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Complete List of Authors:	Cao, Ye-Xuan; State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College Li, Sha; State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia Liu, Hui-Hui; State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, Division of Dyslipidemia Li, Jian-Jun; Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College
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Authors: Ye-Xuan Cao, MD*, Sha Li, MD, PhD *, Hui-Hui Liu, MD*, Jian-Jun Li, MD, PhD*

Affiliations: *Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China.

Correspondence: Professor Jian-Jun Li, MD, PhD, FESC, FACC. Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China. Tel: 86+10+88396077; Fax: 86+10+88396584. E-mail: lijianjun938@126.com.

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ABSTRACT

Objective To evaluate the potential effects of proprotein convertase subtilisin/kexin type 9 monoclonal antibody (PCSK9-mAb) on high-sensitivity C-reactive (hs-CRP) concentrations.

Design A systematic review and meta-analysis of randomized controlled trials.

Data sources PubMed, MEDLINE, The Cochrane Library databases, clinical trials registries websites and recent conferences were searched from inception to January 2018.

Eligibility criteria for selecting studies All randomized controlled trials that reported changes of hs-CRP were included.

Results Ten studies involving 4198 participants were identified. PCSK9-mAbs showed a slight efficacy in reducing hs-CRP (-0.04mg/L, 95%CI:-0.17 to 0.01), but no statistical difference was found. Subgroup analyses showed no significant effect when stratified by PCSK9-mAb types (Alirocumab:0.12mg/L, 95% CI:-0.18 to 0.43; Evolocumab:0.00 mg/L, 95% CI:-0.07 to 0.07; LY3015014:-0.48mg/L, 95% CI:-1.28 to 0.32; RG7652:0.35mg/L, 95% CI:-0.26 to 0.96), treatment duration (≤12w:0.00mg/L, 95% CI:-0.07 to 0.07; >12w:-0.11mg/L, 95% CI:-0.45 to -0.23), participant characteristics (familial hypercholesterolemia:0.00mg/L, 95% CI:-0.07 to 0.07; non-familial hypercholesterolemia:0.07mg/L, 95% CI:-0.12 to 0.26; mix:-0.48mg/L, 95%CI:-1.28 to 0.32) and treatment methods (monotherapy:0.00mg/L, -0.08 to 0.07; combination-therapy:-0.08mg/L, -0.37 to0.21). Meta-regression analyses identified no significant linear correlation between baseline age (p=0.673), sex (p=0.645) and low-density lipoprotein cholesterol reduction (p=0.339).

Conclusions Although previous study showed a slightly reduced effect of PCSK9-mAbs on hs-CRP, the results of this updated meta-analysis suggested that PCSK9-mAbs had no significant impact on circulating hs-CRP levels.

Keywords: PCSK9; monoclonal antibody; hs-CRP; meta-analysis

Strengths and limitations of this study

- This is a comprehensive systematic review and meta-analysis of randomized controlled trials, conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.
- We used a broad search strategy and identified 10 studies that reported changes of high-sensitivity C-reactive during proprotein convertase subtilisin/kexin type 9 monoclonal antibody treatment.
- Studies with moderate heterogeneity and lack of individual level data.

Introduction

Cardiovascular disease is the greatest burden of global health, which is characterized by atherosclerosis[1]. Atherosclerosis is a chronic and progressive inflammatory disease, including endothelial dysfunction, lipid accumulation in the arterial wall and leukocyte infiltration, which leads to luminal stenosis, plaque rupture and acute coronary syndrome (ACS) [2]. Apart from well-established dyslipidaemia theory, inflammation also plays an important role in the initiation and progression of atherosclerosis[3]. Recently, CANTOS trial reported that anti-inflammatory therapy by canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β (IL-1β), significantly reduced the primary cardiovascular end points[4].

Hs-CRP (high-sensitivity C-reactive protein) is the most intensively investigated inflammatory biomarker and shows an extensive clinical application. Hs-CRP is an 11,800 Da molecular synthesized by hepatocytes in response to interleukin 6 (IL-6)[5]. Several studies revealed that hs-CRP concentration was relatively constant in an individual, making it an ideal inflammatory biomarker[5]. Increasing studies have confirmed that hs-CRP is a predictive factor for assessing the progression of atherosclerotic disease and future adverse cardiovascular events (CE) [6,7]. Moreover, previous studies also indicated that hs-CRP may have pro-inflammatory effects and play a direct role in progression of atherosclerosis[8,9]. Many studies demonstrated that administration of statins may modify hs-CRP and other pro-inflammatory cytokine concentrations with decreasing CEs[10,11].

Proprotein convertase subtilisin/kexin type 9 (PCSK9), a 73-kDa protein secreted by liver, regulates the activity of low-density lipoprotein receptor (LDLR) expressing in hepatocellular surface [12]. Multiple studies revealed that PCSK9 binds to LDLR and increases its degradation to block the cholesterol homeostasis, resulting in elevated plasma low-density lipoprotein cholesterol (LDL-C) levels[13,14]. PCSK9 monoclonal antibody (PCSK9-mAb)

decreased LDL-C concentrations by up to 70% and reduced the rate of CEs[15]. Therefore, PCSK9-mAb has emerged as the most powerful lipid-lowering drugs currently. Although the effect of PCSK9 on LDL-C has been established, its role in inflammation has not been fully investigated. A number of experimental studies found that PCSK9 could enhance inflammatory reaction to promote the progression of atherosclerosis and down-regulated PCSK9 expression led to the reduction of inflammation[16,17]. A meta-analysis published two years ago found that PCSK9-mAbs had no effect on serum hs-CRP[18]. However, this meta-analysis did not perform sufficient subgroup analyses to our knowledge. Besides, a recent published study with limited sample size found that initial PCSK9 plasma levels are associated with hs-CRP levels in patients with ACS[19] and some kinds of PCSK9-mAbs showed a reduction in hs-CRP levels[20]. Hence, whether PCSK9-mAbs could decrease circulating hs-CRP levels should be intensively examined.

To further explore the efficiency of PCSK9 inhibitors on serum hs-CRP levels, we performed this meta-analysis including all published randomized controlled trials (RCT) published till January 2018.

Methods

Literature search

The present study was designed according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement[21]. To identify all RCTs that assessed the effects of PCSK9-mAbs on circulating hs-CRP levels, we comprehensively searched PubMed, MEDLINE, and the Cochrane Library database up until January 2018. We also searched the clinical trials registries websites. The search terms we used included the following: (AMG145 or Evolocumab or REGN727 or SAR236553 or Alirocumab or RN316 or bococizumab or RG7652 or LY3015014 or PCSK9 antibody or

anti-PCSK9) AND (randomized controlled trial OR randomized OR randomly). Meanwhile manual search was performed for relevant studies including references lists, relevant review articles and commentaries. No language restriction was used and non-English articles were translated.

Study selection

Original studies met the following criteria would be included: the design was phase 2 or phase 3 double-blind RCTs with longer than 8 weeks treatment duration; human participants were randomly assigned to PCSK9-mAbs group versus control group with or without other lipid-lowering therapy; outcomes included percentage changes of hs-CRP from baseline. Studies were excluded if they were duplicate publications, review articles, non-human studies, observational studies, lack of adequate information on outcomes or lacking control group. Two investigators (YC and SL) independently screened and selected the eligible studies. Disagreements were resolved by discussion with a third investigator.

Data Extraction

A standardized extraction form was used to extract the following items by two investigators (YC and HL) independently: trial name/first author, year of publication, type of intervention, follow-up period, treatment duration, number of patients, participant characteristics, background lipid-lowering therapy, types and doses of PCSK9-mAbs, LDL and hs-CRP levels at baseline and changes. We included the final reported follow-up point if a trial contains several time points. If necessary, further information was required from correspondence author.

Quality Assessment

We used Cochrane Collaboration's tool and Jadad score to assess the data and the methodological quality of included RCTs. For Cochrane Collaboration's tool, the following items were performed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other sources of bias. The judgments were classified as 'low risk', 'high risk', and 'unclear risk' of bias. The 5-point Jadad score included the following items: basis of randomization (0 to 2 points), double blinding (0 to 2 points), and withdrawals and dropouts (0 to 1 points). Studies with a score ≥3 points are considered to be high quality. Two investigators (YC and HL) independently assessed the quality of each study. Disagreements were resolved by discussion with a third investigator.

Data Synthesis and Statistical Analysis

All analyses were analyzed according to the intention-to-treat principle. For all efficacy outcomes, changes in hs-CRP concentrations were expressed as weighted mean difference (WMD) and 95% confidence interval (CI). All the data were standardized and expressed by mg/mL. Standard deviation could be calculated from CI, interquartile range or standard error according to formulas in the Cochrane Handbook for Systematic Reviews of Interventions if not reported.

Heterogeneity was assessed by the Cochran Q test and the I^2 statistic, and we considered I^2<25% as representing low heterogeneity and I^2>75% as representing at high heterogeneity. Outcomes were calculated by fixed-effects model under no or low to moderate inconsistency (I^2<50%); otherwise, the data was pooled based on a random-effects model. Subgroups were applied to reduce the heterogeneity if I^2 ≥50%, such as PCSK9-mAb types, treatment duration and participant characteristics. In order to explore the resource of heterogeneity,

sensitivity analysis was conducted by omitting studies in turn to evaluate the consistency of the results. Meta-regression analyses were performed to the contribution of participant characteristics and reductions in LDL-C concentrations. Publication bias was assessed with a funnel plot and Egger's test.

All analyses were conducted with Review Manager Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, Denmark) and Stata 14.0 (Stata Statistical Software: College Station, TX: Stata Corp LP). P value <0.05 was considered to be statistically significant.

Results

Study selection and Characteristics

The initial search identified 575 articles. After screening the titles and abstracts, 339 were excluded and 236 studies were retrieved for full-text identification. We further excluded 135 studies, of which 58 were pooled or meta-analysis, 2 studies were not RCTs and 13 was phase 1 trials, 16 were open label trails and 42 without adequate information. Finally, ten studies[20,22-30] were included in this meta-analysis. Figure 1 shows flow diagram of selection process.

These ten studies were published between 2012 and 2017 from different countries with low risk of bias, of which 5 were phase 2 studies and 5 were phase 3 studies (Table 1). In total, 4198 participants were included, comprising 2728 individuals in the PCSK9-mAb group and 1470 in the control group. Alirocumab (SAR236553/REGN72 7) was used in 4 arms and 11 arms applied Evolocumab (AMG 145). Five arms managed LY3015014 and 2 arms used RG7652. Participants of 3 studies were heterozygous familial hypercholesterolemia (HeFH), 1 study contained homozygous familial hypercholesterolemia (HoFH) patients, and the remaining 6 trials enrolled non-FH or hypercholesterolemia

individuals. Most of the treatment duration ranged from 12 to 24 weeks and the longest treatment duration was 78 weeks. Apart from DESCARTES trial which co-administered with atorvastatin, another 9 studies used PCSK9-mAb as monotherapy. Baseline characteristics, including circulating hs-CRP levels, were similar between PCSK9-mAbs and control groups within each study. The characteristics of these trials and participants are summarized in Table 1. All these studies had a relatively high quality evaluated by the Jadad score and low risk of bias (online supplementary Table 1 and online supplementary Figure 1, 3 scores=5, 6 scores=4).

Efficiency outcomes of PCSK9-mAbs on hs-CRP

A total of 4198 participants were included in the analysis of efficiency of PCSK9-mAbs on plasma hs-CRP concentrations before and after treatment. When data were pooled, PCSK9-mAbs showed a slight efficacy in reducing hs-CRP (WMD: -0.04mg/L, 95%CI: -0.17 to 0.01), but no statistical difference was found compared with control treatment (Figure 2). There was a moderate heterogeneity between each study (I^2=57.4%, P=0.0001), so the random-effects model was selected.

To assess the potential discrepancy, we applied the subgroup analysis based on the characteristics of trials and participants (Figure 3 and online supplementary Figures 2-5). Although the efficiency of LY3015014 was a mild higher (-0.48 mg/L, 95% CI:-1.28 to 0.32), there was no difference between these four antibodies (Alirocumab: 0.12 mg/L, 95% CI: -0.18 to 0.43; Evolocumab: 0.00 mg/L, 95% CI: -0.07 to 0.07; RG7652: 0.35 mg/L, 95% CI: -0.26 to 0.96). When studies were classified by treatment duration, the hs-CRP reduction showed no difference in less than 12 weeks duration groups (0.00 mg/L, 95% CI: -0.07 to 0.07) and above 12 weeks duration groups (-0.11mg/L, 95% CI: -0.45 to -0.23). There was a statistically non-significant reduction in circulating hs-CRP with use of PCSK9 antibodies

compared with control treatment when categorized to participant characteristics (FH: 0.00 mg/L, 95% CI: -0.07 to 0.07; non-FH: 0.07 mg/L, 95% CI: -0.12 to 0.26; mix: -0.48 mg/L, 95%CI: -1.28 to 0.32). The analysis stratified by treatment method also supported the results that no differential effect of PCSK9-mAb therapy on plasma CRP concentrations was observed (monotherapy: 0.00 mg/L, 95%CI: -0.08 to 0.07 vs combination-therapy: -0.08 mg/L, 95%CI: -0.37 to 0.21).

Sensitivity analysis and publication bias

The sensitivity analysis for all outcomes in the comparison was conducted by gradually removing each study, but the results did not change meaningfully (online supplementary Figure 6). Neither funnel plots nor Egger's regression test (p=0.913) showed publication bias (online supplementary Figure 7).

Meta-regression analyses

We used meta-regression analysis to assess the association between changes in hs-CRP and baseline age, sex and average LDL changes (online supplementary Figure 8). No statistically significant relationship between baseline age, sex and hs-CRP changes were observed. Likewise, LDL lowering effects had no impact on hs-CRP lowering.

Discussion

The results of this comprehensive meta-analysis, based on 10 RCTs encompassing 4198 participants, suggested that short-term PCSK9-mAb therapy had no impact on circulating hs-CRP concentrations. In the subgroup analysis, we found no difference between PCSK9-mAb types, treatment duration, participant characteristics and treatment methods.

Atherosclerosis is a chronic progressive disorder and its pathophysiology is complex. In

addition to well-established imbalanced lipid metabolism, growing evidence has suggested that inflammation played a major role in the formation and progression of atherosclerosis, including vasomotor dysfunction, endothelial cell injury, adhesion and transendothelial migration of monocytes and plaque rupture[2]. Numerous pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), intercellular cell adhesion molecule-1 (ICAM-1), and CRP have been shown to drive the disease progression[31]. However, most anti-inflammatory drugs showed no positive effect in reducing the burden of atherosclerosis in human studies[32]. Fortunately, evidence from CATONS found that canakinumab significantly reduced hs-CRP levels and cardiovascular outcomes after follow-up of 3.7 years, which directly confirmed the inflammatory hypothesis[4].

A number of studies indicated that hs-CRP could independently predict major CEs[6]. Framingham study found that men and women in the highest quartile of CRP respectively had twice and three-times the risk of stroke compared with those in the lowest after more than 10-years follow-up[33]. NOMAS (the Northern Manhattan Study) reported that >3 mg/L CRP was associated with a 1.7-fold increase in cardiovascular outcomes and a 1.55-fold increase in mortality[34]. A recent study showed a positive association between sustained high exposure to hs-CRP and CVD risk[7]. Furthermore, hs-CRP also plays a vital role in the development of atherosclerosis. Zwaka et al[8] found that CRP enhanced the transformation from macrophages to foam cells by increasing the uptake of native LDL. Previous studies also reported that CRP impaired vasodilatation, inhibited the synthesis of nitric oxide synthase, and facilitated adhesion of monocyte[35,36]. Many studies found that statin therapy may reduce hs-CRP and other pro-inflammatory cytokine concentrations with decreasing CEs[10,11]. The JUPITER

study applied rosuvastatin on individuals with LDL-C levels below 130 mg/dl but with hs-CRP levels ≥2 mg/l and found a significant reduction in all vascular events[37]. That is the reason why we chose hs-CRP as an inflammatory biomarker to identify whether PCSK9-mAb has an effect on inflammatory status.

Although the relationship between PCSK9 and LDL-C was well-established, more and more evidence demonstrated its function beyond lipids. In 2010, microarray gene expression analysis suggested that PCSK9 affected not only cholesterol metabolism, but also inflammation. Experimental studies indicated that PCSK9 could participate in vascular and systemic inflammation[16]. PCSK9 enhanced the oxidized low-density lipoprotein (ox-LDL) accumulation in macrophages by up-regulating the expression of CD36 and blocked cholesterol efflux through lowering ATP-binding cassette transporter (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) levels[38]. In transgenic mice expressing human PCSK9 gene, Tavori et al[39] observed that atherosclerosis lesion size and local Ly6C^{hi} monocytes, the precursors of pro-inflammatory M1 macrophages, significantly increased. Clinical studies further supported this hypothesis. Li et al[40] found that serum PCSK9 concentrations were associated with white blood cell count independently in CAD patients, indicating PCSK9 may be involved in the inflammation process. ATHEROREMO-IVUS study reported a positive linearly association between PCSK9 levels and coronary plaque inflammation, including amount of necrotic core tissue and plaque volume[41]. PCSK9 concentration was also reported to be a predictor for carotid atherosclerosis in asymptomatic adults and for the incidence and severity of CAD independent of conventional cardiovascular risk factors[14,42].

Since previous studies showed that PCSK9 directly augmented inflammatory cytokines expression and atherosclerotic lesion, PCSK9 inhibition may exert anti-inflammatory effects. The levels of IL-1, IL-6, TNF-α, and PCSK9 synthesized by THP-1-derived macrophages were increased after treated with ox-LDL and PCSK9 small interfering RNA reduced the expression of these pro-inflammatory genes through nuclear factor kappa B (NF-κB) Pathway[43]. In apoE-/- mice, PCSK9 silencing limited the development of atherosclerosis and decreased the number of macrophages and the expression of vascular inflammation regulators through TLR4/NF-κB signaling pathway[44]. Pro-inflammatory Ly6C^{hi} monocytes and ICAM-1, which enhances the adhesion to the vascular endothelium, were obviously reduced after treatment with Alirocumab[17]. AT04A anti-PCSK9 vaccine also got the same results that anti-PCSK9 therapy could reduce vascular inflammation[45]. Clinically, Bernelot et al[46] found that after 24 weeks of treatment with PCSK9-mAbs, the migratory capacity of monocytes and inflammatory responsiveness showed a significant reduction, and anti-inflammatory cytokine interleukin-10 levels increased in FH patients, which supported the hypothesis that PCSK9-mAb had an anti-inflammatory effect. However, in our study PCSK9 suggested no effect on decreasing hs-CRP in both FH participants and non-FH individuals.

Statins are the cornerstone of cardiovascular drugs because of its pleiotropic effects, including lipid-lowering and anti-inflammatory effect, which notably reduce CAD risks. The CARE trial indicated that pravastatin decreased hs-CRP levels independent of LDL-C[47]. AFCAPS/TexCAPs research also got the similar results that statin was clinically effective in primary prevention of CAD events by reducing LDL-C and hs-CRP levels[48]. Interestingly, unlike statins, although experimental and clinical studies showed that PCSK9 inhibition could

reduce atherosclerotic inflammation, in our study PCSK9-mAbs had no significant impact on plasma hs-CRP levels. Besides, the same results were also observed in combination of statins and PCSK9-mAbs group. Although the results of previous meta-analysis published two years ago consistent with ours, the methods of analyses including subgroup analysis were different and they might be limited by small sample size and insufficient subgroup analyses. Although the exact mechanism between statin and PCSK9-mAb on inflammation is unclear, it is notable that the participants in statins therapy had high levels of hs-CRP at baseline, while, in PCSK9 inhibition therapy, initial hs-CRP levels were at normal range in recruited individuals. Besides, the effective observation of PCSK9-mAb on inflammatory marker in humans may be limited by duration. The treatment duration and follow-up period is long in statin therapy, but the longest follow-up of PCSK9-mAbs was 78 weeks. Furthermore, no confirmative evidence has been found to decline CAD risk by reducing hs-CRP levels alone. Therefore, although this meta-analysis indicated a non-effect of PCSK9 on hs-CRP, a large number of data suggested that PCSK9 plays a vital role in atherosclerotic inflammation. More experimental and clinical researches may be needed to fully understand the impact of PCSK9-mAbs on CRP in the future.

Limitations

First, meta-analysis is a retrospective approach and based on trial level data but not on individual level data. Second, study design, treatment duration, follow-up and baseline characteristics were different in the studies included. Some studies had a statin run-in period which would influence the final results. Third, moderate degree of heterogeneity was obvious in several comparisons. However, there was no publication bias and the results were rather

consistent among different subgroups and sensitivity analyses. Fourth, some studies included in this meta-analysis did not provide adequate information about blinding of participants and personnel.

Conclusions

In conclusion, the current evidence suggests that despite intense effect on lipid-lowering, PCSK9-mAbs have no significant impact on circulating hs-CRP concentrations. Hopefully, more participants with elevated hs-CRP levels at baseline or with CAD will be recruited in large, oncoming RCTs to explore whether PCSK9-mAbs have pleiotropic effects like statins.

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Contributors

JJL contributed to conception and design, acquisition, analysis, and interpretation, and critically revised the manuscript. YXC contributed to design, acquisition, analysis, and interpretation and drafted the manuscript. SL contributed to acquisition, analysis, and critically revised the manuscript. HHL contributed to analysis, interpretation and critically revised the manuscript. All the authors read and approved the final version of the manuscript.

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Tables

Table 1. Study characteristics of included randomized controlled trials.

