Circulating Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Concentration and Risk of Cardiovascular Events — Systematic Review and Meta-Analysis of Prospective Studies —

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Background: Previous studies have not found a consistent association between circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) and the risk of cardiovascular events. The aim of this meta-analysis was to evaluate this association in prospective studies.

Methods and Results: A systematic search of prospective studies published through October 2016 was carried out in order to identify studies that met pre-specified inclusion criteria. After independent data extraction, summary relative risks were calculated using random-effects models. On meta-analysis of 6 cohort and 1 nested case-control study, circulating PCSK9 concentration as a continuous variable was not significantly associated with the risk of cardiovascular events (overall RR, 1.12; 95% CI: 0.98–1.29; $P=0.09$), with significant heterogeneity ($I^2=55.1\%$, $P_{\text{heterogeneity}}=0.038$). The highest but not middle categories of circulating PCSK9 was significantly associated with the risk of cardiovascular events. On subgroup analysis of study design, mean age at baseline, sample size, follow-up time, and pre-existing disease, there was no significant association between PCSK9 and cardiovascular events. Sensitivity analysis with various exclusion and inclusion criteria did not materially change the results.

Conclusions: Circulating PCSK9 concentration as a continuous variable was not significantly associated with the risk of cardiovascular events. More well-designed studies are needed to clarify the role of PCSK9 in cardiovascular risk.

Key Words: Cardiovascular; Event; Meta-analysis; Proprotein convertase subtilisin/kexin type 9 (PCSK9); Prospective study

The proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme plays an essential role in regulating cholesterol homeostasis. PCSK9 enhances the endosomal and lysosomal degradation of hepatic low-density lipoprotein (LDL) receptor (LDL-R), resulting in increased serum LDL cholesterol (LDL-C) concentration.^{1–3}

Mutations in *PCSK9* can influence circulating PCSK9 level.⁴ The coding gene of human PCSK9 protein is located on chromosome 1p32.3; it encompasses 12 exons and encodes a 692-amino acid glycoprotein. To date, 163 mutations in *PCSK9* have been identified, involving both gain of function and loss of function. Mutations in *PCSK9* have been shown to be associated with LDL-C and cardiovascular disease (CVD) in large-scale genome-wide association studies.^{5,6} Hence, PCSK9 has emerged as a novel attractive target in lowering LDL-C. Currently, statins are

Editorial p 1098

the recommended first-line agents for lowering LDL-C but may increase plasma PCSK9, whereas statins combined with PCSK9 inhibitors may counteract the effect of increased PCSK9.^{7,8} In recent years, some early phase clinical trials have shown that PCSK9 inhibitors can reduce plasma LDL-C in patients with familial and non-familial hypercholesterolemia and yield the expected reduction in CVD events without major adverse effect.^{9,10} PCSK9 reduction may also be beneficial to the other lipids and lipoproteins, such as total cholesterol, triglycerides, and high-density lipoprotein cholesterol.

Despite these findings, whether plasma PCSK9 level accurately predicts the risk of cardiovascular events, inde-

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pendent of its effects on LDL-C concentration, remains controversial. The inconsistent results across different studies may have resulted from different measurement methods of plasma PCSK9, variations in study design or differences in clinical outcome. In order to use PCSK9 as a suitable biomarker in CVD prevention trials, it is important to clarify the role of circulating PCSK9 in independently predicting cardiovascular events beyond LDL-C. To address this knowledge gap, we performed a meta-analysis of prospective studies to investigate the pleiotropic effect of circulating PCSK9 level on risk of cardiovascular events by calculating a summary risk estimate.

