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High circulating proprotein convertase subtilisin/Kexin type 9 concentration associates with cardiovascular risk

A meta-analysis of cohort studies

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Abstract

Whether the baseline circulating proprotein convertase subtilisin/Kexin type 9 (PCSK9) concentration associates with cardiovascular risk remains uncertain. This study aimed to investigate the predictive value of circulating PCSK9 in cardiovascular risk prediction.

Relevant studies were searched through the *MEDLINE*, *EMBASE*, and *Cochrane Library* databases. The relative risk (RR) and 95% confidence interval (CI) were pooled to evaluate the association between the circulating PCSK9 concentration and cardiovascular risk. Dose–response meta-analysis was also performed in this study.

A total of 11 cohort studies with 13,761 participants were included. The RR for cardiovascular risk was 1.25 (95% CI: 1.14–1.38, $P < .001$, $I^2 = 25\%$) while compared highest to lowest PCSK9 concentration. Subgroup meta-analysis, which sorted by ethnicity, base risk characteristic, and follow-up time, presented consistent results that there was a pronounced association between highest PCSK9 concentration and cardiovascular risk, such relationship was not significant in the statin-taking subjects. Seven studies were included in dose–response meta-analysis, and a nonlinear association between PCSK9 concentration and cardiovascular risk was observed [(χ^2 test for nonlinearity = 6.7, ($df = 2$), $P = .036$].

This study suggests that high circulating PCSK9 concentration associates with significantly increased cardiovascular risk, and demonstrates for the first time that it is a nonlinear dose–response association between circulating PCSK9 concentration and cardiovascular risk. These results provide the evidence that PCSK9 is an independent risk factor beyond the traditional cardiovascular risk factors and indicates a potential role of PCSK9 measurement for medical decisions. The clinical value of PCSK9 measurement and the identification of risk threshold should be confirmed in appropriately designed clinical trials.

Abbreviations: CAD = coronary artery disease, CI = confidence interval, CVD = cardiovascular disease, GFR = glomerular filtration rate, HR = hazard ratio, LDL-C = low-density lipoprotein-cholesterol, LDLR = low-density lipoprotein receptor, MACE = major adverse cardiovascular event, MOOSE = meta-analysis of observational studies in epidemiology, NOS = Newcastle-Ottawa Scale, PCSK9 = proprotein convertase subtilisin/Kexin type 9, Q = quartile, RR = relative risk, T = tertile.

Keywords: cardiovascular risk, cohort studies, meta-analysis, proprotein convertase subtilisin/Kexin type 9

Editor: Salvatore De Rosa.

Financial support for this study was provided by the National Basic Research Program of China (973 Program) 2014CB542400, the National Science Foundation of China (NSFC) Projects 81370359, 91339204, and 91639301 and the Traditional Chinese Medicine of Hunan province Program 201586, and the Natural Science Foundation of Hunan Province, China 2017JJ3250.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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Medicine (2017) 96:48(e8848)

Received: 19 March 2017 / Received in final form: 13 September 2017 / Accepted: 1 November 2017

http://dx.doi.org/10.1097/MD.0000000000008848

1. Introduction

Proprotein convertase subtilisin/Kexin type 9 (PCSK9), which was identified as the 9th member of the proprotein convertase family in 2003, has attracted considerable attention as a promising target for cholesterol-lowering therapy in the last decade.[1] PCSK9 is synthesized as a 75 kDa precursor; it undergoes autocatalytic cleavage as a 62 kDa mature form in the endoplasmic reticulum, and then releasing to the circulation.[2] PCSK9 plays a vital role in lipid metabolism. Secreted PCSK9 in circulation efficiently binds to the low-density lipoprotein receptor (LDLR) at the live cells surface and destines LDLR for lysosomal degradation. PCSK9-induced LDLR intracellular degradation could lead to reduced clearance of low-density lipoprotein-cholesterol (LDL-C) in plasma, thereby increasing the risk of atherosclerosis and the other cardiovascular diseases.[3,4]

Recently, PCSK9 inhibitors have been rapidly developed. Clinical trials with monoclonal antibodies to PCSK9 showed that there was 60% to 70% LDL-C reductions compared with standard therapy,[5,6] and >50% decrease in major adverse cardiovascular events in high-risk patients.[7] These results revealed the clinical beneficial trends in cardiovascular risk reductions by inhibiting PCSK9 activity.

