Plasma PCSK9 concentrations predict Cardiovascular Events in subjects free of vascular disease and with stable coronary artery disease: Findings from a community-based prospective study and a meta-analysis

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Abstract

Background The secreted protein proprotein convertase subtilisin/kexin type 9 (PCSK9) is a promising new target for lowering plasma low-density lipoprotein cholesterol and preventing cardiovascular disease. Objective We aimed to determine the relationship between PCSK9 levels and cardiovascular (CV) events using a large cohort and a systematic review with meta-analysis.

Methods We analyzed the association of plasma PCSK9 levels with CV events in 1324 subjects (mean age, 61.3 years) from a community-based population in Beijing, China. For the systematic review, we pooled the results of our cohort with other eligible studies in a meta-analysis using a random effects model.

Results After a median follow-up interval of 4.8 years, 79 CV events were identified. Cox regression analysis showed that the presence of CV events (HR: 1.83, 95% CI, 1.07-3.17; P=0.046) was related to PCSK9 levels significantly. Combining another 8 prospective cohorts and our findings in a meta-analysis (9 prospective cohorts with a total of 13013 participants were included) showed a positive association between plasma PCSK9 levels and CV events, with a summary HR of 1.26 (95% CI: 1.07-1.50; P=0.007). When pooled estimates were derived for pre-existent and free of coronary artery disease (CAD) populations, baseline PCSK9 levels predicted total CV events only in subjects free of CAD (pooled HR: 1.34, 95% CI, 1.11-1.61; P=0.003). In patients with pre-existent CAD, further subgroup analysis showed that baseline PCSK9 levels predicted CV events only in subjects with stable CAD (pooled HR: 1.58, 95% CI, 1.04-2.38; P=0.030) but not in patients with unstable CAD (pooled HR: 1.02, 95% CI, 0.76-1.35; P=0.908).

Conclusion Our findings in the Chinese cohort and the meta-analysis support that high plasma PCSK9 level was a predictive factor for future CV events in subjects free of CAD and with stable CAD but not in patients with established unstable CAD. Keywords PCSK9; cardiovascular events; cohort; meta-analysis.

Background

Mutations in proprotein convertase subtilisin/kexin 9 (PCSK9) were discovered to cause autosomal dominant hypercholesterolemia (ADH) in a French family in 2003 firstly [1]. Since then, PCSK9 has been studied thoroughly and these data indicate that PCSK9 strongly expressed in the liver, and was found in rodents plasma as two forms: mature and inactivated [2]. Published articles identified that circulating PCSK9 bound the hepatocyte LDL receptor (LDLR) by targeting the receptor to the lysosome for degradation, leading to reduced LDL-C clearance by preventing recirculation of LDLR onto the hepatocyte surface, which is the major route of clearance of circulating cholesterol [3-5].

Recent observations discovered that rare PCSK9 loss-of-function variants reduced the plasma level of low density lipoprotein-cholesterol (LDL-C) and the incidence of coronary events, suggesting that PCSK9 is related to LDL-C regulation [6]. In clinical studies, over-expression of PCSK9 linked to higher levels of LDL-C and excess of atherosclerosis, conversely poor-expression of PCSK9 induced reduction in mean LDL-C [7,8]. According to these results，PCSK9 was suggested to be a increasingly attractive intervention target

and the subject of intensive study. Several phase III randomized controlled trials reported that PCSK9 inhibition (monoclonal antibodies) with maximal statin therapy or standard therapy decreased levels of LDL-C by about 60% and reduced CVD by 50% [9,10]. In addition, epidemiological studies identified that PCSK9 levels have typically correlations with other atherogenic risk factors positively, such as plasma triglyceride (TG), blood pressure, body mass index (BMI), fasting plasma glucose, insulin levels, white blood cell count, C-reactive protein levels and smoking [3,11-16]. As a result of these landmark observations, changing the function of PCSK9 play a significantly role in the development of atherosclerotic process.

To date, the association of plasma PCSK9 levels and CVD risk is not well characterized. To elucidate the role of circulating PCSK9 levels in CVD development, we investigated the association between prediagnostic plasma PCSK9 and CVD risk in a community-based prospective cohort. Additionally, we systematically reviewed evidence from observational studies on the association between plasma PCSK9 and risk of CVD and carried out a meta-analysis for prospective studies to further clarify any association.

Methods

Subjects

The population of this study including 1324 subjects lived in the Pingguoyuan area of Beijing, China. Baseline data obtained initially from 1680 subjects between September 2007 and January 2009 after a routine health check-up. We followed this population prospectively from February 1 to September 30, 2013 for the first time, and obtained follow-up data from 1499 subjects (181 subjects were lost, follow-up rate was 89.2%). The median follow-up interval for the original 1499 subjects was 4.8 years. Of the 1499 subjects, 175 were excluded from analyses because of because of a history of cardiovascular disease, remaining 1324 subjects for the analysis. No differences other that baseline risk factors were noted in those who completed baseline and follow-up assessments.

The study was approved by the ethics committee of the People's Liberation Army General Hospital, and each subject provided informed written consent.

Clinical data collection

All subjects received a face-to-face questionnaire survey to ascertain new CVD events during these visits. Prevalent diseases, medical histories, lifestyle factors and family history were collected. Urine and fasting blood samples were also obtained. Blood pressure and anthropometric measurements were obtained by trained physicians.