Methods

Data Sources and Literature Search

This study followed the guidelines of the Meta-analysis of Observation Studies in Epidemiology group.¹¹ Databases including PubMed, EMBASE, OVID, Google Scholar, and Cochrane Library were searched through October 2016 for relevant articles that reported the association between circulating PCSK9 level and the risk of cardiovascular events. To avoid missing any relevant study, we also searched the references of retrieved papers and recent reviews in the field. The following medical subject headings (MESH) and key words were used in the literature search: “proprotein convertase subtilisin/kexin type 9”, or “PCSK9”, or “neural apoptosis regulated convertase-1”, or “NARC-1”, and “CVD”, or “cardiovascular events”, or “coronary disease”, or “myocardial infarction”, or “myocardial ischemia”, or “coronary stenosis”, “coronary restenosis”, or “cerebrovascular disorders”, or “stroke”, or “heart failure”, or “death”, “mortality”, or “all-cause mortality”, or “cardiovascular mortality”, and “cohort studies”, or “prospective studies”, or “follow-up studies”. No restrictions were imposed. English and non-English articles were included in the search strategy.

Study Selection

Two reviewers independently screened the titles and abstracts of the searched papers and excluded the articles if they did not clearly study the association between PCSK9 and CVD events using a prospective design. The same 2 reviewers also independently evaluated the remaining full-text articles to assess the study eligibility on the basis of a predefined set of criteria. The inclusion criteria were as follows: (1) full-text study, published prospective cohort or nested case-control study; (2) plasma or serum PCSK9 concentration as exposure of interest; (3) all-cause or cardiovascular mortality or CVD events, myocardial infarction, stroke, or heart failure as outcome of interest; and (4) calculation of relative risk (RR) and the corresponding 95% CI or provision of sufficient data to calculate them. Disagreements on study eligibility were resolved by discussion.

Data Extraction and Quality Assessment

The key exposure variable was circulating PCSK9 concentration at baseline. PCSK9 was measured using enzyme-linked immunosorbent assay (ELISA) kit. With the exception of 1 study,¹² most studies on the incidence of cardiovascular events reported circulating PCSK9 as a continuous variable. Four studies on cardiovascular events reported PCSK9 as a categorical variable. In the present meta-analysis PCSK9 was used as both a continuous and

as a categorical variable.

Outcomes of interest in this meta-analysis included CVD events, defined as fatal and non-fatal coronary artery disease (CAD), fatal and non-fatal stroke, cardiac arrest, heart failure, peripheral artery disease, and sudden death. CAD was defined as acute myocardial infarction, angina pectoris, and other ischemic heart disease.

Two reviewers independently abstracted data on study characteristics and results using a standardized data collection form. Discrepancies in data extraction between reviewers were resolved by consensus. Data extracted included: first author's last name; year of publication; country of origin; study period and duration of follow-up; subject characteristics and age at baseline; number of CVD events, and total number of participants; PCSK9 measurement methods; assessment of outcomes; RR, hazard ratios (HR), OR or incidence density ratio; and adjusted variables. We also systematically assessed the study quality. Briefly, a 9-point system based on the Newcastle Ottawa Scale was used to assess study quality. Each study was evaluated on 3 broad criteria: (1) subject selection; (2) comparability of the subjects; and (3) ascertainment of the exposure or outcome of interest. Two reviewers independently assessed the quality of each study. Disagreements were resolved through discussion to reach a consensus. Studies that scored ≥ 7 (out of a maximum 9 points) were considered to be high quality.

Statistical Analysis

Relative risk was used as a common measure of the association between PCSK9 and the risk of CVD events. HR and incidence density ratios were considered as estimates of RR. RR and corresponding standard errors (SE), which were derived from 95% CI or P-values, were logarithmically transformed for the studies in which the distribution of circulating PCSK9 was skewed, to stabilize variance and normalize the distribution.¹³ To calculate summary RR and its 95% CI, we pooled the results using a random-effects models.¹⁴ The random-effects model was chosen a priori because of the anticipated clinical heterogeneity and because it is considered as more conservative than the fixed-effects model, because it accounts for both within- and between-study heterogeneity.¹⁵ Heterogeneity across studies was evaluated using the Q statistic with a conservative $P=0.10$. We also calculated the I^2 statistic, which describes the total variation across studies attributable to heterogeneity rather than chance; $I^2>50\%$ indicates at least moderate heterogeneity.¹⁶ We assessed the influence of each individual study on the overall risk estimate by excluding 1 study at a time. Because participant characteristics, PCSK9 measurement, and adjustments for confounding factors were not consistent across studies, we further conducted several sensitivity and stratified analyses to explore possible sources of heterogeneity and to examine the influence of various inclusion criteria on the overall risk estimate. Stratified analysis was also carried out for age, number of total participants, duration of follow-up, and presence of pre-existing CAD. Because statins influence PCSK9 concentration, we analyzed the effect of PCSK9 on the risk of CVD events according to statin status. Sensitivity analysis was also carried out for studies with the same ELISA kits for PCSK9 measurement. Potential publication bias was evaluated on visual inspection of the Begg funnel plots in which log RR were plotted against their SE. Begg rank correlation test and Egger