Study	Year	Phase	Inclusion criteria	Patients N	Arm	Mean hs-CRP at baseline mg/L	Mean age (years)	Male %	% LDL-C reduction	Drugs/control	Treatment duration	Jadad Score
RUTHERFORD	2012	II	HeFH	167	(1)	1.09±1.37	47.6	54.5	42.7	E:350mg/PBO, Q4W	12W	5
					(2)	1.07±1.24	51.8	62.5	55.2	E:420mg/PBO, Q4W	12W	
Stein EA 2012	2012	II	HeFH	77	(1)	1·40±1.78	56.3	81.3	67.9	A:150mg /PBO, Q2W	12W	5
					(2)	0·60±0.82	51.3	60.0	28.9	A:150mg /PBO, Q4W	12W	5
					(3)	0·70±1.48	52.9	56.3	31.5	A:200mg /PBO, Q4W	12W	
					(4)	0.70±0.59	54.3	46.7	42.5	A:300mg /PBO, Q4W	12W	
DESCARTES	2014	II	HC	894	(1)	2.00±3.70	50.7	47.3	51.5	E:420 mg/ PBO, Q4W	52W	5
					(2)	1.00±1.48	57.2	42.9	54.7	E:420 mg+ATV10 mg/ PBO, Q4W	52W	
					(3)	1.00±1.48	57.8	52.4	46.7	E:420 mg+ATV80 mg/ PBO, Q4W	52W	
					(4)	1.00±1.48	54.2	55.6	46.8	E:420 mg+ATV80 mg+ Eze10mg / PBO, Q4W	52W	
GAUSS-2	2014	III	HC	307	(1)	1.40±2.00	61.0	55.3	56.1	E:140 mg/ Eze, Q2W	12W	4
					(2)	1.80±1.78	63.0	54.9	52.6	E:420 mg/ Eze, Q4W	12W	
RUTHERFORD-2	2014	III	HeFH	329	(1)	0.92±1.03	52.6	40.0	61.3	E:140 mg /PBO,Q2W	12W	4
					(2)	1.04±1.24	51.9	41.8	55.7	E:420 mg /PBO,Q4W	12W	
TESLA Part B	2014	III	HoFH	49		0.70±1.04	31.0	51.5	23.1	E:140 mg /PBO, Q4W	12W	4
ODYSSEY COMBO II	2015	III	HC	720	(1)	3.58±7.78	61.7	75.2	50.6	A: 75mg /Eze, Q2W	24W	4
					(2)	3.58±7.78	61.7	75.2	51.8	A: 75mg /Eze, Q2W	52W	
GLAGOV	2016	III	HC	968		1.60±1.93	59.8	72.1	34.6	E:420 mg /PBO, Q4W	78W	4
Kastelein 2016	2016	II	HC	519	(1)	1.03±1.41	57.2	51.7	14.9	LY:20mg/PBO, Q4W	16W	4
					(2)	1.34±1.11	57.1	52.3	40.5	LY:120mg/PBO, Q4W	16W	

					(3)	1.63±1.78	59.7	54.7	50.5	LY:300mg /PBO, Q4W	16W	
					(4)	1.39±1.70	59.6	58.1	14.9	LY:100mg /PBO, Q8W	16W	
					(5)	1.10±1.63	58.7	50.6	37.1	LY:300mg /PBO, Q8W	16W	
EQUATOR	2017	II	HC	168	(1)	1.60±2.70	65.0	57.9	23.3	RG:400mg/PBO, Q4W	24W	4
					(2)	2.00±5.90	64.0	51.0	44.3	RG:800mg /PBO,Q8w	24W	

Data presented as mean±SD; LDL-C, low density lipoprotein-cholesterol; hs-CRP, hypersensitive C reactive protein; HC, hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; E, Evolocumab; A, Alirocumab; LY: LY3015014; RG, RG7652; PBO, placebo; ATV, atorvastatin; Eze, ezetimibe; W, weeks; Q2W, every 2 weeks; Q4W, every 4 weeks. N, number; SD, standard deviation.

Figure legends

Figure 1. Flow diagram of selection of studies.

Figure 2. Forest plots depicting the effect of PCSK9-mAbs on hs-CRP.

CI=confidence interval, PCSK9-mAbs=PCSK9 monoclonal antibodies, hs-CRP= hypersensitive C-reactive protein

Figure 3. Subgroup analyses of the effect of PCSK9-mAbs on hs-CRP.

CI=confidence interval. PCSK9-mAb=PCSK9 monoclonal antibody. hs-CRP= hypersensitive C-reactive protein. FH= familial hypercholesterolemia. non-FH= non-familial hypercholesterolemia.

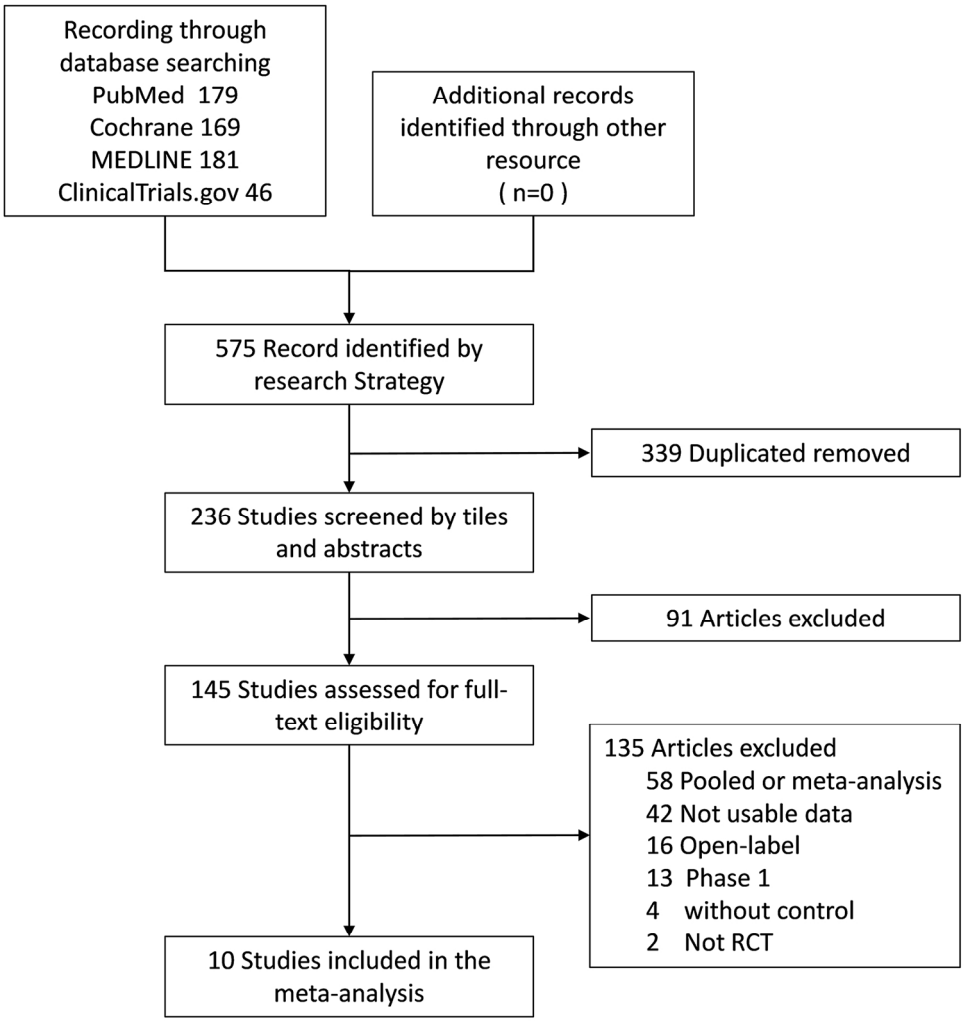

Figure 1. Flow diagram of selection of studies.

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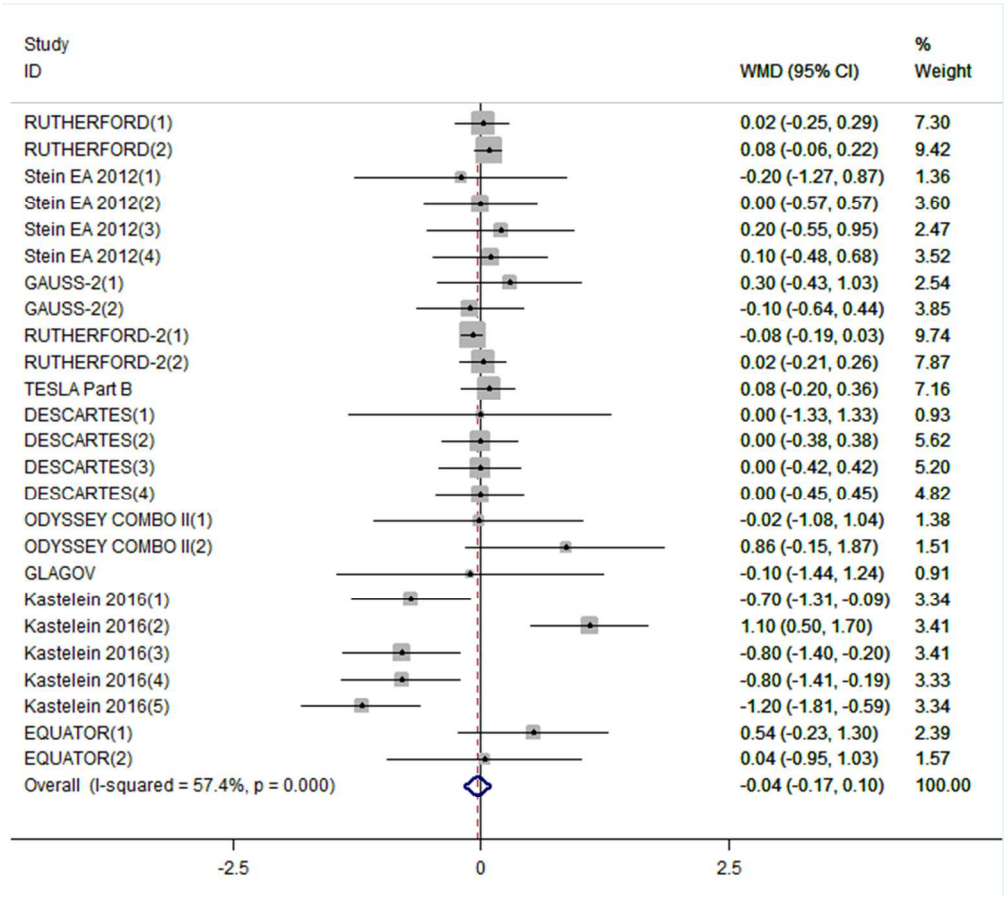


Figure 2. Forest plots depicting the effect of PCSK9-mAbs on hs-CRP.
CI=confidence interval, PCSK9-mAbs=PCSK9 monoclonal antibodies, hs-CRP= hypersensitive C-reactive protein

238x213mm (300 x 300 DPI)

Figure 3. Subgroup analyses of the effect of PCSK9-mAbs on hs-CRP.
CI=confidence interval. PCSK9-mAb=PCSK9 monoclonal antibody. hs-CRP= hypersensitive C-reactive protein. FH= familial hypercholesterolemia. non-FH= non-familial hypercholesterolemia.

256x133mm (300 x 300 DPI)

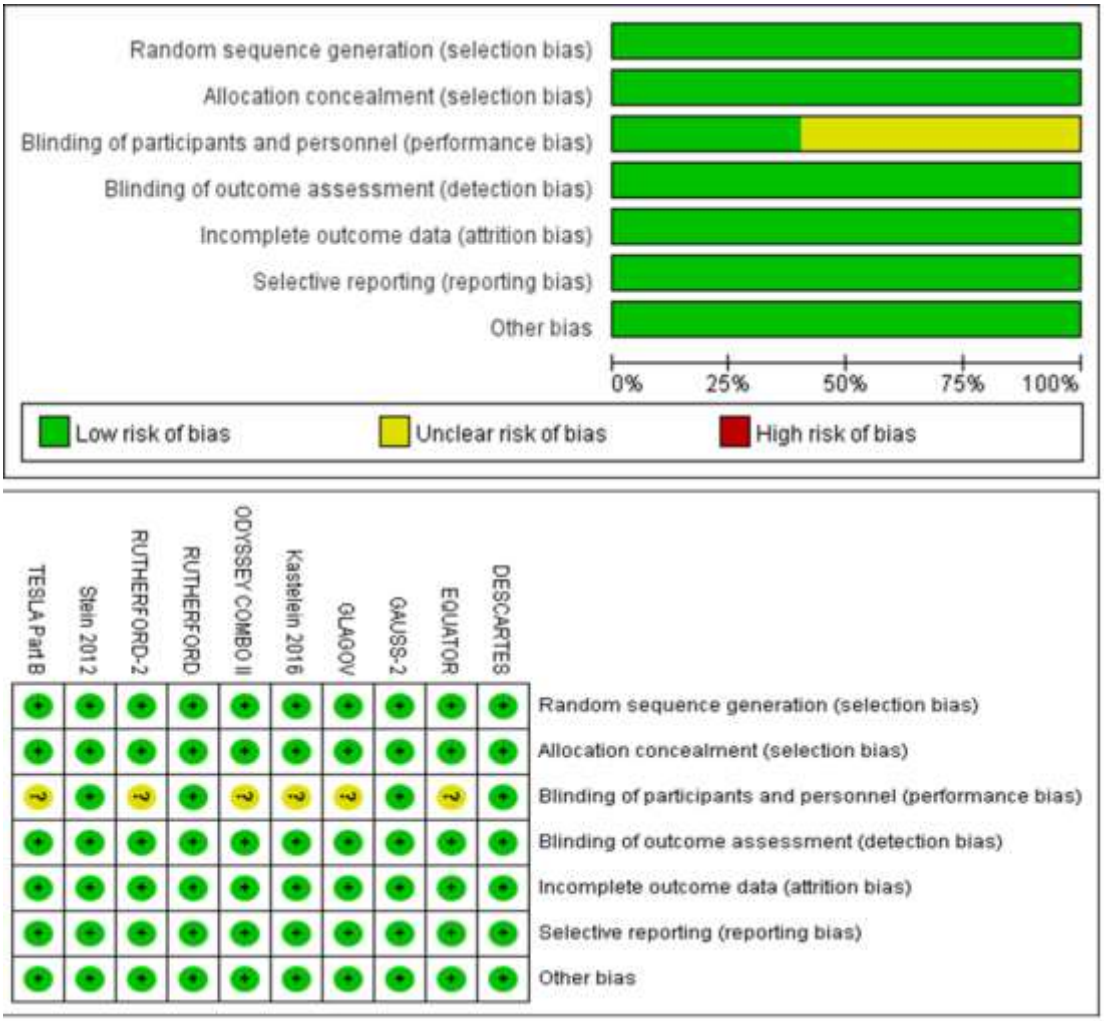
Supplementary material

Supplemental Table 1. Quality assessment of included studies using the Jadad scale

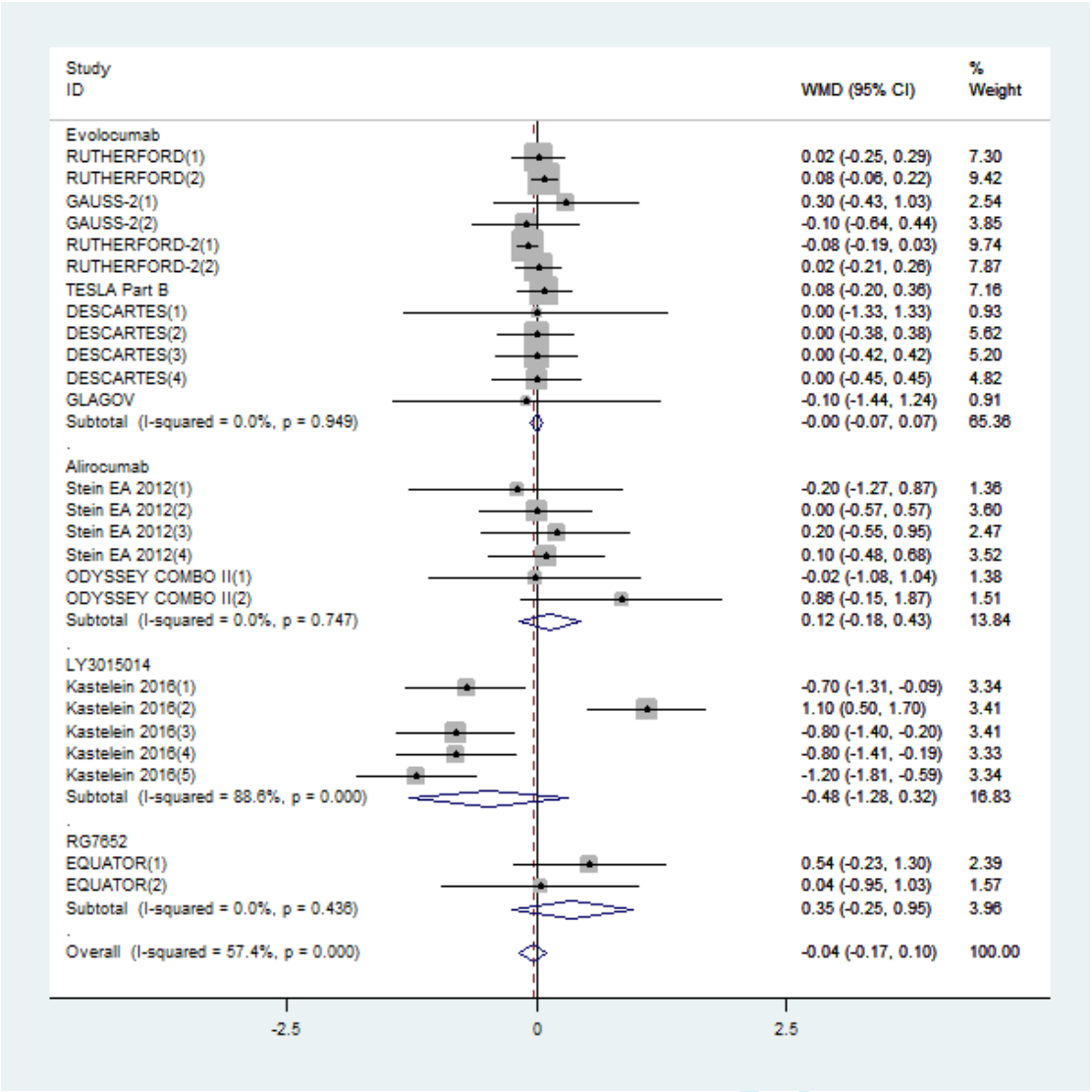
Studies	Representation of randomization	Appropriateness of method for randomization	Representation of double blinding	Appropriateness of method for double blinding	Representation of withdrawals	Total Score
RUTHERFORD	1	1	1	1	1	5
Stein EA 2012	1	1	1	1	1	5
DESCARTES	1	1	1	1	1	5
GAUSS-2	1	1	1	0	1	5
RUTHERFORD-2	1	1	1	0	1	4
TESLA Part B	1	1	1	0	1	4
ODYSSEY COMBO II	1	1	1	0	1	4
GLAGOV	1	1	1	0	1	4
Kastelein 2016	1	1	1	0	1	4
EQUATOR	1	1	1	0	1	4

Representation of randomization:0, not randomized or inappropriate method of randomization; 1, the study was described as randomized.
Appropriateness of method for randomization: 0, no information about the method of randomization;1, the method of randomization was described and it was appropriate.
Representation of double blinding: 0, no blind or inappropriate method of blinding; 1, the study was described as double blinding.
Appropriateness of method for double blinding: 0, no information about the method of double blinding;1, the method of double blinding was described and it was appropriate.
Withdrawals and dropouts:0, not describe the follow-up; 1, a description of withdrawals and dropouts.

Supplementary Figure 1. Evaluation of risk of bias in the studies.

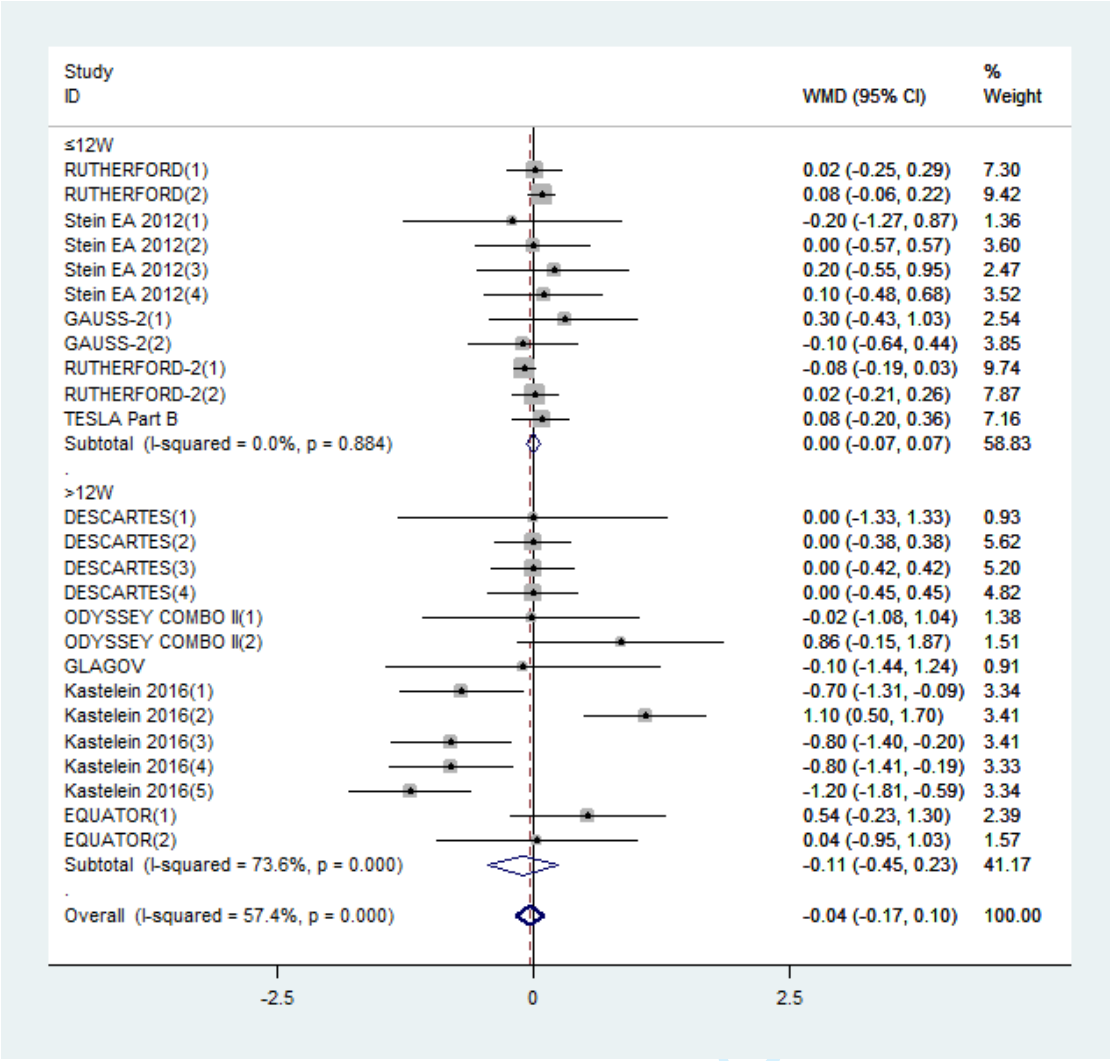


Supplementary Figure 2. Pooled analysis for hs-CRP stratified by PCSK9-mAb types.