Biomarker variable determination

We collected blood samples from all 1324 subjects in centrifuge tubes after overnight fast and centrifuged them for 15 min at 1200×g. Serum aliquots were frozen at −80℃ until assays were performed. Concentrations of PCSK9 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) [17]. Concentrations of fasting blood glucose (FBG), lipids (TC, TG, LDL-C, HDL-C) were measured on a Roche autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA) by the Roche enzymatic assays (Roche Diagnostics GmbH, Mannheim, Germany). Concentrations of serum creatinine were measured on a Hitachi 7600 autoanalyser (Hitachi, Tokyo, Japan) by enzymatic assay (Roche Diagnostics GmbH). All testing was performed in the Department of Biochemistry of Chinese PLA General Hospital by well trained personnel following the criteria of the World Health Organization Lipid Reference Laboratories.

Definition of variables

CVD events were defined as fatal and non-fatal myocardial infarctions, unstable angina, stable angina, deaths from coronary heart disease. Body mass index (BMI) = weight (kg) / height^2 (m^2). The estimated glomerular filtration rate (eGFR) was calculated using the following Chronic Kidney Disease Epidemiology Collaboration equation: eGFR = 141 × min (Scr/κ,1)α× max (Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] × 1.159 [if black], where Scr is plasma creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1. Hypertension was defined as a mean SBP ≥140 mmHg, mean DBP ≥90 mmHg, both, or the use of antihypertensive medication. Diabetes mellitus (DM) was defined as a fasting glucose ≥7.0 mmol/L, glucose ≥11.1 mmol/L at two hours after an oral 75 g glucose challenge, the use of antihyperglycaemic medication, or both.

Statistical analyses

Baseline characteristics were expressed as the median (interquartile range) or mean ± standard deviation (SD) for continuous variables and percentages for dichotomous variables. Subjects were categorized into quartiles according to baseline distribution of PCSK9 levels. Q1 was considered low level group whereas Q2-Q4 was considered high level group.

Differences in the baseline levels of risk factors and clinical characteristics between subjects based follow-up CVD or non-CVD groups were analyzed using a t-test for continuous variables and a chi-square test for categorical variables. Cox proportional hazard regression model was used to evaluated the association between baseline PCSK9 levels and future CVD events. Regression models were adjusted for age and gender as well as hypertension, SBP, DBP, Diabetes (DM), smoking, BMI, levels of glucose, TC, HDL-C, TG, and eGFR (model 1); Model 2 was adjusted for model 1 plus levels of LDL-C; Model 3 was adjusted for model 1 plus levels of sd-LDL-C.

All analyses were conducted using Stata software (version14.0; Stata Corporation, College Station, TX). P-values <0.05 were considered statistically significant.

Systematic Review and meta-analysis

We performed a systematic review and meta-analysis that incorporated results from the current study into findings from previous studies on the association between plasma PCSK9 and risk of CVD. We searched Pubmed and EMBASE from inception until December 1, 2016, with no language restrictions applied. The following full search strategy was used: (proprotein convertase subtilisin/kexin type 9 OR PCSK9) AND (cardiovascular disease OR cardiovascular events OR cardiovascular risk OR coronary heart disease OR myocardial infarction OR stroke OR mortality OR all cause death). Articles describing levels of plasma PCSK9 and risk of CVD retrieved through this search were screened by two investigators (YB and XW) and any disagreement was resolved by consensus. Reference lists of the included articles were hand-searched for potentially relevant articles.

Studies included were prospective population-based cohort which measured plasma PCSK9 levels at baseline and then reported CVD risk during follow-up. Each included cohort study had to report either risk estimates (relative risks, odds ratios, or hazard ratios) with 95% confidence intervals (CI), or provide sufficient data to estimate these.

The following information was obtained from eligible studies: study design, population, risk estimates, and their 95% CIs. In studies not reporting these data, we calculated risk estimates from the survival curves. Data extraction was performed by two authors separately (YB and XW) to ensure accuracy and disagreements were discussed in a consensus conference. Random effects meta-analysis was used due to the presence of high heterogeneity (I^2 >50%). Test for interaction between subgroups was performed using Cochran's Q test. Sensitivity analyses were conducted to evaluate the robustness of our results. We removed each study individually to evaluate that study's effect on the summary estimates. Small study bias was assessed with funnel plots and Egger regression test. Statistical analyses were performed with Stata software (version14.0; Stata Corporation, College Station, TX).

Results

Baseline parameters between follow-up CVD and Non-CVD groups

During a median follow-up interval of 4.8 years, 79 CV events and 36 all-cause mortality were identified, with the cumulative incidence of CV events was 5.97%. Baseline characteristics of all 1324 subjects with or without follow-up CV events were summarized in **Table 1**. Age, male gender, current smoking, BMI and PCSK9 were significantly elevated in subjects with follow-up CV events.

