

Does Evolocumab, as a PCSK9 Inhibitor, Ameliorate the Lipid Profile in Familial Hypercholesterolemia Patients? A Meta-Analysis of Randomized Controlled Trials

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ABSTRACT - Proprotein convertase subtilisin-kexin type 9 (PCSK9) is a member of regulatory serine proteases which is mostly expressed in liver. In the physiological condition, LDL-C binds to LDL receptors (LDLRs) and via endocytosis, LDLRs are degraded. PCSK9 binds to the epidermal growth factor-like repeat A (EGFA) domain of extracellular LDLRs, and then physiological recycling of LDLRs from surface of liver is cancelled, resulting in elevation of circulating LDL-C in plasma. To evaluate whether evolucumab, as PCSK9 inhibitor monoclonal antibody, ameliorates lipid profile in familial hypercholesterolemia (FH) patients, this meta-analysis has been conducted. PubMed, Web of Science (ISI) and Scopus databases were searched for studies which had investigated the efficacy of evolucumab. Types of outcome investigated were percentage changes from baseline of the lipid profile. Our meta-analysis shows that evolucumab at the dosage of 420 mg monthly could decrease LDL-C by 54.71%, TC by 35.08%, VLDL-C by 28.37 %, ratio of TC to HDL-C by 39.14 %, triglycerides by 12.11 %, and increased HDL-C by 6.06% from baseline compared to placebo at the end of study in FH patients. Our findings indicate that evolucumab could be a hopeful agent for challenging patients, such as statin intolerance or patients who fail to attain the target goal of LDL-C despite consumption of maximum doses of statins.

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INTRODUCTION

Familial hypercholesterolemia (FH), the most prevalent inherited disorder, leads to elevations in low-density lipoprotein cholesterol (LDL-C) (1). The predominant underlying reason for FH refers to LDL-C receptors (LDLRs), which over 1288 variants of them have been identified and 79% of these are susceptible to cause disease (2). Initially, it was thought that FH is due to enhanced cholesterol synthesis, but nowadays it is known that catabolic rate of LDL is diminished (3). In heterozygous FH (HeFH), single allele defect leads to moderate accumulation of plasma LDL, while homozygous FH (HoFH) with defect in both of alleles, leads to severe accumulation of plasma LDL without LDL cholesterol clearance. Enhanced circulating LDL-C gradually develops cardiovascular diseases. Mutation in genes which codes for LDLRs, apolipoprotein B and proprotein

convertasesubtilisin-kexin type 9 (PCSK9) causes impaired elimination of LDL-C from the plasma (4, 5).

PCSK9 is a member of regulatory serine proteases which are involved in a broad spectrum of physiologic processes (6). It is mostly expressed in liver and less in kidney, small intestine, and central nervous system (7). This protease is secreted to plasma, existing in free form or associated with plasma proteins (8). PCSK9 affects cholesterol level by altering the number of available LDLRs in the surface of hepatocytes (9, 10). In the physiological condition, LDL-C binds to LDLRs and via endocytosis, LDLRs are degraded by lysosome.

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PCSK9 binds to the epidermal growth factor-like repeat A (EGFA) domain of extracellular LDLRs, and then physiological recycling of LDLRs from surface of liver is cancelled, resulting in elevation of circulating LDL-C in plasma (11-13). It has been observed that lacking PCSK9 in mice causes an increase in LDLRs reserving for LDL-C (14). Mutations, leading to gain of function in PCSK9, give rise to elevation in LDL-C and subsequently increase the cardiovascular events, while loss of function mutations cause diminution in LDL-C, leading to a reduction in coronary heart disease (15). The expression of PCSK9 gene is controlled by intracellular cholesterol content (16). Therefore, cholesterol depletion through treatment with statins, ezetimibe and bile acid-binding resins, leads to PCSK9 upregulation (17-21). The transcription of PCSK9 and LDLRs are co-upregulated through the sterol regulatory element binding protein-2 (SREBP 2) pathway after treatment with statins and this reduces the therapeutic trait of statins (22, 23). Thus, statin treatment increases circulating PCSK9 levels additionally increases the content of LDLRs, explaining why doubling a statin dose, results in only

6 percent added decrement in the level of LDL-C in plasma (24).

On the basis of this evidence, inhibition of PCSK9 by monoclonal antibodies seems to be an impressive approach to reduce LDL-C, mainly in the cases of intolerance or unable to attain desired LDL-C level with statins. Evolocumab, bococizumab and alirocumab as PCSK9 inhibitor monoclonal antibodies are under development and in clinical trials.

Evolocumab (commercial name – Repatha™) has recently received the approval by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), as a therapeutic agent for HoFH, primary hypercholesterolemia or dyslipidemia (25-27). Evolocumab is an IgG2 antibody that binds to PCSK9 and inhibits its interplay with LDLRs (Table 1). Binding of evolocumab to PCSK9 decreases the amount of free PCSK9 and eventually enhances the available LDLRs for LDL in hepatocyte surface.

In the present meta-analysis of randomized controlled trials, we aimed to quantitatively evaluate the therapeutic effect of evolocumab, as a PCSK9 inhibitor on lipid profile in FH patients.