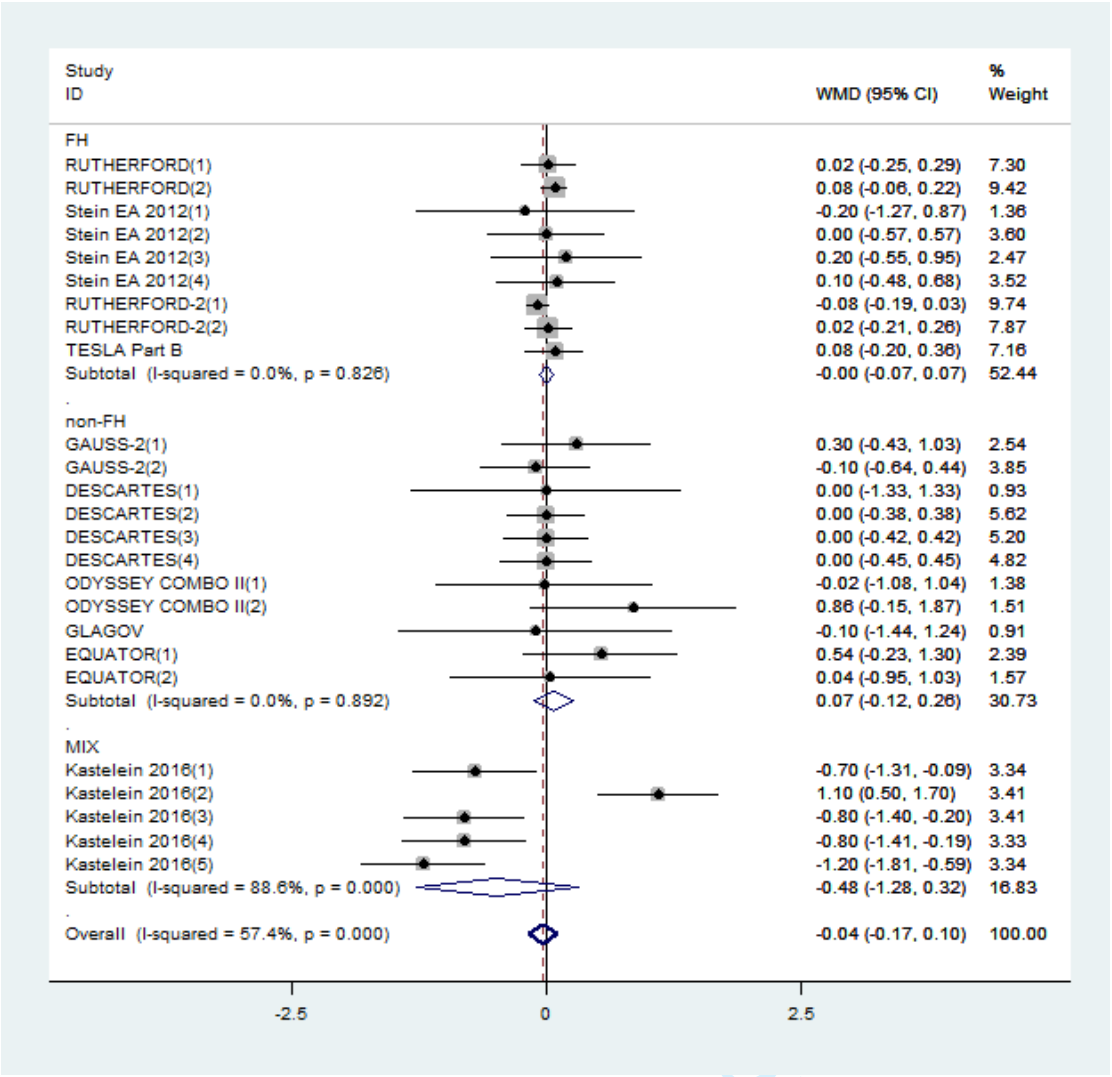
WMD= weighted mean difference, CI = confidence interval, PCSK9-mAb= proprotein convertase subtilisin/kexin type 9 monoclonal antibody, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 3. Pooled analysis for hs-CRP stratified by treatment durations.



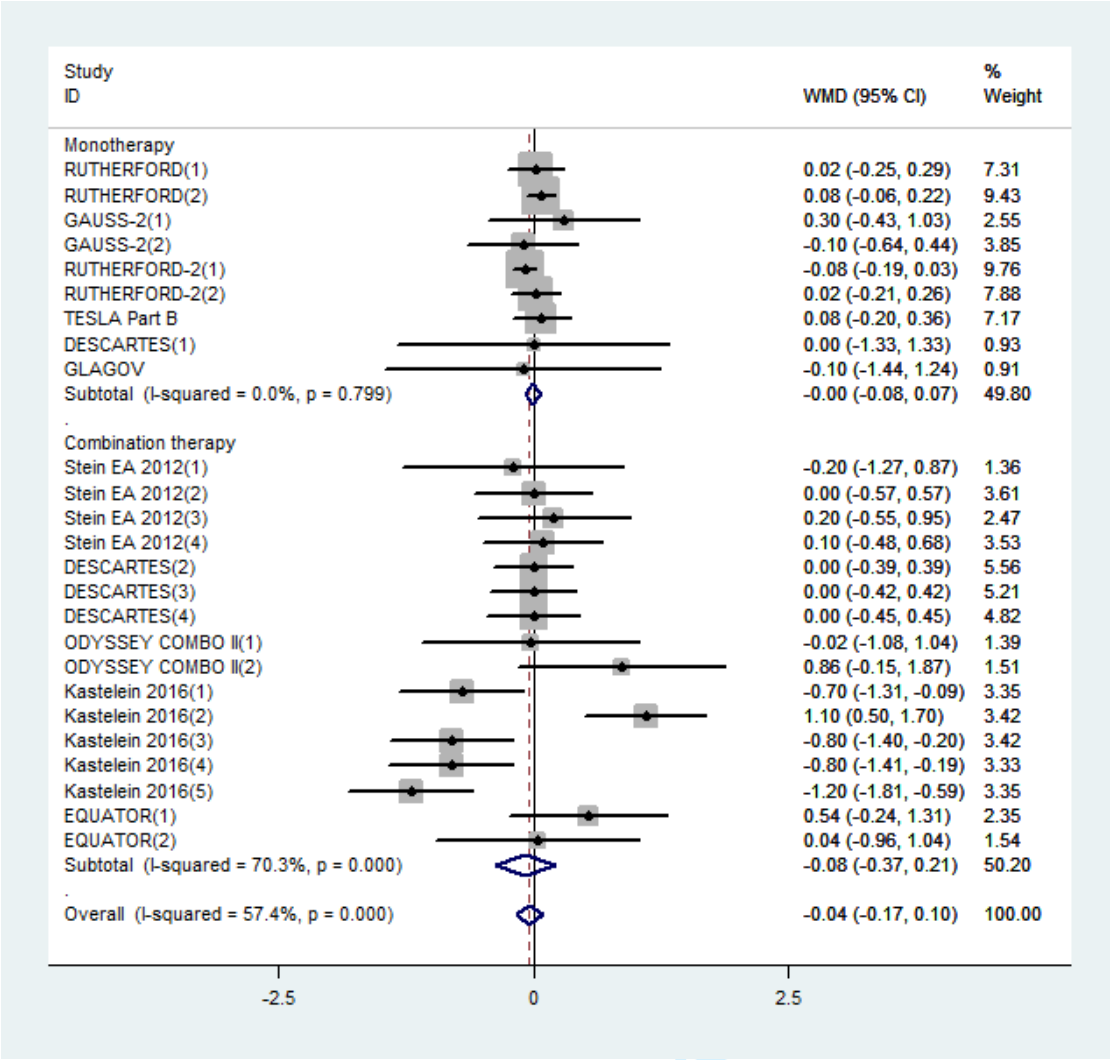
WMD=weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 4. Pooled analysis for hs-CRP stratified by participant characteristics



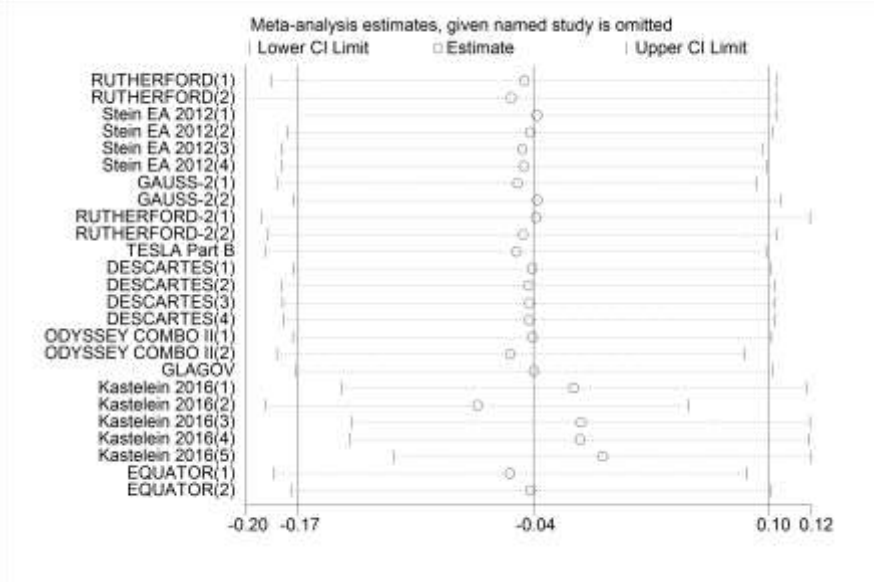
WMD= weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein, FH=familial hypercholesterolemia, non-FH= non-familial hypercholesterolemia

Supplementary Figure 5. Pooled analysis for hs-CRP stratified by of treatment methods.



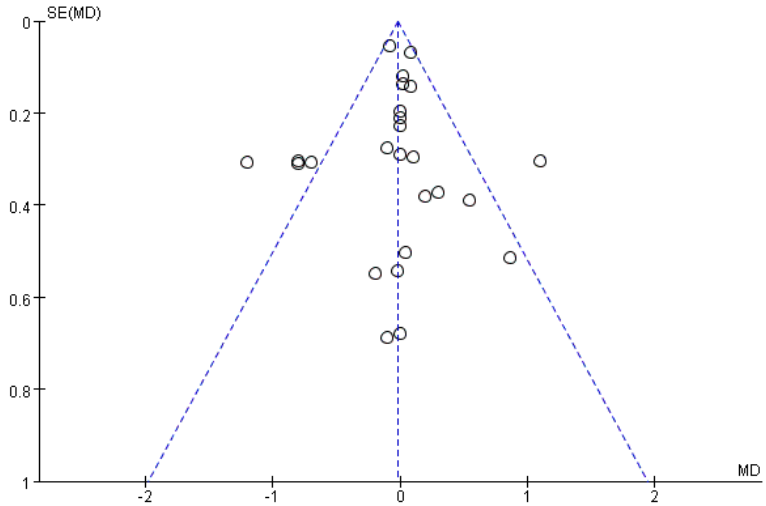
WMD=weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 6. Sensitivity analyses of hs-CRP.

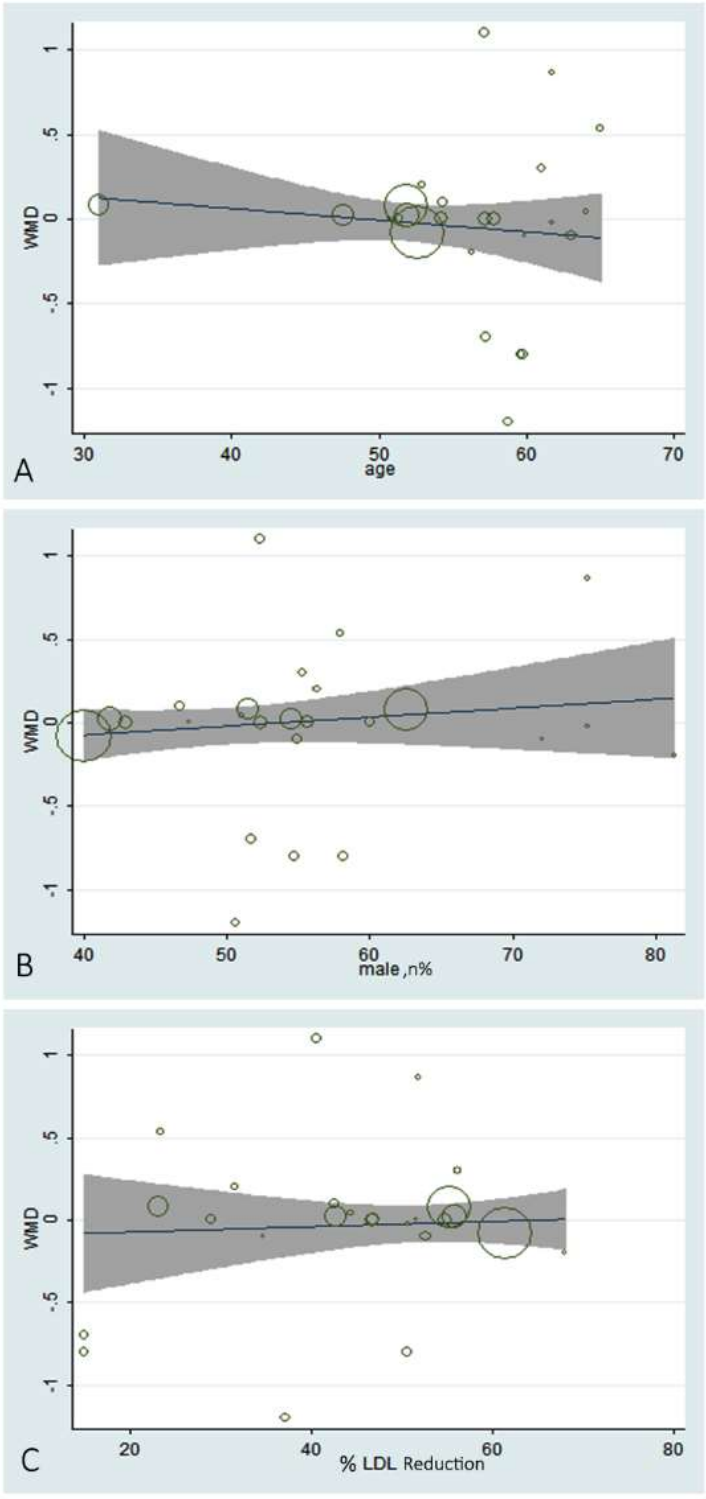


hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 7. Funnel Plots of included studies



Supplementary Figure 8. Meta-regression of baseline age (A), sex (B) and percent change of LDL-C (C).



LDL-C=low density lipoprotein cholesterol

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5-6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6-7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7-8

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7-8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8-9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-10,22
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10-11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Impact of PCSK9-monoclonal antibodies on circulating hs-CRP levels: a systematic review and meta-analysis of randomized controlled trials

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Title: Impact of PCSK9-monoclonal antibodies on circulating hs-CRP levels: a systematic review and meta-analysis of randomized controlled trials

Running Title: PCSK9-mAb and hs-CRP

Authors: Ye-Xuan Cao, MD*, Sha Li, MD, PhD *, Hui-Hui Liu, MD*, Jian-Jun Li, MD, PhD*

Affiliations: *Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China.

Correspondence: Professor Jian-Jun Li, MD, PhD, FESC, FACC. Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China. Tel: 86+10+88396077; Fax: 86+10+88396584. E-mail: lijianjun938@126.com.

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Number of tables/figures: tables 1; figures 3

ABSTRACT

Objective To evaluate the potential effect of proprotein convertase subtilisin/kexin type 9 monoclonal antibody (PCSK9-mAb) on high-sensitivity C-reactive (hs-CRP) concentrations.

Design A systematic review and meta-analysis of randomized controlled trials.

Data sources PubMed, MEDLINE, The Cochrane Library databases, ClinicalTrials.gov and recent conferences were searched from inception to May 2018.

Eligibility criteria for selecting studies All randomized controlled trials that reported changes of hs-CRP were included.

Results Ten studies involving 4198 participants were identified. PCSK9-mAbs showed a slight efficacy in reducing hs-CRP (-0.04mg/L, 95%CI:-0.17 to 0.01) but no statistically different. The results did not altered when subgroup analyses were performed including PCSK9-mAb types (Alirocumab:0.12mg/L, 95% CI:-0.18 to 0.43; Evolocumab:0.00 mg/L, 95% CI:-0.07 to 0.07; LY3015014:-0.48mg/L, 95% CI:-1.28 to 0.32; RG7652:0.35mg/L, 95% CI:-0.26 to 0.96), treatment duration (≤12w:0.00mg/L, 95% CI:-0.07 to 0.07; >12w: -0.11mg/L, 95% CI:-0.45 to -0.23), participant characteristics (familial hypercholesterolemia: 0.00mg/L, 95% CI:-0.07 to 0.07; non-familial hypercholesterolemia:0.07mg/L, 95% CI:-0.12 to 0.26; mix:-0.48mg/L, 95%CI:-1.28 to 0.32) and treatment methods (monotherapy: 0.00mg/L, -0.08 to 0.07; combination-therapy: -0.08mg/L, -0.37 to 0.21). Meta-regression analyses suggested no significant linear correlation between baseline age (p=0.673), sex (p=0.645), and low-density lipoprotein cholesterol reduction (p=0.339).

Conclusions Our updated meta-analysis suggested that PCSK9-mAbs had no significant impact on circulating hs-CRP levels irrespective of PCSK9-mAb types, participant characteristics, and treatment duration or methods.

Keywords: PCSK9; monoclonal antibody; hs-CRP; meta-analysis

Strengths and limitations of this study

- This is a comprehensive systematic review and meta-analysis of randomized controlled trials, which is conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.
- We used a broad search strategy and identified 10 studies that reported the changes of high-sensitivity C-reactive protein when proprotein convertase subtilisin/kexin type 9 monoclonal antibody was applied.
- Studies with moderate heterogeneity and lack of individual level data.

Introduction

Cardiovascular disease is the greatest burden of global health, which is mainly characterized by atherosclerosis[1]. Atherosclerosis is a chronic and progressive inflammatory disease, including endothelial dysfunction, lipid accumulation in the arterial wall and leukocyte infiltration, which leads to luminal stenosis, plaque rupture and acute coronary syndrome (ACS)[2]. Apart from well-established lipid theory, inflammation also plays an important role in the initiation and progression of atherosclerosis[3]. Recently, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) reported that anti-inflammatory therapy by canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β, significantly reduced the primary cardiovascular end points[4]. This study attracted attention to the inflammation intervention in cardiovascular medicine again.

C-reactive protein (CRP), especially high-sensitivity CRP (hs-CRP), is the most intensively investigated inflammatory biomarker in cardiovascular field[5]. Increasing studies have confirmed that hs-CRP is a predictor for the progression of atherosclerotic disease and future major adverse cardiovascular events (MACE)[6,7]. Moreover, previous studies also indicated that hs-CRP played a direct role in the development of atherosclerosis[8,9]. Therefore, reduction of inflammatory markers such as hs-CRP may be a strategy for decreasing MACE[10,11].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is known to target low-density lipoprotein receptor (LDLR) for degradation resulting in elevated plasma low-density lipoprotein cholesterol (LDL-C) levels[12-14]. Recently, it has been reported that PCSK9 monoclonal antibody (PCSK9-mAb), as a novel lipid-lowering drug, can reduce LDL-C by a mean of 70% accompanied with reduction of MACE[15]. Although the relation of PCSK9 to LDL-C has been established, its role in inflammation has not been fully understood. Several experimental studies found that PCSK9 could promote the progression of atherosclerosis by

enhancing inflammatory reaction[16]. On the other hand, PCSK9 deficiency could alleviate the inflammation reaction[17]. Hence, whether PCSK9-mAb can reduce inflammatory marker is clinically of great interest since it was used to treat cardiovascular diseases. A meta-analysis covering 7 randomized controlled trials (RCTs) studies published two years ago suggested that PCSK9-mAbs had no effect on serum hs-CRP[18]. However, this meta-analysis may be limited by insufficient subgroup analyses, sample size, and lack of newly published data[19, 20]. Hence, we performed this meta-analysis including all RCTs published till May 2018 to further explore the efficacy of PCSK9-mAbs on circulating hs-CRP levels.

Methods

Patient and public involvement statement

Patients and the public were not involved.

Literature search

The present study was designed according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement[21]. To identify all RCTs assessing the effect of PCSK9-mAbs on circulating hs-CRP levels, we comprehensively searched PubMed, MEDLINE, and the Cochrane Library database and ClinicalTrials.gov up until May 2018. The search terms we used included the followings: (AMG145 or Evolocumab or REGN727 or SAR236553 or Alirocumab or RN316 or bococizumab or RG7652 or LY3015014 or PCSK9 antibody or anti-PCSK9) AND (randomized controlled trial OR randomized OR randomly). Meanwhile, manual search was performed for relevant studies including references lists, relevant review articles and commentaries. No language restriction was used.