Relationship between baseline PCSK9 levels and CVD events and all-cause mortality

The distribution of PCSK9 levels in the population was slightly skewed (**Fig 1**). PCSK9 concentrations ranged from 83.4 to 362.69 ng/mL，median was 135.87 ng/mL. Subjects were categorized into quartiles according to baseline distribution of PCSK9 levels (Q1 (83.40-125.16 ng/mL, n=331), Q2 (125.27-135.87 ng/mL, n=331), Q3 (135.99-146.70 ng/mL, n=331), and Q4 (146.73-362.69 ng/mL, n=331)). Q1 was considered low level group whereas Q2-Q4 was considered high level group. Kaplan-Meier curves for assessing the baseline PCSK9 level indices as predictors of CV events are presented in **Fig 2.**

Relationships between baseline PCSK9 levels and CVD events and all-cause mortality were shown in **Table 2.** Using cox regression analysis, the presence of CV events (HR:1.943, 95 % CI: 1.112 -3.939, P =0.020) was related to PCSK9 levels significantly in the unadjusted model. In the adjusted models, the association of PCSK9 with CV events remained statistically significant. In participants with higher PCSK9 levels the risk of fatal and nonfatal CV was increased 1.900-fold (HR: 1.900; 95 % CI: 1.052 -3.432; P =0.033) compared with lower PCSK9 levels (Model 1). The HR for PCSK9 levels in predicting 4.8-year incident CVD in model 2 and model 3 were 1.830 (HR: 1.830, 95% CI, 1.011-3.315; P=0.046) and 1.876 (HR: 1.876, 95% CI, 1.034-3.405; P=0.039), respectively. There was no association between baseline PCSK9 levels and follow-up all-cause mortality.

Meta-analysis

Including the present study, nine prospective observational studies [18-24] were identified, with 13,013 participants and 1,983 incident CVD events **(Table 3)**. Pooled random effects meta-analysis showed a positive association between plasma PCSK9 levels and CVD events, with a summary HR of 1.26 (95% CI: 1.07-1.50; P=0.007) (**Fig 3**). When pooled estimates were derived for pre-existent and free of coronary artery disease (CAD) populations, baseline PCSK9 levels predicted total CV events only in subjects free of CAD (pooled HR: 1.34, 95% CI, 1.11-1.61; P=0.003). In patients with pre-existent CAD, further subgroup analysis showed that baseline PCSK9 levels predicted CV events only in subjects with stable CAD (pooled HR: 1.58, 95% CI, 1.04-2.38; P=0.030) but not in patients with unstable CAD (pooled HR: 1.02, 95% CI, 0.76-1.35; P=0.908) (**Fig 4**).

No evidence of small-study bias was found for HR, either by visually at inspection of funnel plots or analytically at Egger test (Egger test for HR, P = 0.209). Sensitivity analysis excluding one study at a time confirmed in direction and magnitude of statistical significance of the results, even after the exclusion of the largest study (Leander et al., 2016) (**Fig 5**).

Discussion

The present study is the first clinical investigation of the significance of plasma levels of PCSK9 regarding the incidence of CVD events in a Chinese community-dwelling population. In a cohort of Chinese people without known previous vascular disease, we found that baseline plasma levels of PCSK9 were found to continuously relate to risk of CVD events, independent of age, gender and other conventional cardiovascular risk factors including LDL-C and sd-LDL.

Previous observational analyses have been conflicted on the relationship between plasma PCSK9 levels and CVD events. In line with our study, Leander K et al. investigated the association of serum PCSK9 concentration with incident CVD in a vascular disease-free cohort including 4,232 men and women, founding that PCSK9 concentration was positively associated with future risk of CVD [24]. However, in the other two studies enrolled initially healthy subjects [19,22], no association was observed between baseline PCSK9 and future CVD events. In patients with stable coronary heart disease, Li et al. found a positively relation between PCSK9 and future risk of MACE. While in patients with unstable CVD, none of

the three studies [21,23] indicated association of plasma PCSK9 levels with CVD events. These qualitatively and quantitatively discrepant findings are most likely because of the difference in study populations and the limited power of individual studies to reliably measure modest risk associations, as supported by our systematic review.

In view of the ongoing uncertainty surrounding the role of plasma PCSK9 levels in the prediction of CVD risk, we supplemented our analysis with a meta-analysis of 9 prospective observational studies, showed a positive association between plasma PCSK9 levels and CVD events. Notably, in the predefined subgroup analysis, we demonstrated that high plasma PCSK9 level was a predictive factor for future CV events only in subjects free of vascular disease and patients with stable CAD but not in patients with established unstable CAD.

There are several ways that plasma PCSK9 levels related to CVD risk. First, published articles identified that circulating PCSK9 bound the hepatocyte LDL receptor (LDLR) by targeting the receptor to the lysosome for degradation, leading to reduced LDL-C clearance by preventing recirculation of LDLR onto the hepatocyte surface, which is the major route of clearance of circulating cholesterol [3-5]. Second, it has been identified that PCSK9 levels have typically correlations with other atherogenic risk factors positively, such as plasma triglyceride (TG), blood pressure, body mass index (BMI), fasting plasma glucose, insulin levels, white blood cell count, C-reactive protein levels and smoking [3,11-16]. Third, PCSK9 was also involved in the development of atherosclerosis by inducing endothelial injury, cell apoptosis and several other pathways as well [25].