Starting in 2012, many concerns have been raised about the association between baseline circulating PCSK9 concentration

and cardiovascular risk. However, these findings remain inconsistent. One published meta-analysis included 12,081 samples reported that PCSK9 levels predicted the future cardiovascular risk in healthy population, but not in high-risk subjects.[8] Aimed to fully explore the predictive value of PCSK9 concentration in cardiovascular risk prediction, this meta-analysis was conducted by including 11 cohort studies comprising 13,761 subjects, and a dose–response meta-analysis was also performed to identify the dose–response association between circulating PCSK9 concentration and cardiovascular risk. Notably, some different and important results were found in this study.

2. Materials and methods

The current meta-analysis was based on the previously published studies, and thus ethical approval and patient consent are not necessary. We followed the principles proposed by meta-analysis of observational studies in epidemiology (MOOSE) group for performing and reporting the present meta-analysis study.[9]

2.1. Data source and search strategy

Studies dealing with the relationship of circulating PCSK9 concentration and cardiovascular events were considered eligible. Relevant studies were systematically searched in *PubMed*, *EMBASE*, and *Cochrane Library* databases. In additional, manual searches were also performed through the reference lists of review articles. The literature search was last updated on December 1, 2016. The following 3 groups of keywords were used in the process of searching *MEDLINE* (via the *PubMed* gateway): [("proprotein convertase subtilisin/kexin type 9" OR "Pro-protein convertase subtilisin-kexin type 9" OR "proprotein convertase subtilisin kexin 9" OR PCSK9 OR "neural apoptosis-regulated convertase 1" OR NARC1 OR "NARC-1") AND ("cardiovascular events" OR "mortality" OR "all cause death" OR "cardiovascular risk" OR "acute coronary syndromes" OR "myocardial infarction" OR "cardiovascular disease" OR "risk" OR "death" OR "mortality" OR "outcome" OR "stroke" OR "transient ischemic attacks" OR "intracranial hemorrhage" OR "events") AND ("cohort" OR "cohort study" OR "cohort analysis" OR "incidence study" OR "longitudinal studies" OR "follow-up studies" OR "prospective studies")].

2.2. Study selection and data extraction

The eligible studies for the meta-analysis must meet all the following inclusion criteria: study type was a cohort study; risk factor of interest was circulating PCSK9 level, and the clinical outcomes were identified as combined cardiovascular events, including fatal and nonfatal myocardial infarctions, unstable angina, deaths from coronary heart disease, fatal and nonfatal ischemic strokes; and adequate raw data, including hazard ratios (HRs) or relative risk (RR) with the corresponding 95% confidence intervals (Cis) were provided to pooled. Exclusion criteria were as follows: studies did not provide sufficient data to extract the information we needed; case report or cross-sectional study; and repeated publications about the same population.

Study selection and data extraction were conducted by 2 investigators independently (Qiu and Zhou). For all phases, disagreements were resolved in consultation with the third investigator (Li). The following information was recorded for each of the eligible studies: first author's name, publication year, country, sample size, age, male/female, PCSK9 level, follow-up time, characteristic of participants, and events.

2.3. Quality assessment

The Newcastle-Ottawa Scale15 (NOS)[10,11] was used to assess the quality of the included studies by 2 investigators (Cao and Pan). This scale involves 3 parts including selection, comparability, and outcome. Assessment score ranges from 0 to 9 stars: a study could be awarded a maximum of one star for each numbered item within the selection and outcome categories; a maximum of 2 stars could be given for comparability.

2.4. Statistical analysis

In the current meta-analysis, multi-adjusted RRs and HRs with corresponding 95% CIs were pooled to calculate the cardiovascular risk of circulating PCSK9 concentration. The HR was approximately considered as estimate of RR which have commonly used in meta-analysis.[12] In addition, sensitivity analysis was conducted using the leave-one-out method in each turn to investigate the influence of single study on the overall risk estimate,[13] subgroup meta-analyses which sorted by ethnicity, statin use, follow-up time, and the baseline risk characteristic were performed to test the robustness of the observed associations.