Table 1. Drug information

Trade names	Repatha
Type	Fully human IgG2 monoclonal antibody
Company	Amgen
Protein structure	
Indication	For treatment of heterozygous/homozygous familial hypercholesterolemia or clinical atherosclerotic cardiovascular disease in patients on maximum tolerated statin therapy
Pharmacology description	Evolocumab binds on the catalytic site of PCSK9 next to the binding site for the LDL receptor, resulting in the inhibition of binding between PCSK9 and the LDL receptor
Route of administration	Subcutaneous injection
Protein chemical formula	C6242H9648N1668O1996S56
Half-life	11–17 days

The provided information are adapted from <http://www.drugbank.ca/drugs/DB09303>

IgG2: Immunoglobulin G2, PCSK9: Proprotein convertase subtilisin/kexin type 9, LDL: Low-density lipoprotein,

METHODS

Data sources

PubMed, Web of Science, and Scopus were searched for studies which investigated the efficacy of evolucumab in reducing lipid parameters. The search terms were “Evolucumab [Title/Abstract] OR PCSK9 inhibitor [Title/Abstract] AND Lipid profile (Total Cholesterol, LDL-C, HDL-C, VLDL-C, Triglycerides) [Title/Abstract] AND Hypercholesterolemia [Title/Abstract]”. An expert librarian was consulted for assistance in conducting a comprehensive search with appropriate search terms. We limited our search to studies written in English and there was no limitation for the years. At last, article reference lists underwent a review for additional applicable studies. In PubMed, our search was limited to clinical trials while in Scopus and Web of Science, all articles were reviewed. Studies were included if they met the following criteria: placebo-controlled, randomized, included outcomes of changes in lipid parameters in patients receiving evolucumab 420 mg every 4 weeks versus placebo.

Study selection

The randomized controlled trials investigated the effect of evolucumab and its efficacy to ameliorate lipid profile in hypercholesterolemia patients receiving evolucumab 420 mg every 4 weeks versus placebo, were considered. Two reviewers independently examined the title and abstract of each article to eliminate duplicates, reviews, case studies, and uncontrolled trials. Trials were excluded if they were not placebo-controlled or their outcomes did not possess the outcomes of interest. Percentage change of LDL-C was the primary outcome of interest for assessment of efficacy of evolucumab. The reviewers independently extracted data on patients' characteristics, therapeutic regimens, dosage, trial duration, and outcome measures. Disagreements, if any, were resolved by consensus.

Assessment of trial quality

The quality of evidence was determined using Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology (28). The GRADE system assesses risk of bias, imprecision, inconsistency, indirectness of study results, and publication bias across the body of

evidence to derive an overall summary of the quality of evidence (Table 3).

STATISTICAL ANALYSIS

Data from selected studies were extracted in the form of 2×2 tables by study characteristics. Included studies were weighted by effect size and pooled. Data were analyzed using StatsDirect software version 3.0.190. Standardized effect size and 95% confidence intervals (95% CI) were calculated using Mulrow-Oxman (for fixed effects) and Der Simonian-Laird (for random effects) methods. The Cochran Q test and I^2 inconsistency were used to test heterogeneity and $P < 0.05$ considered significant. When there is heterogeneity that cannot readily be explained or few included studies, the random effects model was used. Egger and Begg-Mazumdar tests were used to evaluate publication bias indicators in funnel plot.

RESULTS

A total of 8 eligible studies were included in the meta-analysis (Figure 1). Characteristics of included studies are shown in Table 2. An assessment of quality is shown in Table 3.

Total Cholesterol (TC)

Effect of evolucumab in comparison to placebo therapy in TC in FH patients

The summary for the standardized effect size of mean differences of %change from baseline at the end of study for TC in FH patients “ ΔTC ” for evolucumab therapy for six included studies comparing to placebo (29-34) was -35.08 with 95% CI= -38.34 to -31.81 ($P < 0.0001$, Figure 2a). The Cochran Q test for heterogeneity indicated that the studies are heterogeneous ($P = 0.003$), I^2 (inconsistency) of 71.8% with 95% CI = 6.6% to 85.9% and could not be combined, thus the random effects for individual and summary of effect size for standardized mean differences was applied. For evaluation of publication bias Egger regression of normalized effect vs. precision for all included studies for “ ΔTC ” in FH patients among evolucumab vs. placebo therapy was -1.68 (95% CI= -6.69 to 3.33, $P = 0.4$) and Begg-Mazumdar Kendall's test on the standardized effect vs. variance indicated tau= -0.2, $P = 0.47$ (Figure 2b).

