9 and sdLDL in a community-dwelling cohort, but not in type 2 diabetic subpopulation, after confounder adjustment.

Abbreviations: APOB = apolipoprotein B, BMI = body mass index, CVD = cardiovascular diseases, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, lbLDL = large boyant LDL, LDL-C = low density lipoprotein-cholesterol, LDL-R = LDL receptor, PCSK9 = proprotein convertase subtilisin/kexin type 9, PLAGH = People's Liberation Army General Hospital, SBP = systolic blood pressure, TG = triglyceride, VLDL = very low density lipoprotein, WHR = waist-to-hip ratio.

Keywords: confounder adjustment, diabetes, plasma proprotein convertase subtilisin kexin 9, small dense low-density lipoprotein

1. Introduction

Elevated plasma low density lipoprotein-cholesterol (LDL-C) has been found as a major risk factor of cardiovascular diseases (CVD),^[1–3] but the plasma LDL-C in some CVD patients is still within the normal range.^[4–6] Several studies have shown that the physical properties of LDL particles are more important risk factors than LDL-C concentration in CVD.^[7–12] These properties include but not limited to the density, size, lipid and protein contents, and chemical composition. Among all LDL particles,

the small dense LDL particles (sdLDL), in contrast to the large boyant LDL (lbLDL) particles, are proven to be more important in the process of atherosclerosis.^[7,13] The sdLDL particles are the subcategories of LDL-C with density ranging from 1.034 g/ml to 1.066 g/ml. The particles contain less cholesterol and have a lower affinity to the LDL receptor (LDL-R). Moreover, they possess a long half-time which potentiates a greater possibility of oxidative reaction^[14,15] and a stronger ability to infiltrate the subendothelium. Increased sdLDL has been reported in chronic inflammatory conditions such as kidney disease, rheumatoid and psoriatic arthritis as well as in metabolic diseases, including obesity, metabolic syndrome, and diabetes.^[8,16,17]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is the 9th member of the proprotein convertase family that degrades the LDL-R. The uptake of LDL-C by hepatic cells is reduced, which leads to an increased concentration of LDL-C in the circulation. Therefore, antibodies against PCSK9 as therapeutic intervention have been developed and approved by the US Food and Drug Administration. The antibodies are shown in studies to reduce the plasma LDL-C and the risk of cardiovascular events.^[18–20] Several studies have revealed that serum PCSK9 increases in individuals with insulin resistance related diseases, metabolic syndrome, and type 2 diabetes.^[21–25] However, some other studies show that there is no significant difference in serum PCSK9 level between diabetic and non-diabetic subjects.^[26,27] Moreover, the relationship between blood PCSK9 and sdLDL remains unclear. Thus, this study was undertaken to investigate the correlation between PCSK9 and sdLDL in community-dwelling individuals with and without type 2 diabetes.

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2. Material and methods

2.1. Study subjects

This cohort study was conducted in Pingguoyuan community, Shijingshan district of Beijing, China. A public recruiting announcement was issued in the community by the People's Liberation Army General Hospital (PLAGH) and the Community Management Committee. Subjects were voluntary to participate in this study which aimed to recruit a community-dwelling population. Bedridden patients or patients with mental disorders or severe systemic diseases (including myocardial infarction, coronary artery diseases, cerebrovascular events, severe liver insufficiency, immunological, endocrine or metabolic disorders [except for type 2 diabetes mellitus]) were excluded from this study. Initially, all the subjects received physical examination at the community medical center. A total of 1828 subjects aged ≥ 18 years were recruited between September 2007 and January 2009. After recruitment, blood samples were collected for future assay. However, 62 subjects were excluded from this study due to either unqualified blood specimens or missing data. The remaining 1766 subjects were included for final analysis. The whole protocol of this study was approved by the Ethics Committee of the PLAGH, and written consent was confidentially obtained from each subject (Fig. 1).

2.2. Questionnaire and anthropometric measurements

Survey with questionnaires and anthropometric measurements were carried out by qualified physicians from the PLAGH. The face-to-face questionnaires used in our study were specially developed to collect following information: family history, lifestyle related factors, prevalent diseases, and medications used at baseline. Height was measured with a tape and weight with a digital scale. Waist circumference was defined as the circumference at the middle part between the last rib and the iliac crest. Hip circumference was defined as the circumference at the widest part of the hips. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were detected in a sitting position after resting for

at least 5 min. Blood pressure were measured at least twice and then average was calculated for analyses.

