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Effect of monoclonal antibodies to PCSK9 on high-sensitivity C-reactive protein levels: a meta-analysis of 16 randomized controlled treatment arms

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ABSTRACT

AIMS. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are an emerging class of low-density lipoprotein cholesterol (LDL-C)-lowering agents. In spite of their known effects on lipids, the impact of these drugs on systemic inflammation is less known. We aimed to investigate the effect of PCSK9 inhibitors on high-sensitivity C-reactive protein (hs-CRP) levels through a meta-analysis of randomized controlled trials (RCTs).

METHODS. A systematic literature search of Medline, SCOPUS and Google Scholar was conducted up to December 2015 to identify RCTs assessing changes in hs-CRP concentrations during treatment with PCSK9 inhibitors. Quantitative data synthesis was performed using a random-effects model, with weighed mean difference (WMD) and 95% confidence interval (CI) as summary statistics.

RESULTS. Sixteen treatment arms, with a total of 2546 participants, were included. Random-effects meta-analysis did not show any significant effect of PCSK9 inhibitors on hs-CRP levels (WMD: 0.002 mg l–1,CI:–0.017, 0.021;P= 0.807;I2= 37.26%). This effect size was robust, not sensitive to any single study, and not affected by the type of PCSK9 inhibitor (evolocumab: WMD: 0.002 mg l–1,CI:–0.02, 0.02;P= 0.855; alirocumab WMD: 0.15 mg l–1,CI:–0.11, 0.40;P=0.259;I2= 0%), or dosing frequency (biweekly:WMD: 0.13 mg l–1,CI:–0.20, 0.46;P=0.433;I2= 55.19%; monthly: WMD: 0.003 mg l–1,CI:–0.01, 0.01;P=0.59;I2=0%).Random-effects meta-regression did not suggest any association of changes in hs-CRP levels with changes in plasma LDL-C concentrations (P= 0.697) or cumulative dosage of the drug (P= 0.980).

CONCLUSIONS. This meta-analysis of RCTs did not suggest an effect of PCSK9 inhibitors on hs-CRP concentrations

INTRODUCTION

Atherosclerosis is the primary risk factor for several conditions, such as coronary artery disease, stroke and peripheral vascular disease, and is the leading cause of morbidity and mortality worldwide [1]. Hypercholesterolaemia is a major cardiovascular (CV) risk factor that leads the process of atherosclerotic plaque formation [2]. Besides being a disorder of lipid accumulation, atherosclerosis is known to have major inflammatory properties that contribute to plaque rupture and clinical events [3]. Low-density lipoprotein cholesterol (LDL-C) is the primary atherogenic lipoprotein, and LDL-C reduction is the target of primary or secondary prevention of CV diseases (CVDs) [4]. Currently, LDL reduction with statins is considered as the corner-stone of hypolipidaemic drug therapy to reduce CV risk [5, 6]. Although statins were developed specifically to decrease cholesterol synthesis, many studies have suggested that their beneficial effects extend beyond lipid lowering. However, there is no evidence that they reduce the risk of additional CVD events, be-yond that expected from the degree of LDL-C lowering, beyond the reduction of CV risk expected from the reduction of LDL-C levels [7, 8]. The use of statins in primary and secondary prevention has suggested that the magnitude of their efficacy in the prevention of CV events is not linearly or simply related to their hypolipidaemic action; in addition, statin efficacy may be ascribed, in part, to their ability to attenuate systemic inflammation and high-sensitivity C-reactive protein (hs-CRP) levels [9, 10].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have recently emerged as an elegant strategy to lower LDL-C and other lipid parameters [11–15]. They have been evaluated comprehensively in several phase 2 and 3 trials [16–22] exploring dose ranging, dose frequency and different patient populations, and tested as monotherapy or as add on therapy to conventional lipid-lowering strategies. Furthermore, experimental studies showed that, beyond its role in LDL-C homeostasis, PCSK9 is expressed in atherosclerotic plaques [23] and might pro-mote atherosclerosis by stimulating inflammation, endothelial dysfunction and hypertension [13]. Moreover, it has been observed that PCSK9 down regulates gene expression of the stress response and inflammation in liver cells, indicating that PCSK9affects metabolic pathways beyond cholesterol metabolism [24]. Although an evaluation of systemic inflammation has been performed in most of the trials conducted to date, there has been no meta-analysis to ascertain the overall effect size ofPCSK9 inhibition on systemic inflammation. In this context, hs-CRP has been the most widely used method for quantifying inflammation in CVDs [9, 25, 26]. Therefore, the purpose of the present meta-analysis was to investigate the impact ofPCSK9 inhibitors on plasma hs-CRP concentrations.

METHODS

Search strategy

The present study was designed according to the guidelines of the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [27]. SCOPUS (http://www.scopus.com), Medline (http://www.ncbi.nlm.nih.gov/pubmed) and Google Scholar (http://www.scholar.google.com) were searched using the following search terms in titles and abstracts (also in combination with MESH terms): (evolocumab OR alirocumab OR AMG145 OR‘AMG 145’ OR SAR236553/REGN727 OR SAR236553 OR ‘SAR236553’ OR REGN727 OR ‘REGN 727’ORREGN727/SAR236553) AND (CRP OR C-reactive protein OR ‘C reactive protein’ OR hsCRP OR hs-CRP). The search was limited to human studies and the wild-card term‘*’was used to increase the sensitivity of the search strategy. No language restriction was used in the literature search. The literature was searched from inception to 24 December 2015.

Study selection

Original studies were included if they met the following inclusion criteria: (i) being a randomized controlled trial (RCT)in a parallel, crossover or single-arm design; (ii) investigating the impact of a PCSK9 inhibitor on plasma/serum concentrations hs-CRP; (iii) having a treatment duration of at least 2weeks; (iv) presenting sufficient information on hs-CRP concentrations at the end of the treatment period or providing a value for net changes; and (v) including a control group. Exclusion criteria were: (i) nonclinical studies; (ii) observational studies with a case-control, cross-sectional or cohort design; iii) single-arm trials; and (iv) lack of sufficient information on baseline or follow-up PCSK9 concentrations.

Data extraction

Eligible studies were reviewed and the following data were abstracted: (i) first author's name; (ii) year of publication; (iii)study location; (iv) study design; (v) number of participants in the PCSK9 inhibitor and control groups; (vi) dose, frequency of injections, and duration of intervals between the injections of PCSK9 inhibitor; (vii) age, gender and body mass index (BMI) of study participants; (viii) baseline levels of total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides and glucose; (ix) systolic and diastolic blood pressures; and (x) data regarding baseline and follow-up concentrations of hs-CRP.

