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Association between plasma PCSK9 levels and 10-year progression of carotid atherosclerosis beyond LDL-C: A cohort study

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ABSTRACT

Background: To evaluate the association of plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) levels with the progression of carotid atherosclerosis and identify additional PCSK9-lipoprotein-atherosclerosis pathway beyond low-density lipoprotein cholesterol (LDL-C).

Methods: Among 643 participants (aged from 45 to 74 years) free of cardiovascular disease at baseline, carotid ultrasound examinations were performed in 2002 (baseline) and 2012 (follow-up). None of the participants were taking lipid-lowering drugs or had detectable carotid plaques at baseline. Carotid plaque formation and total plaque area (TPA) were used to reflect 10-year progression of atherosclerosis.

Results: Baseline plasma PCSK9 levels have a wide variation, ranged from 64.60–532.20 ng/mL (median: 192.57 ng/mL). PCSK9 levels were significantly associated with new plaque formation after adjusting for LDL-C levels and other risk factors (relative risk for per quartile increase = 1.09, 95% confidence interval: 1.03–1.15). PCSK9 levels were also linearly associated with TPA after multivariate adjustment including LDL-C ($P = 0.008$). Among participants with the lowest or second tertile of LDL-C, PCSK9 quartiles were linearly associated with TPA ($P = 0.021$), but the association lost significance after additional adjustment for very low-density lipoprotein cholesterol (VLDL-C) tertiles ($P = 0.072$). Further stepwise linear regression (entry, 0.05; removal, 0.05) indicated that VLDL-C tertiles could be entered into the model but PCSK9 quartiles could not.

Conclusions: Plasma PCSK9 levels are associated with 10-year progression of atherosclerosis. The LDL-independent association of PCSK9 levels may through its ability to regulate VLDL-C levels. Further research is needed to systematically investigate the role of PCSK9 for the pathogenesis of atherosclerosis, beyond LDL-C metabolism.

1. Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a crucial protein in low-density lipoprotein cholesterol (LDL-C) metabolism because it promotes the degradation of low-density lipoprotein receptor (LDLR) and prevents it from recycling to the membrane [1]. Two recent randomized trials reported that PCSK9 inhibition, when added to maximal statin therapy or standard therapy, significantly decreased LDL-C levels by 60%, and then reduced cardiovascular events by about 50% [2,3].

Recent studies raised the issue that PCSK9 may have multiple roles beyond LDL-C associated function in the progression of atherosclerosis. The Dallas Heart Study indicated that even plasma concentrations of PCSK9 and LDL-C correlate with each other, less than 10% of the variance of PCSK9 was explained by plasma LDL-C concentration [4]. A recent review by Urban et al. summarized evidence from experimental studies and reported that PCSK9 might accelerate atherosclerosis by mechanisms independent of the LDLR [5]. Furthermore, an increasing amount of studies in humans also have identified correlations between circulating PCSK9 levels and other atherogenic risk factors, which support the possibility that PCSK9 has a broader physiological role than originally thought [4,6–10].

Recent studies found that very low-density lipoprotein cholesterol (VLDL-C), also known as remnant cholesterol or triglyceride-rich lipoproteins, is even more atherogenic than LDL-C [11]. Interesting, published articles indicated that PCSK9 may affect VLDL metabolism. As reported by Poirier et al. the activity of PCSK9 is not restricted to the LDLR but appears to contribute to the degradation of very low-density lipoprotein receptor (VLDLR) [12]. An observational cohort study found that plasma PCSK9 levels were associated with future cardiovascular events in 504 patients with coronary heart disease, but the

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association lost significance after adjustment for triglycerides [13]. The results from clinical trials also found that PCSK9 inhibition can not only reduce LDL-C levels but also decrease VLDL-C levels in an apparent dose–response fashion [14,15].

It is very important to further investigate the LDL-independent effects of PCSK9 and understand other potential beneficial therapeutic effects from PCSK9 inhibition. Therefore, the aims of this study are to test two hypotheses that plasma PCSK9 levels may have an LDL-independent association with the progression of atherosclerosis, reflected by carotid plaque formation and total plaque area (TPA), and PCSK9 may be associated with the progression of atherosclerosis through other lipoprotein pathways.

2. Methods

2.1. Study population

The Chinese Multi-provincial Cohort Study (CMCS) is a study that followed the Sino-MONICA Project [16]. A detailed description of the goals, design, and methods of the CMCS has been published elsewhere [17]. The current study is part of the CMCS and aimed to investigate the progression and determinants of subclinical atherosclerosis [18,19]. Supplementary Fig. S1 shows the sampling frame and this study design. Briefly, an age- and sex-stratified random sample of 1982 participants aged from 35 to 64 years was selected from a community in Beijing in 1992. Face-to-face follow-up interviews were carried out with the participants or family members to ascertain new cardiovascular events at the end of each year [17,20]. Of the 1982 participants, 53 died before September 2002. A total of 1324 subjects (69% of the remaining participants) took part in an examination of cardiovascular risk factors and a carotid B-mode ultrasound examination in September 2002. In September 2012, we invited all of the participants for a follow-up ultrasound re-examination. Of the 1324 participants, 440 individuals were excluded because of a history of cardiovascular disease (n = 68), presence of carotid plaques (n = 209), missing laboratory results (n = 21), or taking lipid-lowering drugs (n = 142) at baseline. An additional 241 individuals were excluded because an ultrasound re-examination was not performed (67 participants died before re-examination and 174 lost to follow-up). The remaining 643 participants (269 men and 374 women) with complete data were used for the analysis.