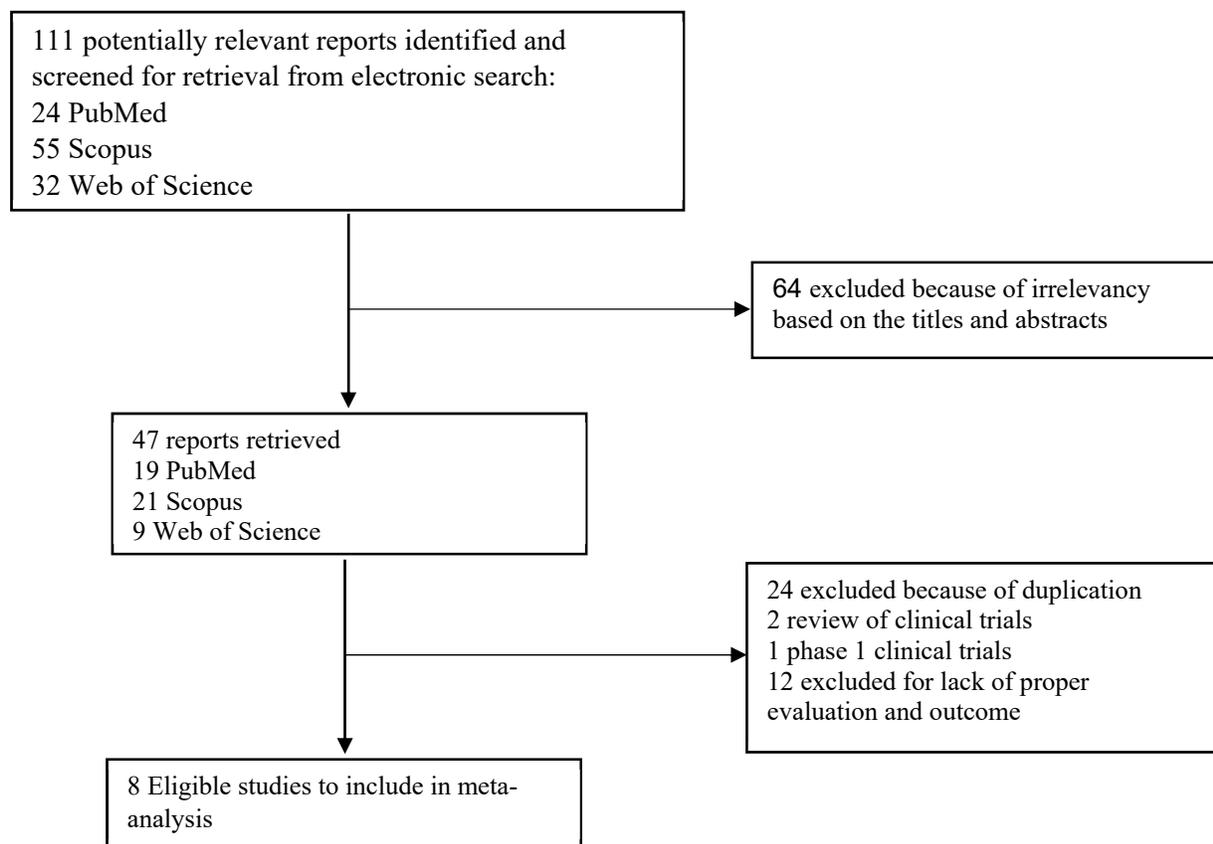


Figure 1. Study flow diagram

Low-density lipoprotein Cholesterol (LDL-C) Effect of evolocumab in comparison to placebo therapy in LDL-C in FH patients

The summary for the standardized effect size of mean differences of %change from baseline at the end of the study for LDL-C in FH patients “ Δ LDL-C” for evolocumab therapy for eight included studies comparing to placebo (29-36) was -54.71 with 95% CI= -59.39 to -50.03 ($P < 0.0001$, Figure 2c). The Cochrane Q test for heterogeneity indicated that the studies are heterogeneous ($P < 0.0001$), I^2 (inconsistency) of 94.7% with 95% CI = 92.2% to 96.1% and could not be combined, thus the random effects for individual and summary of effect size for standardized mean differences was applied. For evaluation of publication bias Egger regression of normalized effect vs. precision for all included studies for “ Δ LDL-C” in FH patients among evolocumab vs. placebo therapy was -0.61 (95% CI= -6 to 4.77, $P = 0.79$) and Begg-Mazumdar Kendall’s test on the standardized effect vs. variance indicated $\tau = 0$, $P = 0.91$ (Figure 2d).

High-density lipoprotein Cholesterol (HDL-C) Effect of evolocumab in comparison to placebo therapy in HDL-C in FH patients

The summary for the standardized effect size of mean differences of %change from baseline at the end of the study for HDL-C in FH patients “ Δ HDL-C” for evolocumab therapy for eight included studies comparing to placebo (29-36) was 6.06 with 95% CI= 4.7 to 7.43 ($P < 0.0001$, Figure 3a). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous ($P = 0.25$), I^2 (inconsistency) of 22.1% with 95% CI = 0% to 65.3% and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean differences was applied. For evaluation of publication bias, Egger regression of normalized effect vs. precision for all included studies for “ Δ HDL-C” in FH patients among evolocumab vs. placebo therapy was 0.78 (95% CI= -1.12 to 2.68, $P = 0.35$) and Begg-Mazumdar Kendall’s test on the standardized effect vs. variance indicated $\tau = 0.21$, $P = 0.55$ (Figure 3b).

Table 2. Characteristics of the 8 studies included in the meta-analysis

Study	Year	Study population	No.	Phase of trial	Duration of trial	Type of design	Ref
MENDEL	2012	FH	406	2	12 week	52 Centers, randomized, double-blind	(32)
RUTHERFORD	2012	HeFH	167	2	12 week	Multicenter, double-blind, randomized, placebo controlled, dose-ranging	(33)
OSLER	2014	FH, Completed Phase II parent study	1104	2	52 week	Open-Label study of long term evaluation, randomized trial	(31)
YUKAWA	2014	FH	310	2	12 week	Randomized, double-blind, placebo-controlled	(30)
MENDEL-2	2014	FH	614	3	12 week	Compare biweekly and monthly evolocumab with placebo and ezetimibe	(35)
RUTHERFORD-2	2014	HeFH	329	3	12 week	Multicentre, randomised, double-blind, placebo-controlled trial	(36)
TESLA Part B	2014	HoFH	49	3	12 week	Randomised, double-blind, placebo-controlled	(34)
DESCARTES	2014	FH	901	3	52 week	Randomized, double-blind, placebo-controlled	(29)

No: Number of patients, FH: Familial hypercholesterolemia, Ref: References, HeFH: Heterozygous familial hypercholesterolemia, HoFH: Homozygous familial hypercholesterolemia