Quantitative data synthesis

Meta-analysis was conducted using Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ, USA) [28]. hs-CRP concentrations were collated in mg l–1. A division by 9.524 was used to convert hs-CRP values expressed in nmol l^{-1} into mg l^{-1}. Standard deviations (SDs) of the mean difference were calculated using the following formula: SD = square root $[(SD_{pre\text{-}treatment})^2+(SD_{post\text{-}treatment})^2-(2R \times SD_{pre\text{-}treatment} \times SD_{post\text{-}treatment})]$, assuming a correlation coefficient (R) = 0.5. In cases where the standard error of the mean (SEM) was reported, SD was estimated using the following formula: SD = SEM × square root(n), where *n* is the number of subjects. If the outcome measures were reported as the median and inter-quartile range, the mean and SD values were estimated using the method described by Hozoet al. [29]. To convert interquartile range into min–max range, the following equations were used: A = median + 2 ×(Q3–median) and B = median–2 × (median–Q1), where a, b, Q1and Q3 are upper and lower ends of the range, upper and lower ends of the interquartile range, respectively. Net changes in measurements (change scores) were calculated for parallel and crossover trials, as follows: (measure at end of follow-up in the treatment group-measure at baseline in the treatment group)-(measure at end of follow-up in the control group-measure at baseline in the control group). A random-effects model (using the DerSimonian–Laird method) and the generic inverse variance method were used to compensate for the heterogeneity of studies in terms of demographic characteristics of populations being studied and also differences in study design and type of fibrate being studied [30]. Heterogeneity was assessed quantitatively using theI2index. Effect sizes were expressed as WMD and 95% confidence interval (CI). Interstudy heterogeneity was assessed using the Cochran Q test andI2index. In order to evaluate the influence of each study on the overall effect size, sensitivity analysis was conducted using the leave-one-out method that is, removing one study each time and repeating the analysis [31, 32].

Meta-regression

*R*andom-effects meta-regression was performed using the unrestricted maximum likelihood method to evaluate the association between the changes in plasma hs-CRP concentrations following

treatment with PCSK9 inhibitors and respective reductions in LDL-C concentrations and cumulative dosage of PCSK9 inhibitor received during the trial.

Publication bias

Potential publication bias was explored using visual inspection of Begg's funnel plot asymmetry, Begg's rank correlation and Egger's weighted regression tests. The Duval and Tweedietrim–and-fill correction was used to adjust the analysis for the effects of publication bias [33]

RESULTS

Flow and characteristics of included studies

A summary of the study selection process is shown in Figure 1. The initial literature search yielded 279 articles. The screening for potential relevance removed the articles for which the titles and/or abstracts were obviously irrelevant. Among the 27 full-text articles that were assessed for eligibility, 21 studies were excluded: 18 because hs-CRP was not measured, two because they were not original studies and one because it was not a clinical study. After careful assessment, seven articles, with 16 treatment arms, met the inclusion criteria and were selected for the final meta-analysis [16–22]. In total, 2546 participants were randomized, of whom1707 were allocated to PCSK9 inhibitor intervention and839 to control groups. The number of participants in these trials ranged from 49 to 901. All the included studies were published between 2012 and 2015, involving several countries worldwide. The following PCSK9 inhibitor doses were administered in the included trials: evolocumab 350 mg monthly, evolocumab 420 mg monthly, evolocumab 140mg biweekly, alirocumab 150 mg biweekly, alirocumab 150mg monthly, alirocumab 200 mg monthly, alirocumab 300mg monthly and alirocumab 420 mg monthly. The duration of the intervention ranged between 3 months and 12months. All studies were designed as multicentre, double-blind RCTs, comprising a total of 16 treatment arms. Demo-graphic and baseline laboratory parameters of the included studies are shown in Table 1.

Risk of bias assessment

The systematic assessment of bias in the included studies using Cochrane criteria [34] and the Jadad scale is shown in Table 2. Overall, studies included in the meta-analysis provided sufficient data regarding the methods applied for random sequence generation, allocation concealment, blinding, as well other sources of bias. All studies reported de-tailed information about financial support from a commercial source.

Effect of PCSK9 inhibitors on plasma hs-CRP concentrations

Meta-analysis of data from 16 randomized controlled treatment arms did not suggest any significant effect of PCSK9 inhibitors on plasma hs-CRP concentrations (WMD: 0.002mg l^{-1},CI:–0.017, 0.021;P= 0.807;I2= 37.26%) (Figure 2).This effect size was robust and not sensitive to any single study included in the analysis (Figure 2). When the included studies were stratified according to the type of PCSK9 inhibitor used, no significant difference was observed with either evolocumab (WMD: 0.002 mg l^{-1},CI:–0.02, 0.02;P= 0.855;I2= 50.98%) or alirocumab (WMD: 0.15 mg l^{-1},CI:–0.11,0.40;P= 0.259;I2= 0%) (Figure 3). When the studies were categorized according to the number of PCSK9 inhibitor injections received during the course of the trial, there was no effect in the subgroup of trials with ≥ 6 injections (WMD:–0.01 mg l^{-1},CI:–0.02, 0.01;P= 0.531;I2=45.07%) but a slight increase in plasma hs-CRP was observed in the subgroup of trials with <6 PCSK9 inhibitor injections (WMD: 0.06 mg l^{-}

^{1}, CI: 0.01, 0.11;P=0.011;I2=0%)(Figure4).There was no effect of dosing strategy on the effect size as no significant alteration of hs-CRP levels was seen in trials with either biweekly (WMD: 0.13 mg l–1,CI:–0.20,0.46;P= 0.433;I2= 55.19%) or monthly (WMD: 0.003 mgl^{-1},CI:–0.01, 0.01;P= 0.59;I2= 0%) injection strategies(Figure 5). When the studies were categorized according to the studied population, no significant change in hs-CRP was observed either in familial hypercholesterolaemia (FH)(WMD: 0.03 mg l^{-1},CI:–0.04, 0.11;P= 0.396;I2=57.30%) or non-FH (WMD: 0.0 mg l^{-1},CI:–0.01, 0.01;P=0.979;I2= 0%) populations (Figure 6).

Meta-regression

Random-effects meta-regression did not suggest an association between changes in plasma hs-CRP and LDL-C concentrations following treatment with PCSK9 inhibitors (slope:0.0002; 95% CI:–0.001, 0.001;P= 0.698). Likewise, the impact of PCSK9 inhibitors on plasma hs-CRP concentrations was not influenced by the cumulative dosage of drug received during the course of the trial (slope: 0; 95% CI:–0.00001,0.00001;P= 0.980) (Figure 7).

Publication bias

The results of Begg's rank correlation (Kendall's tau with continuity correction = 0.26;Z=1.40; two-tailed P-value = 0.163) and Egger's linear regression (intercept = 0.59; standard error =0.39; 95% CI =–0.25, 1.43;t= 1.50; df = 14; two-tailed P=0.156) tests did not suggest publication bias in the meta-analysis of the effect of PCSK9 inhibitors on plasma CRP concentrations. However, the funnel plot of study precision (inverse standard error) by effect size (mean difference) was asymmetric and indicative of potential publication bias. This potential bias was addressed using trim-and-fill correction, the results of which attributed this bias to four potentially missing studies on the left-hand side of the funnel plot, yielding a corrected effect size of 0.001 mg l^{-1} (95% CI:–0.02, 0.02) (Figure 8).