In the present prospective cohort study and meta-analysis, plasma PCSK9 level was found to be related to future CV events in low- and moderate-CV risk subjects (free of vascular disease and patients with stable CAD) but not in high-CV risk patients (with established unstable vascular disease). This difference can be attributed to several factors. First, the high inflammatory burden in high-CV risk patients may bias the prognostic value of PCSK9 levels. It has been found that hepatic PCSK9 expression is enhanced in the context of MI and inflammation [26-27], indicating that blood sampling in the milieu of an unstable vascular disease with increased inflammatory burden may lead to a prognostic bias. Second, as it was previously shown in 504 stable CHD patients under statin therapy that high PCSK9 levels were no longer predictive of CV complications when adjusted for triglyceride levels.as the present study confirms our previous observation, indicating that PCSK9 levels are higher in patients classified as having familial hypercholesterolaemia compared with no familial hypercholesterolaemia,10 we cannot exclude that assessing PCSK9 could represent an additional tool for detection for familial hypercholesterolaemia in top of conventional lipid and genetic profiling. This hypothesis requires further investigation.

Conclusion

These findings provide novel insights into the predictive value of PCSK9 concentrations for the risk of CVD. In a cohort of Chinese people without known previous vascular disease and meta-analysis, we demonstrate for the first time that plasma PCSK9 level was related to future CV events in low- and

moderate-CV risk subjects (free of vascular disease and patients with stable CAD) but not in high-CV risk patients (with established unstable vascular disease).

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the People's Liberation Army General Hospital, and each subject provided informed written consent.

Competing interests

The authors have no conflicts of interest to declare regarding the publication of this manuscript.

Authors' contribution

XW and PY designed the study; YB made the meta-analysis; RC, XY, WX, YZ and HW participated in acquisition of data; XW and PY researched and evaluated the literature; XW undertook the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Baseline characteristics between follow-up CVD and Non-CVD groups

	CVD (n=79)	Non-CVD (n=1245)	P value
Age (years)	63.46±0.39	57.93±10.95	<0.001
Men, n (%)	24(30.38)	530(42.57)	<0.001
Smoking, n (%)	15(18.99)	328(26.35)	<0.001
BMI (kg/m^2)	26.07±4.28	24.96±2.03	0.033
SBP (mmhg)	120.54±17.76	132.26±18.54	0.754
DBP (mmhg)	75.45±11.23	76.11±12.30	0.604
PCSK9 (ng/dl)	140.77±26.37	135.93±18.36	0.019
TC (mmol/l)	4.98±0.91	5.00±1.01	0.882
TG (mmol/l)	1.87±1.34	1.78±1.30	0.497
HDL-C (mmol/l)	1.39±0.38	1.39±0.37	0.957
LDL-C (mmol/l)	3.01±0.67	2.87±0.73	0.081
FBG (mmol/l)	5.32±1.86	5.26±1.53	0.714
eGFR (ml/min/1.73 m^2)	93.71±13.68	94.67±13.35	0.499

Notes: Characteristics are reported as percentages for categorical variables and means (±SD) or median (with interquartile range) for continuous variables. Categorical variables are presented as counts and percentages. The values outside the parentheses are the number of subjects, and the values inside the parentheses are prevalence.

Abbreviations: BMI, body-mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; SBP, Systolic blood pressure; DBP, diastolic blood pressure; PCSK9, proprotein convertase subtilisin/kexin 9; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate.

Table 2 Relationship between baseline PCSK9 levels and deaths and CVD events

	PCSK9	
	HR(95%CI)	P-value
Incident of CVD		
Unadjusted	1.943(1.112-3.393)	0.020
Model 1	1.900(1.052-3.432)	0.033
Model 2	1.830(1.011-3.315)	0.046
Model 3	1.876(1.034-3.405)	0.039
Incident of all cause mortality		
Unadjusted	1.596(0.751-3.394)	0.224
Model 1	0.979(0.419-2.888)	0.960
Model 2	0.954(0.378-2.406)	0.920
Model 3	0.892(0.352-2.260)	0.809

Model 1：adjusted for age, sex, smoking, Hypertension、BMI、DM、TC、TG、HDL-C and eGFR

Model 2：adjusted for age, sex, smoking, Hypertension、BMI、DM、TC、TG、HDL-C、eGFR and LDL-C

Model 3：adjusted for age, sex, smoking, Hypertension、BMI、DM、TC、TG、HDL-C、eGFR and sd-LDL-C

Table 3 Main features of included studies

Studies	Publication year	Number of subjects	Population	Age (mean)	Follow-up years (mean or median)	Events
Werner et al.	2014	504	patients with stable CAD	68	4	composite of cardiovascular death and unplanned cardiovascular hospitalization
Zhu et al.	2015	1527	middle-aged men free of vascular disease	49	7.2	cardiovascular events (nonfatal myocardial infarctions, revascularizations, cerebrovascular or peripheral vascular events)
Li et al.	2015	616	patients with stable CAD	58	3	cardiovascular events (cardiac deaths, nonfatal strokes, MI, revascularization, or unstable angina)
Rogacev et al. (CFH)	2016	443	CKD 2–4 Patients from CARE FOR HOMe Cohort	68	3	acute MI, surgical or interventional coronary/cerebrovascular/peripheral-arterial revascularization; stroke, amputation above the ankle; or death of any cause
Rogacev et al. (LURIC)	2016	1450	CKD 2–4 Patients from LURIC Cohort	67	10	acute MI, surgical or interventional coronary/cerebrovascular/peripheral-arterial revascularization; stroke, amputation above the ankle; or death of any cause
Ridker et al.	2016	712	initially healthy American women	63	17	cardiovascular events (MI, thromboembolic stroke, or cardiovascular death)
Gencer et al.	2016	2030	ACS patients undergoing coronary angiography	64	1	cardiovascular-cause death
Leander et al.	2016	4232	subjects free of vascular disease	60	15	Composite endpoint of the following incident ischemic cardiovascular events (fatal or non-fatal myocardial infarction, angina pectoris, chronic ischemic heart disease, sudden cardiac death, and fatal or non-fatal ischemic stroke)
Ye et al. (The present study)	Un-published	1324	subjects free of vascular disease	63	5	the combined cardiovascular events of fatal and non-fatal myocardial infarctions, unstable angina, stable angina or deaths from coronary heart disease.