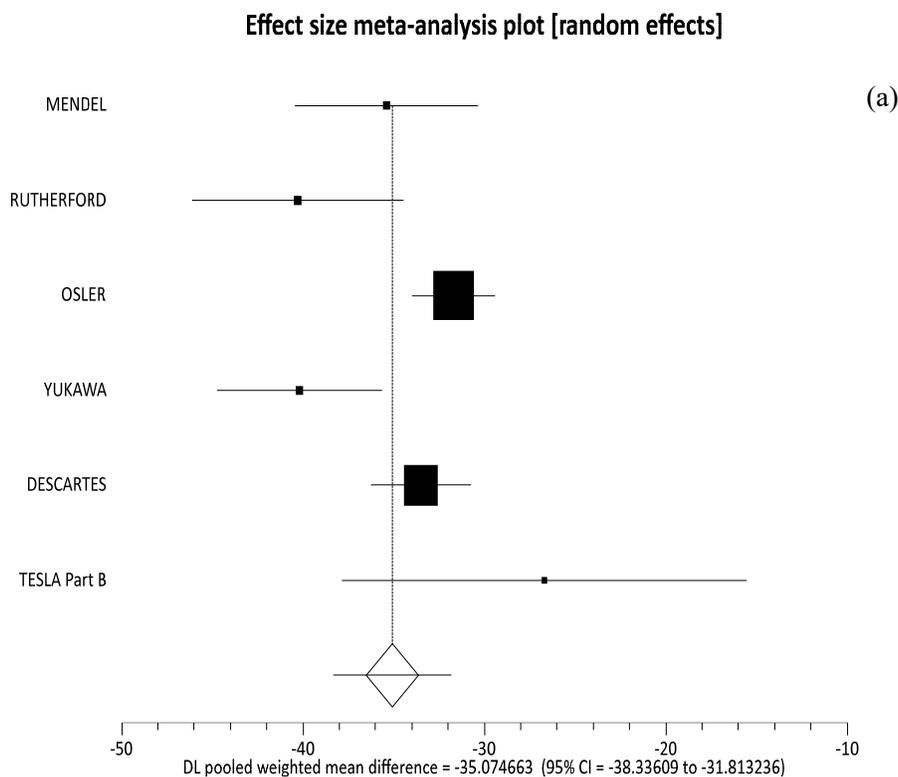


Figure 2

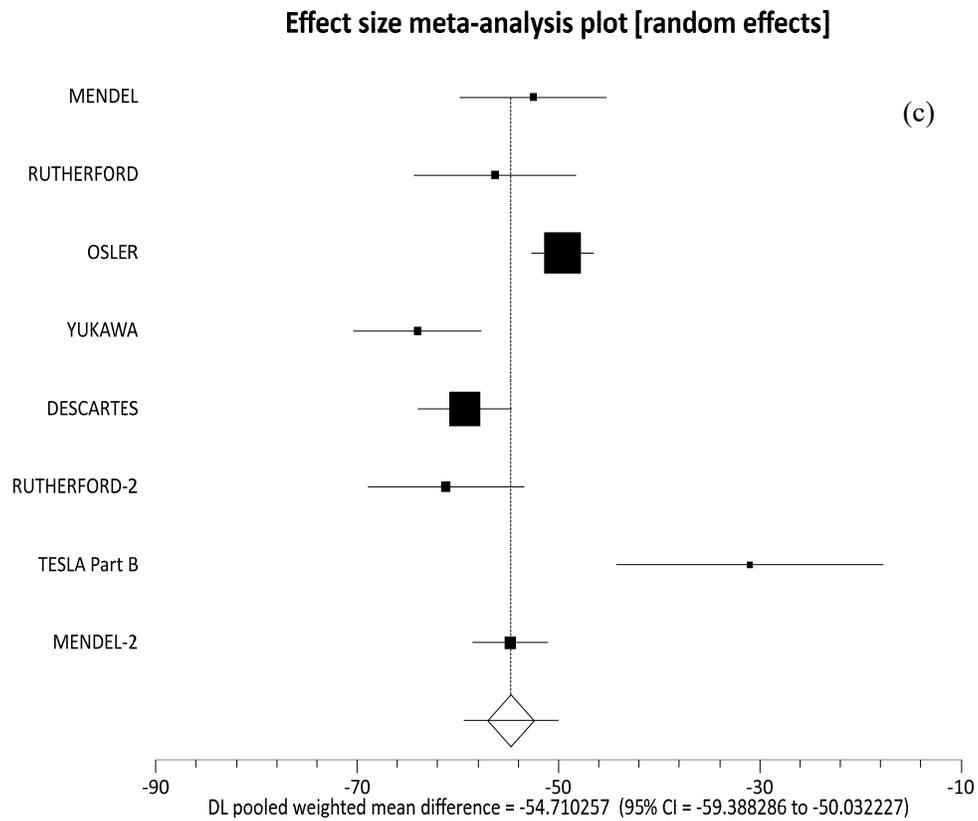
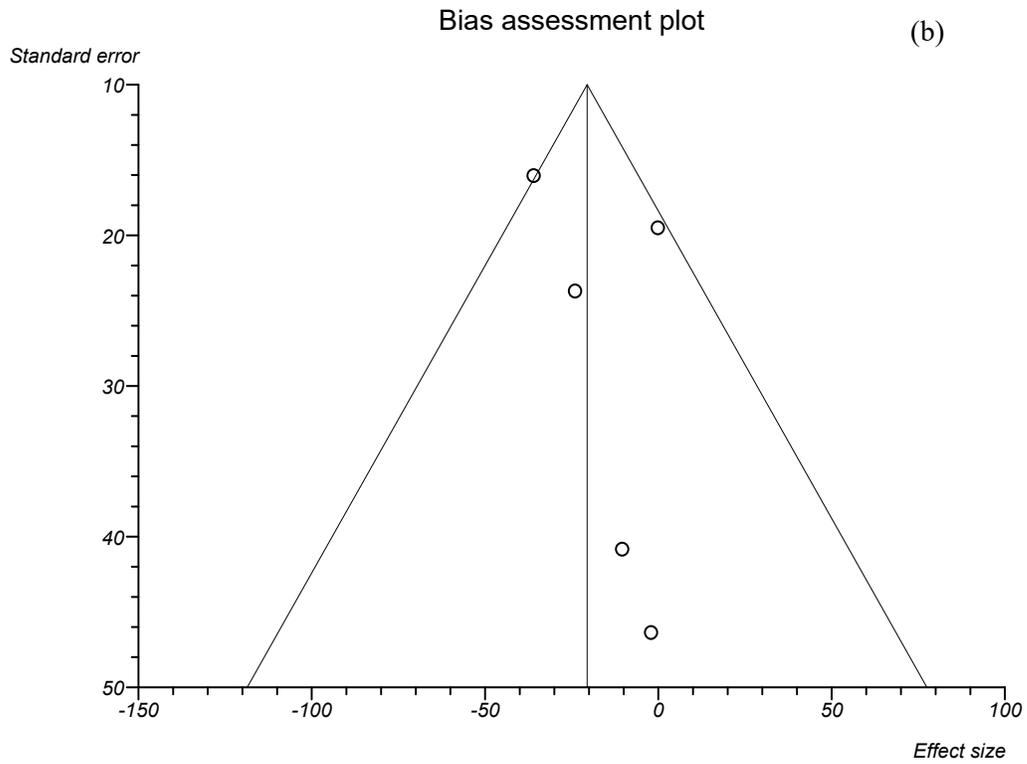