For the dose–response meta-analysis, the "generalized least squares for trend estimation" method proposed by Greenland and Longnecker[14,15] was performed to assess the cardiovascular risk change cross the elevation of PCSK9 level. This method requires the cases and cohort size/control subjects of each category and the RR with its variance estimate for at least 3 quantitative exposure categories be known.[16] The dosage value assigned to each level of PCSK9 concentration was the median or mean in each category provided by the original research. For the studies not containing median/mean, the midpoint was used for closed category and the same amplitude as the neighborhood category for open-ended one. The potential nonlinear dose–response relationship was estimated in 2 stages[17,18]: at the first stage, a restricted cubic spline model with 3 knots at percentiles 10%, 50%, and 90% of the concentration distribution of PCSK9 value was estimated; at the second stage, the 2 regression coefficients (3 knots minus 1) and the variance/covariance matrix within each study were combined in a multivariate random-effects meta-analysis. *P* value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline is equal to 0.

2.5. Heterogeneity and publication bias assessment

Between-study heterogeneity was assessed with I^2 statistics.[19] I^2 statistics quantified the heterogeneity and yielded results ranged from 0% to 100% (0%–25%: no heterogeneity; 25%–50%: moderate heterogeneity; 50%–75%: large heterogeneity; and 75%–to 100%: extreme heterogeneity). Meanwhile, publication bias was assessed by visual inspection of funnel plots for asymmetry and statistical evaluation with Begg rank correlation test[20] and Egger linear regression test.[21] Two-tailed α level of significance was set at 0.05.

All statistical analyses were performed with STATA/SE.12.0 (StataCorp, College station, TX), Review Manager Version 5.3 (The Nordic Cochrane Center, Copenhagen, Denmark), and R version 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

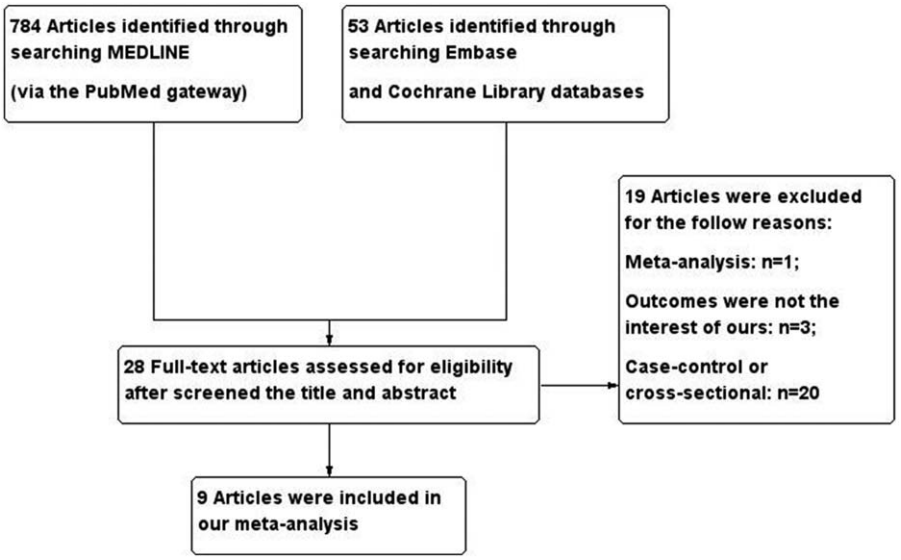

Figure 1. Flow diagram of the study selection process.

3. Results

3.1. Characteristics and quality of eligible studies

A total of 837 published articles were identified as potentially relevant studies via searching in *PubMed*, *EMBASE*, and *Cochrane Library* databases. After the initial screening of all titles and abstracts, 809 articles were excluded; finally, 9 articles were included by performing a critical full-text review. Figure 1 presents the process of study selection. A total of 13,761 subjects were included in the meta-analysis. The endpoints were defined as combined cardiovascular event as detailed in inclusion criteria section. The characteristics of each individual study were demonstrated in Table 1.

Quality of included studies was assessed by NOS, and the score ranged from 6 to 9 stars as shown in Table 1. The most reasons for lowering quality were the insufficient follow-up time and insufficient adjustment for the potential baseline confounders (more details about NOS score descrambled in supplementary materials Table S1, http://links.lww.com/MD/B972).