DISCUSSION

RCTs have indicated that the PCSK9 inhibitors significantly reduced LDL-C levels with or without other lipid-lowering therapies [16–22]. However, a possible pleiotropic effect of these agents, in terms of anti-inflammatory actions, remains elusive. To the best of our knowledge, this meta-analysis was the first with the aim of elucidating the role of PCSK9 inhibitors in reducing systemic inflammation. Overall, our findings clearly showed that PCSK9 inhibitors have no significant influence on hs-CRP levels, independently from the type and dose of PCSK9 inhibitor, as well changes in LDL-C levels. In spite of this observation, there are some issues that should be considered. Firstly, it is worth noting that the populations studied would be expected to have normal levels of hs-CRP. In patients with FH, the marked increase in LDL-C seems to be associated with vascular events, without an important underlying inflammatory state [35]. Even patients with homozygous FH, who have extremely high levels of LDL-C and therefore a very high risk of early atherosclerosis, were found to have only slightly increased levels of CRP compared with control subjects [36]. It has also been argued that in FH patients, high LDL-C levels cause atherosclerosis without a major inflammatory component, whereas an inflammatory response is driven by elevated remnant cholesterol concentrations [35]. However, among subjects enrolled in the present meta-analysis without FH–that is, statin-intolerant patients–the design of the studies included a run-in phase with statin therapy, which is known to mitigate the vascular inflammatory response. Taken together, the above observations could explain, at least in part, both the low hs-CRP levels at baseline and the

lack of reduction in this biomarker after treatment. Secondly, CRP has been the most widely used inflammatory biomarker in statin trials, in view of the fact that this is the only reproducible biomarker for which a standardized assay has been developed [37, 38].Although the association between elevated CRP levels and CVDs is well established, the lack of CRP-lowering effect does not call into question the clinical benefit of PCSK9 inhibitors as these agents have been shown to reduce clinical outcomes and have been recently approved for patients with heterozygous FH [39–41]. The recent success of these agents could be principally ascribed to the magnitude of the lipid-lowering effect, and emphasizes the primacy of LDL-C lowering as a strategy to prevent coronary heart disease

It would be of paramount importance to elucidate further the role of PCSK9 beyond LDL-C modulation and homeostasis. It is evident that PCSK9 is expressed in atherosclerotic plaques [23], and pathways connecting PCSK9 to vascular inflammation and apoptosis are being explored[42].Furthermore, an *in vivo* study revealed that infection and inflammation stimulate PCSK9 expression [43], and small interfering RNA-mediated knockdown of PCSK9 inhuman macrophages has been found to reduce inhibitor of nuclear factor kappa B (NF-κB)-αdegradation and NF-κB nuclear translocation, thereby reducing the expression of proinflammatory genes [44]. In patients with an acute myocardial infarction, plasma PCSK9 concentration is elevated compared with those with stable coronary artery disease [45]. According to these findings, it is conceivable thatPCSK9 inhibition might have a positive impact on plaque stability and growth, and the effects of these inhibitors on clinical outcomes have been recently established [39–41]. The present meta-analysis had several limitations. Firstly, the trials tested different types and doses of PCSK9 inhibitors, which may have introduced heterogeneity to the results. However, a random-effects model and subgroup analyses were conducted to minimize the impact of heterogeneity. Secondly, included the studies differed in terms of baseline characteristics, study design and duration. In particular, most of the subjects underwent a run-in phase with high-dose statin therapy, and this could have influenced the results in terms of hs-CRP-levels. In addition, most of the evolocumab trials lasted 12 weeks, so we cannot exclude a time-dependent effect of these agents on hs-CRP. Thirdly, none of the studies considered elevated baseline hs-CRP as an inclusion criterion and none was conducted on patients with acute coronary syndromes, who might have been more prone to having high hs-CRP levels and, in turn, to responding to the effects of lipid-lowering agents on systemic inflammation.

In conclusion, the findings of the present meta-analysis did not suggest an effect of PCSK9-inhibitors in decreasing hs-CRP levels. Further investigations are needed to elucidate whether these agents have pleiotropic actions that go beyond their established hypolipidemic effects. However, as preliminary data suggest that PCSK-9 inhibitors reduce cardiovascular events without lowering hs-CRP levels, the question arises as to whether inflammation plays a major causal role in atherosclerosis. These findings underline the priority of LDL-C lowering as a strategy for preventing cardiovascular events [46]. Other studies, enrolling patients after acute coronary syndromes, are currently ongoing, and it will be important to evaluate the effect of PCSK9inhibitors on the inflammatory response in populations with elevated baseline hs-CRP levels, to shed light on their possible anti-inflammatory actions.

COMPETING INTERESTS

The authors declare no conflict of interests. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declare no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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SUPPORTING INFORMATION

Appendix S1. Excluded trials' list with reason for exclusion

Figure 1 Flow chart of the studies identified and included into the meta-analysis. CRP, C-reactive proteinPCSK9 inhibitors and hs-CRP levels

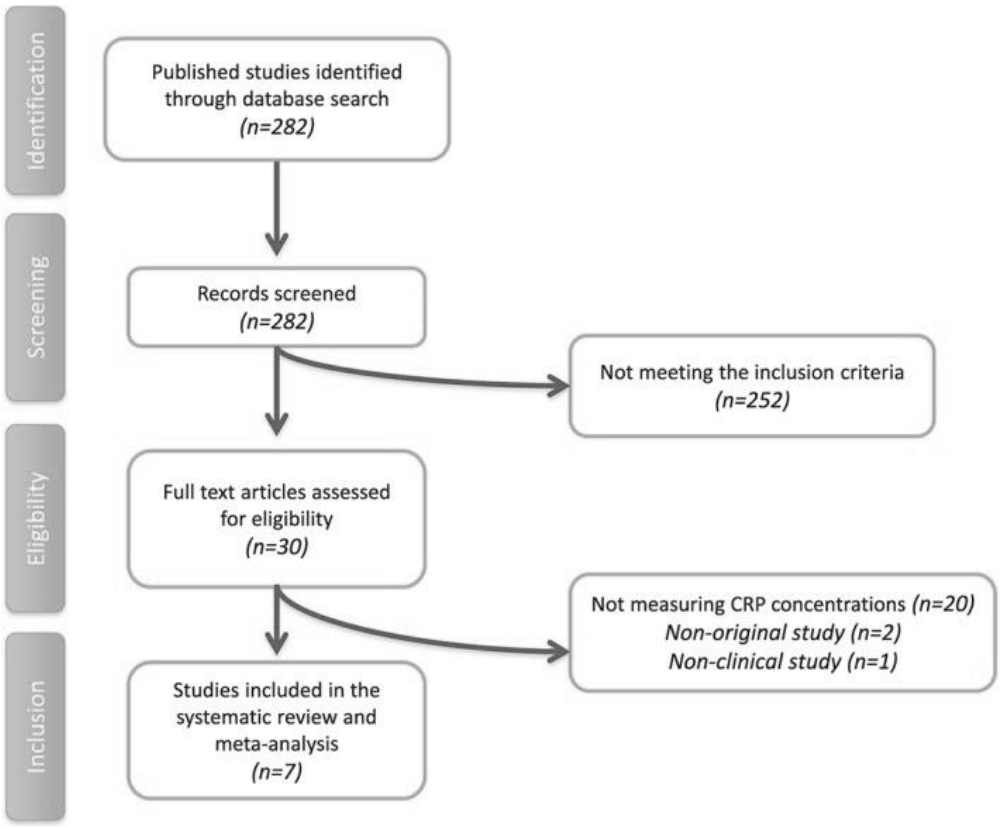
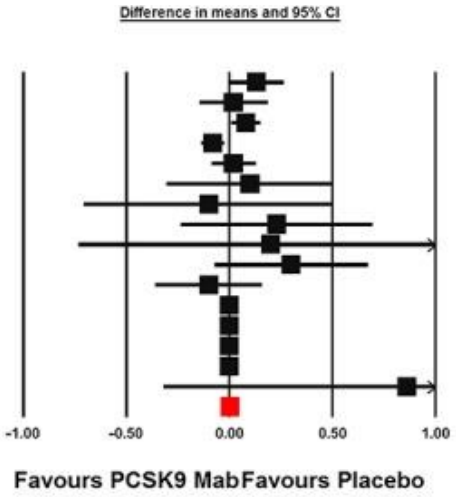