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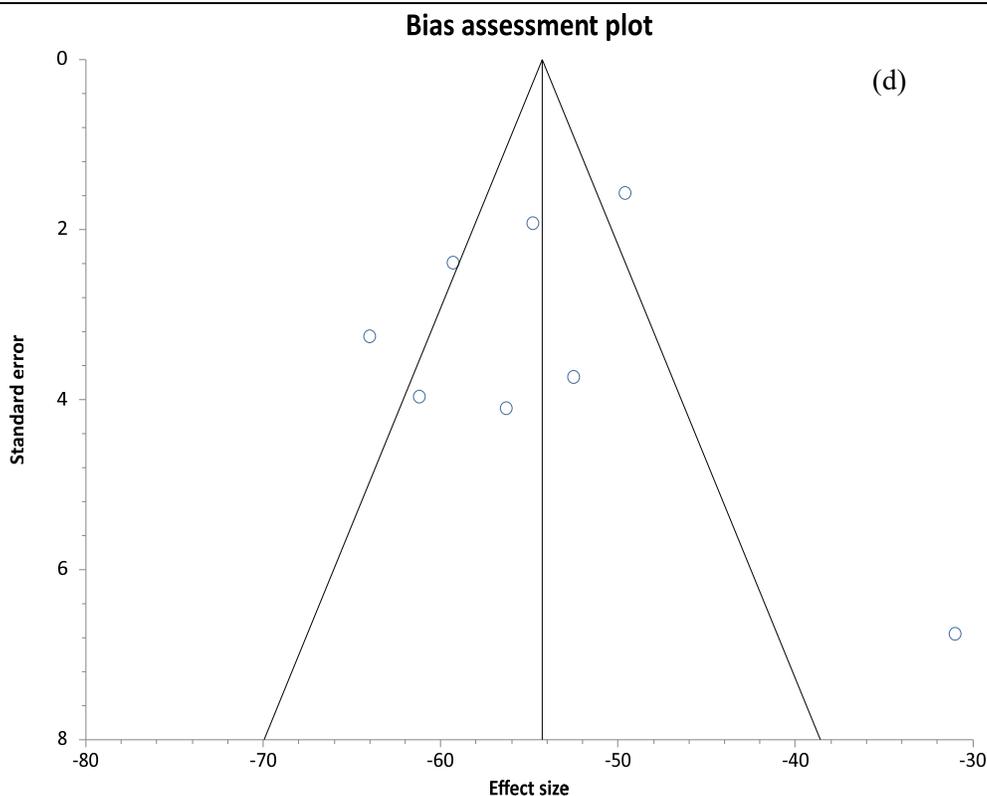


Figure 2. (a) Individual and pooled effect size for the outcome of “ Δ TC” in the studies considering evolocumab comparing to placebo therapy in FH patients (Heterogeneous Cochrane Q test for $P = 0.003$ and $I^2 = 71.8\%$ with 95% CI of 6.6% to 85.9%). (b) Publication bias indicators for the outcome of “ Δ TC” in the studies considering evolocumab comparing to placebo therapy in FH patients. (c) Individual and pooled effect size for the outcome of “ Δ LDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients (Heterogeneous Cochrane Q test for $P < 0.0001$ and $I^2 = 94.7\%$ with 95% CI of 92.2% to 96.1%). (d) Publication bias indicators for the outcome of “ Δ LDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients.

Very Low-density Lipoprotein Cholesterol (VLDL-C)

Effect of evolocumab in comparison to placebo therapy in VLDL-C in FH patients

The summary for the standardized effect size of mean differences of % change from baseline at the end of the study for VLDL-C in FH patients “ Δ VLDL-C” for evolocumab therapy for five included studies comparing to placebo (29, 32-35) was -28.37 with 95% CI= -36.7 to -20.04 ($P < 0.0001$, Figure 3c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous ($P = 0.82$), I^2 (inconsistency) of 0% with 95% CI = 0% to 64.1% and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean differences was applied. For evaluation of publication bias Egger regression of normalized effect vs. precision for all included studies for “ Δ VLDL-C” in FH patients among evolocumab vs. placebo therapy was 0.07 (95% CI=

-2.16 to 2.29, $P = 0.93$) and Begg-Mazumdar Kendall’s test on the standardized effect vs. variance indicated tau= 0, $P = 0.82$ (Figure 3d).