Study selection

Original studies met the following criteria would be included: the design was phase 2 or phase 3 double-blind RCTs with longer than 8 weeks treatment duration; human participants were randomly assigned to PCSK9-mAbs group versus control group with or without other lipid-lowering therapy; outcomes included percentage changes of hs-CRP from baseline. Studies were excluded if they were duplicate publications, review articles, non-human studies, observational studies, and lack of adequate information on outcomes or lacking control group. Two investigators (YC and SL) independently screened and selected the eligible studies. Disagreements were resolved by discussion with a third investigator.

Data Extraction

A standardized extraction form was used to extract the following items by two investigators (YC and HL) independently: trial name/first author, year of publication, type of intervention, follow-up period, treatment duration, number of patients, participant characteristics, background lipid-lowering therapy, types and doses of PCSK9-mAbs, LDL and hs-CRP levels at baseline and changes. We included the final reported follow-up point if a trial contains several time points. If necessary, further information was required from correspondence author.

Quality Assessment

We used Cochrane Collaboration's tool and Jadad score to assess the data and the methodological quality of included RCTs. For Cochrane Collaboration's tool, the following items were performed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective

reporting (reporting bias), and other sources of bias. The judgments were classified as 'low risk', 'high risk', and 'unclear risk' of bias. The 5-point Jadad score included the following items: basis of randomization (0 to 2 points), double blinding (0 to 2 points), and withdrawals /dropouts (0 to 1 points). Studies with a score ≥3 points are considered to be high quality. Two investigators (YC and HL) independently assessed the quality of each study. Disagreements were resolved by discussion with a third investigator.

Data Synthesis and Statistical Analysis

All analyses were conducted according to the intention-to-treat principles. For all efficacy outcomes, changes in hs-CRP concentrations were expressed as weighted mean difference (WMD) and 95% confidence interval (CI). All the data were standardized and expressed by mg/mL. Standard deviation could be calculated from CI, interquartile range or standard error according to formulas in the Cochrane Handbook for Systematic Reviews of Interventions if not reported.

Heterogeneity was assessed by the Cochran Q test and the I^2 statistic. We considered I^2<25% as representing low heterogeneity and I^2>75% as representing at high heterogeneity. Outcomes were calculated by fixed-effects models under no or low to moderate inconsistency (I^2<50%); otherwise, the data was pooled based on random-effects models. Subgroups were applied to reduce the heterogeneity if I^2 ≥50%, such as PCSK9-mAb types, treatment duration, and participant characteristics. In order to explore the resource of heterogeneity, sensitivity analysis was conducted by omitting studies in turn to evaluate the consistency of the results. Meta-regression analyses were performed to evaluate the contribution of participant characteristics and reductions in LDL-C concentrations. Publication bias was assessed with a funnel plot and Egger's test.

All analyses were conducted with Review Manager Version 5.3 (Copenhagen: The

Nordic Cochrane Centre, The Cochrane Collaboration, Denmark) and Stata 14.0 (Stata Statistical Software: College Station, TX: Stata Corp LP). P value <0.05 was considered to be statistically significant.

Results

Study selection and Characteristics

The initial search identified 575 articles. After excluding duplicate publications and screening the titles and abstracts, 430 were excluded and 145 studies were retrieved for full-text identification. We further excluded 135 studies, of which 58 were pooled or meta-analysis, 2 studies were not RCTs and 13 was phase 1 trials, 16 were open label trails and 42 without adequate information. Finally, ten studies were included in this meta-analysis[20,22-30]. Figure 1 shows flow diagram of selection process.

These ten studies were published between 2012 and 2017 from different countries with low risk of bias, of which 5 were phase 2 studies and 5 were phase 3 studies (Table 1). A total of 4198 participants were included, comprising 2728 individuals in the PCSK9-mAb group and 1470 in the control group. Alirocumab (SAR236553/REGN727) was used in 4 arms and 11 arms applied Evolocumab (AMG 145). Five arms managed LY3015014 and 2 arms used RG7652. Four trials included patients with familial hypercholesterolemia (FH), of which 3 were heterozygous FH (HeFH) and 1 was homozygous FH (HoFH). Most of the treatment duration ranged from 12 to 24 weeks and the longest treatment duration was 78 weeks. Apart from DESCARTES trial which co-administered with atorvastatin, another 9 studies used PCSK9-mAb as monotherapy. Baseline characteristics including circulating hs-CRP levels were similar between PCSK9-mAbs and control groups within each study. The characteristics of these trials and participants are summarized in Table 1. All these studies had a relatively high quality evaluated by the Jadad score and low risk of bias (online supplementary Table 1

and online supplementary Figure 1, 3 scores=5, 6 scores=4).

Efficacy outcomes of PCSK9-mAbs on hs-CRP

A total of 4198 participants were included in the analysis of efficacy of PCSK9-mAbs on plasma hs-CRP concentrations before and after treatment. When data were pooled, PCSK9-mAbs showed a slight efficacy in reducing hs-CRP (WMD: -0.04mg/L, 95%CI: -0.17 to 0.01), while no statistical difference was found compared with control treatment (Figure 2). There was a moderate heterogeneity between each study (I^2=57.4%, P=0.0001), so the random-effect model was selected.

To assess the potential discrepancy, we applied the subgroup analyses based on the characteristics of trials and participants (Figure 3 and online supplementary Figures 2-5). Although the efficacy of LY3015014 was a mild higher (-0.48 mg/L, 95% CI:-1.28 to 0.32), there was no difference between these four antibodies (Alirocumab: 0.12 mg/L, 95% CI: -0.18 to 0.43; Evolocumab: 0.00 mg/L, 95% CI: -0.07 to 0.07; RG7652: 0.35 mg/L, 95% CI: -0.26 to 0.96). When studies were classified by treatment duration, the hs-CRP reduction showed no difference in less than 12-week duration group (0.00 mg/L, 95% CI: -0.07 to 0.07) and above 12-week duration group (-0.11mg/L, 95% CI: -0.45 to -0.23). There was no significant reduction in circulating hs-CRP with use of PCSK9 antibodies compared with control treatment when categorized to participant characteristics (FH: 0.00 mg/L, 95% CI: -0.07 to 0.07; non-FH: 0.07 mg/L, 95% CI: -0.12 to 0.26; mix: -0.48 mg/L, 95%CI: -1.28 to 0.32). The analysis stratified by treatment method also supported the results that no differential effect of PCSK9-mAb therapy on plasma hs-CRP concentrations was observed (monotherapy: 0.00 mg/L, 95%CI: -0.08 to 0.07 vs combination-therapy: -0.08 mg/L, 95%CI: -0.37 to 0.21).

Sensitivity analysis and publication bias

The sensitivity analysis for all outcomes was conducted by gradually removing each study. However, the results did not change meaningfully (online supplementary Figure 6). Neither funnel plots (online supplementary Figure 7) nor Egger's regression test (p=0.913) showed publication bias.

Meta-regression analyses

We used meta-regression analyses to assess the relationship between changes in hs-CRP and baseline age, sex, and average LDL changes (online supplementary Figure 8). No statistically significant relationship between baseline age (p=0.673), male sex (p=0.645), and hs-CRP changes were observed. Likewise, LDL-C lowering effects by PCSK9-mAb therapy had no impact on hs-CRP lowering (p=0.339, online supplementary Figure 8).

Discussion

The results of this updated, comprehensive meta-analysis, based on 10 RCTs encompassing 4198 participants, suggested that short-term PCSK9-mAb therapy had no impact on circulating hs-CRP concentrations. In the subgroup analyses, no difference was found between PCSK9-mAb types, participant characteristics, and treatment duration or methods.

Atherosclerosis, a chronic progressive disorder, is characterized by lipid accumulation and chronic inflammation in the arterial wall[2]. Although previous data indicated a positive effects of anti-inflammatory drugs on atherosclerosis in animal studies but no positive data was available in human studies[31]. Fortunately, recent evidence from CANTOS found that canakinumab significantly reduced hs-CRP levels and MACE after follow-up of 3.7 years which may support the inflammatory hypothesis of atherosclerosis[4]. Moreover, ongoing Cardiovascular Inflammation Reduction Trial (CIRT) was also designed to directly test the

inflammatory hypothesis of atherosclerosis by evaluating the effect of methotrexate on adverse cardiovascular outcomes without substantive impact on lipids[32]. Hence, focusing on inflammation in the development of atherosclerosis may be an unsolved issue and great of interest clinically.

In fact, increasing studies indicated that hs-CRP could independently predict MACE[6]. Framingham study found that men and women in the highest quartile of CRP respectively had twice and three-times the risk of stroke compared with those in the lowest ones after more than 10-years follow-up[33]. The Northern Manhattan Study reported that >3 mg/L CRP was associated with a 1.7-fold increase in cardiovascular outcomes and a 1.55-fold increase in mortality[34]. Furthermore, it has been demonstrated that hs-CRP also plays a direct and vital role in the development of atherosclerosis. Zwaka et al[8] found that CRP enhanced the transformation from macrophages to foam cells by increasing the uptake of LDL. CRP was also reported to impair vasodilatation, inhibit the synthesis of nitric oxide synthase, and facilitate the adhesion of monocyte[35,36]. Based on these evidence, reduction of hs-CRP may be associated with a decrease in MACE. Interestingly, the JUPITER study applied rosuvastatin on individuals with LDL-C levels below 130 mg/dL and hs-CRP levels ≥2 mg/L, and suggested a significant reduction in all vascular events[37]. Although a fact that statins lower LDL-C and proportionately reduce MACE is widely accepted, the hs-CRP reduction by statin administration is also an attractive phenomenon, called as pleiotropic effect of statin. That is the reason why we chose hs-CRP as an inflammatory biomarker to identify whether PCSK9-mAb has an effect on inflammatory status.

Although the relationship between PCSK9 and LDL-C was well-established, more and more evidence demonstrated its function beyond lipids. In 2010, microarray gene expression analysis suggested that PCSK9 affected not only cholesterol metabolism, but also inflammation[38]. Experimental studies indicated that PCSK9 could participate in vascular and

systemic inflammation[16]. In transgenic mice expressing human PCSK9 gene, Tavori et al[39] observed that atherosclerosis lesion size and local Ly6C^{hi} monocytes, the precursors of pro-inflammatory M1 macrophages, significantly increased. Clinical studies further supported this hypothesis. Our previous study found that serum PCSK9 concentrations were independently associated with white blood cell count in patients with stable coronary artery disease, indicating PCSK9 might be involved in the inflammation process[40]. ATHEROREMO-IVUS study reported a positive linearly association between PCSK9 levels and coronary plaque inflammation including amount of necrotic core tissue and plaque volume[41]. On the other hand, data also showed that PCSK9 inhibition could exert an anti-inflammatory effect. Tang et al[42] reported that PCSK9 small interfering RNA reduced the expression of pro-inflammatory genes through nuclear factor kappa B (NF-κB) pathway. In apoE-/- mice, PCSK9 silencing limited the development of atherosclerosis and decreased the number of macrophages via TLR4/NF-κB signaling pathway[43]. Besides, AT04A anti-PCSK9 vaccine also got the same results that anti-PCSK9 therapy could reduce vascular inflammation[44]. Recently, Bernelot et al[45] found that after 24 weeks of treatment with PCSK9-mAbs, the migratory capacity of monocytes and inflammatory responsiveness reduced significantly while anti-inflammatory cytokine levels increased in FH patients. Therefore, we hypothesized that PCSK9-mAbs treatment could reduce hs-CRP in randomized clinical studies. Unfortunately, in our meta-analysis the results showed that PCSK9-mAbs therapy had no effect on decreasing hs-CRP in both FH participants and non-FH individuals.

To explore the potential reasons why PCSK9-mAbs therapy was not benefit from inflammatory marker, named as the reduction of circulating hs-CRP levels, we further performed subgroup analyses. Firstly, we did not observe an impact of PCSK9-mAbs types on hs-CRP, which may exclude the influence of PCSK9-mAbs itself on the inflammatory marker in our meta-analysis. Besides, the same results were also observed in combination of

statins and PCSK9-mAbs group, suggesting that the effects of PCSK9-mAbs therapy on hs-CRP is not linked with treatment methods. It is notable that the participants in JUPITER trial had high levels of hs-CRP at baseline, while in PCSK9-mAb therapy, initial hs-CRP levels were at normal range in recruited individuals. Finally, we did not find a positive effects of PCSK9-mAbs therapeutic duration on hs-CRP in this updated analysis. Taken together, we may conclude that although PCSK9-mAbs had a powerful ability in lowering LDL-C, they had no impact on circulating hs-CRP concentration despite of PCSK9-mAb types, participant characteristics, and treatment duration or methods.

Limitations

There were several limitations in our meta-analysis. Firstly, our results were based on study data but not individual data as in most meta-analyses. Secondly, although our meta-analysis is an updated one, it is still limited by study numbers, sample size, and therapy duration. Moreover, moderate degree of heterogeneity was observed in several comparisons. However, there was no publication bias and the results were rather consistent among different subgroups. Sensitivity analysis suggested that the pooled WMD were robust. Finally, some studies included in this meta-analysis did not provide adequate information about blinding of participants and personnel.

Conclusions

In conclusion, the current updated evidence suggested that PCSK9-mAb, a novel powerful lipid-lowering drug, had no significant impact on circulating hs-CRP concentrations, whose effect did not influenced by PCSK9-mAb types, participant characteristics, and treatment duration or methods. Long-term observation may be needed.

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Contributors

JJL contributed to conception and design, acquisition, analysis, and interpretation, and critically revised the manuscript. YXC contributed to design, acquisition, analysis, and interpretation and drafted the manuscript. SL contributed to acquisition, analysis, and critically revised the manuscript. HHL contributed to analysis, interpretation and critically revised the manuscript. All the authors read and approved the final version of the manuscript.

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Tables

Table 1. Study characteristics of included randomized controlled trials.

Study	Year	Phase	Inclusion criteria	Patients N	Arm	Mean hs-CRP at baseline mg/L	Mean age (years)	Male %	% LDL-C reduction	Drugs/control	Treatment duration	Jadad Score
RUTHERFORD[22]	2012	II	HeFH	167	(1)	1.09±1.37	47.6	54.5	42.7	E:350mg/PBO, Q4W	12W	5
					(2)	1.07±1.24	51.8	62.5	55.2	E:420mg/PBO, Q4W		
Stein 2012[24]	2012	II	HeFH	77	(1)	1·40±1.78	56.3	81.3	67.9	A:150mg /PBO, Q2W	12W	5
					(2)	0·60±0.82	51.3	60.0	28.9	A:150mg /PBO, Q4W		
					(3)	0·70±1.48	52.9	56.3	31.5	A:200mg /PBO, Q4W		
					(4)	0.70±0.59	54.3	46.7	42.5	A:300mg /PBO, Q4W		
DESCARTES[25]	2014	II	HC	894	(1)	2.00±3.70	50.7	47.3	51.5	E:420 mg/ PBO, Q4W	52W	5
					(2)	1.00±1.48	57.2	42.9	54.7	E:420 mg+ATV10 mg/ PBO, Q4W		
					(3)	1.00±1.48	57.8	52.4	46.7	E:420 mg+ATV80 mg/ PBO, Q4W		
					(4)	1.00±1.48	54.2	55.6	46.8	E:420 mg+ATV80 mg+ Eze10mg / PBO, Q4W		
GAUSS-2[26]	2014	III	HC	307	(1)	1.40±2.00	61.0	55.3	56.1	E:140 mg/ Eze, Q2W	12W	4
					(2)	1.80±1.78	63.0	54.9	52.6	E:420 mg/ Eze, Q4W		
RUTHERFORD-2[23]	2014	III	HeFH	329	(1)	0.92±1.03	52.6	40.0	61.3	E:140 mg /PBO,Q2W	12W	4
					(2)	1.04±1.24	51.9	41.8	55.7	E:420 mg /PBO,Q4W		
TESLA Part B[27]	2014	III	HoFH	49		0.70±1.04	31.0	51.5	23.1	E:140 mg /PBO, Q4W	12W	4
ODYSSEY COMBO II[28]	2015	III	HC	720	(1)	3.58±7.78	61.7	75.2	50.6	A: 75mg /Eze, Q2W	24W	4
					(2)	3.58±7.78	61.7	75.2	51.8	A: 75mg /Eze, Q2W	52W	
GLAGOV[29]	2016	III	HC	968		1.60±1.93	59.8	72.1	34.6	E:420 mg /PBO, Q4W	78W	4

Kastelein 2016[20]	2016	II	HC	519	(1)	1.03±1.41	57.2	51.7	14.9	LY:20mg/PBO, Q4W	16W	4
					(2)	1.34±1.11	57.1	52.3	40.5	LY:120mg/PBO, Q4W		
					(3)	1.63±1.78	59.7	54.7	50.5	LY:300mg /PBO, Q4W		
					(4)	1.39±1.70	59.6	58.1	14.9	LY:100mg /PBO, Q8W		
					(5)	1.10±1.63	58.7	50.6	37.1	LY:300mg /PBO, Q8W		
EQUATOR[30]	2017	II	HC	168	(1)	1.60±2.70	65.0	57.9	23.3	RG:400mg/PBO, Q4W	24W	4
					(2)	2.00±5.90	64.0	51.0	44.3	RG:800mg /PBO,Q8W		

Data presented as mean±SD; LDL-C, low density lipoprotein-cholesterol; hs-CRP, hypersensitive C reactive protein; HC, hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; E, Evolocumab; A, Alirocumab; LY: LY3015014; RG, RG7652; PBO, placebo; ATV, atorvastatin; Eze, ezetimibe; W, weeks; Q2W, every 2 weeks; Q4W, every 4 weeks. N, number; SD, standard deviation.

Figure legends

Figure 1. Flow diagram of selection of studies.

Figure 2. Forest plots depicting the effect of PCSK9-mAbs on hs-CRP.

CI=confidence interval, PCSK9-mAb=PCSK9 monoclonal antibody, hs-CRP= hypersensitive C-reactive protein

Figure 3. Subgroup analyses of the effect of PCSK9-mAbs on hs-CRP.

CI=confidence interval. PCSK9-mAb=PCSK9 monoclonal antibody. hs-CRP= hypersensitive C-reactive protein. FH= familial hypercholesterolemia. non-FH= non-familial hypercholesterolemia.

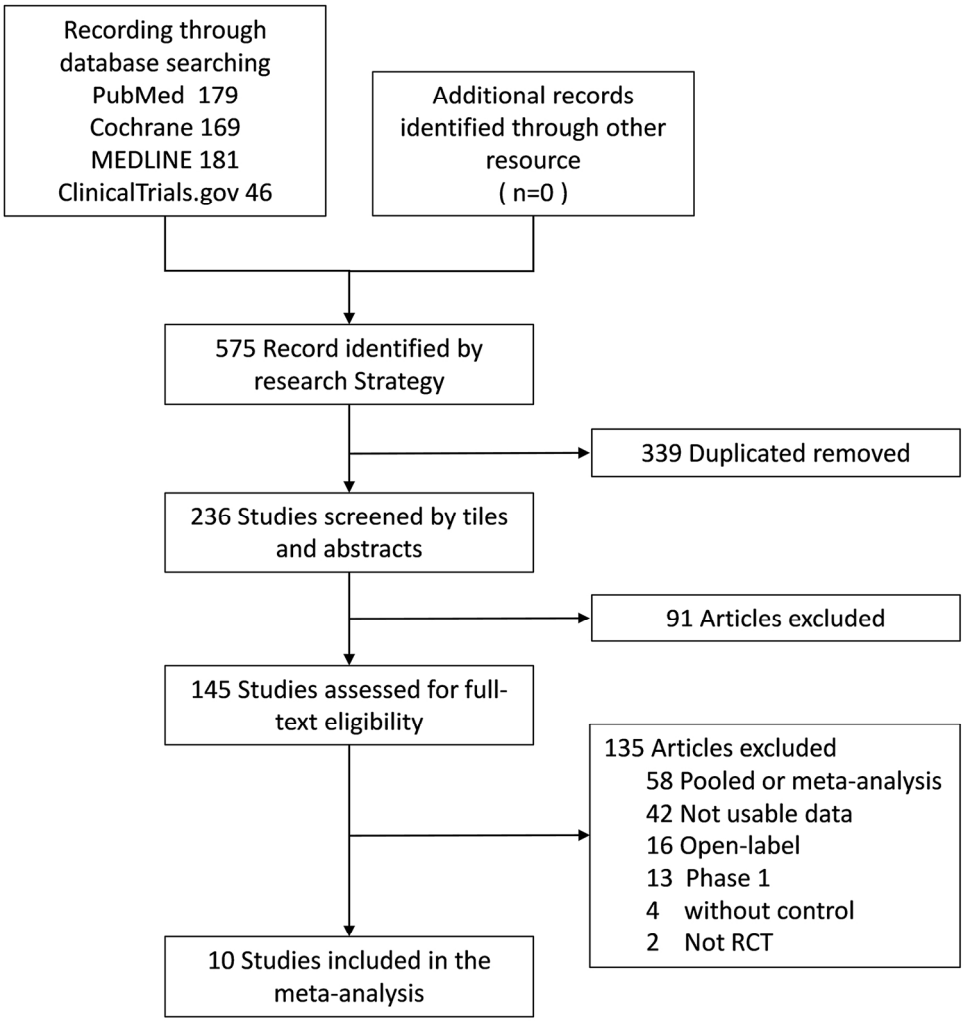

Figure 1. Flow diagram of selection of studies.