linear regression test were also carried at the $P < 0.10$ level.^{17,18} All analysis was performed using STATA version 11.0 (Stata Corp LP, College Station, TX, USA). $P < 0.05$ was considered statistically significant, except where otherwise specified.

Results

Study Selection

Of the 1,270 reports retrieved from the initial search, we excluded 1,181 studies unrelated to the association between PCSK9 and the risk of CVD events after first screening based on abstracts or titles. A full-text review of the remaining 89 papers further excluded 82 studies that were not prospective cohort studies or nested case-control studies. Fifteen studies that did not report CVD outcomes and an additional 5 studies that did not report circulating PCSK9 concentration were also excluded. Furthermore, a retracted paper that reported PCSK9 and CVD outcomes was also excluded.¹⁹ Seven studies were ultimately included in the present meta-analysis (Figure 1).

Study Characteristics

Table 1 lists the characteristics of the 7 prospective studies including 6 cohort^{20–25} and 1 nested case-control study.¹² These studies were published between 2014 and 2016 from different countries. The subject age range was 49.4 to 68 years old. One study was limited to male respondents;²² another was conducted in women only,¹² and the other 5 studies reported combined results for both men and women. The mean length of follow-up ranged from 1 to 17 years. Study sample size ranged from 443 to 4,232 (total, 10,068). Of the 6 cohort studies, 1 study was conducted in patients with chronic kidney disease,²⁵ 1 among community-based adults,²⁴ 3 in CAD patients, and 1 in male fire-

fighters.²² Mean PCSK9 in these 6 studies was 303 ng/mL and ranged from 94.3 to 546.5 ng/mL. Of the 7 studies included, only 1 reported all-cause mortality,²³ and the other studies reported CVD events including similar CVD outcomes. None of the 7 studies, however, reported associations between PCSK9 and the risk of discrete CVD events (e.g., cardiovascular mortality, myocardial infarction, stroke, or heart failure). CVD outcomes were assessed using a variety of sources, including medical records, death certificates, and hospital database. All the studies were adjusted for a group of conventional cardiovascular risk factors, and the RR or HR from the model adjusted for the maximum number of risk factors were used to calculate summary estimates. The quality of the included 8 studies was high; 2 studies scored a maximum of 9 points and the other 5 studies scored 8 points, using the Newcastle–Ottawa Scale evaluation system (Table 1).

Circulating PCSK9 as a Continuous Variable

Figure 2 presents the results from the random-effects model combining the RR for the risk of CVD events. RR varied from 0.33 to 1.48 across the different studies but only one of the 7 studies showed a significantly positive association between PCSK9 and the risk of CVD events (RR, 1.48; 95% CI: 1.12–1.95; $P < 0.001$).²⁴ Overall, circulating PCSK9 concentration as a continuous variable was not significantly associated with the risk of CVD events (RR, 1.12; 95% CI: 0.98–1.29, $P = 0.09$). Significant heterogeneity across the studies was observed ($I^2 = 55.1\%$, $P_{\text{heterogeneity}} = 0.038$). Stratified analysis by study design shows similar results. The combined RR was 1.12 (95% CI: 0.97–1.31, $P = 0.117$) in the 6 cohort studies, with moderate heterogeneity ($I^2 = 61.5\%$, $P_{\text{heterogeneity}} = 0.023$). Similarly, the association between PCSK9 and the risk of CVD events in the 1 nested case-control study was also not significant (RR, 1.15; 95% CI: 0.74–1.79, $P = 0.535$).