The protocol was approved by the Ethics Committee of Beijing Anzhen Hospital. Written informed consent was obtained from all of the participants.

2.2. Laboratory assays, carotid ultrasound protocol and risk factor survey

Plasma PCSK9 levels were measured after a second thaw using a commercially available quantitative sandwich ELISA assay by following the manufacturer's instructions (CY-8079; CycLex Co., Nagano, Japan) at the laboratory of MBL (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). A new carotid plaque is defined as the presence of focal wall thickening that is at least 50% greater than that of the surrounding vessel wall or as a focal region with intima-media thickness (IMT) greater than 1.5 mm [21]. In individuals with new carotid plaques in the six segments (bilateral common carotid arteries, bifurcations, and internal carotid arteries), the area of the maximum plaque at each segment was measured offline using commercial software (Carotid Analyzer; Vascular Research Tools 6, Medical Imaging Applications, Coralville, IA, USA. Supplementary Fig. S2). TPA was calculated as the sum of the areas of maximum plaques and a value of 0 was assigned to those with no plaque formation.

The details of laboratory assays, the carotid ultrasound protocol, the reproducibility study, and risk factor survey are available in Supplementary Methods.

2.3. Statistical analysis

The results are presented as percentages for categorical variables or mean ± standard deviation for continuous variables with a normal distribution. Triglycerides, fasting insulin, high-sensitivity C-reactive protein (hsCRP), and PCSK9 levels are shown as medians with interquartile ranges because their distribution was highly skewed. Correlations between PCSK9 levels and cardiovascular risk factors were evaluated using the Spearman correlation method by sex. The baseline characteristics of the participants with and without new carotid plaque formation were compared using the *t*-test, Wilcoxon rank test, or chi-square test.

Modified Poisson regression was performed to assess the strength of associations between baseline PCSK9 levels and plaque formation [22]. Modified Poisson regression was used rather than Logistic regression because plaque formation was not rare. The mean values of TPA by baseline LDL-C or low-density lipoprotein particle (LDL-P) tertiles and PCSK9 quartiles were also calculated by using analysis of covariance after multivariable adjustment. The linear trend was tested using multiple linear regression analysis. The 3 × 4 subgroup sample sizes were all greater than 30 (Supplementary Fig. S3). To evaluate the modifying effects of baseline risk factors on the association between PCSK9 levels and atherosclerosis progression, we used Z-test to compare the difference between the two regression coefficients from subgroup analysis by using the following equation: $Z = \frac{\beta 1 - \beta 2}{\sqrt{SE(\beta 1)^2 + SE(\beta 2)^2}}$. [23]

According to the data collected at re-examination (2012), 105 individuals (16.3%) reported having taken statins. Because statin therapy during the follow-up period may affect our results, estimates were adjusted for baseline risk factors as well as the status of statin therapy at re-examination.

Statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA). All analyses were two-sided, with a *P* value of 0.05 considered to indicate statistical significance.

3. Results

3.1. Baseline characteristics

All of the 643 participants without carotid plaque were free of cardiovascular disease or lipid-lowering medication use at baseline.

Table 1
Baseline characteristics of the study participants.

Characteristic	Men (n = 269)	Women (n = 374)
Age (years)	59.8 ± 7.5	55.9 ± 7.4
PCSK9 (ng/mL)	174.20 (138.82–231.59)	198.40 (157.78–247.07)
LDL-C (mmol/L)	3.28 ± 0.75	3.34 ± 0.79
LDL-P number (nmol/L)	1055.8 ± 243.6	1064.0 ± 255.2
HDL-C (mmol/L)	1.31 ± 0.26	1.49 ± 0.31
VLDL-C (mmol/L)	0.75 ± 0.43	0.75 ± 0.42
TG (mmol/L)	1.23 (0.93–1.72)	1.17 (0.87–1.72)
FBG (mmol/L)	4.89 ± 0.97	4.79 ± 0.88
Fasting insulin (pmol/L)	42.49 (32.04–58.51)	41.79 (31.34–57.11)
HsCRP (mg/L)	0.69 (0.33–1.31)	0.80 (0.37–1.83)
SBP (mm Hg)	129.3 ± 17.6	125.0 ± 17.8
BMI (kg/m^2)	24.9 ± 2.8	24.9 ± 3.6
Current smoking (%)	68 (25.3)	3 (0.8)
Hypertension (%)	130 (48.3)	141 (37.7)
Antihypertensive medication (%)	71 (26.4)	83 (22.2)
Type 2 diabetes (%)	11 (4.1)	17 (4.6)

The results are presented as mean ± SD, median (quartile 1–quartile 3), or n (%).
PCSK9 indicates proprotein convertase subtilisin/kexin type 9; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particle; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TG, triglycerides; FBG, fasting blood glucose; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; BMI, body mass index.