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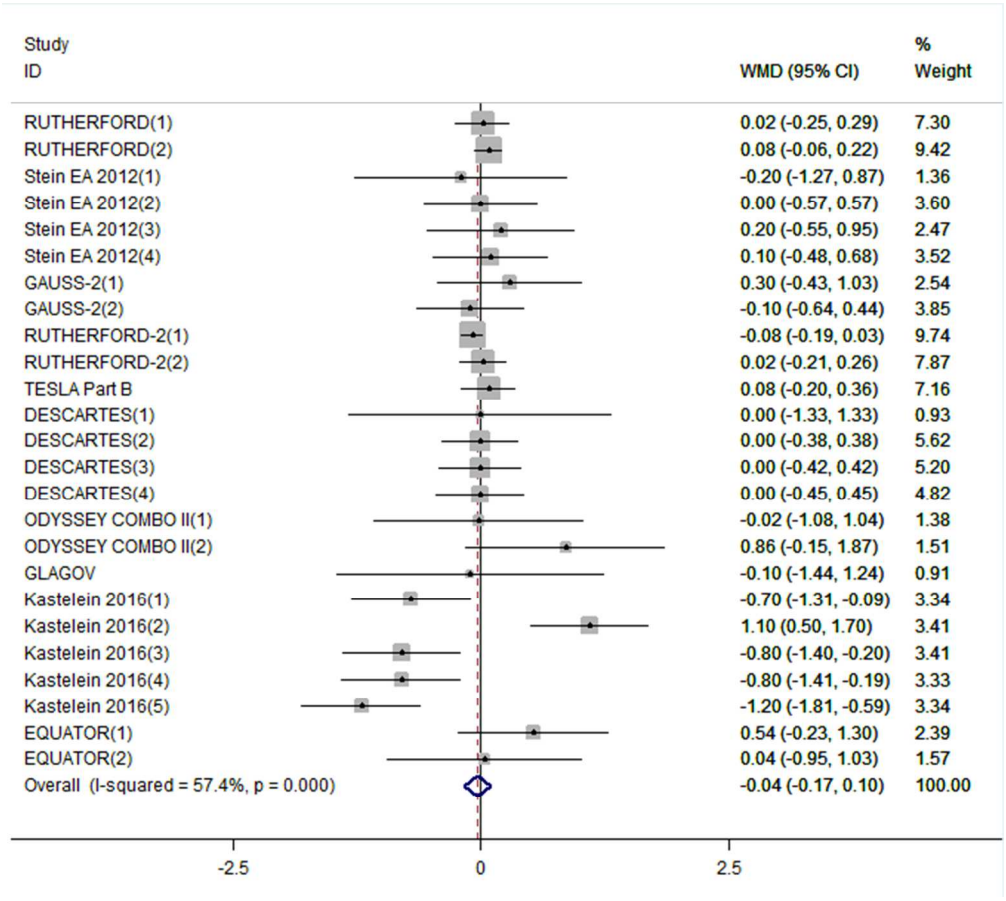


Figure 2. Forest plots depicting the effect of PCSK9-mAbs on hs-CRP.
CI=confidence interval, PCSK9-mAbs=PCSK9 monoclonal antibodies, hs-CRP= hypersensitive C-reactive protein

238x213mm (300 x 300 DPI)

Figure 3. Subgroup analyses of the effect of PCSK9-mAbs on hs-CRP.
CI=confidence interval. PCSK9-mAb=PCSK9 monoclonal antibody. hs-CRP= hypersensitive C-reactive protein. FH= familial hypercholesterolemia. non-FH= non-familial hypercholesterolemia.

256x133mm (300 x 300 DPI)

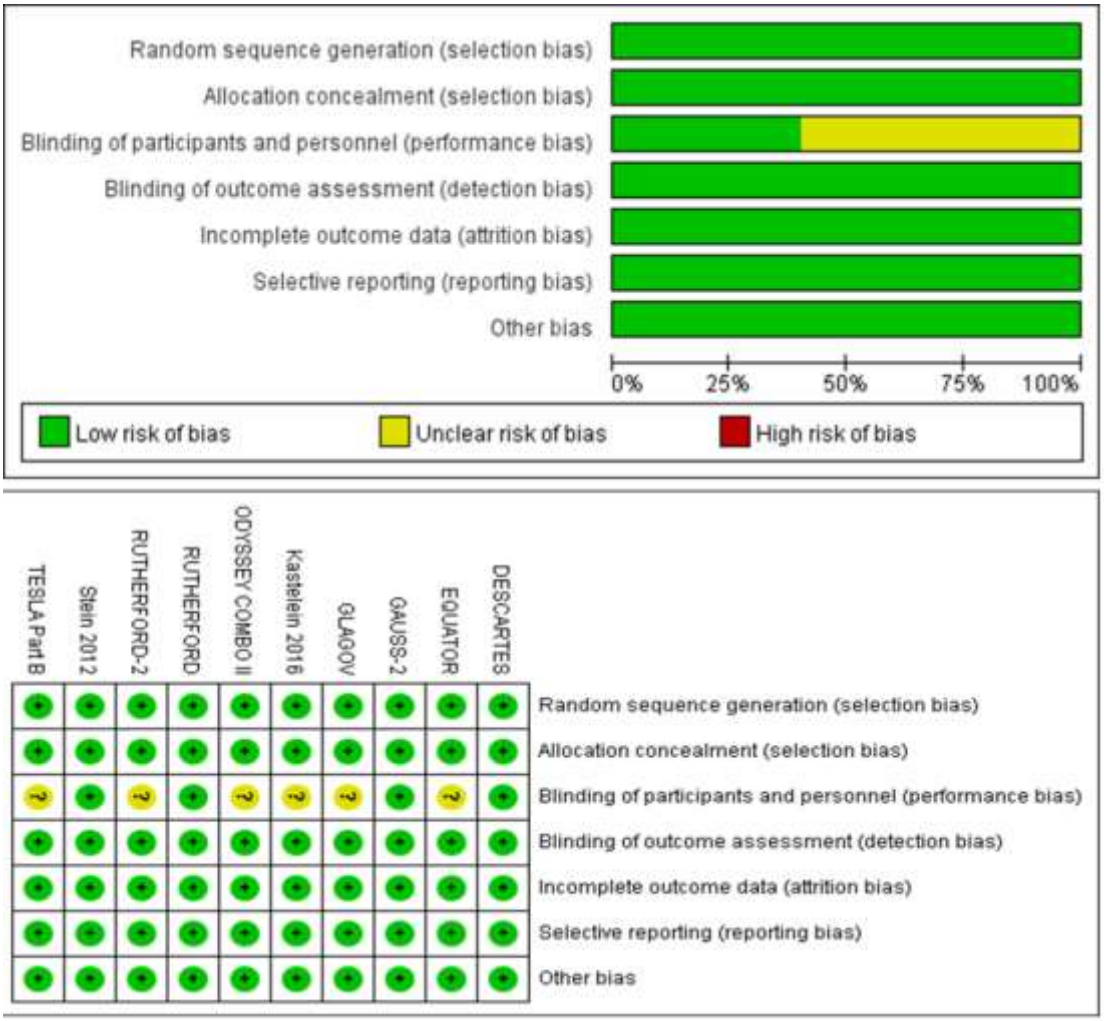
Supplementary material

Supplemental Table 1. Quality assessment of included studies using the Jadad scale

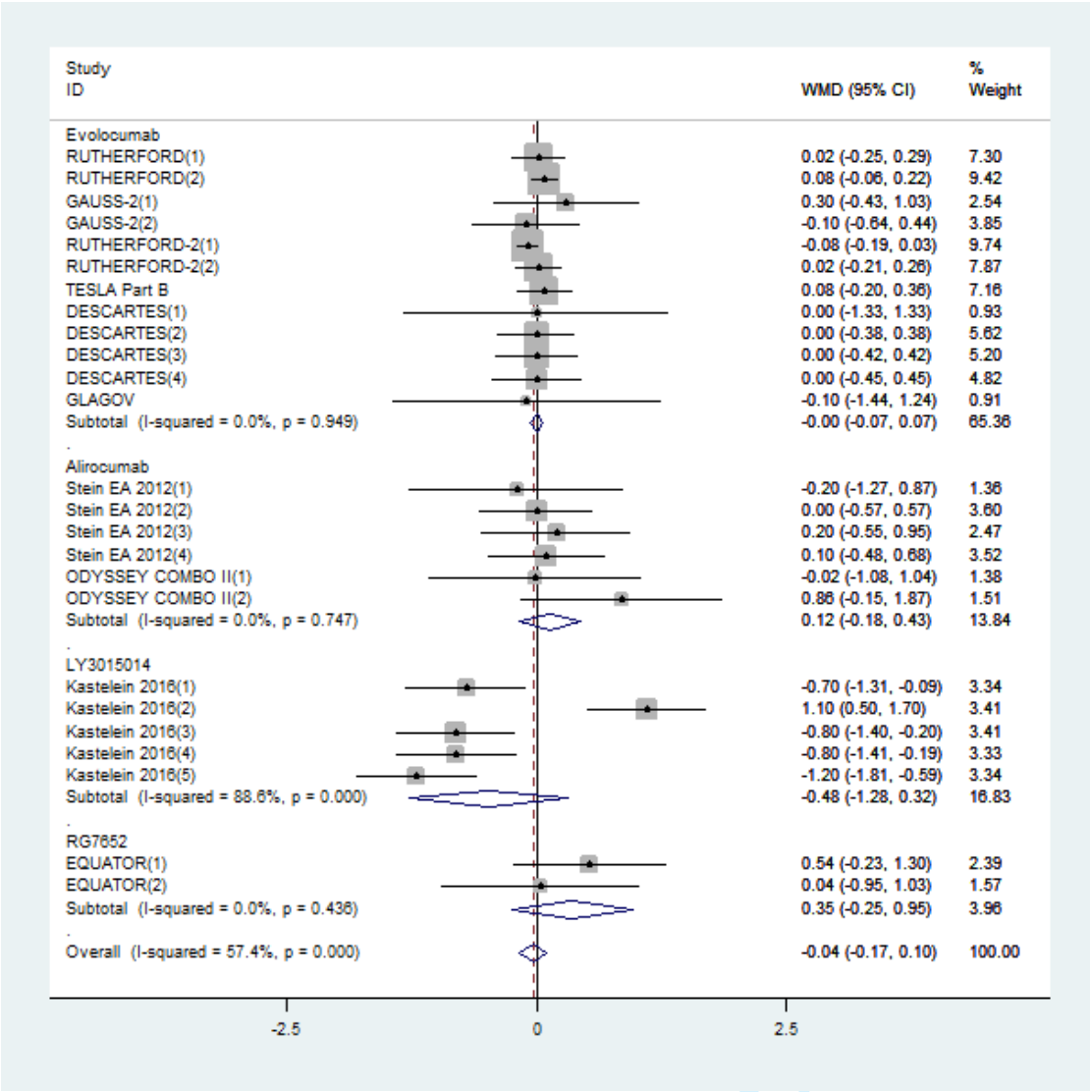
Studies	Representation of randomization	Appropriateness of method for randomization	Representation of double blinding	Appropriateness of method for double blinding	Representation of withdrawals	Total Score
RUTHERFORD	1	1	1	1	1	5
Stein EA 2012	1	1	1	1	1	5
DESCARTES	1	1	1	1	1	5
GAUSS-2	1	1	1	0	1	5
RUTHERFORD-2	1	1	1	0	1	4
TESLA Part B	1	1	1	0	1	4
ODYSSEY COMBO II	1	1	1	0	1	4
GLAGOV	1	1	1	0	1	4
Kastelein 2016	1	1	1	0	1	4
EQUATOR	1	1	1	0	1	4

Representation of randomization:0, not randomized or inappropriate method of randomization; 1, the study was described as randomized.
Appropriateness of method for randomization: 0, no information about the method of randomization;1, the method of randomization was described and it was appropriate.
Representation of double blinding: 0, no blind or inappropriate method of blinding; 1, the study was described as double blinding.
Appropriateness of method for double blinding: 0, no information about the method of double blinding;1, the method of double blinding was described and it was appropriate.
Withdrawals and dropouts:0, not describe the follow-up; 1, a description of withdrawals and dropouts.

Supplementary Figure 1. Evaluation of risk of bias in the studies.

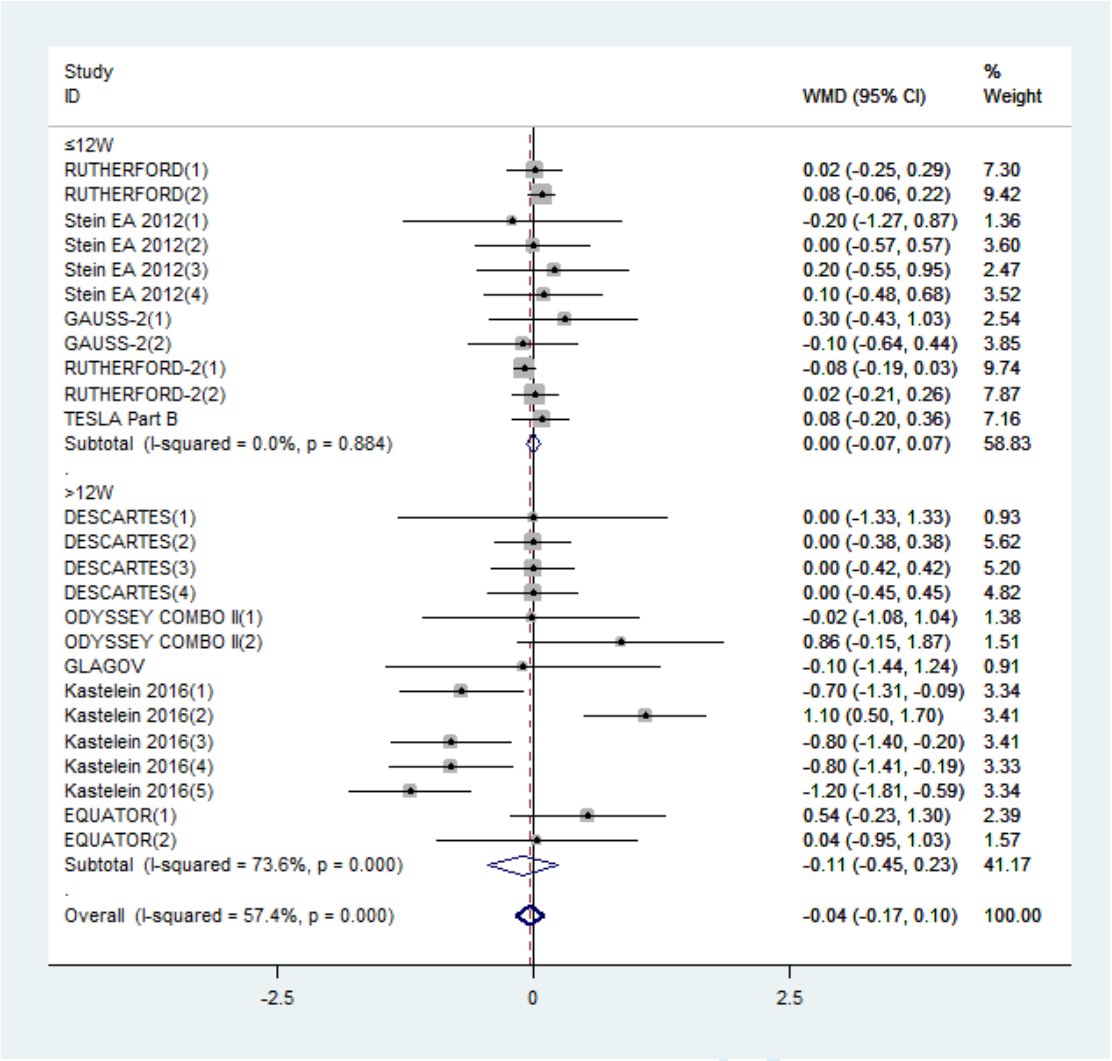


Supplementary Figure 2. Pooled analysis for hs-CRP stratified by PCSK9-mAb types.



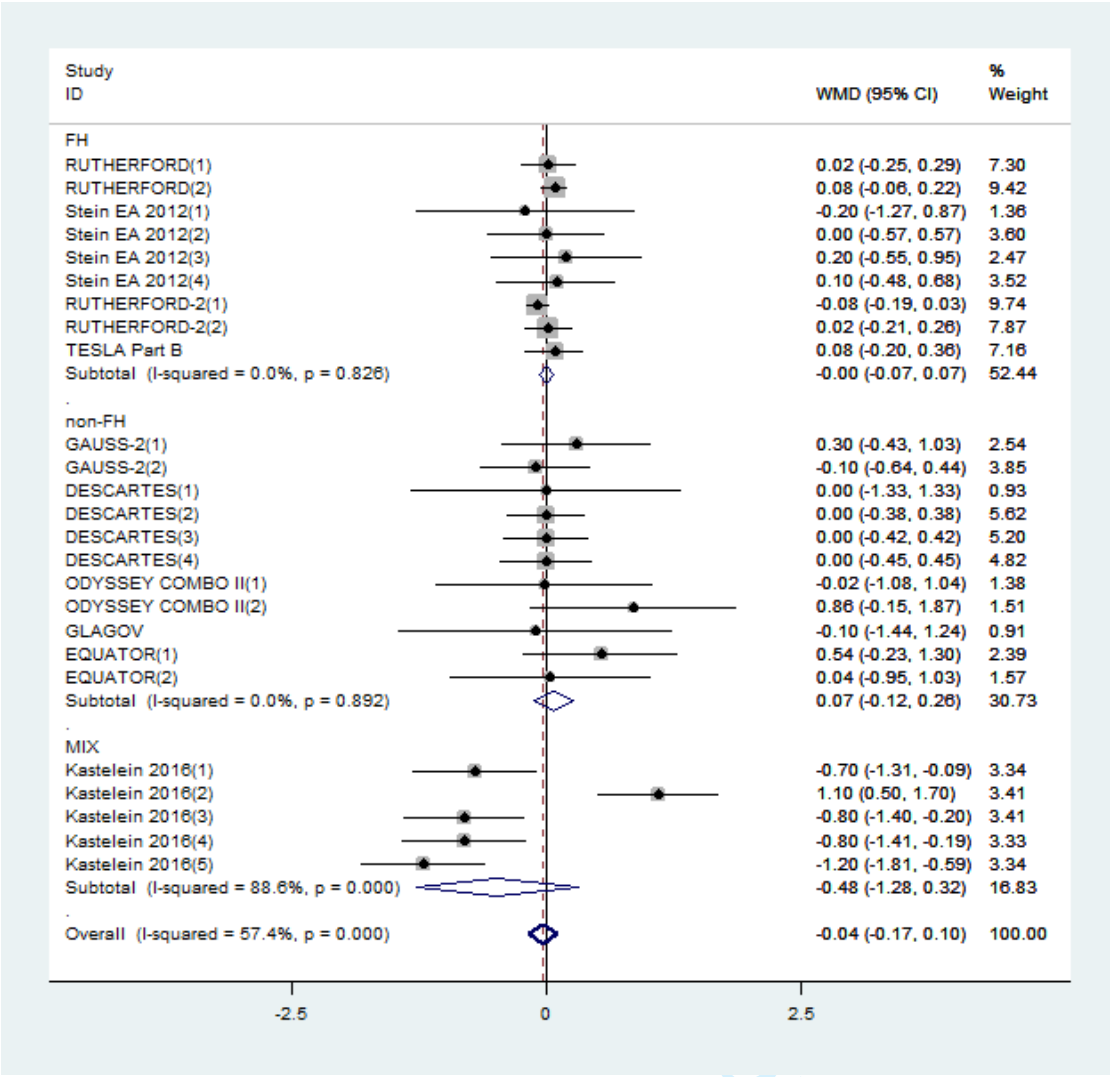
WMD= weighted mean difference, CI = confidence interval, PCSK9-mAb= proprotein convertase subtilisin/kexin type 9 monoclonal antibody, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 3. Pooled analysis for hs-CRP stratified by treatment durations.



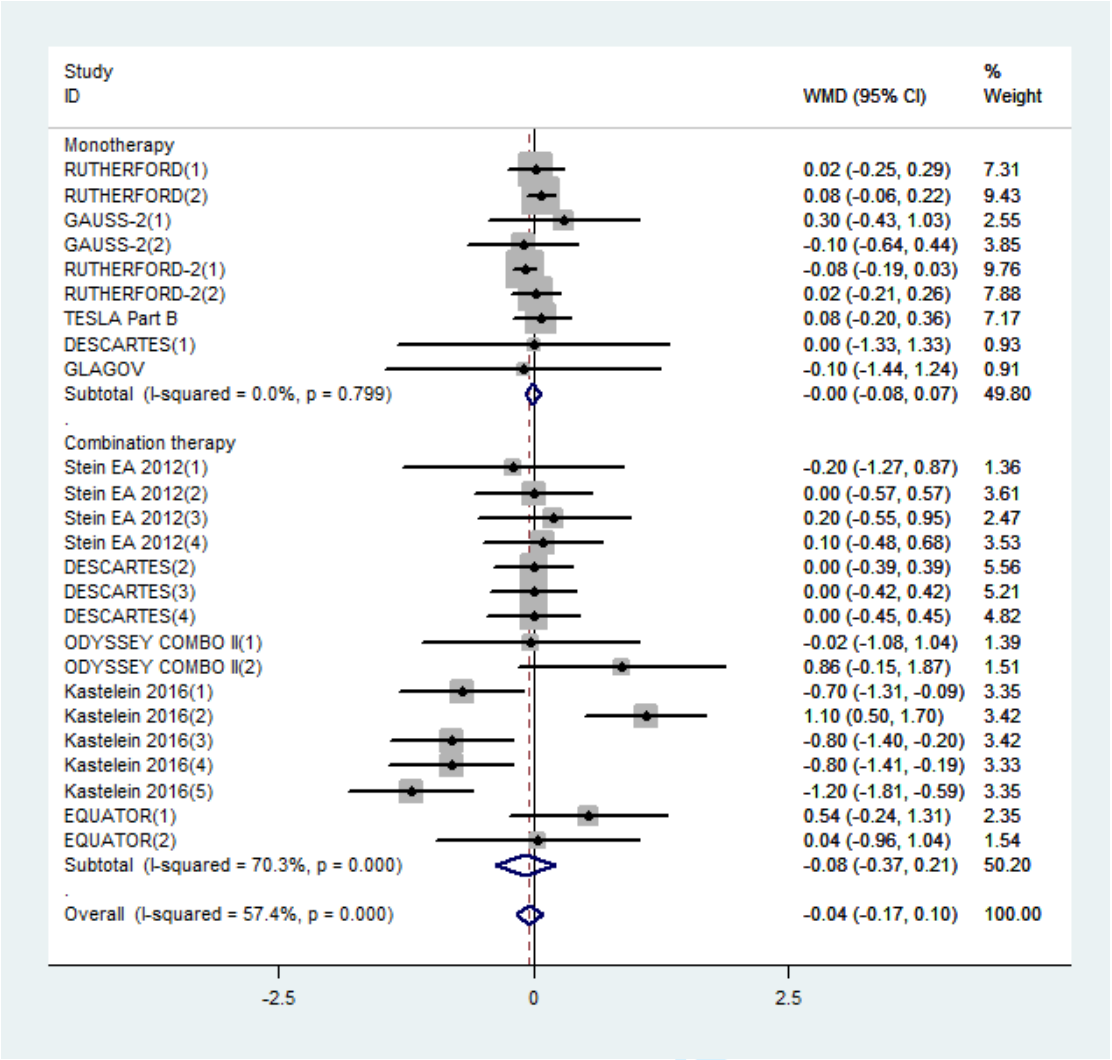
WMD=weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 4. Pooled analysis for hs-CRP stratified by participant characteristics



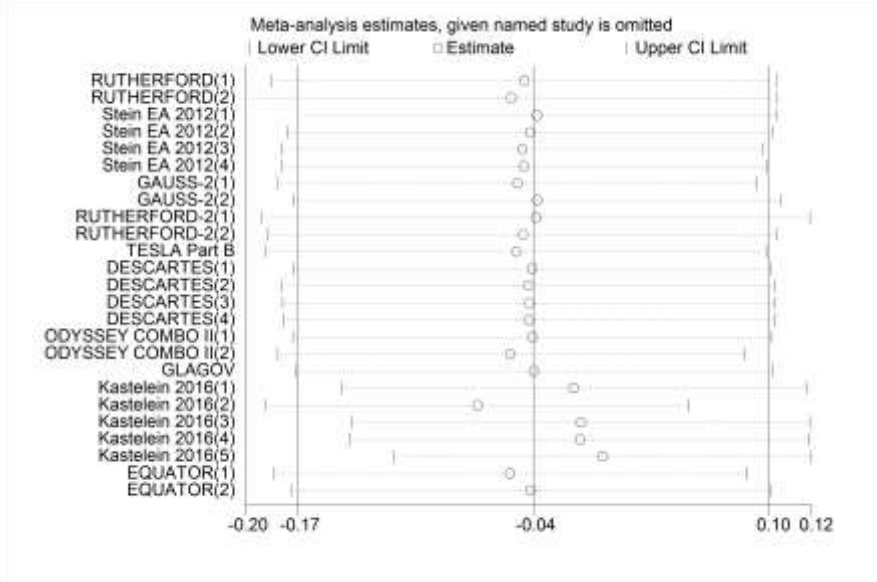
WMD= weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein, FH=familial hypercholesterolemia, non-FH= non-familial hypercholesterolemia

Supplementary Figure 5. Pooled analysis for hs-CRP stratified by of treatment methods.