3.2. Quantitative synthesis of data

3.2.1. Assessment of the association between circulating PCSK9 concentration and cardiovascular risk. A total of 11 cohort studies with 2,222 events among 13,761 subjects were finally included in the meta-analysis to investigate the relationship of PCSK9 concentration with cardiovascular risk, the pooled RR was 1.25 (95% CI: 1.14–1.38, $P < .001$) while compared the highest to lowest PCSK9 concentration (Fig. 2). Moderate between-heterogeneity was observed, while I^2 was 25%. Results of sensitivity testing showed that the combined estimate of cardiovascular risk of high PCSK9 concentration was robust (Fig. 3). As presented in Figure 4, there was no obvious publication bias be observed, the statistical results of Begg correlation test ($P = .68$) and Egger linear regression test ($P = .42$) suggested the inexistence of potential publication bias in the meta-analysis.

3.2.2. Subgroup meta-analysis. As shown in Figure 5, subgroup meta-analysis, which sorted by ethnicity, base risk characteristic, and follow-up time, presented consistent results that there was a statistically significant association between high PCSK9 concentration and cardiovascular risk. However, such significant association was not found in the population with satin therapy (RR = 1.14, 95% CI: 0.85–1.53, $I^2 = 60\%$). After excluded one "Treating to New Targets" trial[30] that performed in patients accepted statin with extreme high dose, a pronounced association was observed with statistically significant RR of 1.33 and corresponding 95% CI was 1.06 to 1.66, no heterogeneity was observed. No significant difference was found in subgroups.

3.2.3. Dose–response meta-analysis. Seven studies provided data for dose–response meta-analysis. (Data for dose-response meta-analysis was presented in Supplemental digital content 2 http://links.lww.com/MD/B989). Using a restricted cubic spline model, a nonlinear dose–response relationship between PCSK9 level and risk of cardiovascular events [χ^2 test for nonlinearity = 6.7 ($df = 2$), $P = .036$] was observed. The dose–response curve (Fig. 6) indicated that with the increasing of PCSK9 level; the cardiovascular risk increased gradually. Referring to the lowest concentration of PCSK9 (62.5 ng/mL) among the 7 studies as reference, the RR values for 484 ng/mL, 500 ng/mL, 550 ng/mL, 600 ng/mL were 1.78 (95% CI: 1.00–3.15), 1.81 (95% CI: 1.02–3.21), 1.90 (95% CI: 1.06–3.40), and 2.00 (95% CI: 1.11–3.62), respectively. The heterogeneity among studies was not significant [multivariate Cochran Q test for heterogeneity = 8.1247 ($df = 12$), $P = .77$].

4. Discussion

To the best of our knowledge, the present study is the first time to comprehensively evaluate the categorical association and the continuous dose–response association between circulating PCSK9 concentration and cardiovascular risk. Two important results were found in this study. First, highest levels of circulating PCSK9 were more likely to lead to the incidence of cardiovascular events with 25% increased risk while compared with lowest levels. Consistent results stayed significant in subgroup analysis which stratified by ethnicity, base risk characteristic, and follow-up time. However, the association was not significant among

Table 1

Main characteristics of included studies.

Study ID	Country	Sample size	Age	Male/female	PCSK9 level (ng/mL)	Follow-up time (y)	Characteristic of participants	Events (n)	NOS*
Leander, 2016[22]	Sweden	4232	60	2039/2193	Q1: ≤73; Q2: 73–122; Q3: 94-≤122; Q4: >122	15	60-year-olds population	CV events (491)	9
Rogacev, (HOMe) 2016[23]	Germany	443	67 (57–74)	265/178	343 (270–413)	5	Patients with decreased GFR	CV events (91)	6
Rogacev, (LURIC) 2016[23]	Germany	1450	67 (59–72)	925/525	208 (161–264)	10	Patients with decreased GFR	CV events (335)	7
Ridker, 2016[24]	USA	716	63 (58–68)	0/716	304 (252–365)/300 (253–359)	17	Healthy American women	CV events (358)	8
Xie, 2016[25]	China	643	57±7.68	269/374	192±29	10	Patients were free of cardiovascular disease at baseline	Carotid plaque formation (NA)	9
Gencer, 2016[26]	Italy	2030	63±12	1501/529	T1: 203+40; T2: 302+27; T3: 478+134	1	Patients with acute coronary syndromes	All-cause death (90)	8
Zhu, 2015[27]	Canada	1527	49±10	1527/0	286 (231–355)	7.2±1.7	Middle-aged men	CVD events (111)	8
Li, 2015[28]	China	603	57±10	434/169	230 (191–278)	3	Patients with stable CAD	MACE (72)	8
Werner, 2014[29]	Germany	504	68 (59,74)	420/84	T1: <471; T2: 471–622; T3: >622	4	Patients with stable CAD	MACE (178)	8
Huijgen, 2012[30]	The Netherlands	1613	62±8	1339/74	309 (228–396)	5	Patients with stable CAD	MACE (496)	8

* A total number of stars examined by NOS.