Figure 2. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the impact of proprotein convertase subtilisin/kexin type 9 inhibitors on plasma high-sensitivity C-reactive protein concentrations (upper plot). Lower plot shows the result of leave-one-out sensitivity analysis

Study	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	*P*-value
Raal *et al*., 2014c	0.130	0.067	0.005	−0.002	0.262	1.932	0.053
Raal *et al*., 2012a	0.020	0.084	0.007	−0.144	0.184	0.239	0.811
Raal *et al*., 2012b	0.080	0.035	0.001	0.011	0.149	2.268	0.023
Raal *et al*., 2014a	−0.080	0.028	0.001	−0.135	−0.025	−2.866	0.004
Raal *et al*., 2014b	0.020	0.054	0.003	−0.087	0.127	0.367	0.714
Stein *et al*., 2012a	0.100	0.207	0.043	−0.305	0.505	0.484	0.628
Stein *et al*., 2012b	−0.100	0.309	0.095	−0.705	0.505	−0.324	0.746
Stein *et al*., 2012c	0.230	0.236	0.056	−0.233	0.693	0.973	0.330
Stein *et al*., 2012d	0.200	0.475	0.225	−0.730	1.130	0.421	0.673
Stroes *et al*., 2014a	0.300	0.189	0.036	−0.069	0.689	1.591	0.112
Stroes *et al*., 2014b	−0.100	0.131	0.017	−0.357	0.157	−0.763	0.445
Blom *et al*., 2014a	0.000	0.022	0.000	−0.044	0.044	0.000	1.000
Blom *et al*., 2014b	0.000	0.008	0.000	−0.016	0.016	0.000	1.000
Blom *et al*., 2014c	0.000	0.010	0.000	−0.020	0.020	0.000	1.000
Blom *et al*., 2014d	0.000	0.011	0.000	−0.021	0.021	0.000	1.000
Cannon *et al*., 2015	0.860	0.601	0.361	−0.318	2.038	1.430	0.153
	0.002	0.010	0.000	−0.017	0.021	0.244	0.807



Study	Statistics with study removed						
	Point	Standard error	Variance	Lower limit	Upper limit	Z-Value	*P*-value
Raal *et al*., 2014c	−0.000	0.009	0.000	−0.018	0.017	−0.043	0.966
Raal *et al*., 2012a	0.002	0.010	0.000	−0.017	0.022	0.236	0.814
Raal *et al*., 2012b	−0.002	0.008	0.000	−0.019	0.014	−0.297	0.767
Raal *et al*., 2014a	0.005	0.006	0.000	−0.008	0.017	0.712	0.476
Raal *et al*., 2014b	0.002	0.010	0.000	−0.018	0.022	0.205	0.838
Stein *et al*., 2012a	0.002	0.010	0.000	−0.017	0.022	0.235	0.814
Stein *et al*., 2012b	0.003	0.010	0.000	−0.017	0.022	0.270	0.787
Stein *et al*., 2012c	0.002	0.010	0.000	−0.017	0.021	0.206	0.837
Stein *et al*., 2012d	0.002	0.010	0.000	−0.017	0.022	0.249	0.803
Stroes *et al*., 2014a	0.001	0.009	0.000	−0.017	0.019	0.141	0.888
Stroes *et al*., 2014b	0.003	0.010	0.000	−0.016	0.022	0.308	0.758
Blom *et al*., 2014a	0.003	0.011	0.000	−0.018	0.025	0.294	0.768
Blom *et al*., 2014b	0.005	0.013	0.000	−0.021	0.032	0.408	0.683
Blom *et al*., 2014c	0.005	0.013	0.000	−0.020	0.030	0.383	0.702
Blom *et al*., 2014d	0.005	0.013	0.000	−0.020	0.029	0.370	0.711
Cannon *et al*., 2015	0.002	0.009	0.000	−0.016	0.020	0.204	0.839
	0.002	0.010	0.000	−0.017	0.021	0.244	0.807

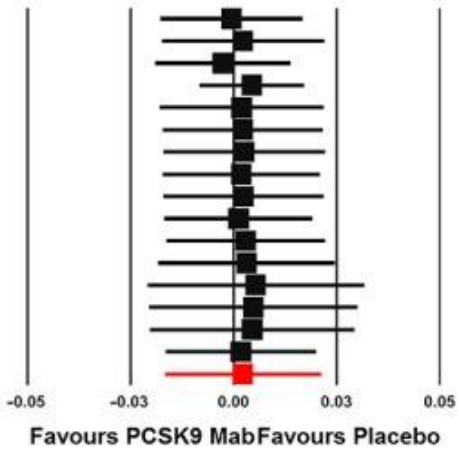
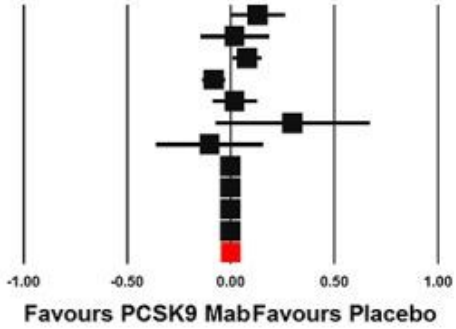


Figure 3. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the impact of evolocumab (upper plot) and alirocumab (lower plot) on plasma high-sensitivity C-reactive protein concentrations

Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Raal et al., 2014c	0.130	0.067	0.005	-0.002	0.262	1.932	0.053
Raal et al., 2012a	0.020	0.084	0.007	-0.144	0.184	0.239	0.811
Raal et al., 2012b	0.080	0.035	0.001	0.011	0.149	2.268	0.023
Raal et al., 2014a	-0.080	0.028	0.001	-0.135	-0.025	-2.866	0.004
Raal et al., 2014b	0.020	0.054	0.003	-0.087	0.127	0.367	0.714
Stroes et al., 2014a	0.300	0.189	0.036	-0.069	0.669	1.591	0.112
Stroes et al., 2014b	-0.100	0.131	0.017	-0.357	0.157	-0.763	0.445
Blom et al., 2014a	0.000	0.022	0.000	-0.044	0.044	0.000	1.000
Blom et al., 2014b	0.000	0.008	0.000	-0.016	0.016	0.000	1.000
Blom et al., 2014c	0.000	0.010	0.000	-0.020	0.020	0.000	1.000
Blom et al., 2014d	0.000	0.011	0.000	-0.021	0.021	0.000	1.000
	0.002	0.010	0.000	-0.018	0.022	0.183	0.855



Study name	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Stein et al., 2012a	0.100	0.207	0.043	-0.305	0.505	0.484	0.628
Stein et al., 2012b	-0.100	0.309	0.095	-0.705	0.505	-0.324	0.746
Stein et al., 2012c	0.230	0.236	0.056	-0.233	0.693	0.973	0.330
Stein et al., 2012d	0.200	0.475	0.225	-0.730	1.130	0.421	0.673
Cannon et al., 2015	0.860	0.601	0.361	-0.318	2.038	1.430	0.153
	0.147	0.130	0.017	-0.108	0.402	1.130	0.259

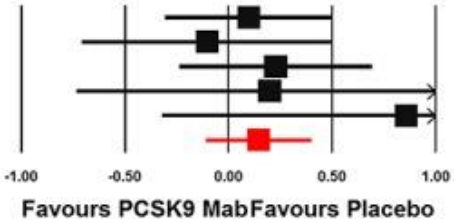
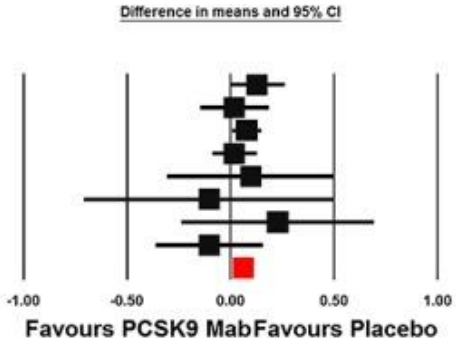


Figure 4. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the impact of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors on plasma high-sensitivity

C-reactive protein concentrations in trials with <6 (upper plot) and ≥6 (lower plot) injections of PCSK9 inhibitor

Study name	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Raal et al., 2014c	0.130	0.067	0.005	-0.002	0.262	1.932	0.053
Raal et al., 2012a	0.020	0.084	0.007	-0.144	0.184	0.239	0.811
Raal et al., 2012b	0.080	0.035	0.001	0.011	0.149	2.268	0.023
Raal et al., 2014b	0.020	0.054	0.003	-0.087	0.127	0.367	0.714
Stein et al., 2012a	0.100	0.207	0.043	-0.305	0.505	0.484	0.628
Stein et al., 2012b	-0.100	0.309	0.095	-0.705	0.505	-0.324	0.746
Stein et al., 2012c	0.230	0.236	0.056	-0.233	0.693	0.973	0.330
Stroes et al., 2014b	-0.100	0.131	0.017	-0.357	0.157	-0.763	0.445
	0.063	0.025	0.001	0.015	0.112	2.544	0.011



Study name	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Raal et al., 2014a	-0.080	0.028	0.001	-0.135	-0.025	-2.866	0.004
Stein et al., 2012d	0.200	0.475	0.225	-0.730	1.130	0.421	0.673
Stroes et al., 2014a	0.300	0.189	0.036	-0.069	0.669	1.591	0.112
Blom et al., 2014a	0.000	0.022	0.000	-0.044	0.044	0.000	1.000
Blom et al., 2014b	0.000	0.008	0.000	-0.016	0.016	0.000	1.000
Blom et al., 2014c	0.000	0.010	0.000	-0.020	0.020	0.000	1.000
Blom et al., 2014d	0.000	0.011	0.000	-0.021	0.021	0.000	1.000
Cannon et al., 2015	0.860	0.601	0.361	-0.318	2.038	1.430	0.153
	-0.006	0.009	0.000	-0.024	0.012	-0.626	0.531

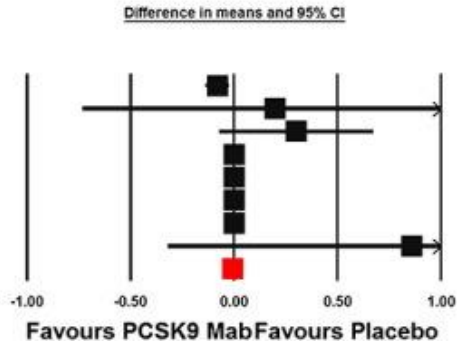
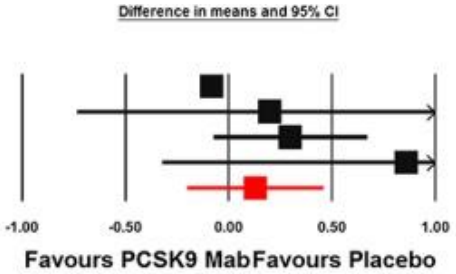


Figure 5. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the impact of proprotein convertase subtilisin/kexin type 9 inhibitors on plasma high-sensitivity C-reactive protein concentrations in trials with biweekly (upper plot) and monthly (lower plot) dosing strategy

Study name	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Raal et al., 2014a	-0.080	0.028	0.001	-0.135	-0.025	-2.866	0.004
Stein et al., 2012d	0.200	0.475	0.225	-0.730	1.130	0.421	0.673
Stroes et al., 2014a	0.300	0.189	0.036	-0.069	0.669	1.591	0.112
Cannon et al., 2015	0.860	0.601	0.361	-0.318	2.038	1.430	0.153
	0.131	0.167	0.028	-0.197	0.460	0.785	0.433



Study name	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Raal et al., 2014c	0.130	0.067	0.005	-0.002	0.262	1.932	0.053
Raal et al., 2012a	0.020	0.084	0.007	-0.144	0.184	0.239	0.811
Raal et al., 2012b	0.080	0.035	0.001	0.011	0.149	2.268	0.023
Raal et al., 2014b	0.020	0.054	0.003	-0.087	0.127	0.367	0.714
Stein et al., 2012a	0.100	0.207	0.043	-0.305	0.505	0.484	0.628
Stein et al., 2012b	-0.100	0.309	0.095	-0.705	0.505	-0.324	0.746
Stein et al., 2012c	0.230	0.236	0.056	-0.233	0.693	0.973	0.330
Stroes et al., 2014b	-0.100	0.131	0.017	-0.357	0.157	-0.763	0.445
Blom et al., 2014a	0.000	0.022	0.000	-0.044	0.044	0.000	1.000
Blom et al., 2014b	0.000	0.008	0.000	-0.016	0.016	0.000	1.000
Blom et al., 2014c	0.000	0.010	0.000	-0.020	0.020	0.000	1.000
Blom et al., 2014d	0.000	0.011	0.000	-0.021	0.021	0.000	1.000
	0.003	0.005	0.000	-0.007	0.013	0.534	0.593