Circulating PCSK9 as a Categorical Variable

The association between PCSK9 and the risk of CVD events was further analyzed using results from the 3 studies that assessed this association using PCSK9 as a categorical variable. Among the 3 studies, 2 divided PCSK9 into quartiles, and the other used tertiles. We first combined the HR of quartiles 2 and 3 from the 2 quartile studies into the middle PCSK9 category. Then, we calculated the summary HR for the middle and highest PCSK9 categories, respectively, in relation to the risk of CVD events, with the lowest category as the reference. The combined RR for the risk of CVD events from the random-effects models are shown in Figure 3. Overall, there was no significant association between the middle PCSK9 category and the risk of CVD events (RR, 1.07; 95% CI: 0.89–1.29, $P = 0.485$; $I^2 = 26.2\%$, $P_{\text{heterogeneity}} = 0.258$). The highest PCSK9 category, however, was significantly associated with the risk of CVD events (RR, 1.38; 95% CI: 1.12–1.72, $P = 0.003$; $I^2 = 0.0\%$, $P_{\text{heterogeneity}} = 0.636$).

Stratified and Sensitivity Analyses

The overall combined RR were not significant when stratified according to mean age (≤ 60 and > 60 years), sample size ($< 1,000$ and $> 1,000$), duration of follow-up (≤ 4 and > 4 years), and presence of pre-existing CAD. The association between PCSK9 and the risk of CVD events was stronger in studies with sample size $> 1,000$ (RR, 1.08; 95% CI: 1.00–1.15) than in studies with $< 1,000$ participants (RR,

Table 1. Circulating PCSK9 and Risk of CVD Events: Characteristics of 7 Prospective Studies

Source	Location and study design	Subjects	Sex/age (years)	PCSK9 concentration (ng/mL) Median (range)	Duration (years)	Adjusted risk factors	Outcomes	Quality score
Leander et al (2016) ²⁴	Sweden, cohort	4,232 population-based adults	M/F 60-year-olds	94.3 (73.1–122.3)	15	Sex, LDL-C, HDL-C, lipoprotein(a), triglycerides, hypertension, DM, obesity, overweight, physical inactivity, current smoking, and statin use.	CVD events: fatal or non-fatal MI, angina pectoris, chronic ischemic heart disease, sudden cardiac death, and fatal or non-fatal ischemic stroke.	9
Li et al (2015) ²¹	China, cohort	616 patients with stable CAD	M/F 57.88±9.82	230.11 (190.45–277.83)	1.5	Age, sex, BMI, hypertension, diabetes, hypercholesterolemia, hypo-HDL cholesterol, current smoking, family history of CAD, uric acids, and hs-CRP.	MACE: cardiac death, stroke, MI, post-discharge revascularization, or unstable angina.	8
Rogacev et al (2016) ²⁵	Germany, cohort	443 CKD patients	M/F 67.7 (57.0–74.3)	343 (270–413)	3.0	Age, GFR, total cholesterol, Apo A-I, Apo B and mean BP, gender, prevalent CVD and DM, statin intake.	Acute MI; surgical or interventional coronary/cerebrovascular/peripheral-arterial revascularization; stroke with symptoms ≥24 h, amputation above the ankle; or death from any cause.	8
Gencer et al (2016) ²³	Switzerland, cohort	2,030 ACS patients undergoing coronary angiography	M/F 63.6±12.5	323 (374–396)	1	Cardiovascular risk factor (sex, history of hypertension, history of DM, smoking status, baseline total cholesterol, and baseline use of statin). GRACE (ST-segment deviation, age, heart rate, SBP, creatinine, Killip class, cardiac arrest at admission, and elevated biomarkers of necrosis).	All-cause death (cardiac, vascular, and non-cardiovascular) major adverse cardiovascular events: cardiac death or recurrent MI events, cardiac death or recurrent MI or stroke events, cardiac death or recurrent MI or coronary revascularization events.	8
Zhu et al (2015) ²²	Canada, cohort	1,527 male firefighters	M 49.4±9.9	286 (231–355)	7.2	Age, Framingham risk score, metabolic syndrome, DM, fasting glucose, insulin, hypertension, current smoking status, family history, on hypertension medication, on statin therapy, waist circumference, BMI, total cholesterol, HDL-C, LDL-C, triglycerides, hs-CRP.	Composite of cardiovascular death; resuscitated cardiac arrest; non-fatal MI; revascularization in the coronary, carotid, or peripheral circulation; symptomatic vascular disease with >50% stenosis; and incidences of stroke or transient ischemic attack.	8
Werner et al (2014) ²⁰	Germany, cohort	504 consecutive patients who had a documented clinically stable CAD	M/F 68 (59–74)	546.5 (471–622)	4	Age, gender, risk factors, medication, glucose metabolism and lipids.	Cardiovascular death and cardiovascular hospitalization for ACS or hospitalization for unplanned, symptom-induced coronary angiography and coronary revascularization.	8
Ridker et al (2016) ¹²	USA, nested case-control	358 first ever cardiovascular events case and 358 control	F 63 (58–68)	299.7 (252.9–358.8)	17	Age, smoking, and HRT.	First ever cardiovascular events: MI, thromboembolic stroke, or cardiovascular death.	9