CAD = coronary artery disease, CVD = cardiovascular disease, GFR = glomerular filtration rate, MACE = major adverse cardiovascular event, NA = nonassessment, NOS = the Newcastle-Ottawa Scale, Q = quartile, T = tertile.

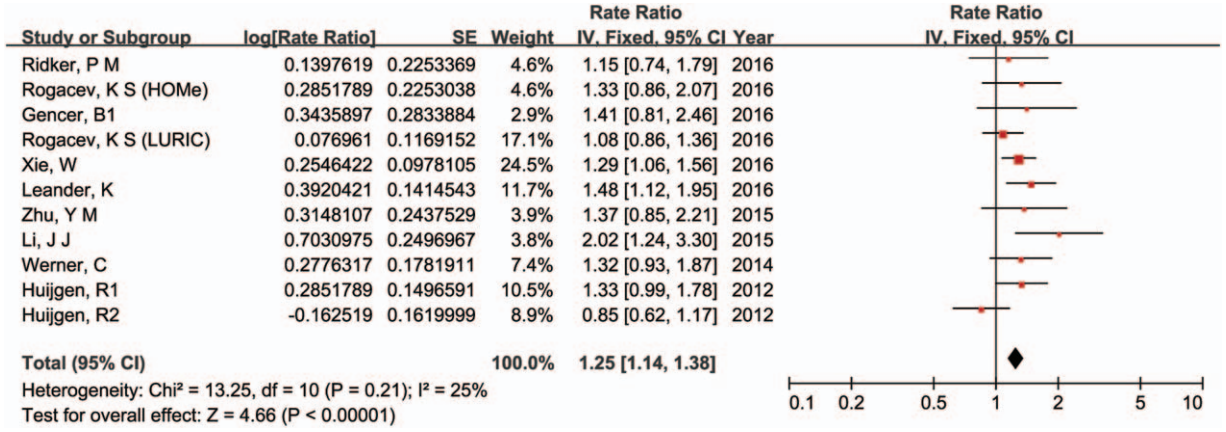

Figure 2. Forest plot of relative risks and 95% confidence intervals for the association between the circulating PCSK9 concentration and cardiovascular risk while compared highest to lowest PCSK9 concentration.

statin-taken patients. Second, a nonlinear dose–response relationship between circulating PCSK9 concentration and cardiovascular risk was found via dose–response analysis. These results opened a new potential clinical application of circulating PCSK9 measurement in cardiovascular risk prediction.

The association between circulating PCSK9 concentration and cardiovascular risk has been reported by 2 published meta-analyses. Vlachopoulos et al[8] found that PCSK9 levels are associated with increased risk of total cardiovascular events in general populatiion but not in high-risk population. Xiao et al[31] observed a significant association between highest circulating PCSK9 and cardiovascular risk; however, when the circulating PCSK9 concentration is presented as a continuous variable, the association was not statically significant. In the present study, a similar association between highest PCSK9 levels and cardiovascular risk was demonstrated, such association was observed both in low- and high-risk subjects via performing a subgroup analysis sorted by the base risk characteristic, which is different from the results that Vlachopoulos et al reported. Subgroup meta-analysis based on ethnicity and follow-up time also showed a consistent increased cardiovascular risk with the high circulating PCSK9 concentration. It is well recognized that PCSK9 regulates LDL-C metabolism by escorts LDLR to lysosomal degradation.[32,33] With the exception of system regulation on serum LDL-C level, PCSK9 action have a direct role on vascular system. PCSK9 increases the oxidized low-density lipoprotein (ox-LDL) uptake and may lead to endothelial cells dysfunction under inflammatory status.[34,35] PCSK9 secreted from smooth muscle cells and macrophages directly affects plaque composition by altering plaque morphology and increasing inflammation.[36] Given the important roles of PCSK9 on LDL-C regulation and vascular health, it is speculated that high circulating PCSK9 concentration may be a causal risk factor for increased cardiovascular risk. However, further studies are needed to support this concept.