2.3. Laboratory measurements

Blood samples were collected in pre-cooled EDTA tubes from all subjects after fasting for at least 12 hours and stored at 4°C. After centrifugation at 3000 rpm for 15 minutes at 4°C, the supernatant (plasma) was harvested and then kept at -80°C. Fasting blood glucose (FBG), triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C) and LDL-C were detected with corresponding Roche enzymatic assay kits (Roche Diagnostics GmbH, Mannheim, Germany) on the Roche auto-analyzer (Roche Diagnostics, Indianapolis, IN, USA). Serum PCSK9 was detected by ELISA (CycLex Co., Nagano, Japan). Plasma sdLDL was detected with sdLDL "Seiken" kit (Denka Seiken Co. Ltd, Tokyo, Japan) on the HITACHI 7180 automatic biochemical analyzer (HITACHI, Japan).^[28] All the blood samples were analyzed in the same lab according to the criteria of the World Health Organization Lipid Reference Laboratories.^[29]

2.4. Definition of variables

Current smoking was defined as smoking at least 1 cigarette per day for at least 1 year. Body mass index (BMI) was defined as the body weight in kilograms divided by the square of body height in meters (kg/m^2). The waist-to-hip ratio (WHR) was defined as the waist circumference divided by the hip circumference. Renal function was assessed by the estimated glomerular filtration rate (eGFR), which was calculated using the Modified glomerular filtration rate estimating equation in renal disease for Chinese patients^[30]: $\text{eGFR} (\text{ml}/\text{min}/1.73 \text{ m}^2) = 75 \times \text{standard creatinine} (\text{mg}/\text{dl}) - 1.234 \times \text{age} (\text{year}) - 0.179 \times (0.79 \text{ if female})$. All the subjects without a history of DM received a standard 75 g oral glucose tolerance test. DM was diagnosed if

1. fasting venous blood glucose was ≥ 7.1 mmol/L;
2. 2 hours venous blood glucose was ≥ 11.1 mmol/L; or
3. subjects received glucose-lowering therapy.

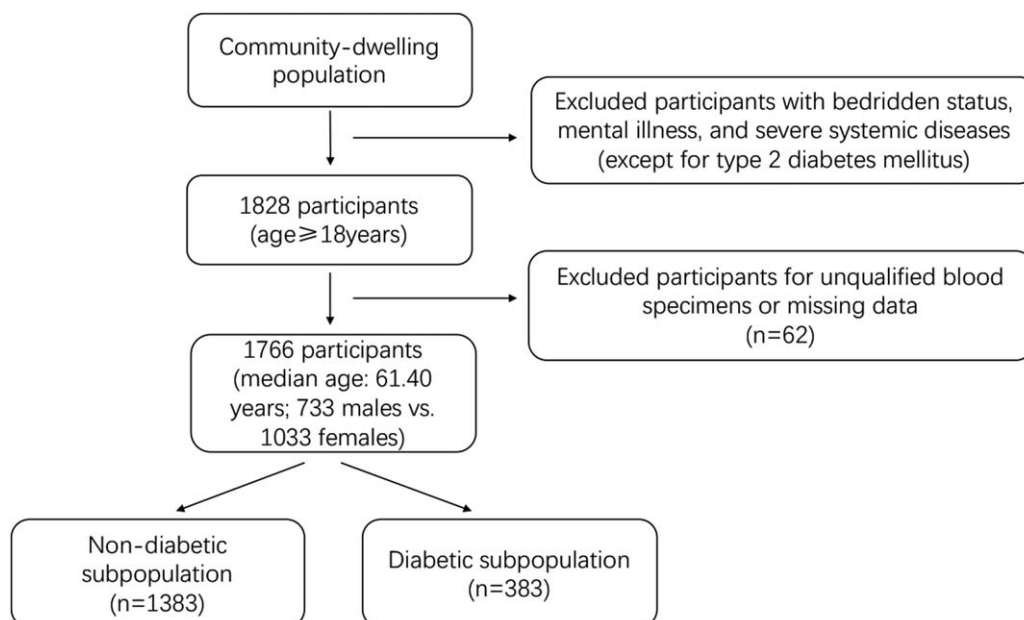


Figure 1. Study flowchart.