ACS, acute coronary syndrome; Apo, apolipoprotein; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; CKD, chronic kidney disease; CVD, cardiovascular disease; DM, diabetes mellitus; GFR, glomerular filtration rate; GRACE, Global Registry of Acute Coronary Events; HDL-C, high-density lipoprotein cholesterol; HRT, hormone replacement therapy; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MACE, major adverse cardiovascular events; MI, myocardial infarction; PCSK9, proprotein convertase subtilisin/kexin type 9; SBP, systolic blood pressure.

Figure 2. Random-effects model combining the relative risk (RR) for cardiovascular disease events and circulating proprotein convertase subtilisin/kexin type 9 as a continuous variable.

Figure 3. Association between high and middle circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) levels and risk of cardiovascular disease (CVD) events. RR, relative risk.

1.04; 95% CI: 0.83–1.25) and in studies with follow-up >4 years than in those ≤4 years (follow-up >4 years: RR, 1.08; 95% CI: 1.00–1.17; follow-up ≤4 years: RR, 1.01; 95% CI: 0.89–1.14). No significant effect of PCSK9 on CVD risk was observed with regard to statins status (Table 2). Sensitivity analysis was also conducted with different inclusion criteria. Werner et al found that PCSK9 was significantly associated with the risk of CVD events in the model unadjusted for fasting triglyceride concentration (RR, 1.44; 95% CI: 1.02–2.04; P=0.038).²⁰ The association, however, became non-significant after adjustment for fasting triglyceride concentration (RR, 1.32; 95% CI: 0.93–1.87; P=0.126).²¹ In the present meta-analysis, we found that using the HR from the model unadjusted for fasting triglyceride concentration did not change the overall risk estimate (RR, 1.04; 95% CI: 0.99–1.10; I²=48.3%, P=0.060). Furthermore, Li et al reported that the association was significant in CAD patients with medication alone but not in those with percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG).²¹ We assessed the risk estimates in these 2 subgroups and the results essentially remained the same. Sensitivity analysis according to type of ELISA kit used for PCSK9 measure-

ment also did not change the results (Table 2). In addition, exclusion of any single study did not materially alter the combined estimate. The combined RR ranged from 1.008 to 1.082, and all the 95% CI included 1, suggesting that the association between PCSK9 and the risk of CVD events was not statistically significant.

Publication Bias

Although the funnel plot was slightly asymmetric, after using the trim-and-fill method, visual inspection of the Begg funnel plot did not identify substantial asymmetry (Figure 4). In addition, there was no evidence of publication bias (Begg test, P=0.764; Egger test, P=0.205).