Ratio of TC to HDL-C

Effect of evolocumab in comparison to placebo therapy in Ratio of TC to HDL-C in FH patients

The summary for the standardized effect size of mean differences of % change from baseline at the end of the study for the Ratio of TC to HDL-C in FH patients “ Δ Ratio of TC to HDL-C” for evolocumab therapy for seven included studies comparing to placebo (29-34, 36) was -39.14 with 95% CI= -43.34 to -34.95 ($P < 0.0001$, Figure 4a). The Cochrane Q test for heterogeneity indicated that the studies are heterogeneous ($P = 0.0002$), I^2 (inconsistency) of 77% with 95% CI= 41% to 87.4% and could not be combined, thus the random effects for individual and summary of effect size for standardized mean differences was applied. For evaluation of

publication bias Egger regression of normalized effect vs. precision for all included studies for “ Δ Ratio of TC to HDL-C” in FH patients among evolocumab vs. placebo therapy was -1.99 (95% CI=

-6.41 to 2.42, P= 0.3) and Begg-Mazumdar Kendall’s test on the standardized effect vs. variance indicated tau= -0.05, P= 0.77 (Figure 4b).

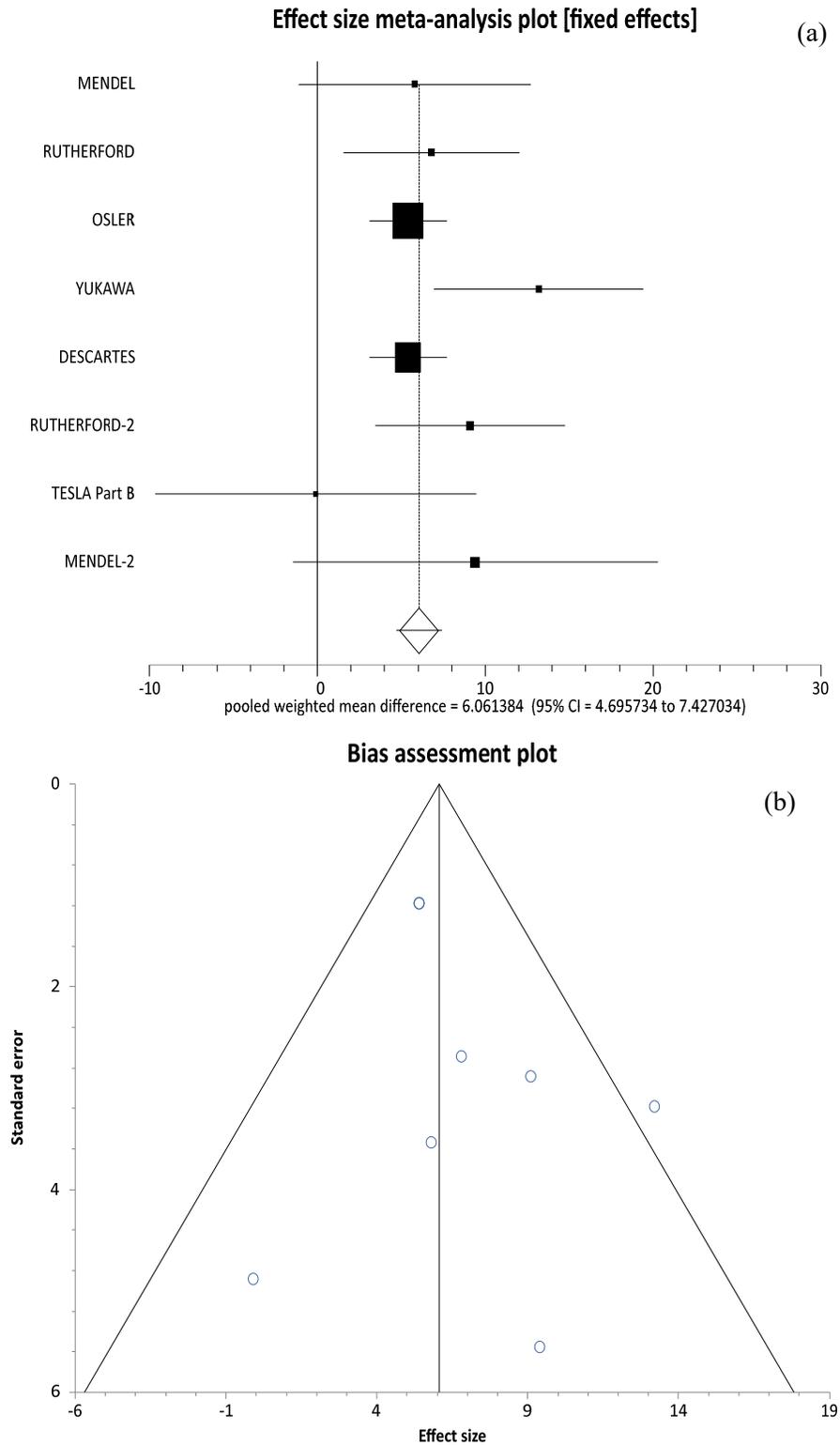


Figure 3

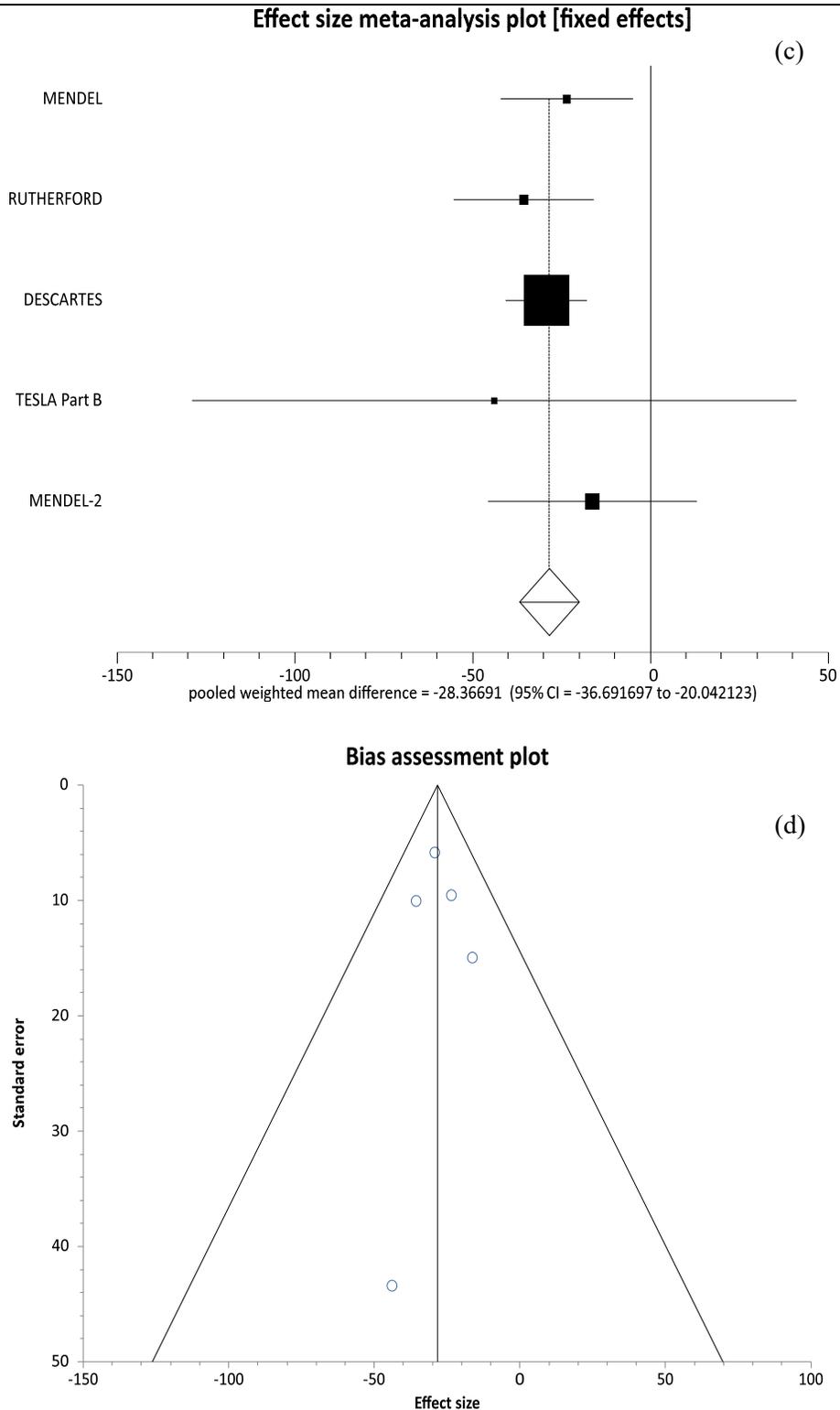


Figure 3. (a) Individual and pooled effect size for the outcome of “ Δ HDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients (Heterogeneous Cochrane Q test for $P=0.25$ and $I^2=22.1\%$ with 95% CI of 0% to 65.3%). (b) Publication bias indicators for the outcome of “ Δ HDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients. (c) Individual and pooled effect size for the outcome of “ Δ VLDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients (Heterogeneous Cochrane Q test for $P=0.82$ and $I^2=0\%$ with 95% CI of 0% to 64.1%). (d) Publication bias indicators for the outcome of “ Δ VLDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients.