Table 1**Baseline profiles of the subjects.**

Items	All subjects (n=1766)	DM (n=383)	Non-DM (n=1383)	P value
Age (years)	61.45 ± 11.2	63.14 ± 9.9	58.1 ± 11.4	<.001
Male sex (n (%))	733 (41.5)	133 (34.72)	600 (43.38)	.021
BMI (kg/m ⁻²)	25.40 ± 3.12	25.78 ± 6.7	24.8 ± 5.5	.962
WHR	0.80 ± 0.53	0.79 ± 0.29	0.90 ± 2.98	.631
Smoker (n (%))	464 (26.3)	103 (26.8)	361 (26.1)	.347
SBP (mm Hg)	125.74 ± 17.71	129.18 ± 16.68	118.32 ± 19.24	.017
DBP (mm Hg)	76.42 ± 10.23	76.92 ± 11.12	75.79 ± 12.48	.549
TC (mmol/L ⁻¹)	5.01 ± 0.91	4.88 ± 1.14	5.00 ± 0.95	.184
TG (mmol L ⁻¹)	1.89 ± 1.14	1.97 ± 1.09	1.74 ± 1.31	.048
HDL-C (mmol/L ⁻¹)	1.38 ± 0.42	1.25 ± 0.32	1.41 ± 0.37	<.001
LDL-C (mmol/L ⁻¹)	2.91 ± 0.62	2.93 ± 0.83	2.88 ± 0.71	.406
FBG (mmol/L ⁻¹)	5.17 ± 1.54	7.14 ± 2.90	4.88 ± 0.66	<.001
eGFR (ml/min ⁻¹ /1.73 m ²)	94.10 ± 14.3	94.26 ± 13.34	94.01 ± 13.65	.838
PCSK9 (ng/ml ⁻¹)	137.08 ± 18.33	138.99 ± 20.33	136.19 ± 17.27	.095
sdLDL (mmol/L ⁻¹)	40.293 ± 5.18	43.52 ± 7.08	39.64 ± 6.47	.026
Hypertension (n (%))	922 (52.2)	209 (54.5)	713 (51.5)	.163

Continuous variables (Age, BMI, WHR, SBP, DBP, TC, TG, HDL-C, LDL-C, FBG, pcsk9, sdLDL and eGFR) were expressed as mean ± SD, and categorical variables (Male, Current smoking, and Hypertension) were expressed as counts and percentages.

BMI=body mass index, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate, FBG=fasting blood glucose, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, PCSK9=proprotein convertase subtilisin/kexin type 9, SBP=systolic blood pressure, sdLDL=small dense low-density lipoprotein, TC=total cholesterol, TG=triglyceride, WHR=waist-to-hip ratio.

2.5. Statistical analysis

The dichotomous data are expressed as numbers and percentages, and continuous data as the median or mean ± standard deviation. After testing the normality, TG, HDL-C, eGFR, and PCSK9 were normalized by natural logarithm transformation as necessary. Correlation between plasma PCSK9 and sdLDL was evaluated by Pearson correlation analysis and multiple linear regression analysis. The SPSS version 17.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analysis, and a value of $P < .05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics of subjects included

A total of 1766 subjects were included in this study, including 733 (41.5%) males and 1033 (58.49%) females. The mean age was 61.40 ± 11.4 years. Among these subjects, 383 (21.7%) were diagnosed with DM. The baseline characteristics and laboratory findings of 1766 subjects are summarized in Table 1.

3.2. Correlation between plasma PCSK9 and sdLDL in the whole study population

Pearson correlation analysis showed age ($r = -0.119$; $P < .001$), BMI ($r = 0.095$; $P = .004$), TC ($r = 0.492$; $P < .001$), TG ($r = 0.426$; $P < .001$), HDL-C ($r = -0.209$; $P < .001$), LDL-C ($r = 0.505$; $P < .001$), FBG ($r = 0.095$; $P = .005$), and PCSK9 ($r = 0.263$; $P < .001$) were closely related to plasma sdLDL. In multiple linear regression analysis, the plasma sdLDL served as the dependent variable, and some confounders were adjusted (such as age, male, smoking status, BMI, WHR, SBP, DBP, TC, TG, LDL-C, HDL-C, FBG, and eGFR). Results showed the plasma PCSK9 had a positive correlation with plasma sdLDL (Table 2).