CKD: chronic kidney disease; CAD: coronary artery disease; ACS: acute coronary syndrome; MI: myocardial infarction

Figures

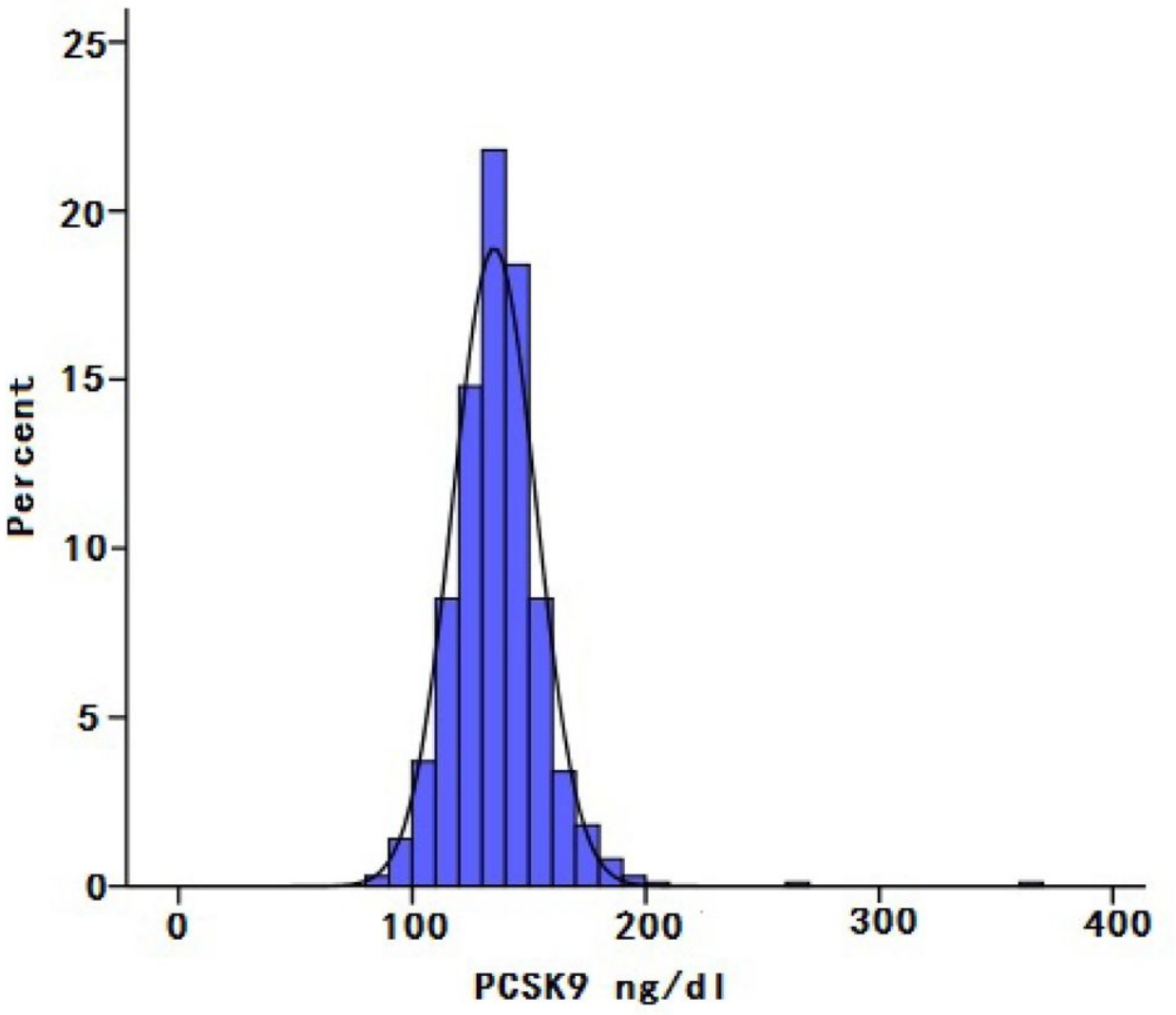

Figure 1

Distribution of baseline plasma PCSK9 levels. PCSK9: Proprotein convertase subtilisin/kexin 9.