Triglycerides (TG)

Effect of evolocumab in comparison to placebo therapy in TG in FH patients

The summary for the standardized effect size of mean differences of %change from baseline at the end of the study for TG in FH patients “ΔTG” for evolocumab therapy for seven included studies compared to placebo (29, 30, 32-36) was -12.11 with 95% CI= -16.05 to -8.16 (P< 0.0001, Figure 4c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (P= 0.29), I² (inconsistency) of 18.9% with 95% CI = 0% to 65.9% and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean differences was applied. For evaluation of publication bias Egger regression of normalized effect vs. precision for all included studies for “ΔTG” in FH patients among evolocumab vs. placebo therapy was 0.1 (95% CI= -2.69 to 2.88, P= 0.93) and Begg-Mazumdar Kendall’s test on the standardized effect vs. variance indicated tau= 0.14, P= 0.77 (Figure 4d).

DISCUSSION

This is the first time that a meta-analysis has been carried out to investigate the efficacy of evolocumab, as a lipid lowering agent in FH patients. In the present meta-analysis, 8 randomized controlled trials consisting of 3880 patients were included. Our meta-analysis shows that evolocumab at the dosage of 420 mg monthly could decrease 54.71% of LDL-C, 35.08% of TC, 28.37% of VLDL-C, 39.14% of ratio of TC to HDL-C, 12.11% of TG, and increased 6.06% of HDL-C from baseline compared to placebo at the end of study in FH patients.