As commonly prescribed in LDL-cholesterol lowering therapy, statins induce the PCSK9 transcription via activating sterol-regulatory element binding protein-2 (SREBP2), which, in turn, alleviates their cholesterol lowering effect.[37] Whether

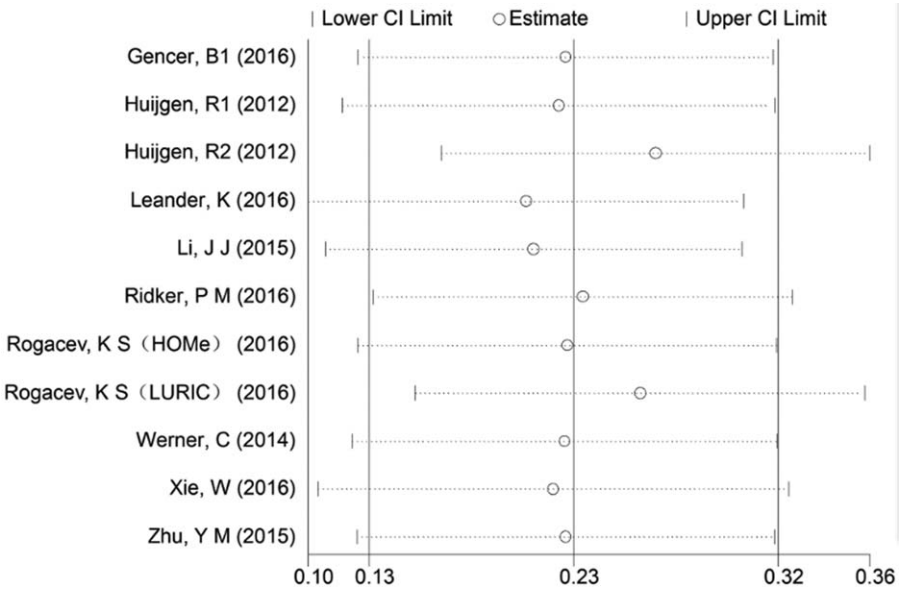

Figure 3. Sensitivity test of single study on combined effect estimation.

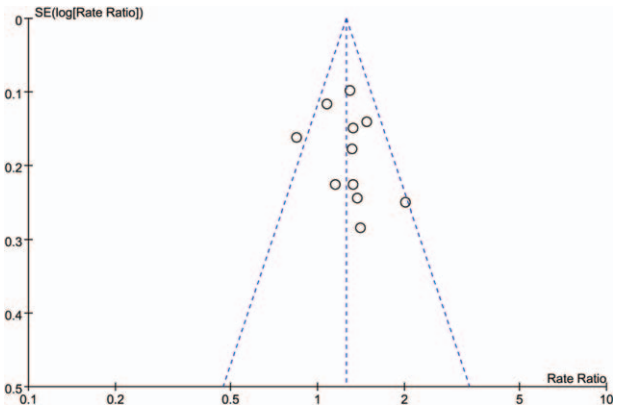

Figure 4. Funnel plot of circulating PCSK9 concentration and cardiovascular risk.

statin-induced PCSK9 upregulation leads to high cardiovascular risk is important to guide clinical application. In this study, we did not observe a significant association between PCSK9 levels and cardiovascular risk in the statin-taking subjects. However, a statistically significant association was found after excluded a study which performed in the subjects who took extreme high-dose statin.[30] The possible reasons for this are as follows: first, statin-based LDL-C lowering therapy has reduced the cardiovascular risk; second, statins induce the expression of PCSK9 in a dose-dependent manner, the upregulation of PCSK9 may confound the real association, this may explain the pronounced association was found after excluded a group with extreme high-dose statin. In fact, it is critical to determine whether satin-induced PCSK9 elevation will take more risk for the patients, it remains to be determined because of limited data now, and further clinical studies are strongly encouraged to give the answer.

The dose–response association between circulating PCSK9 concentration and cardiovascular risk has not been reported previously. We comprehensively assessed the quantitative dose–response association by included 7 studies, and found an important nonlinear association between PCSK9 concentration and cardiovascular risk. These findings suggest that considerably higher levels of circulating PCSK9 concentration are a risk factor for cardiovascular event; the lower levels of PCSK9 are not considered as the equal significance in cardiovascular risk prediction. However, we could hardly identify the threshold point of PCSK9 for cardiovascular risk based on the current data. We expect more research to be published in the future with the aim to get a stable threshold point.