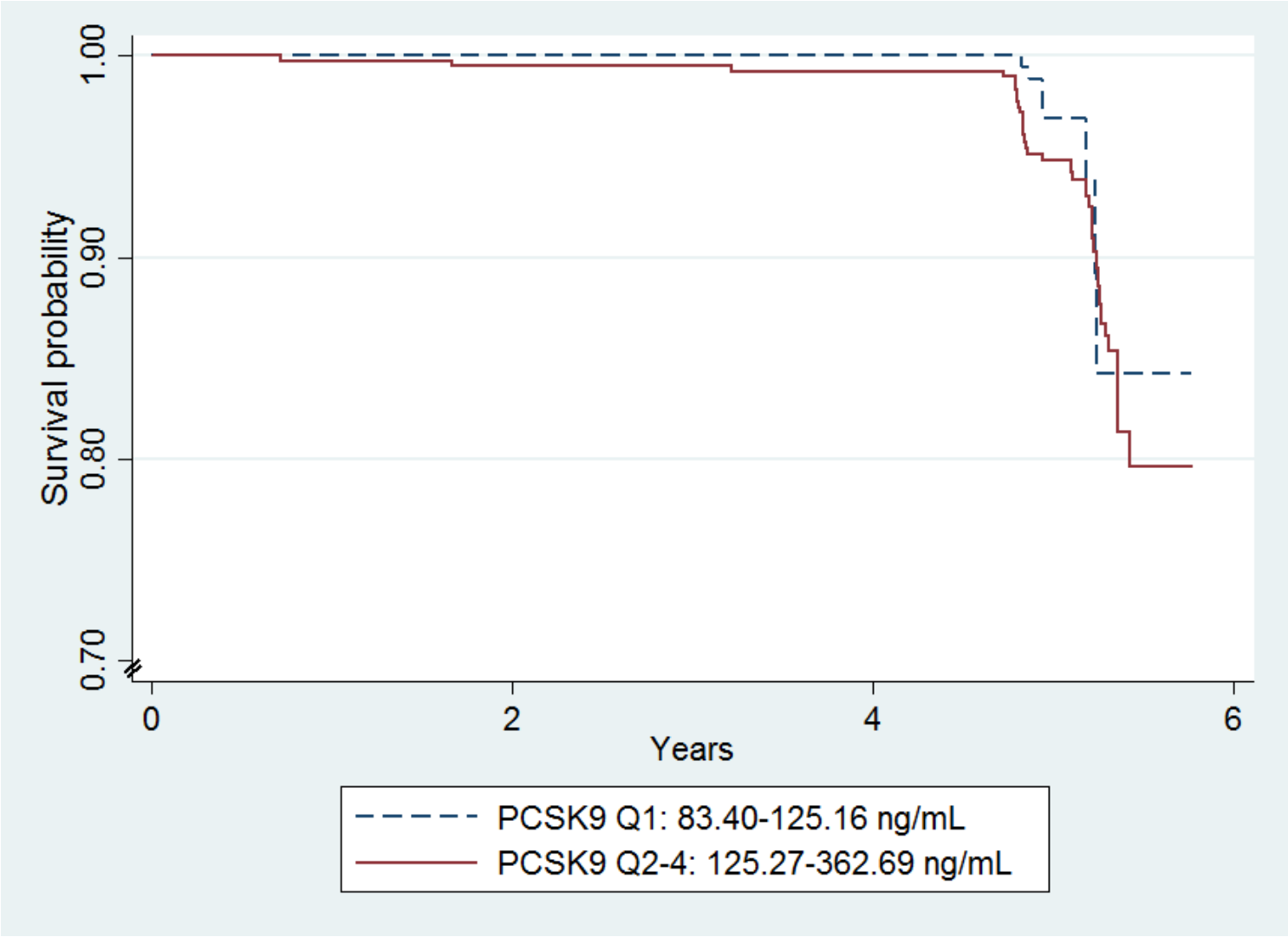

Figure 2

Kaplan–Meier curve showing cardiovascular disease event–free survival stratified by proprotein convertase subtilisin/kexin type 9 (PCSK9) tertiles in a Chinese community-dwelling cohort.