Discussion

To our knowledge, this is the first meta-analysis of prospective studies to summarize the evidence of the association between circulating PCSK9 concentration and the risk of CVD events. Circulating PCSK9 as a continuous variable was not significantly associated with the risk of CVD events. Moreover, subgroup and sensitivity analysis did not materially change the results.

Table 2. Studies on Circulating PCSK9 and Risk of CVD Events: Stratified Analysis

	No. studies	RR	Low 95% CI	High 95% CI	I ² (%)	P value
Overall	7	1.03	0.98	1.09	37.9	0.127
Stratified analysis						
Mean age at baseline (years)						
≤60	3	1.08	0.96	1.20	76.9	0.013
>60	4	1.03	0.97	1.10	1.0	0.400
Sample size						
<1,000	3	1.04	0.83	1.25	29.1	0.238
>1,000	4	1.08	1.00	1.15	32.2	0.219
Follow-up						
≤4 years	4	1.01	0.89	1.14	24.1	0.267
>4 years	3	1.08	1.00	1.17	32.0	0.220
Pre-existing disease						
CAD	3	1.10	1.00	1.24	0.0	0.566
No CAD	4	1.08	0.98	1.18	43.0	0.135
Statin status						
Statin used	3	1.17	0.97	1.41	23.6	0.27
No statin therapy	4	1.02	0.96	1.08	6.4	0.361
Sensitivity analysis						
Werner et al (2014) ²⁰ model						
Unadjusted for fasting triglyceride	7	1.04	0.99	1.10	48.3	0.060
Adjusted for fasting triglyceride	7	1.05	0.99	1.11	52.1	0.041
Li et al (2015) ²¹ model						
PCI/CABG	7	1.04	0.98	1.10	50.3	0.050
Medication alone	7	1.04	0.99	1.10	46.1	0.072
ELISA kit used						
R&D systems	4	1.01	0.96	1.08	6.4	0.361
Cyclex Japan	2	0.86	0.25	3.00	58.4	0.121

CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention. Other abbreviations as in Table 1.

The methodological discrepancies, for example, sample size, duration of follow-up, and other aspects of the included studies may at least partially explain the negative results. Further, fasting status and type of blood sample (serum or plasma) also differed across the studies, resulting in a large range of mean PCSK9 (94.3–546.5 ng/mL).^{24,20}

Differences in treatment regimen may have also contributed to the different results. Plasma PCSK9 predicted CVD events in CAD patients with medication alone but not in those with PCI/CABG treatment.²¹ Another study observed a significant association between PCSK9 and CVD risk in patients treated with low-dose but not high-dose atorvastatin.¹⁹ Statins can upregulate PCSK9 expression and modify the association between PCSK9 concentration and CVD risk.^{7,8,26} This upregulation of PCSK9 has the potential to limit the efficacy of statin-induced LDL-C reduction.^{27,28} PCSK9 binds to LDL-R and directs it toward lysosomal degradation rather than to normal recycling.^{29,30} Hence, inhibiting PCSK9 is a logical strategy to enhance statin-induced LDL-C reduction to decrease cardiovascular risk.³¹ The PCSK9 monoclonal antibody is of great interest because it blocks binding to LDL-R via an allosteric mechanism.³² PCSK9 inhibitors have been found to reduce plasma LDL-C in some early-phase clinical trials,^{9,10} but it remains unknown whether the reduced LDL-C induced by PCSK9 inhibitors can lower CVD mortality in the long term.

Recently, there has been increasing interest in the role of PCSK9 variants in lipid homeostasis and the pathogenesis

Figure 4. Funnel plot of the associations between circulating proprotein convertase subtilisin/kexin type 9 and risk of cardiovascular disease events.

of CAD. A linear relationship was observed between PCSK9 variants and LDL-C or CAD risk in Mendelian randomized studies. Gain-of-function mutation of PCSK9 (E670G) was associated with increased LDL-C and severity of CAD hypercholesterolemia,^{33–35} whereas loss-of-function variants (C679X, Y142X, and R46L) were associated with reduced mean LDL-C and CAD risk.⁶ Furthermore, clinical trials have shown that inhibition of