Together with these results, it indicates a potential clinical practice of circulating PCSK9 monitors for cardiovascular risk prediction. There are some strengths of our study. First, the included sample size was large. Second, effect size from multiadjusted models was pooled to estimate cardiovascular risk, which reduces the potential confounders. Third, any significant heterogeneity was presented in the current meta-analysis. Fourth, sensitivity analysis was used to confirm the robustness of these findings; we did not observe a material change in the magnitude or the direction of the pooled effect size.

Several limitations of the present study should also be acknowledged. First, included studies used the combined cardiovascular event to estimate the cardiovascular risk, thus taking difficult to identify the risk of specific cardiovascular event, such as myocardial infarctions and ischemic strokes. We expect further clinical trials to study the association between PCSK9 concentration and specific cardiovascular event; second, serum level of PCSK9 was measured using ELISA. Testing sensitivity of ELISA Kits are varied in different bands, which may result in between-studies various. In the final, though an important nonlinear association was found between circulating PCSK9 concentration and cardiovascular risk, the threshold value of PCSK9 was not identified in the present study.

Subgroup	Sample	RR(95%CI)	P*	I^2	Begg (P†)	Egger's (P‡)	P§
Overall	13761	1.25(1.14,1.38)	<0.001	24.5	0.39	0.42	
Statin-therapy							0.68
Mix	9474	1.32(1.11,1.58)	<0.001	27	0.35	0.28	
Yes	2177	1.14(0.85,1.53)	0.37	60	0.60	1.00	
No	3577	1.31(1.10,1.56)	<0.001	0	0.12	0.11	
Ethnicity							
European	9768	1.19(1.05,1.35)	0.01	41	0.85	0.66	0.39
Asian	3277	1.36(1.17,1.58)	<0.001	0	0.04	0.31	
USA	716	1.15(0.74,1.79)	0.54				
Base risk							0.28
High risk	6643	1.20(1.05,1.36)	0.01	45	0.29	0.16	
Low risk	7118	1.33(1.15,1.53)	<0.001	0	1.00	0.93	
Follow-up (y)							0.13
>=5	10624	1.22(1.10,1.35)	<0.001	22	0.46	0.97	
<5	3137	1.50(1.16,1.93)	<0.001	0	0.60	0.64	

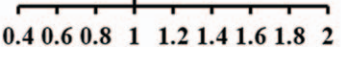

Figure 5. Subgroup analyses of circulating PCSK9 concentration and cardiovascular risk which sorted by ethnicity, base risk of participants, and follow-up time. *P was utilized to assess the pooled effects; †P and ‡P were utilized to assess the publication bias by Begg rank correlation test and Egger linear regression test, respectively; §P was utilized to assess the subgroup differences.

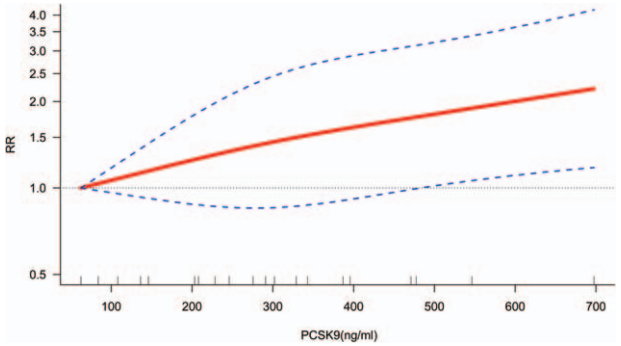

Figure 6. Nonlinear dose–response of relationship between circulating PCSK9 concentration and cardiovascular risk. The bars above the horizontal axe are the original PCSK9 value used to fit the dose response curve.

5. Conclusions

This study suggests that high circulating PCSK9 concentration associates with significantly increased cardiovascular risk, and demonstrates for the first time that it is a nonlinear dose–response association between circulating PCSK9 concentration and cardiovascular risk. These results provide the evidence that PCSK9 is an independent risk factor beyond the traditional cardiovascular risk factors and indicates a potential role of PCSK9 measurement for medical decisions. The clinical value of PCSK9 measurement and the identification of risk threshold should be confirmed in appropriately designed clinical trials.

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