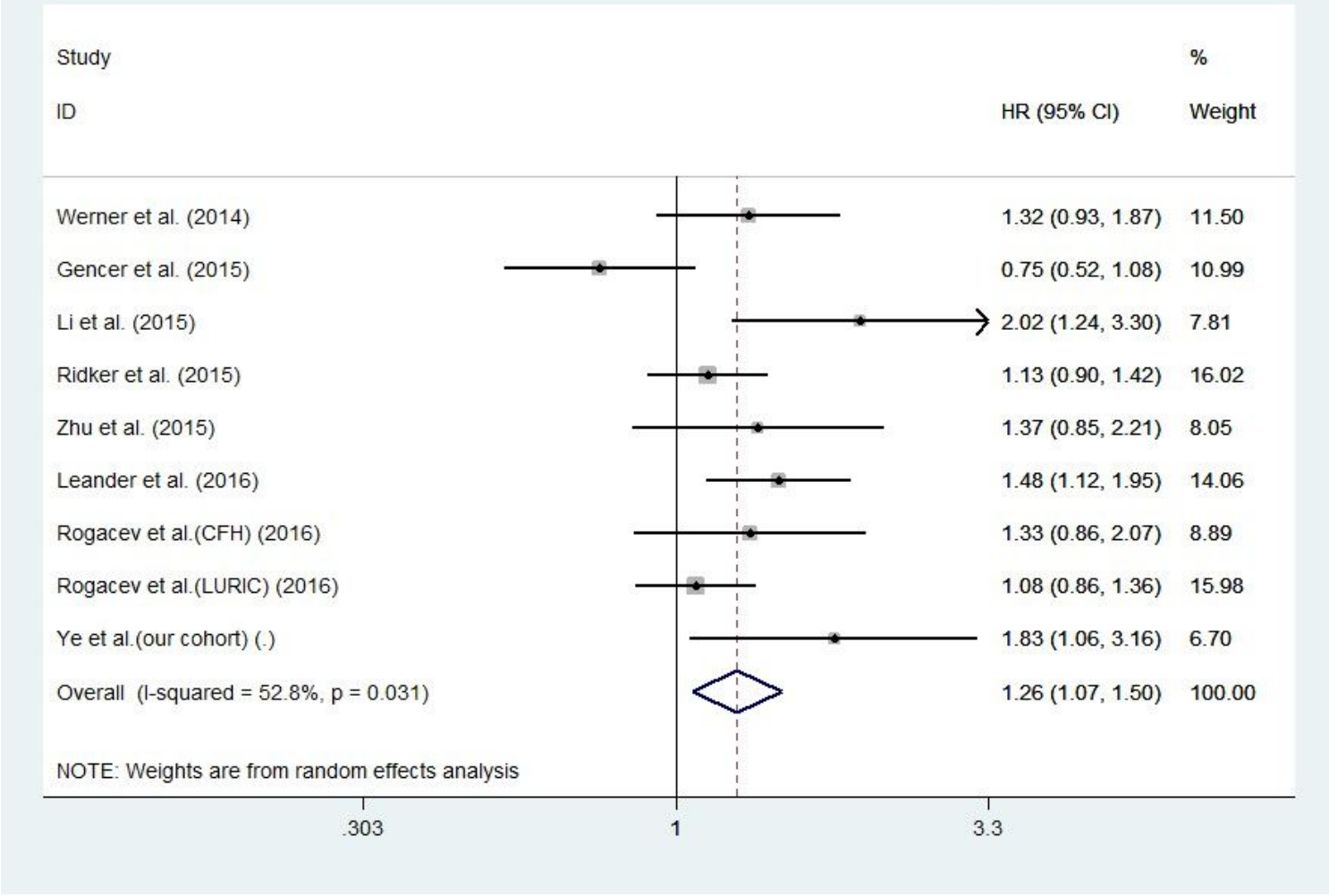

Figure 3

Random-effects meta-analysis of HR and 95% CI for cardiovascular events according to baseline PCSK9 levels (high versus low tertile). HR, hazard ratios; CI, confidence interval.