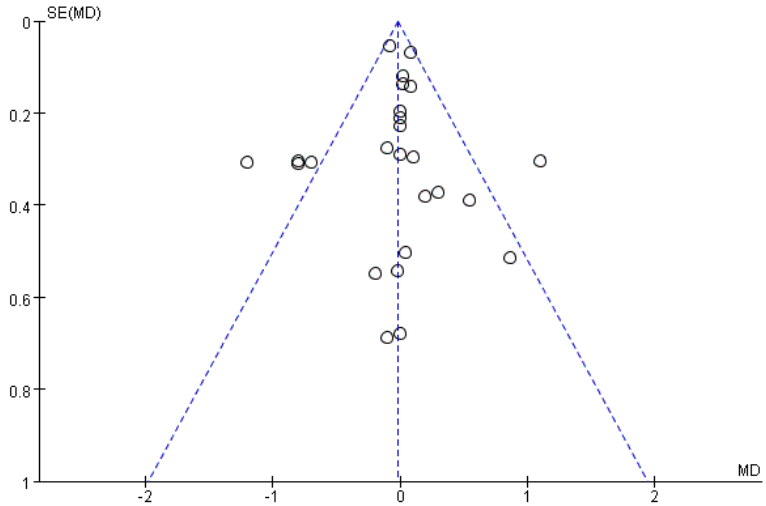
WMD=weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 6. Sensitivity analyses of hs-CRP.

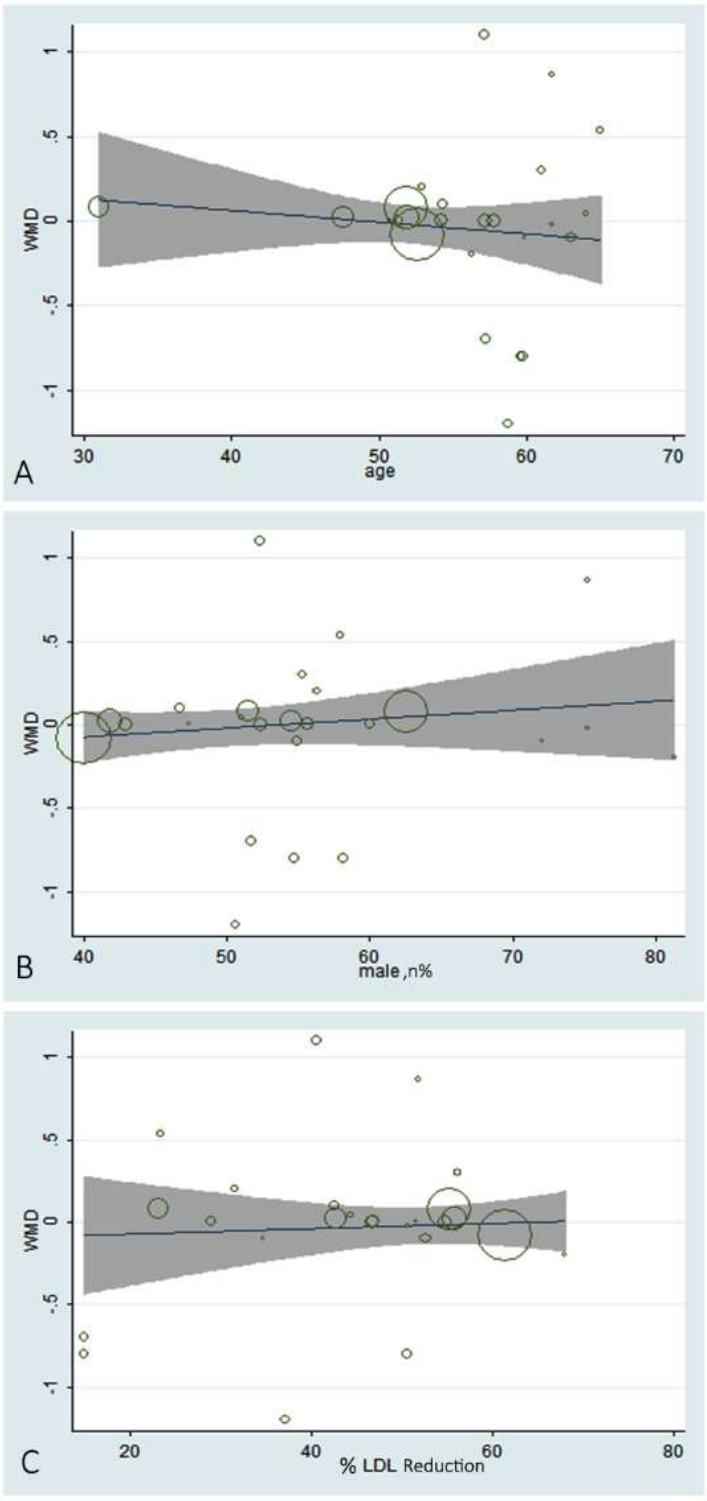


hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 7. Funnel Plots of included studies



Supplementary Figure 8. Meta-regression of baseline age (A), sex (B) and percent change of LDL-C (C).



LDL-C=low density lipoprotein cholesterol

PRISMA 2009 Checklist

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PRISMA 2009 Checklist

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Impact of PCSK9-monoclonal antibodies on circulating hs-CRP levels: a systematic review and meta-analysis of randomized controlled trials

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Title: Impact of PCSK9-monoclonal antibodies on circulating hs-CRP levels: a systematic review and meta-analysis of randomized controlled trials

Running Title: PCSK9-mAb and hs-CRP

Authors: Ye-Xuan Cao, MD*, Sha Li, MD, PhD *, Hui-Hui Liu, MD*, Jian-Jun Li, MD, PhD*

Affiliations: *Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China.

Correspondence: Professor Jian-Jun Li, MD, PhD, FESC, FACC. Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences, Peking Union Medical College, BeiLiShi Road 167, Beijing 100037, China. Tel: 86+10+88396077; Fax: 86+10+88396584. E-mail: lijianjun938@126.com.

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ABSTRACT

Objective To evaluate the potential effects of proprotein convertase subtilisin/kexin type 9 monoclonal antibody (PCSK9-mAb) on high-sensitivity C-reactive (hs-CRP) concentrations.

Design A systematic review and meta-analysis of randomized controlled trials.

Data sources PubMed, MEDLINE, The Cochrane Library databases, ClinicalTrials.gov and recent conferences were searched from inception to May 2018.

Eligibility criteria for selecting studies All randomized controlled trials that reported changes of hs-CRP were included.

Results Ten studies involving 4198 participants were identified. PCSK9-mAbs showed a slight efficacy in reducing hs-CRP (-0.04mg/L, 95%CI:-0.17 to 0.01) which was not statistically different. The results did not altered when subgroup analyses were performed including PCSK9-mAb types (Alirocumab:0.12mg/L, 95% CI:-0.18 to 0.43; Evolocumab:0.00 mg/L, 95% CI:-0.07 to 0.07; LY3015014:-0.48mg/L, 95% CI:-1.28 to 0.32; RG7652:0.35mg/L, 95% CI:-0.26 to 0.96), treatment duration (≤12w:0.00mg/L, 95% CI:-0.07 to 0.07; >12w: -0.11mg/L, 95% CI:-0.45 to -0.23), participant characteristics (familial hypercholesterolemia: 0.00mg/L, 95% CI:-0.07 to 0.07; non-familial hypercholesterolemia:0.07mg/L, 95% CI:-0.12 to 0.26; mix:-0.48mg/L, 95%CI:-1.28 to 0.32) and treatment methods (monotherapy: 0.00mg/L, -0.08 to 0.07; combination-therapy: -0.08mg/L, -0.37 to 0.21). Meta-regression analyses suggested no significant linear correlation between baseline age (p=0.673), sex (p=0.645), and low-density lipoprotein cholesterol reduction (p=0.339).

Conclusions Our updated meta-analysis suggested that PCSK9-mAbs had no significant impact on circulating hs-CRP levels irrespective of PCSK9-mAb types, participant characteristics, and treatment duration or methods.

Keywords: PCSK9; monoclonal antibody; hs-CRP; meta-analysis

Strengths and limitations of this study

- This is a comprehensive systematic review and meta-analysis that gives an overview of the effect of proprotein convertase subtilisin/kexin type 9 monoclonal antibody (PCSK9-mAb) on inflammation.
- An extensive systematic literature search identified all available randomized controlled trials that reported the changes of high-sensitivity C-reactive protein using PCSK9-mAb.
- Studies with moderate heterogeneity and lack of individual level data may limit the quality of evidence for this meta-analysis.

Introduction

Cardiovascular disease is the greatest burden of global health, which is mainly characterized by atherosclerosis[1]. Atherosclerosis is a chronic and progressive inflammatory disease, including endothelial dysfunction, lipid accumulation in the arterial wall and leukocyte infiltration, which leads to luminal stenosis, plaque rupture and acute coronary syndrome (ACS)[2]. Apart from well-established lipid theory, inflammation also plays an important role in the initiation and progression of atherosclerosis[3]. Recently, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) reported that anti-inflammatory therapy by canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β, significantly reduced the primary cardiovascular end points[4]. This study attracted attention to the inflammation intervention in cardiovascular medicine again.

C-reactive protein (CRP), especially high-sensitivity CRP (hs-CRP), is the most intensively investigated inflammatory biomarker in cardiovascular field[5]. Increasing studies have confirmed that hs-CRP is a predictor for the progression of atherosclerotic disease and future major adverse cardiovascular events (MACE)[6,7]. Moreover, previous studies also indicated that hs-CRP played a direct role in the progression of atherosclerosis[8,9]. Therefore, reduction of inflammatory markers such as hs-CRP may be a strategy for decreasing MACE[10,11].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is known to target low-density lipoprotein receptor (LDLR) for degradation resulting in elevated plasma low-density lipoprotein cholesterol (LDL-C) levels[12-14]. Recently, it has been reported that PCSK9 monoclonal antibody (PCSK9-mAb), as a novel lipid-lowering drug, can reduce LDL-C by a mean of 70% accompanied with reduction of MACE[15]. Although the relation of PCSK9 to LDL-C has been established, its role in inflammation has not been fully understood. Several

experimental studies found that PCSK9 could promote the progression of atherosclerosis by enhancing inflammatory reaction[16]. On the other hand, PCSK9 deficiency could alleviate the inflammation reaction[17]. Hence, whether PCSK9-mAb can reduce inflammatory marker is clinically of great interest since it was used to treat cardiovascular diseases. A meta-analysis covering 7 randomized controlled trials (RCTs) studies published two years ago suggested that PCSK9-mAbs had no effect on serum hs-CRP[18]. However, this meta-analysis may be limited by insufficient subgroup analyses, sample size, and lack of newly published data[19, 20]. Hence, we performed this meta-analysis including all RCTs published till May 2018 to further explore the efficacy of PCSK9-mAbs on circulating hs-CRP levels.

Methods

Literature search

The present study is reported according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement[21]. To identify all RCTs assessing the effect of PCSK9-mAbs on circulating hs-CRP levels, we comprehensively searched PubMed, MEDLINE, and the Cochrane Library database and ClinicalTrials.gov up until May 2018. The search terms we used included the followings: (AMG145 or Evolocumab or REGN727 or SAR236553 or Alirocumab or RN316 or bococizumab or RG7652 or LY3015014 or PCSK9 antibody or anti-PCSK9) AND (randomized controlled trial OR randomized OR randomly). Meanwhile, manual search was performed for relevant studies including references lists, relevant review articles and commentaries (see online supplementary Appendix 1). No language restriction was used.

Study selection

Original studies met the following criteria would be included: the design was phase 2 or

phase 3 double-blind RCTs with longer than 8 weeks treatment duration; human participants were randomly assigned to PCSK9-mAbs group versus control group with or without other lipid-lowering therapy; outcomes included percentage changes of hs-CRP from baseline. Studies were excluded if they were duplicate publications, review articles, non-human studies, observational studies, and lack of adequate information on outcomes or lacking control group. Two investigators (YC and SL) independently screened and selected the eligible studies. Disagreements were resolved by discussion with a third investigator.

Data Extraction

A standardized extraction form was used to extract the following items by two investigators (YC and HL) independently: trial name/first author, year of publication, type of intervention, follow-up period, treatment duration, number of patients, participant characteristics, background lipid-lowering therapy, types and doses of PCSK9-mAbs, LDL-C and hs-CRP levels at baseline and changes. We included the final reported follow-up point if a trial contains several time points. If necessary, further information was required from correspondence author.

Quality Assessment

We used Cochrane Collaboration's tool and Jadad score to assess the data and the methodological quality of included RCTs. For Cochrane Collaboration's tool, the following items were performed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other sources of bias. The judgments were classified as 'low risk', 'high risk', and 'unclear risk' of bias. The 5-point Jadad score included the following

items: basis of randomization (0 to 2 points), double blinding (0 to 2 points), and withdrawals /dropouts (0 to 1 points). Studies with a score ≥3 points are considered to be high quality. Two investigators (YC and HL) independently assessed the quality of each study. Disagreements were resolved by discussion with a third investigator.

Data Synthesis and Statistical Analysis

All analyses were conducted according to the intention-to-treat principles. For all efficacy outcomes, changes in hs-CRP concentrations were expressed as weighted mean difference (WMD) and 95% confidence interval (CI). All the data were standardized and expressed by mg/mL. Standard deviation could be calculated from CI, interquartile range or standard error according to formulas in the Cochrane Handbook for Systematic Reviews of Interventions if not reported.

Heterogeneity was assessed by the Cochran Q test and the I^2 statistic. We considered I^2<25% as representing low heterogeneity and I^2>75% as representing at high heterogeneity. Outcomes were calculated by fixed-effects models under no or low to moderate inconsistency (I^2<50%); otherwise, the data was pooled based on random-effects models. Subgroups were applied to reduce the heterogeneity if I^2 ≥50%, such as PCSK9-mAb types, treatment duration, and participant characteristics. In order to explore the resource of heterogeneity, sensitivity analysis was conducted by omitting studies in turn to evaluate the consistency of the results. Meta-regression analyses were performed to evaluate the contribution of participant characteristics and reductions in LDL-C concentrations. Publication bias was assessed with a funnel plot and Egger's test.

All analyses were conducted with Review Manager Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, Denmark) and Stata 14.0 (Stata Statistical Software: College Station, TX: Stata Corp LP). P value <0.05 was considered to be

statistically significant.

Patient and public involvement statement

Participants and the public sector were not directly involved in the design and conduct of this study.

Results

Study selection and Characteristics

The initial search identified 575 articles. After excluding duplicate publications and screening the titles and abstracts, 430 were excluded and 145 studies were retrieved for full-text identification. We further excluded 135 studies, of which 58 were pooled or meta-analysis, 2 studies were not RCTs and 13 was phase 1 trials, 16 were open label trails and 42 without adequate information. Finally, ten studies were included in this meta-analysis[20,22-30]. Figure 1 shows flow diagram of selection process.

These ten studies were published between 2012 and 2017 from different countries with low risk of bias, of which 5 were phase 2 studies and 5 were phase 3 studies (Table 1). A total of 4198 participants were included, comprising 2728 individuals in the PCSK9-mAb group and 1470 in the control group. Alirocumab (SAR236553/REGN727) was used in 4 arms and 11 arms applied Evolocumab (AMG 145). Five arms managed LY3015014 and 2 arms used RG7652. Four trials included patients with familial hypercholesterolemia (FH), of which 3 were heterozygous FH (HeFH) and 1 was homozygous FH (HoFH). Most of the treatment duration ranged from 12 to 24 weeks and the longest treatment duration was 78 weeks. Apart from DESCARTES trial which co-administered with atorvastatin, another 9 studies used PCSK9-mAb as monotherapy. Baseline characteristics including circulating hs-CRP levels were similar between PCSK9-mAbs and control groups within each study. The characteristics

of these trials and participants are summarized in Table 1. All these studies had a relatively high quality evaluated by the Jadad score and low risk of bias (online supplementary Table 1 and online supplementary Figure 1, 3 scores=5, 6 scores=4).

Efficacy outcomes of PCSK9-mAbs on hs-CRP

A total of 4198 participants were included in the analysis of efficacy of PCSK9-mAbs on plasma hs-CRP concentrations before and after treatment. When data were pooled, PCSK9-mAbs showed a slight efficacy in reducing hs-CRP (WMD: -0.04mg/L, 95%CI: -0.17 to 0.01), while no statistical difference was found compared with control treatment (Figure 2). There was a moderate heterogeneity between each study (I^2=57.4%, p=0.0001), so the random-effect model was selected.

To assess the potential discrepancy, we applied the subgroup analyses based on the characteristics of trials and participants (Figure 3 and online supplementary Figures 2-5). Although the efficacy of LY3015014 was a mild higher (-0.48 mg/L, 95% CI:-1.28 to 0.32), there was no difference between these four antibodies (Alirocumab: 0.12 mg/L, 95% CI: -0.18 to 0.43; Evolocumab: 0.00 mg/L, 95% CI: -0.07 to 0.07; RG7652: 0.35 mg/L, 95% CI: -0.26 to 0.96). When studies were classified by treatment duration, the hs-CRP reduction showed no difference in less than 12-week duration group (0.00 mg/L, 95% CI: -0.07 to 0.07) and above 12-week duration group (-0.11mg/L, 95% CI: -0.45 to -0.23). There was no significant reduction in circulating hs-CRP with use of PCSK9 antibodies compared with control treatment when categorized to participant characteristics (FH: 0.00 mg/L, 95% CI: -0.07 to 0.07; non-FH: 0.07 mg/L, 95% CI: -0.12 to 0.26; mix: -0.48 mg/L, 95%CI: -1.28 to 0.32). The analysis stratified by treatment method also supported the results that no differential effect of PCSK9-mAb therapy on plasma hs-CRP concentrations was observed (monotherapy: 0.00 mg/L, 95%CI: -0.08 to 0.07 vs combination-therapy: -0.08 mg/L, 95%CI:

-0.37 to 0.21).

Sensitivity analysis and publication bias

The sensitivity analysis for all outcomes was conducted by gradually removing each study. However, the results did not change meaningfully (online supplementary Figure 6). Neither funnel plots (online supplementary Figure 7) nor Egger's regression test (p=0.913) showed publication bias.

Meta-regression analyses

We used meta-regression analyses to assess the relationship between changes in hs-CRP and baseline age, sex, and average LDL-C changes (online supplementary Figure 8). No statistically significant relationship between baseline age (p=0.673), male sex (p=0.645), and hs-CRP changes were observed. Likewise, LDL-C lowering effects by PCSK9-mAb therapy had no impact on hs-CRP lowering (p=0.339, online supplementary Figure 8).

Discussion

The results of this updated, comprehensive meta-analysis, based on 10 RCTs encompassing 4198 participants, suggested that short-term PCSK9-mAb therapy had no impact on circulating hs-CRP concentrations. In the subgroup analyses, no difference was found between PCSK9-mAb types, participant characteristics, and treatment duration or methods.

Atherosclerosis, a chronic progressive disorder, is characterized by lipid accumulation and chronic inflammation in the arterial wall[2]. Although previous data indicated a positive effects of anti-inflammatory drugs on atherosclerosis in animal studies but no positive data was available in human studies[31]. Fortunately, recent evidence from CANTOS found that canakinumab significantly reduced hs-CRP levels and MACE after follow-up of 3.7 years

which may support the inflammatory hypothesis of atherosclerosis[4]. Moreover, ongoing Cardiovascular Inflammation Reduction Trial (CIRT) was also designed to directly test the inflammatory hypothesis of atherosclerosis by evaluating the effect of methotrexate on adverse cardiovascular outcomes without substantive impact on lipids[32]. Hence, focusing on inflammation in the development of atherosclerosis may be an unsolved issue and great of interest clinically.