PCSK9 by a monoclonal antibody significantly reduced LDL-C and reduced the incidence of cardiovascular events.^{9,10} This suggests that the effect of PCSK9 variants or PCSK9 inhibitors on CVD risk is mainly due to its regulation of LDL-C. The negative results of the present meta-analysis also indicate that there may be no pleiotropic effect of PCSK9 variants and level on CVD risk beyond LDL-C. No study, however, has simultaneously investigated the effect of PCSK9 variants on circulating PCSK9 level and of genotype-phenotype interaction on the risk of CVD events. Because genotype distribution differs with ethnicity, the association between circulating PCSK9 and the risk of CVD events might be affected by the prevalence of PCSK9 polymorphisms. Further studies are needed in order to test this hypothesis.

The present study has several limitations. First, we noted substantial heterogeneity among the included studies, which may affect the interpretation of the results. The between-study heterogeneity may be influenced differing subject characteristics. Despite our efforts to identify the sources of heterogeneity on stratified and sensitivity analyses, the heterogeneity was not explained entirely. Second, uncontrolled or unmeasured risk factors may potentially introduce bias. Although all the included studies were adjusted for a group of conventional risk factors, the possibility of residual confounding cannot be completely ruled out, because these risk factors do not explain all of the risk of CVD events. Furthermore, the influencing factors of circulating PCSK9 and the correlations between PCSK9 and other risk factors differed between studies. In addition, because the distribution of circulating PCSK9 was skewed in some studies, PCSK9 was log transformed in those studies and the combined risk estimates included both log-transformed and non-transformed HR. Finally, although we used broad inclusion criteria to minimize selection bias, and confirmed that this was unlikely on statistical analysis, the possibility cannot be completely ruled out. The capacity to detect publication bias is reduced when meta-analyses are based on a relatively small number of included studies.^{17,18} Selective reporting also cannot be excluded.

Conclusions

Although PCSK9 is a target for LDL-C reduction, its circulating level as a continuous variable is not associated with the risk of cardiovascular events. It seems that there is no pleiotropic effect of PCSK9 on CVD risk. A well-designed multicenter study with standardized methodologies is needed to clarify whether PCSK9 is a biomarker for cardiovascular risk.

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Disclosures

The authors declare no conflicts of interest.