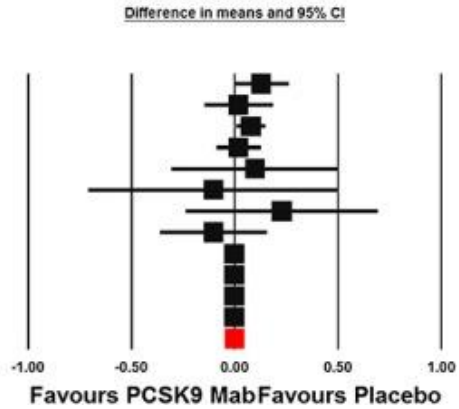
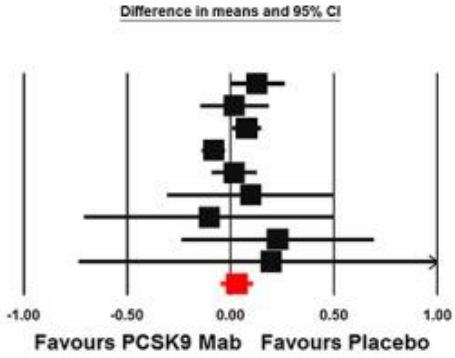


Figure 6. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the impact of proprotein convertase subtilisin/kexin type 9 inhibitors on plasma high-sensitivity C-reactive protein concentrations in trials in populations with (upper plot) and without (lower plot) familial hypercholesterolaemia

Study name	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Raal et al., 2014c	0.130	0.067	0.005	-0.002	0.262	1.932	0.053
Raal et al., 2012a	0.020	0.084	0.007	-0.144	0.184	0.239	0.811
Raal et al., 2012b	0.080	0.035	0.001	0.011	0.149	2.268	0.023
Raal et al., 2014a	-0.080	0.028	0.001	-0.135	-0.025	-2.866	0.004
Raal et al., 2014b	0.020	0.054	0.003	-0.087	0.127	0.367	0.714
Stein et al., 2012a	0.100	0.207	0.043	-0.305	0.505	0.484	0.628
Stein et al., 2012b	-0.100	0.309	0.095	-0.705	0.505	-0.324	0.746
Stein et al., 2012c	0.230	0.236	0.056	-0.233	0.693	0.973	0.330
Stein et al., 2012d	0.200	0.475	0.225	-0.730	1.130	0.421	0.673
	0.033	0.039	0.002	-0.043	0.109	0.849	0.396



Study name	Statistics for each study						
	Difference in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Stroes et al., 2014a	0.300	0.189	0.036	-0.069	0.669	1.591	0.112
Stroes et al., 2014b	-0.100	0.131	0.017	-0.357	0.157	-0.763	0.445
Blom et al., 2014a	0.000	0.022	0.000	-0.044	0.044	0.000	1.000
Blom et al., 2014b	0.000	0.008	0.000	-0.016	0.016	0.000	1.000
Blom et al., 2014c	0.000	0.010	0.000	-0.020	0.020	0.000	1.000
Blom et al., 2014d	0.000	0.011	0.000	-0.021	0.021	0.000	1.000
Cannon et al., 2015	0.860	0.601	0.361	-0.318	2.038	1.430	0.153
	0.000	0.005	0.000	-0.010	0.011	0.027	0.979

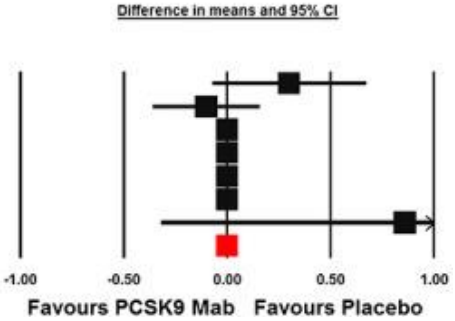


Figure 7. Meta-regression plots for the association between mean changes in plasma high-sensitivity C-reactive protein concentrations with changes in plasma low-density lipoprotein cholesterol (LDL-C) concentrations (upper plot) and cumulative dose of proprotein convertase subtilisin/kexin type 9 inhibitor received during the course of the trial (lower plot)