In fact, increasing studies indicated that hs-CRP could independently predict MACE[6]. Framingham study found that men and women in the highest quartile of CRP respectively had twice and three-times the risk of stroke compared with those in the lowest ones after more than 10-years follow-up[33]. The Northern Manhattan Study reported that >3 mg/L CRP was associated with a 1.7-fold increase in cardiovascular outcomes and a 1.55-fold increase in mortality[34]. Furthermore, it has been demonstrated that hs-CRP also plays a direct and vital role in the development of atherosclerosis. Zwaka et al[8] found that CRP enhanced the transformation from macrophages to foam cells by increasing the uptake of LDL-C. CRP was also reported to impair vasodilatation, inhibit the synthesis of nitric oxide synthase, and facilitate the adhesion of monocyte[35,36]. Based on these evidence, reduction of hs-CRP may be associated with a decrease in MACE. Interestingly, the JUPITER study applied rosuvastatin on individuals with LDL-C levels below 130 mg/dL and hs-CRP levels ≥2 mg/L, and suggested a significant reduction in all vascular events[37]. Although a fact that statins lower LDL-C and proportionately reduce MACE is widely accepted, the hs-CRP reduction by statin administration is also an attractive phenomenon, called as pleiotropic effect of statin. That is the reason why we chose hs-CRP as an inflammatory biomarker to identify whether PCSK9-mAb has an effect on inflammatory status.

Although the relationship between PCSK9 and LDL-C was well-established, more and more evidence demonstrated its function beyond lipids. In 2010, microarray gene expression

analysis suggested that PCSK9 affected not only cholesterol metabolism, but also inflammation[38]. Experimental studies indicated that PCSK9 could participate in vascular and systemic inflammation[16]. In transgenic mice expressing human PCSK9 gene, Tavori et al[39] observed that atherosclerosis lesion size and local Ly6C^{hi} monocytes, the precursors of pro-inflammatory M1 macrophages, significantly increased. Clinical studies further supported this hypothesis. Our previous study found that serum PCSK9 concentrations were independently associated with white blood cell count in patients with stable coronary artery disease, indicating PCSK9 might be involved in the inflammation process[40]. ATHEROREMO-IVUS study reported a positive linearly association between PCSK9 levels and coronary plaque inflammation including amount of necrotic core tissue and plaque volume[41]. On the other hand, data also showed that PCSK9 inhibition could exert an anti-inflammatory effect. Tang et al[42] reported that PCSK9 small interfering RNA reduced the expression of pro-inflammatory genes through nuclear factor kappa B (NF-κB) pathway. In apoE-/- mice, PCSK9 silencing limited the development of atherosclerosis and decreased the number of macrophages via TLR4/NF-κB signaling pathway[43]. Besides, AT04A anti-PCSK9 vaccine also got the same results that anti-PCSK9 therapy could reduce vascular inflammation[44]. Recently, Bernelot et al[45] found that after 24 weeks of treatment with PCSK9-mAbs, the migratory capacity of monocytes and inflammatory responsiveness reduced significantly while anti-inflammatory cytokine levels increased in FH patients. Therefore, we hypothesized that PCSK9-mAbs treatment could reduce hs-CRP in randomized clinical studies. Unfortunately, in our meta-analysis the results showed that PCSK9-mAbs therapy had no effect on decreasing hs-CRP in both FH participants and non-FH individuals.

To explore the potential reasons why PCSK9-mAbs therapy was not benefit from inflammatory marker, named as the reduction of circulating hs-CRP levels, we further performed subgroup analyses. Firstly, we did not observe an impact of PCSK9-mAbs types

on hs-CRP, which may exclude the influence of PCSK9-mAbs itself on the inflammatory marker in our meta-analysis. Besides, the same results were also observed in combination of statins and PCSK9-mAbs group, suggesting that the effects of PCSK9-mAbs therapy on hs-CRP is not linked with treatment methods. It is notable that the participants in JUPITER trial had high levels of hs-CRP at baseline, while in PCSK9-mAb therapy, initial hs-CRP levels were at normal range in recruited individuals. Finally, we did not find a positive effects of PCSK9-mAbs therapeutic duration on hs-CRP in this updated analysis. Taken together, we may conclude that although PCSK9-mAbs had a powerful ability in lowering LDL-C, they had no impact on circulating hs-CRP concentration despite of PCSK9-mAb types, participant characteristics, and treatment duration or methods.

Limitations

There were several limitations in our meta-analysis. Firstly, our results were based on study data but not individual data as in most meta-analyses. Secondly, although our meta-analysis is an updated one, it is still limited by study numbers, sample size, and therapy duration. Moreover, moderate degree of heterogeneity was observed in several comparisons. However, there was no publication bias and the results were rather consistent among different subgroups. Sensitivity analysis suggested that the pooled WMD were robust. Finally, some studies included in this meta-analysis did not provide adequate information about blinding of participants and personnel.

Conclusions

In conclusion, the current updated evidence suggested that PCSK9-mAb, a novel powerful lipid-lowering drug, had no significant impact on circulating hs-CRP concentrations, whose effect did not influenced by PCSK9-mAb types, participant characteristics, and treatment

duration or methods. Long-term observation may be needed.

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Not any.

Contributors

JJL contributed to conception and design, acquisition, analysis, and interpretation, and critically revised the manuscript. YXC contributed to design, acquisition, analysis, and interpretation and drafted the manuscript. SL contributed to acquisition, analysis, and critically revised the manuscript. HHL contributed to analysis, interpretation and critically revised the manuscript. All the authors read and approved the final version of the manuscript.

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Competing interests: None declared.

Patient consent: Not required.

Ethics approval: This research is exempt from ethical approval.

Data sharing statement: No additional data available.

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Tables

Table 1. Study characteristics of included randomized controlled trials.

Study	Year	Phase	Inclusion criteria	Patients N	Arm	Mean age (years)	Male %	Mean hs-CRP at baseline mg/L	hs-CRP reduction (mg/L)	% LDL-C reduction	Drugs/control	Treatment duration	Jadad Score
RUTHERFORD[22]	2012	II	HeFH	167	(1)	47.6	54.5	1.09±1.37	-0.06 ± 0.94	42.7	E:350mg/PBO, Q4W	12W	5
					(2)	51.8	62.5	1.07±1.24	0.00 ± 0.30	55.2	E:420mg/PBO, Q4W		
Stein 2012[24]	2012	II	HeFH	77	(1)	56.3	81.3	1·40±1.78	-0.40 ± 2.04	67.9	A:150mg /PBO, Q2W	12W	5
					(2)	51.3	60.0	0·60±0.82	-0.20 ± 0.81	28.9	A:150mg /PBO, Q4W		
					(3)	52.9	56.3	0·70±1.48	0.00 ± 1.29	31.5	A:200mg /PBO, Q4W		
					(4)	54.3	46.7	0.70±0.59	-0.10± 0.84	42.5	A:300mg /PBO, Q4W		
DESCARTES[25]	2014	II	HC	894	(1)	50.7	47.3	2.00±3.70	0.00 ± 2.57	51.5	E:420 mg/ PBO, Q4W	52W	5
					(2)	57.2	42.9	1.00±1.48	0.00 ± 1.48	54.7	E:420 mg+ATV10 mg/ PBO, Q4W		
					(3)	57.8	52.4	1.00±1.48	0.00 ± 1.48	46.7	E:420 mg+ATV80 mg/ PBO, Q4W		
					(4)	54.2	55.6	1.00±1.48	0.00 ± 1.48	46.8	E:420 mg+ATV80 mg+ Eze10mg / PBO, Q4W		
GAUSS-2[26]	2014	III	HC	307	(1)	61.0	55.3	1.40±2.00	0.30 ± 2.67	56.1	E:140 mg/ Eze, Q2W	12W	4
					(2)	63.0	54.9	1.80±1.78	-0.30 ± 1.78	52.6	E:420 mg/ Eze, Q4W		
RUTHERFORD-2[23]	2014	III	HeFH	329	(1)	52.6	40.0	0.92±1.03	-0.05 ± 0.39	61.3	E:140 mg /PBO,Q2W	12W	4
					(2)	51.9	41.8	1.04±1.24	0.03 ± 0.73	55.7	E:420 mg /PBO,Q4W		
TESLA Part B[27]	2014	III	HoFH	49		31.0	51.5	0.70±1.04	-0.02 ± 0.52	23.1	E:140 mg /PBO, Q4W	12W	4
ODYSSEY COMBO II[28]	2015	III	HC	720	(1)	61.7	75.2	3.58±7.78	-0.39 ± 6.95	50.6	A: 75mg /Eze, Q2W	24W	4
					(2)	61.7	75.2	3.58±7.78	-0.07 ± 8.57	51.8	A: 75mg /Eze, Q2W	52W	
GLAGOV[29]	2016	III	HC	968		59.8	72.1	1.60±1.93	-0.40 ± 10.67	60.8	E:420 mg /PBO, Q4W	78W	4

Kastelein 2016[20]	2016	II	HC	519	(1)	57.2	51.7	1.03±1.41	-0.20 ± 2.07	14.9	LY:20mg/PBO, Q4W	16W	4
					(2)	57.1	52.3	1.34±1.11	1.60 ± 2.00	40.5	LY:120mg/PBO, Q4W		
					(3)	59.7	54.7	1.63±1.78	-0.30 ± 2.00	50.5	LY:300mg /PBO, Q4W		
					(4)	59.6	58.1	1.39±1.70	-0.30 ± 2.07	14.9	LY:100mg /PBO, Q8W		
					(5)	58.7	50.6	1.10±1.63	-0.70 ± 2.07	37.1	LY:300mg /PBO, Q8W		
EQUATOR[30]	2017	II	HC	168	(1)	65.0	57.9	1.60±2.70	0.34 ± 1.93	23.3	RG:400mg/PBO, Q4W	24W	4
					(2)	64.0	51.0	2.00±5.90	-0.16 ± 2.92	44.3	RG:800mg /PBO,Q8W		

Data presented as mean±SD; LDL-C, low density lipoprotein-cholesterol; hs-CRP, hypersensitive C reactive protein; HC, hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; E, Evolocumab; A, Alirocumab; LY: LY3015014; RG, RG7652; PBO, placebo; ATV, atorvastatin; Eze, ezetimibe; W, weeks; Q2W, every 2 weeks; Q4W, every 4 weeks. N, number; SD, standard deviation.

Figure legends

Figure 1. Flow diagram of selection of studies.

Figure 2. Forest plots depicting the effect of PCSK9-mAbs on hs-CRP.

CI=confidence interval, PCSK9-mAb=PCSK9 monoclonal antibody, hs-CRP= hypersensitive C-reactive protein

Figure 3. Subgroup analyses of the effect of PCSK9-mAbs on hs-CRP.

CI=confidence interval. PCSK9-mAb=PCSK9 monoclonal antibody. hs-CRP= hypersensitive C-reactive protein. FH= familial hypercholesterolemia. non-FH= non-familial hypercholesterolemia.

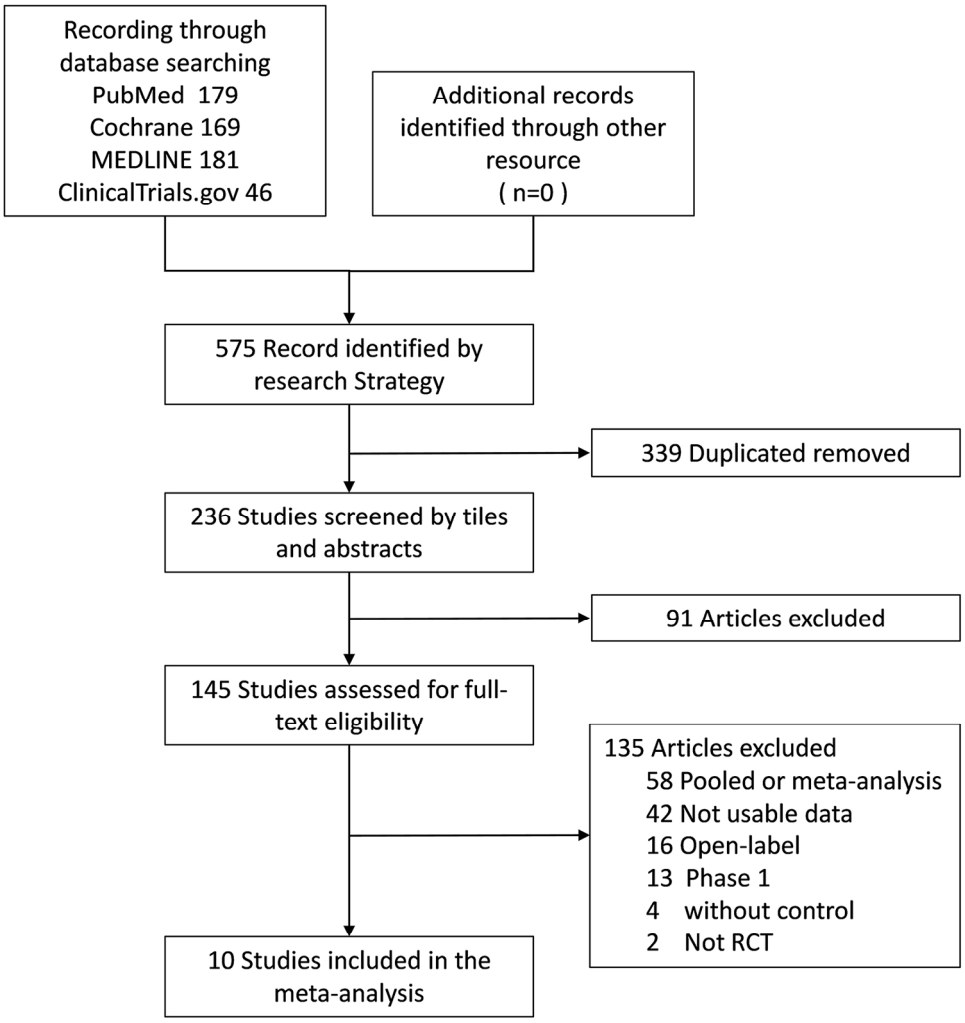

Figure 1. Flow diagram of selection of studies.

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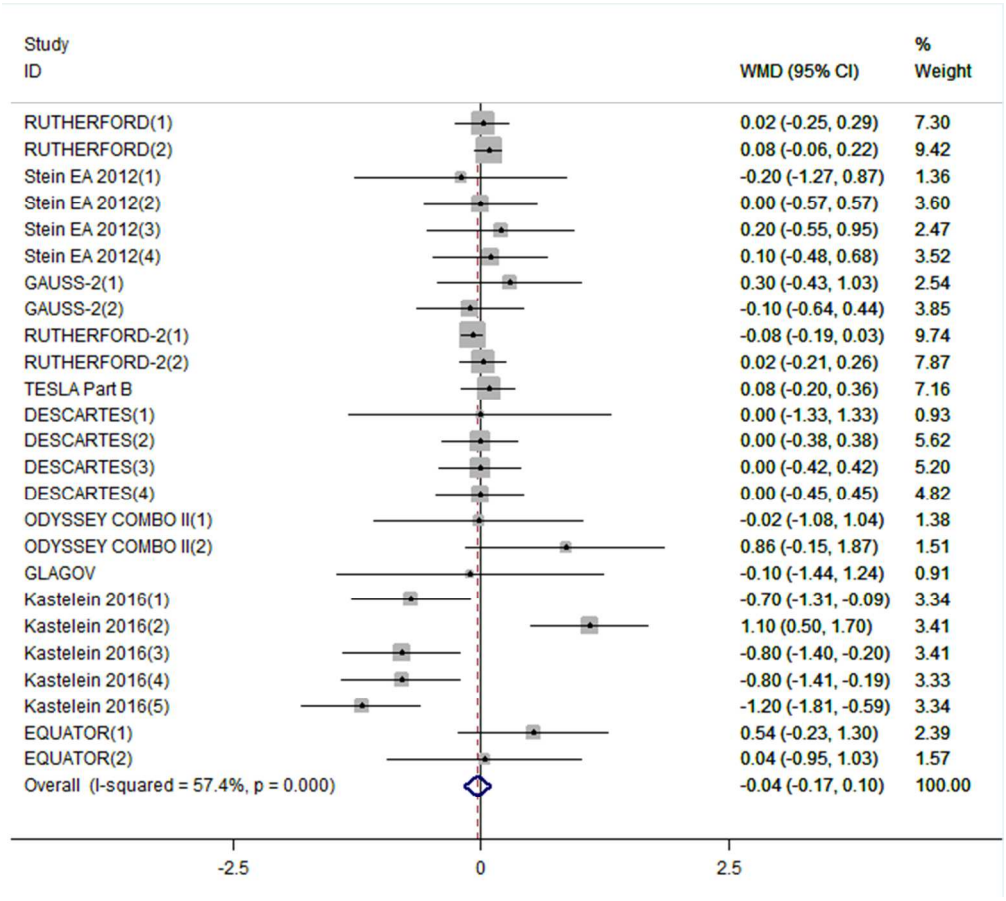


Figure 2. Forest plots depicting the effect of PCSK9-mAbs on hs-CRP.
CI=confidence interval, PCSK9-mAbs=PCSK9 monoclonal antibodies, hs-CRP= hypersensitive C-reactive protein

238x213mm (300 x 300 DPI)

Figure 3. Subgroup analyses of the effect of PCSK9-mAbs on hs-CRP.
CI=confidence interval. PCSK9-mAb=PCSK9 monoclonal antibody. hs-CRP= hypersensitive C-reactive protein. FH= familial hypercholesterolemia. non-FH= non-familial hypercholesterolemia.

256x133mm (300 x 300 DPI)

Supplementary material

Appendix 1. The search string

Medline via Ovid

1.exp AMG 145/

2.exp Evolocumab/

3.exp REGN727/

4.exp SAR236553/

5.exp Alirocumab/

6.exp RN316/

7.exp Bococizumab/

8.exp RG7652/

9.exp LY3015014/

10.exp ALN-PCSS/

11.exp PCSK9 antibodies/

12.exp anti-PCSK9/

13.exp Clinical Trial/

14.exp Randomized Controlled Trial/

15.exp Controlled Clinical Trial/

16.exp Random Allocation/

17.1 OR 2 OR 3 OR 4 OR 5 OR 6 OR7 OR 8 OR 9 OR 10 OR 11 OR 12

18.13 OR 14 OR 15 OR 16

19.17 AND 18

20. limit 19 to (humans)

Pubmed

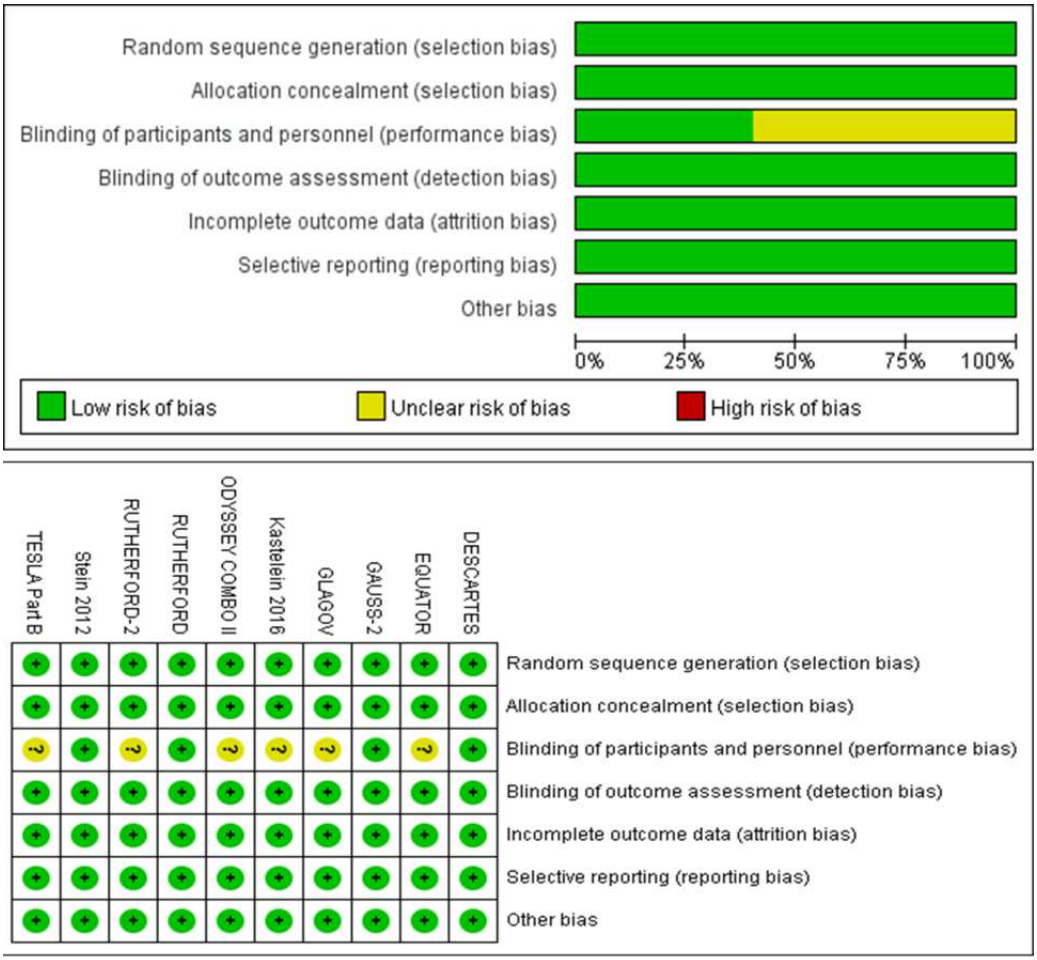
"amg145"[Title/Abstract]) OR "Evolocumab"[Title/Abstract]) OR "REGN727"[Title/Abstract]) OR "SAR236553"[Title/Abstract]) OR "Alirocumab"[Title/Abstract]) OR "RN316"[Title/ Abstract]) OR "bococizumab"[Title/Abstract]) OR "RG7652"[Title/Abstract]) OR "LY3015014" [Title/Abstract]) OR "ALN-PCSSC"[Title/Abstract]) OR "PCSK9 antibodies"[Title/Abstract]) OR "anti-PCSK9"[Title/ Abstract]) AND randomized controlled trial[Publication Type]) AND "humans"[MeSH Terms]