References

- Levy E, Ben Djoudi Ouadda A, Spahis S, Sane AT, Garofalo C, Grenier E, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. *Atherosclerosis* 2013; **227**: 297–306.
- Lagace TA, Curtis DE, Garuti R, McNutt MC, Park SW, Prather HB, et al. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. *J Clin Invest* 2006; **116**: 2995–3005.
- Zhang DW, Lagace TA, Garuti R, Zhao Z, McDonald M, Horton JD, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. *J Biol Chem* 2007; **282**: 18602–18612.
- Chernogubova E, Strawbridge R, Mahdessian H, Malarstig A, Krapivner S, Gigante B, et al. Common and low-frequency genetic variants in the PCSK9 locus influence circulating PCSK9 levels. *Arterioscler Thromb Vasc Biol* 2012; **32**: 1526–1534.
- Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; **34**: 154–156.
- Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006; **354**: 1264–1272.
- Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G, Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. *J Lipid Res* 2008; **49**: 394–398.
- Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ. High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. *J Lipid Res* 2010; **51**: 2714–2721.
- Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, et al. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015; **372**: 1500–1509.
- Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, et al. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med* 2015; **372**: 1489–1499.
- Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: A proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008–2012.
- Ridker PM, Rifai N, Bradwin G, Rose L. Plasma proprotein convertase subtilisin/kexin type 9 levels and the risk of first cardiovascular events. *Eur Heart J* 2016; **37**: 554–560.
- Walter SD, Cook RJ. A comparison of several point estimators of the odds ratio in a single 2x2 contingency table. *Biometrics* 1991; **47**: 795–811.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177–188.
- Riley RD, Higgins JP, Deeks JJ. Interpretation of random effects meta-analyses. *BMJ* 2011; **342**: d549.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557–560.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629–634.
- Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088–1101.
- Huijgen R, Boekholdt SM, Arsenault BJ, Bao W, Davaine JM, Tabet F, et al. Plasma PCSK9 levels and clinical outcomes in the TNT (Treating to New Targets) trial: A nested case-control study. *J Am Coll Cardiol* 2012; **59**: 1778–1784.
- Werner C, Hoffmann MM, Winkler K, Bohm M, Laufs U. Risk prediction with proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with stable coronary disease on statin treatment. *Vascul Pharmacol* 2014; **62**: 94–102.
- Li JJ, Li S, Zhang Y, Xu RX, Guo YL, Zhu CG, et al. Proprotein convertase subtilisin/kexin type 9, C-reactive protein, coronary severity, and outcomes in patients with stable coronary artery disease: A prospective observational cohort study. *Medicine* 2015; **94**: e2426.
- Zhu YM, Anderson TJ, Sikdar K, Fung M, McQueen MJ, Lonn EM, et al. Association of proprotein convertase subtilisin/kexin type 9 (PCSK9) with cardiovascular risk in primary prevention. *Arterioscler Thromb Vasc Biol* 2015; **35**: 2254–2259.
- Gencer B, Montecucco F, Nanchen D, Carbone F, Klingenberg R, Vuilleumier N, et al. Prognostic value of PCSK9 levels in patients with acute coronary syndromes. *Eur Heart J* 2016; **37**: 546–553.
- Leander K, Malarstig A, Van't Hooft FM, Hyde C, Hellenius ML, Troutt JS, et al. Circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) predicts future risk of cardiovascular events independently of established risk factors. *Circulation* 2016; **133**: 1230–1239.
- Rogacev KS, Heine GH, Silbernagel G, Kleber ME, Seiler S,

- Emrich I, et al. PCSK9 plasma concentrations are independent of GFR and do not predict cardiovascular events in patients with decreased GFR. *PLoS One* 2016; **11**: e0146920.
26. Berge KE, Ose L, Leren TP. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. *Arterioscler Thromb Vasc Biol* 2006; **26**: 1094–1100.
27. Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L, et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1454–1459.
28. Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D, Park SW. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. *J Lipid Res* 2008; **49**: 399–409.
29. Lambert G. Unravelling the functional significance of PCSK9. *Curr Opin Lipidol* 2007; **18**: 304–309.
30. Lambert G, Charlton F, Rye KA, Piper DE. Molecular basis of PCSK9 function. *Atherosclerosis* 2009; **203**: 1–7.
31. Steinberg D, Witztum JL. Inhibition of PCSK9: A powerful weapon for achieving ideal LDL cholesterol levels. *Proc Natl Acad Sci USA* 2009; **106**: 9546–9547.
32. Fitzgerald K, Frank-Kamenetsky M, Shulga-Morskaya S, Liebow A, Bettencourt BR, Sutherland JE, et al. Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: A randomised, single-blind, placebo-controlled, phase 1 trial. *Lancet* 2014; **383**: 60–68.
33. Huang CC, Fornage M, Lloyd-Jones DM, Wei GS, Boerwinkle E, Liu K. Longitudinal association of PCSK9 sequence variations with low-density lipoprotein cholesterol levels: The Coronary Artery Risk Development in Young Adults Study. *Circ Cardiovasc Genet* 2009; **2**: 354–361.
34. Slimani A, Harira Y, Trabelsi I, Jomaa W, Maatouk F, Hamda KB, et al. Effect of E670G polymorphism in PCSK9 gene on the risk and severity of coronary heart disease and ischemic stroke in a Tunisian cohort. *J Mol Neurosci* 2014; **53**: 150–157.
35. Hsu LA, Teng MS, Ko YL, Chang CJ, Wu S, Wang CL, et al. The PCSK9 gene E670G polymorphism affects low-density lipoprotein cholesterol levels but is not a risk factor for coronary artery disease in ethnic Chinese in Taiwan. *Clin Chem Lab Med* 2009; **47**: 154–158.