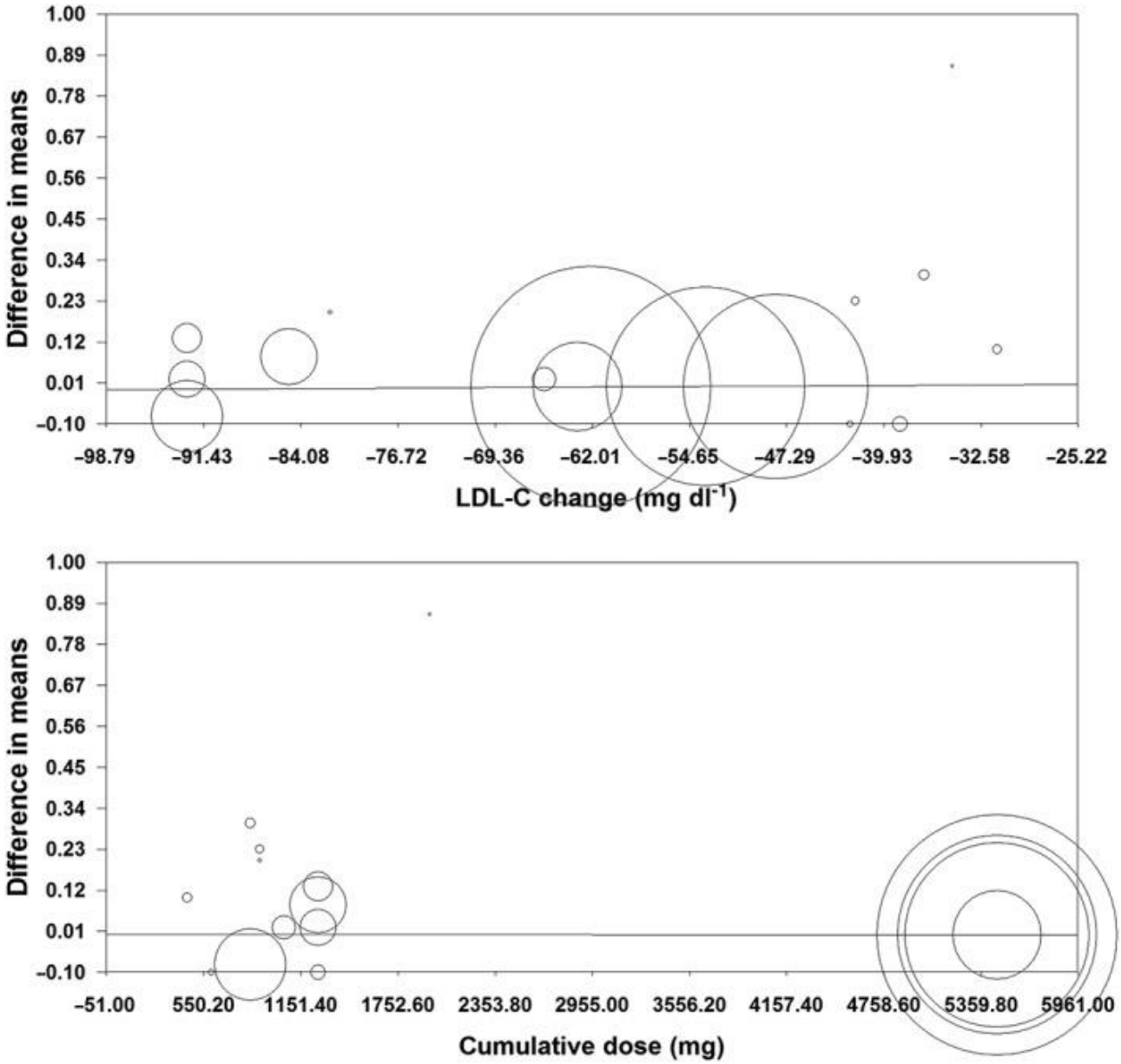


Figure 8. Funnel plot detailing publication bias in the studies reporting the impact of proprotein convertase subtilisin/kexin type 9 inhibitors on plasma sensitivity C-reactive protein concentrations. Open circles represent observed published studies; closed circles represent imputed unpublished studies. Std Err, standard error

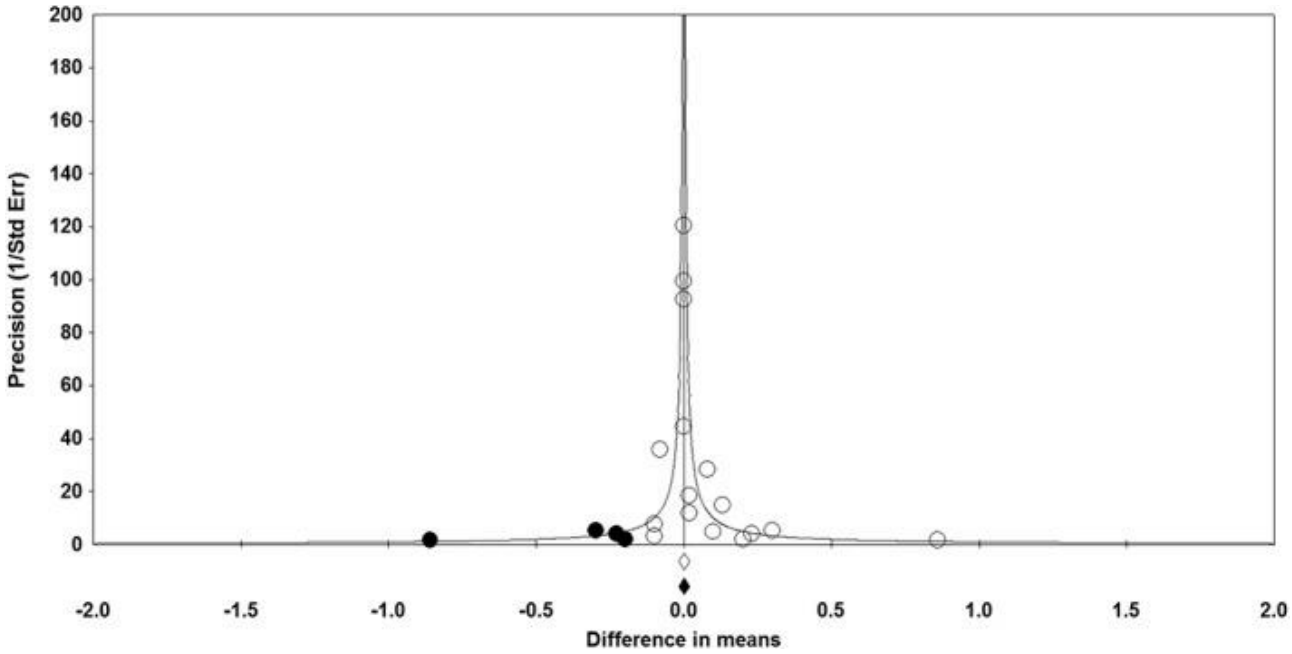


Table 1. Demographic characteristics of the included studies

Study	Raal 2012, RUTHERFORD [16]			Raal 2014, RUTHERFORD-2 [17]			
Treatment arm	A	B		A		B	
Year	2012			2014			
Jadad score	5			5			
Location	Lipid clinics at 24 sites in North America, Western Europe, Hong Kong, Singapore, South Africa			39 sites (mostly specialized lipid clinics, mainly attached to academic centres) in Australia, Asia, Europe, New Zealand, North America and South Africa			
Design	Phase 2, global, multicentre, double-blind, placebo-controlled			Phase 3, multicentre, randomized, double-blind, placebo-controlled			
Length of trial	August 2011 –February 2012			7 February – 19 December 2013			
Inclusion criteria	M and F aged 18–75 years, with a diagnosis of heterozygous FH and LDL-C ≥2.6 mmol l^{-1} (100 mg dl^{-1}), TRIG ≤4.5 mmol l^{-1} (400 mg dl^{-1}), at least 4 weeks of stable statin and other lipid-lowering therapy			Patients 18–80 years of age with a diagnosis of heterozygous FH and on a stable dose of a statin with or without other approved lipid-modifying therapy			
Treatment	Evolocumab 350 mg 4 weeks	Evolocumab 420 mg 4 weeks	Placebo 4 weeks	Evolocumab 140 mg 2 weeks	Placebo 2 weeks	Evolocumab 420 mg 4 weeks	Placebo 4 weeks
Length of treatment	12 weeks	12 weeks	12 weeks	12 weeks	12 weeks	12 weeks	12 weeks
N participants	55	56	56	110	53	108	54
Age (years)	47.6	51.8	49.3	52.6	51.1	51.9	46.8
Male (%)	54.5	62.5	42.9	60	54	58	56
BMI (kg m^{-2})							
hs-CRP baseline (mg l^{-1})	1.09	1.07	0.62	0.2	0.2	0.2	0.3
hs-CRP median change at the end of treatment (mg l^{-1})	–0.06	0	–0.08	0.01	0.01	0.01	0
hs-CRP median percentage change from baseline (%)	–7.99	0	–20.34	–7.1	9.2	4.3	1.4