Supplemental Table 1. Quality assessment of included studies using the Jadad scale

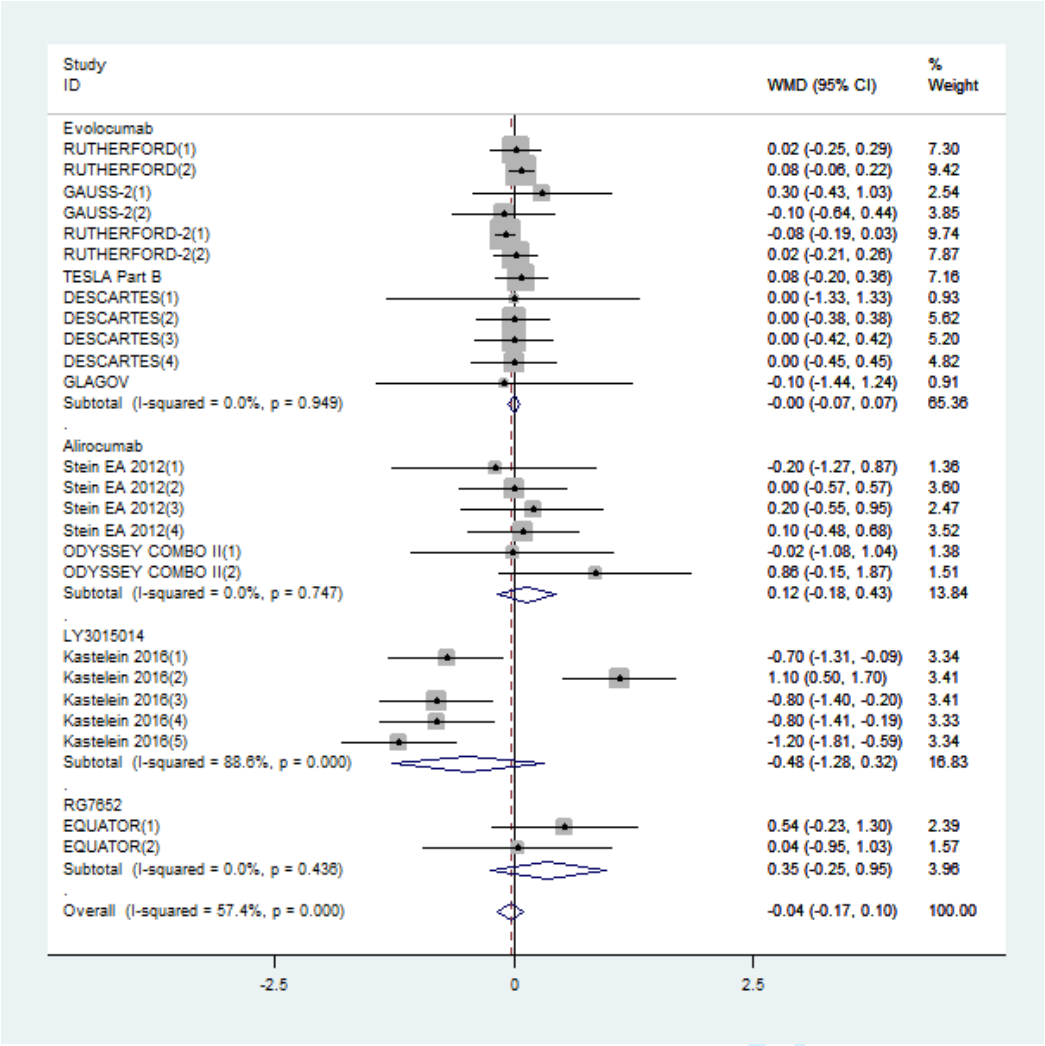
Studies	Representation of randomization	Appropriateness of method for randomization	Representation of double blinding	Appropriateness of method for double blinding	Representation of withdrawals	Total Score
RUTHERFORD	1	1	1	1	1	5
Stein EA 2012	1	1	1	1	1	5
DESCARTES	1	1	1	1	1	5
GAUSS-2	1	1	1	0	1	5
RUTHERFORD-2	1	1	1	0	1	4
TESLA Part B	1	1	1	0	1	4
ODYSSEY COMBO II	1	1	1	0	1	4
GLAGOV	1	1	1	0	1	4
Kastelein 2016	1	1	1	0	1	4
EQUATOR	1	1	1	0	1	4

Representation of randomization:0, not randomized or inappropriate method of randomization; 1, the study was described as randomized.
Appropriateness of method for randomization: 0, no information about the method of randomization;1, the method of randomization was described and it was appropriate.
Representation of double blinding: 0, no blind or inappropriate method of blinding; 1, the study was described as double blinding.
Appropriateness of method for double blinding: 0, no information about the method of double blinding;1, the method of double blinding was described and it was appropriate.
Withdrawals and dropouts:0, not describe the follow-up; 1, a description of withdrawals and dropouts.

Supplementary Figure 1. Evaluation of risk of bias in the studies.

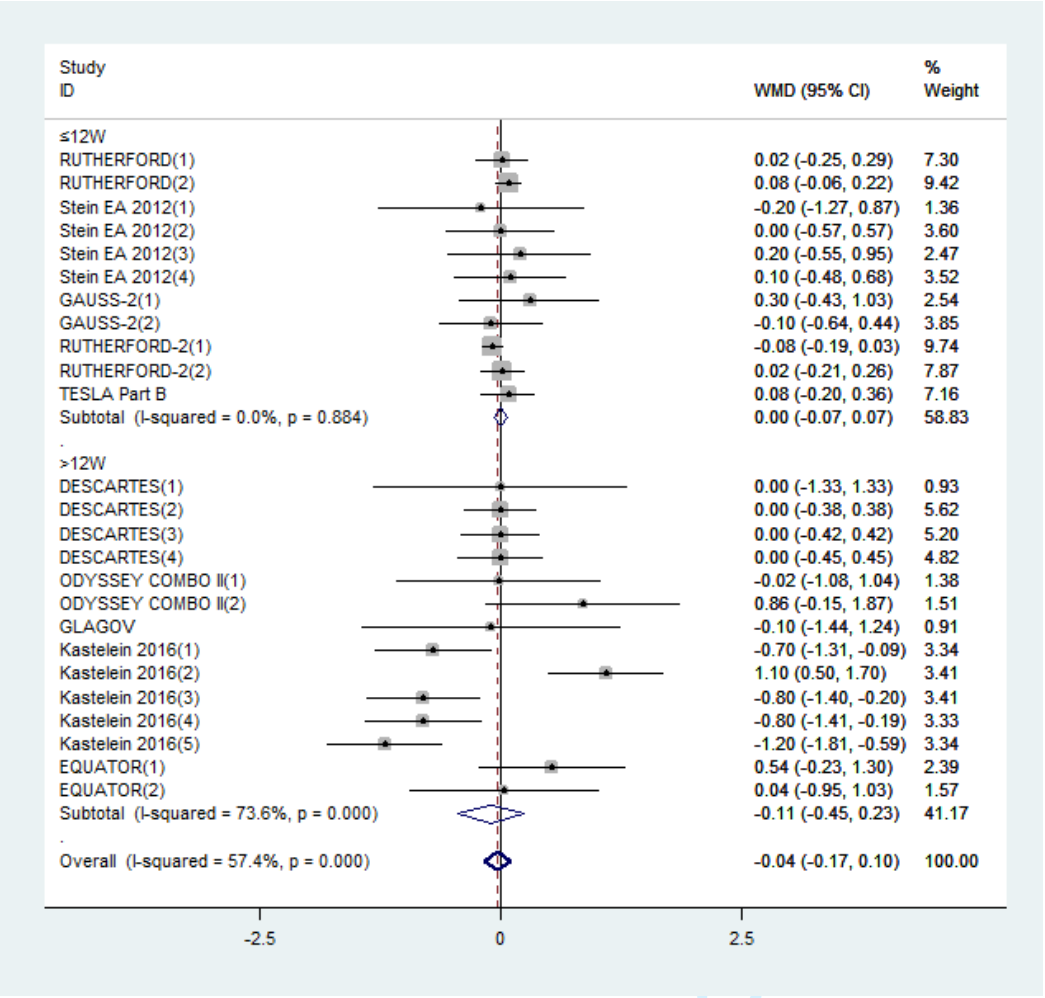


Supplementary Figure 2. Pooled analysis for hs-CRP stratified by PCSK9-mAb types.



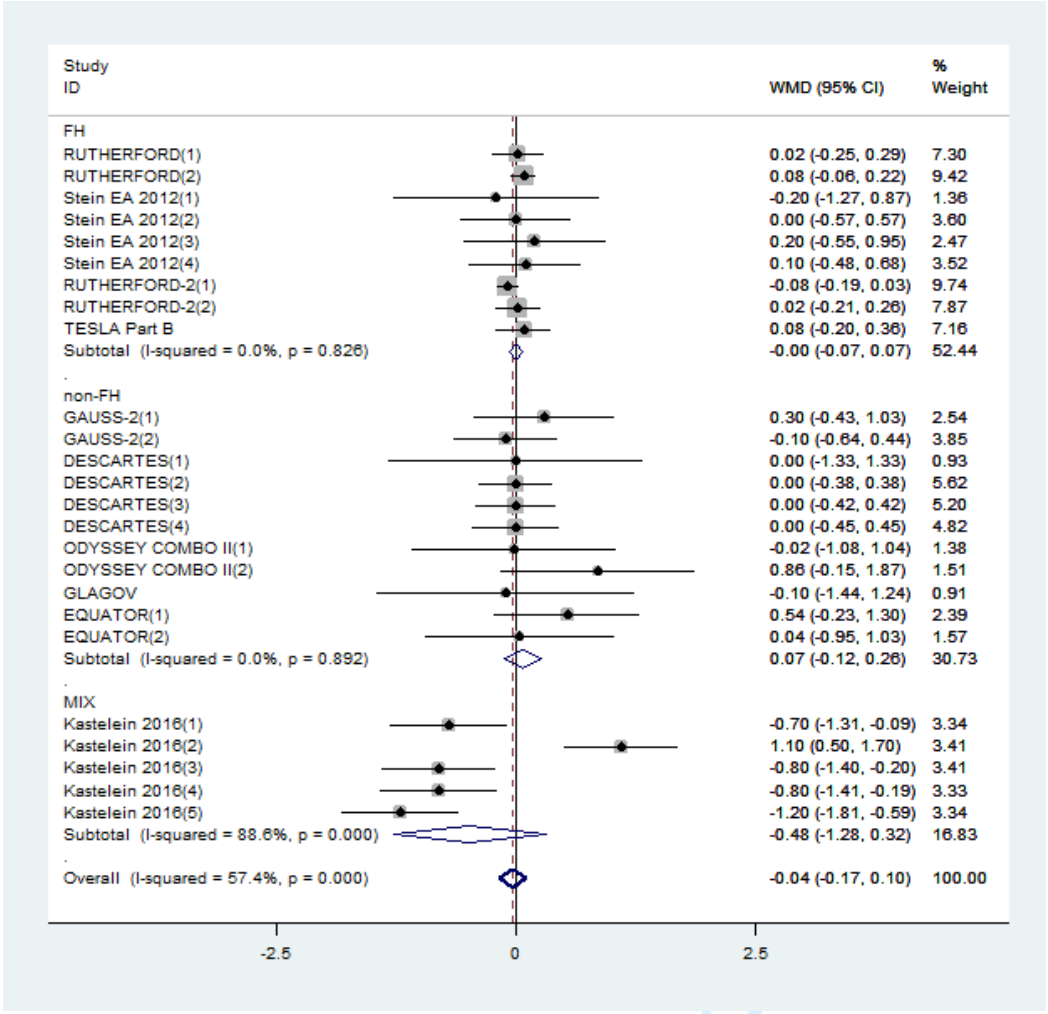
WMD= weighted mean difference, CI = confidence interval, PCSK9-mAb= proprotein convertase subtilisin/kexin type 9 monoclonal antibody, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 3. Pooled analysis for hs-CRP stratified by treatment durations.



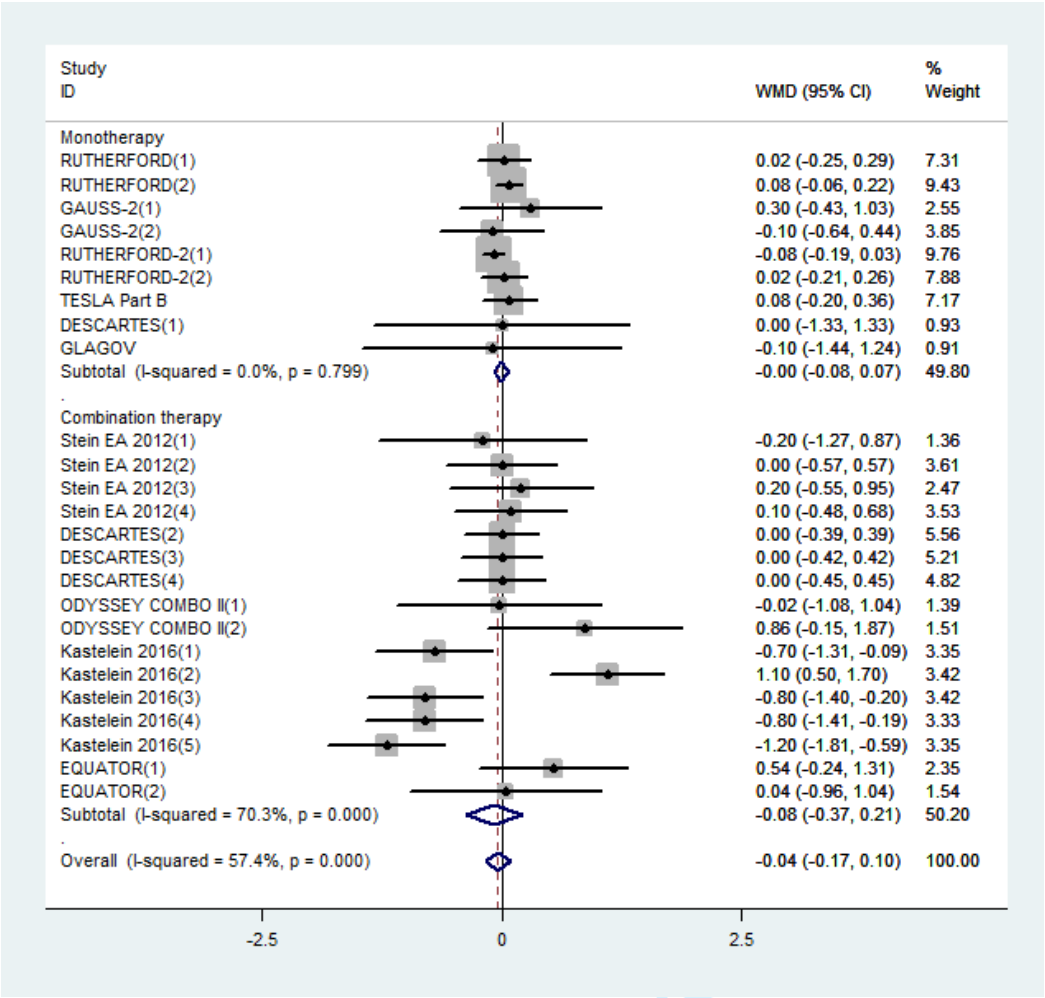
WMD=weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 4. Pooled analysis for hs-CRP stratified by participant characteristics



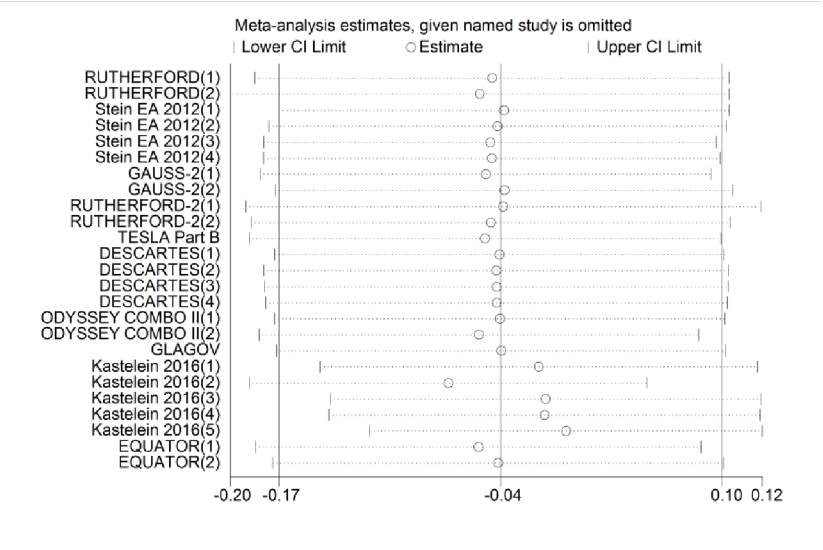
WMD= weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein, FH=familial hypercholesterolemia, non-FH= non-familial hypercholesterolemia

Supplementary Figure 5. Pooled analysis for hs-CRP stratified by of treatment methods.



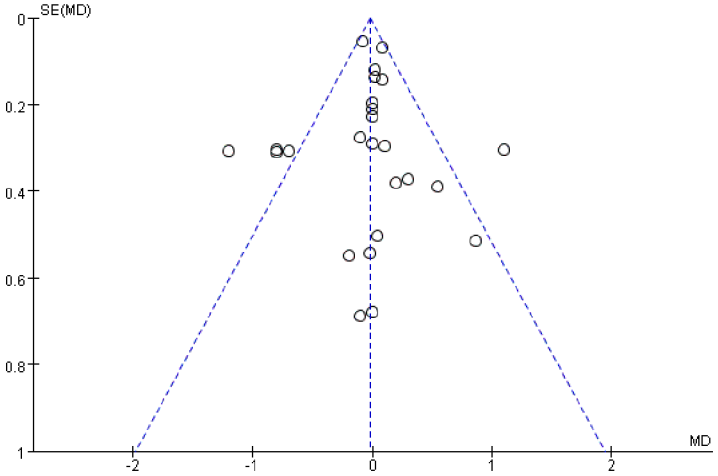
WMD=weighted mean difference, CI = confidence interval, hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 6. Sensitivity analyses of hs-CRP.

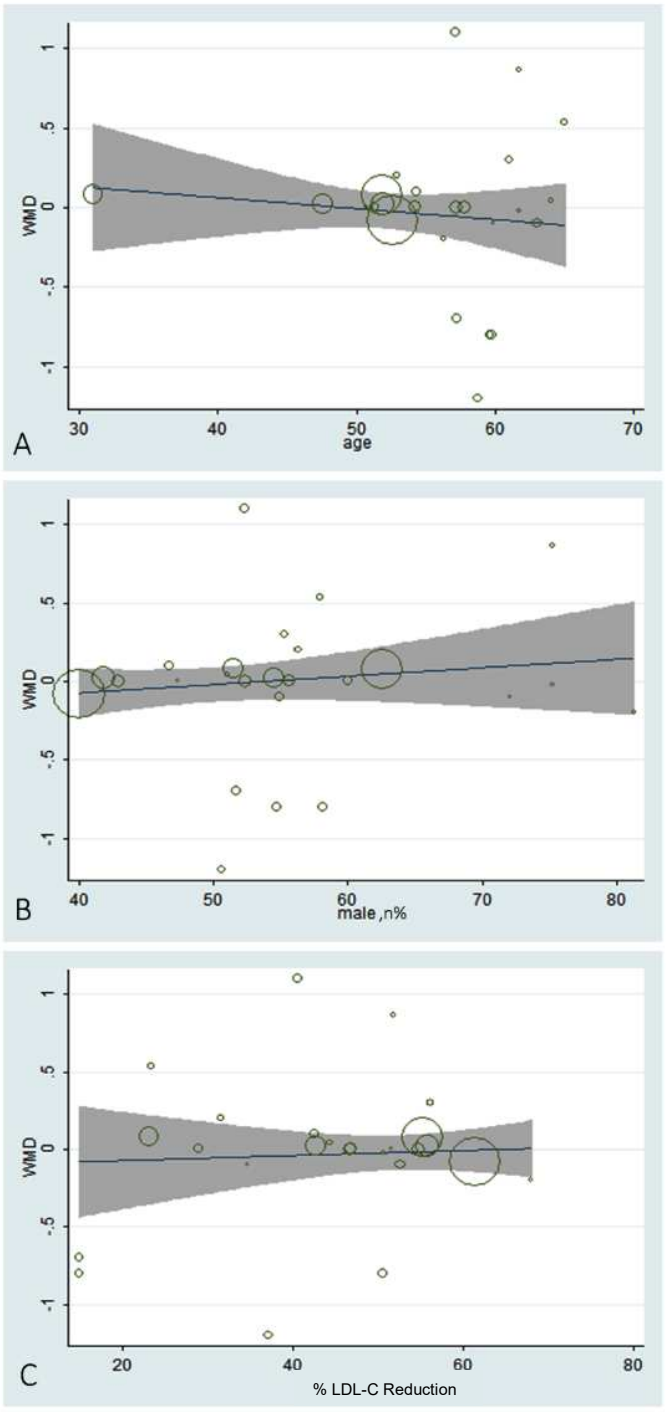


hs-CRP=hypersensitive C-reactive protein

Supplementary Figure 7. Funnel Plots of included studies



Supplementary Figure 8. Meta-regression of baseline age (A), sex (B) and percent change of LDL-C (C).



LDL-C=low density lipoprotein cholesterol

PRISMA 2009 Checklist

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PRISMA 2009 Checklist

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