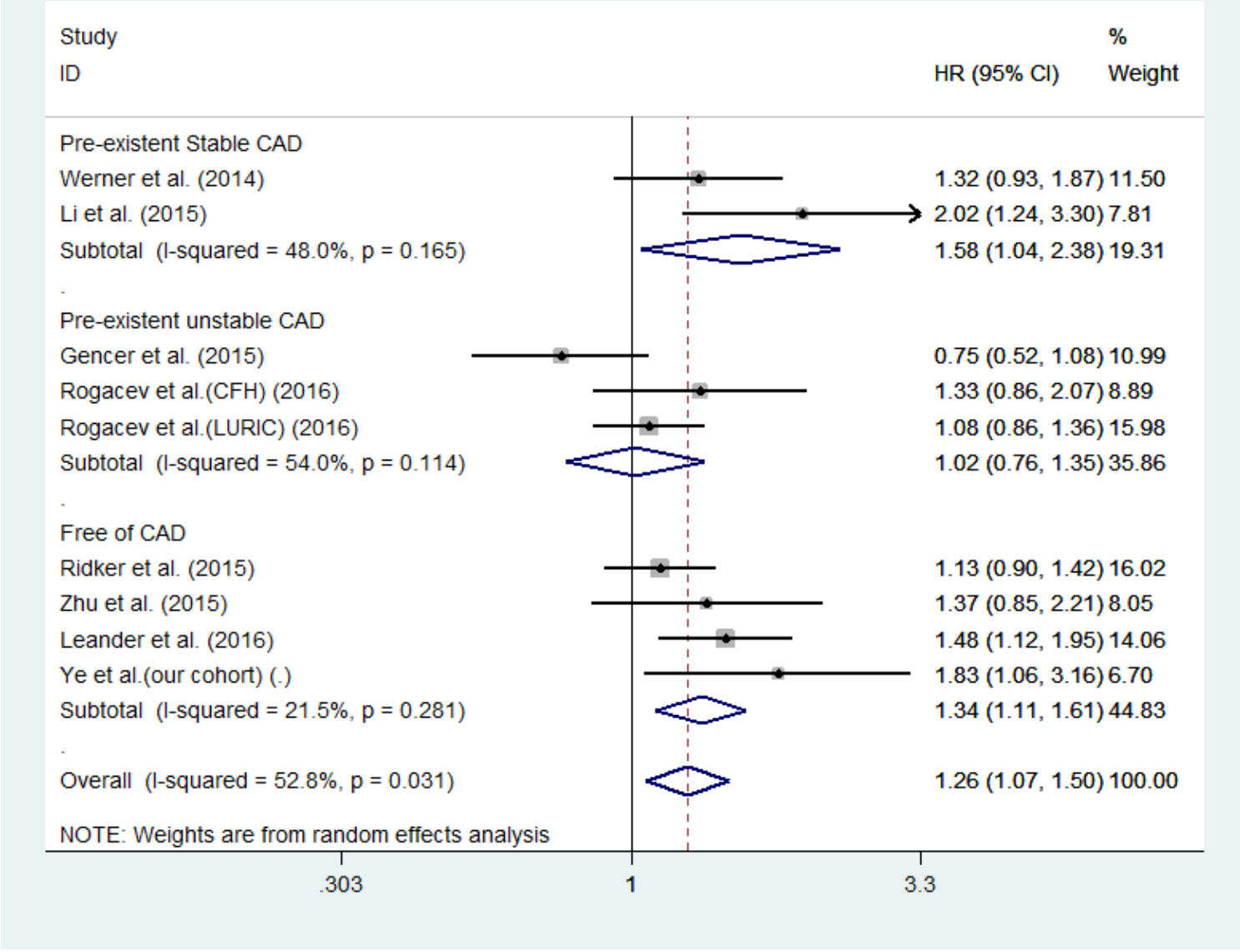

Figure 4

Subgroup meta-analysis of HR and 95% CI for cardiovascular events according to baseline PCSK9 levels (high versus low tertile). Subgroups were differentiated by the clinical states of the recruited population: free of CAD, suffered from stable or unstable CAD. HR, hazard ratios; CI, confidence interval; CAD, coronary artery disease.

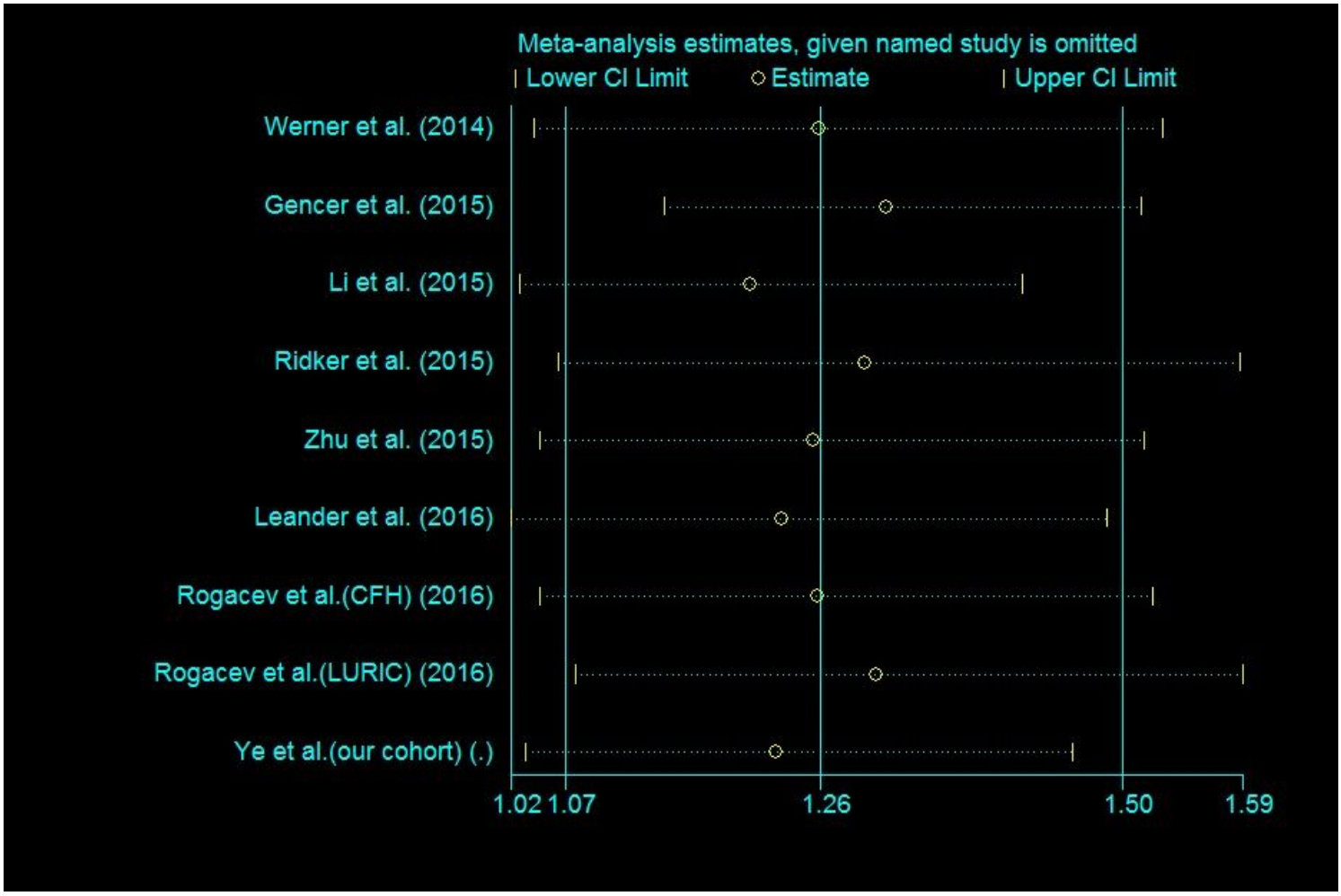

Figure 5

Sensitivity analysis investigating the influence of each individual study on the meta-analysis of hazard ratios (HR) for cardiovascular events according to baseline PCSK9 levels. The meta-analysis of all studies except the “omitted” study named on the left margin is presented as a horizontal confidence interval (CI). The full, “combined” results are shown as the solid vertical lines.