3.3. Correlation between plasma PCSK9 and sdLDL in the non-diabetic subpopulation

Pearson correlation analysis showed age ($r = -0.129$; $P < .001$), BMI ($r = 0.118$; $P = .001$), TC ($r = 0.517$; $P < .001$), TG ($r = 0.407$; $P < .001$), HDL-C ($r = -0.222$; $P < .001$), LDL-C ($r = 0.529$; $P < .001$), FBG ($r = 0.089$; $P = .015$), and PCSK9 ($r =$

Table 2**Correlation between PCSK9 and sdLDL in total study population.**

	Pearson's correlation		Multiple linear regression	
	r	P value	β	P value
Age (years)	-0.119	<.001	-0.097	.092
Male sex	-0.022	.501	0.647	.542
Current smoking	-0.009	.784	0.682	.591
BMI (kg/m ⁻²)	0.095	.004	0.140	.139
TC (mmol/L ⁻¹)	0.492	<.001	9.930	<.001
TG (mmol/L ⁻¹)	0.426	<.001	2.948	<.001
HDL-C (mmol/L ⁻¹)	-0.209	<.001	-10.903	<.001
LDL-C (mmol/L ⁻¹)	0.505	<.001		
FBG (mmol/L ⁻¹)	0.095	.005	-0.275	.489
eGFR (ml/min ⁻¹ /1.73 m ²)	0.075	.025	0.068	.145
PCSK9	0.263	<.001	15.749	<.001

BMI=body mass index, DBP=diastolic blood pressure, eGFR=estimated glomerular filtration rate, FBG=fasting blood glucose, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, PCSK9=proprotein convertase subtilisin/kexin type 9, SBP=systolic blood pressure, sdLDL=small dense low-density lipoprotein, TC=total cholesterol, TG=triglyceride, WHR=waist-to-hip ratio.

Table 3**Correlation between plasma PCSK9 and sdLDL levels in the non-diabetic subpopulation.**

	Pearson's correlation		Multiple linear regression	
	<i>r</i>	<i>P</i> value	β	<i>P</i> value
Age (years)	-0.129	<.001	-0.065	.537
Male sex	0.003	.93	0.056	.985
Current smoking	-0.024	.521	-1.677	.243
BMI (kg/m ⁻²)	0.118	.001	0.183	.149
TC (mmol/L ⁻¹)	0.517	<.001	9.894	<.001
TG (mmol/L ⁻¹)	0.407	<.001	2.456	<.001
HDL-C (mmol/L ⁻¹)	-0.222	<.001	-11.537	<.001
LDL-C (mmol/L ⁻¹)	0.529	<.001		
FBG (mmol/L ⁻¹)	0.089	.015	0.759	.395
eGFR (ml/min ⁻¹ /1.73 m ²)	0.081	.028	0.083	.625
PCSK9	0.267	.001	18.464	<.001

BMI = body mass index, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, PCSK9 = proprotein convertase subtilisin/kexin type 9, SBP = systolic blood pressure, sdLDL = small dense low-density lipoprotein, TC = total cholesterol, TG = triglyceride, WHR = waist-to-hip ratio.

0.263; $P < .001$) were closely related to plasma sdLDL. In multiple linear regression analysis, plasma sdLDL served as the dependent variable and several confounders were adjusted (such as age, male, current smoking, BMI, WHR, SBP, DBP, TC, TG, LDL-C, HDL-C, FBG, and eGFR). Results showed the plasma PCSK9 still had a close relationship with plasma sdLDL (Table 3).

3.4. Correlation between plasma PCSK9 and sdLDL in the diabetic subpopulation

Pearson correlation analysis indicated TC ($r = 0.421$; $P < .001$), TG ($r = 0.526$; $P < .001$), LDL-C ($r = 0.404$; $P < .001$), and PCSK9 ($r = 0.269$; $P = .001$) were closely related to plasma sdLDL. Age, sex, BMI, HDL-C and FBG had no relationship with plasma sdLDL in the diabetic subpopulation. In the multiple linear regression analysis, plasma sdLDL served as the dependent variable and several confounders were adjusted (such as age, male, current smoking, BMI, WHR, SBP, DBP, TC, TG, HDL-C, FBG and eGFR). Results showed plasma PCSK9 had no relationship with plasma sdLDL (Table 4).

4. Discussion

The present study showed a positive correlation between plasma PCSK9 and sdLDL in a community-based population. However, in subpopulation analysis, this correlation was only found in the non-diabetic population, but not in the type 2 DM patients after adjustment of several confounders.

The correlation between PCSK9 and sdLDL has increasingly attracted attentions in the past few years. PCSK9 directly mediates the degradation of LDL-R in lysosome, which in turn increases plasma LDL level. PCSK9 also regulates the synthesis and secretion of apolipoprotein B (APOB) through a LDL-R independent mechanism. There is evidence showing that elevated APOB is able to inhibit intracellular APOB autophagosome/lysosomal degradation,^[31,32] which will eventually increase the secretion of lipoproteins containing APOB, including VLDL, LDL and sdLDL.