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Study	Raal 2014, TESLA Part B [18]		Stein 2012 [19]				
Treatment arm	**C**		**A**	**B**	**C**	**D**	
Year	2014		2012				
Jadad score	5		5				
Location	17 sites in ten countries in North America, Europe, the Middle East and South Africa		16 lipid clinics in the USA and Canada				
Design	Phase 3, randomized, double-blind, placebo-controlled		Phase 2, randomized, double-blind, placebo-controlled				
Length of trial	February 2013 – January 2014		18 January – 7 November 2011				
Inclusion criteria	M and F, aged ≥ 12 years, with homozygous FH and LDL-C ≥3.4 mmol l^{-1} after at least 4 weeks of a stable, low-fat diet and baseline lipid-lowering therapies; TRIG ≤4.5 mmol l^{-1}; and bodyweight ≥40 kg		M and F with a diagnosis of FH and LDL-C ≥2.6 mmol l^{-1}. at least 6 weeks of stable statin therapy				
Treatment	Evolocumab 420 mg 4 weeks	Placebo 4 weeks	Alirocumab 150 mg 2 weeks	Alirocumab 150 mg 4 weeks	Alirocumab 200 mg 4 weeks	Alirocumab 300 mg 4 weeks	Placebo 2 weeks
Length of treatment	12 weeks	12 weeks	12 weeks	12 weeks	12 weeks	12 weeks	12 weeks
***N* participants**	33	16	16	15	16	15	15
Age (years)	30	32	56.3	51.3	52.9	54.3	51.9
Male (%)	52	50	61	60	56	47	60
BMI (kg m^{-2})			30,7	28,3	28,8	28,3	29,4
hs-CRP baseline (mg l^{-1})	0.7	0.6	1.4	0.6	0.7	0,7	0.9
hs-CRP median change at the end of treatment (mg l^{-1})	0	0.02	1	0.4	0,7	0.6	0.7
hs-CRP median percentage change from baseline (%)							

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Study	Stroes 2014, GAUSS-2 [20]			
Treatment arm	A	B	C	D
Year	2014			
Jadad score	5			
Location	50 sites in North America, Europe, Asia, Africa and Australia			
Design	Phase 3, randomized, double-blind, placebo- and ezetimibe-controlled			
Length of trial	January – August 2013			
Inclusion criteria	Patients aged 18–80 years, on no or low-dose statins, LDL-C above their NCEP Adult Treatment Panel III goal, previous intolerance to ≥2 statins			
Treatment	Daily ezetimibe 10 mg + placebo 2 weeks	Evolocumab 140 mg 2 weeks + daily placebo	Daily ezetimibe 10 mg + placebo 4 weeks	Evolocumab 420 mg 4 weeks + daily placebo
Length of treatment	12 weeks	12 weeks	12 weeks	12 weeks
N participants	51	103	51	102
Age (years)	62	61	60	63
Male (%)	47	55	57	55
BMI (kg m^{-2})				
hs-CRP baseline (mg l^{-1})	1.7	1.4	1.8	1.8
hs-CRP median change at the end of treatment (mg l^{-1})	1.7	1.7	1.6	1.5
hs-CRP median percentage change from baseline (%)				

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Study	Blom 2014 [21]							
Treatment arm	**A**		**B**		**C**		**D**	
Year	2014							
Jadad score	5							
Location	88 centres in nine countries: USA, South Africa, Hungary, Denmark, Czech Republic, Canada, Belgium, Austria, Australia							
Design	Phase 3, randomized, double-blind, placebo- and ezetimibe-controlled							
Length of trial	January 2012 – November 2013							
Inclusion criteria	Adults 18–75 years, LDL-C ≥75 mg dl^{-1} (1.94 mmol l^{-1}), TRIG ≤400 mg dl^{-1} (4.52 mmol l^{-1}). On the basis of stratification according to the risk categories outlined by the ATP III of the NCEP, patients were started on background lipid-lowering therapy with diet alone or diet plus atorvastatin at a dose of 10 mg daily, or a dose of 80 mg daily, or atorvastatin at a dose of 80 mg daily plus ezetimibe at a dose of 10 mg daily, for a run-in period of 4–12 weeks. Patients with an LDL-C level ≥75 mg dl^{-1} (1.9 mmol l^{-1}) were then randomly assigned in a 2 : 1 ratio to receive either evolocumab (420 mg) or placebo every 4 weeks.							
Treatment	Diet + evolocumab 420 mg 4 weeks	Diet + placebo 420 mg 4 weeks	Diet + atorvastatin 10 mg + evolocumab 420 mg 4 weeks	Diet + atorvastatin 10 mg + placebo 4 weeks	Diet + atorvastatin 80 mg + evolocumab 420 mg 4 weeks	Diet + atorvastatin 80 mg + placebo 4 weeks	Diet + atorvastatin 80 mg + ezetimibe 10 mg + evolocumab 420 mg 4 weeks	Diet + atorvastatin 80 mg + ezetimibe 10 mg + placebo 4 weeks
Length of treatment	52 weeks	52 weeks	52 weeks	52 weeks	52 weeks	52 weeks	52 weeks	52 weeks
***N* participants**	74	37	254	129	145	73	126	63
Age (years)	50.7	53.5	57.2	57	57.8	58.4	54.2	55.9
Male (%)	47.3	40.5	42.9	45.7	52.4	45.2	55.6	52.4
BMI (kg m^{-2})	31.1	29.4	29.6	30.2	30.3	31.6	29.6	30.2
hs-CRP baseline (mg l^{-1})	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
hs-CRP median change at the end of treatment (mg l^{-1})	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
hs-CRP median percentage change from baseline (%)	13.1	27.4	0	0.7	0	0.9	0	10.2

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Study	Cannon 2015 – ODYSSEY COMBO II	
Treatment arm	A	
Year	2015	
Jadad score	5	
Location	126 sites (Europe, Israel, North America, South Africa, South Korea)	
Design	Double-blind, double-dummy, active-controlled, parallel-group	
Length of trial	August 2012 – May 2013	
Inclusion criteria	High cardiovascular risk patients with inadequately controlled hypercholesterolaemia on maximally tolerated doses of statins	
Treatment	Alirocumab 75 mg biweekly. The dose in the alirocumab arm (only) was automatically increased, per protocol, at Week 12, to 150 mg biweekly if the Week-8 LDL-C value was ≥1.8 mmol l^{-1}.	Ezetimibe 10 mg daily
Length of treatment	52 weeks The study is ongoing at the time of writing, and randomized treatment will continue until Week 104, followed by an 8-week post-treatment observational period	
N participants	479	241
Age (years)	61.7	61.3
Male (%)	75.2	70.5
BMI (kg m^{-2})	30.0	30.3
CRP baseline (mg l^{-1})	0.8	0.9
CRP median change at the end of treatment (mg l^{-1})	0.8	0.6
CRP median percentage change from baseline (%)		

ATP, Adult Treatment Panel; BMI, body mass index; F, female; HF, familial hypercholesterolaemia; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; M, male; NCEP, National Cholesterol Education Program; PCSK9, proprotein convertase subtilisin/kexin type 9; TRIG, triglyceride.

Table 2. Systematic assessment of bias in the included studies, using Cochrane criteria

	Random sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective reporting	Free of other bias
Raal *et al.* [16], **RUTHERFORD**	YES	YES	YES	YES	YES	YES
Raal *et al.* [17], **RUTHERFORD-2**	YES	YES	YES	YES	YES	YES
Raal *et al.* [17], **TESLA Part B**	YES	YES	YES	YES	YES	YES
Stein *et al.* [19]	YES	YES	YES	YES	YES	YES
Stroes et al. [20], **GAUSS-2**	YES	YES	YES	YES	YES	YES
Blom *et al.* [21] **2014**	YES	YES	YES	YES	YES	YES
Cannon *et al.* [22] **2015 ODISSEY COMBO-2**	YES	YES	YES	YES	YES	YES