But the correlation between plasma PCSK9 and sdLDL remains controversial among epidemiological studies. Our finding indicated that PCSK9 positively correlated with sdLDL in the community population, which was consistent with previously reported that plasma sdLDL was positively related

Table 4**Correlation between plasma PCSK9 and sdLDL levels in the diabetic subpopulation.**

	Pearson's correlation		Multiple linear regression	
	<i>r</i>	<i>P</i> value	β	<i>P</i> value
Age (years)	-0.148	.068	0.12	.827
Male sex	-0.149	.067	-5.362	.607
Current smoking	0.032	.692	0.889	.775
BMI (kg/m ⁻²)	0.010	.900	0.112	.600
Hypertension	0.044	.591	-2.245	.373
TC (mmol/L ⁻¹)	0.421	<.001	9.260	<.001
TG (mmol/L ⁻¹)	0.526	<.001	6.640	<.001
HDL-C (mmol/L ⁻¹)	-0.079	.335	-7.338	.165
LDL-C (mmol/L ⁻¹)	0.404	<.001		
FBG (mmol/L ⁻¹)	0.075	.356	-0.735	.118
eGFR (ml/min ⁻¹ /1.73 m ²)	0.046	.578	0.364	.628
PCSK9	0.269	.001	9.591	.142

BMI = body mass index, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, FBG = fasting blood glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, PCSK9 = proprotein convertase subtilisin/kexin type 9, SBP = systolic blood pressure, sdLDL = small dense low-density lipoprotein, TC = total cholesterol, TG = triglyceride, WHR = waist-to-hip ratio.

to Hetero-dimer PCSK9 concentration in CAD patients (n=164).^[33] Similar results were reported by Zhang et al that plasma sdLDL was only positively related to PCSK9 in patients with stable CAD (n=490).^[12] However, Kwakernaak et al reported the plasma PCSK9 was not associated with sdLDL in healthy subjects (n=52, n=124).^[34] This discrepancy might be explained as follows: Firstly, the study population was different among studies, such as sample size, race, and health status. Secondly, the lack of standardized method for sdLDL measurement might also cause the inconsistency among studies. Thirdly, although plasma PCSK9 positively correlated with sdLDL and total LDL-C, it played a relatively minor role in the complicated metabolic regulation of LDL-C.^[35]

The TG-rich very low density lipoprotein (VLDL) secreted by the liver is delipidated by lipoprotein lipase. The remaining TG in the VLDL is then transferred into LDL particles by cholesterol ester transfer protein. The sdLDL is the product of the LDL particles delipidated by hepatic lipase.^[4,36] The sdLDL has a longer half-life than ldLDL (3.10 d vs 1.95 d),^[37] and thus it is more susceptible to oxidative reaction.^[15] Therefore, the sdLDL is more potent to cause atherosclerosis, especially in metabolic syndrome, obesity, and diabetes.^[38-42]

Diabetic dyslipidemia is characterized by the elevated TG and sdLDL, decreased HDL-C and slightly elevated or normal LDL-C. A study shows that the ratio of sdLDL/LDL in healthy male adults is about 30% to 35%, whereas it reaches as high as 45% to 50% in diabetic subjects.^[43,44] Similar results were reported in a Japanese study.^[45] In our study, results showed no significant difference in LDL-C within the general population, but higher sdLDL was found in the diabetic subgroup. Moreover, the positive correlation between PCSK9 and sdLDL was not observed after adjusting multiple confounders, especially after adjusting TG in the diabetic subgroup. Higher sdLDL was mainly as a result of the abnormality of lipid metabolism in DM. Thus, it may account for the discrepancy in the association of PCSK9 with sdLDL between whole population and diabetic subjects when accounting for other lipid parameters, and might also be a main reason for the difference as compared to previous studies.

Our study for the first time reported the direct relationship between plasma PCSK9 and sdLDL in a community-dwelling population with a large sample size after consideration of confounders, but this correlation was absent in type 2 DM patients. A complete understanding of this discrepancy will facilitate the understanding of the importance of the relationship between sdLDL and PCSK9. However, this was a community-based study with limited ethnical and geographical population. In addition, PCSK9 might be altered by posttranslational modification, which might alter its function and affect the subsequent measurement of plasma PCSK9 by antibody-based system such as ELISA (used in our study).^[46]

In conclusion, our study shows a direct positive correlation between plasma PCSK9 and sdLDL in a community-dwelling population, but not in the type 2 DM patients after adjustment of multiple confounders.

Author contributions

Conceptualization: Yan Liu, Ping Ye.

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Supervision: Ping Ye.

Writing – original draft: Yan Liu.

Writing – review & editing: Ping Ye.

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