Although plenty of medications have been used by the aim of managing lipid profile and reducing the risk of cardiovascular diseases in FH patients, still it is not under control (37). Statins, which are the most efficient agents for alleviating LDL-C level, many of the patients treating with statins are incapable to tolerate them mainly because of muscle-related side effects or higher dose is required to attain the target LDL-C (38). Nevertheless, statin intolerant patients have a need for more effective LDL-C lowering therapies

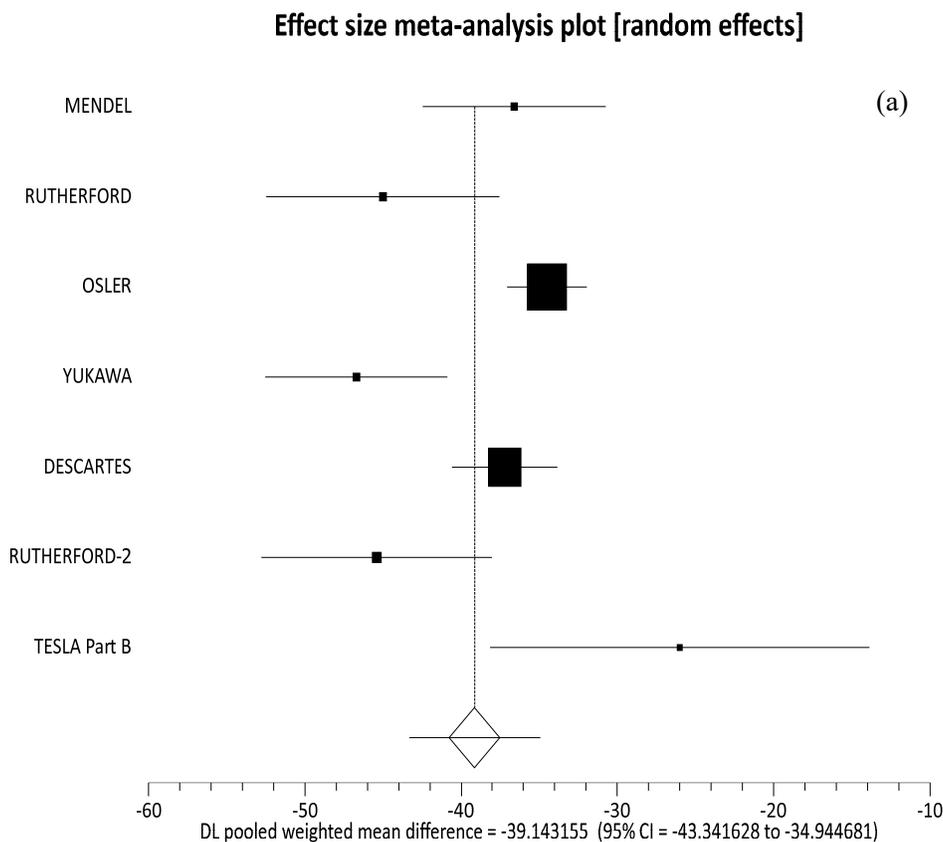


Figure 4

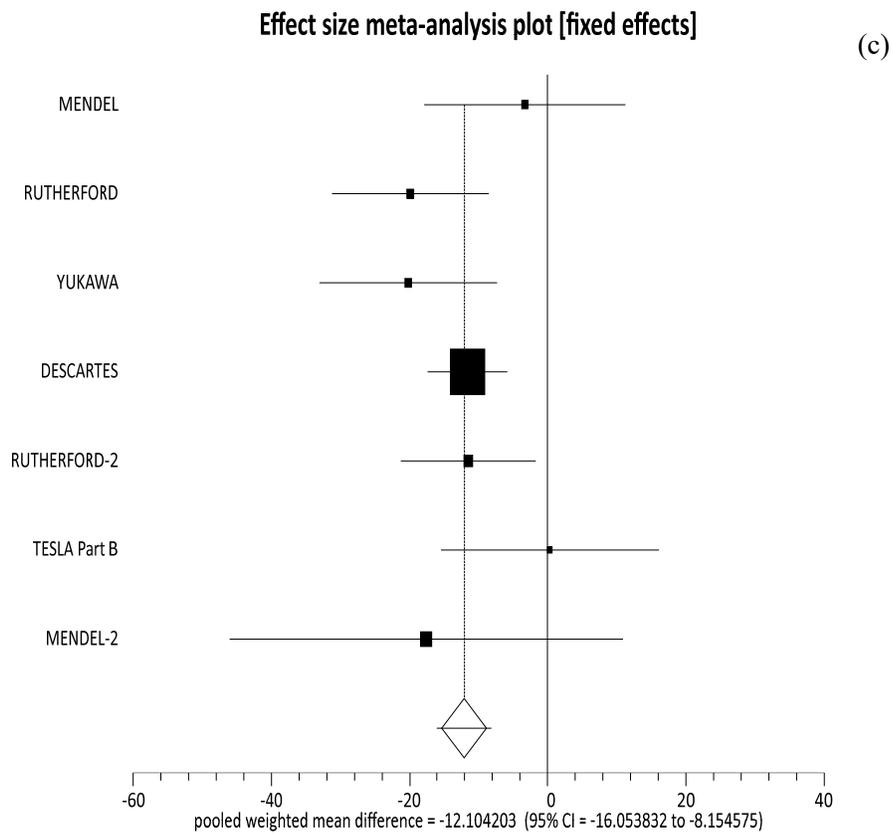
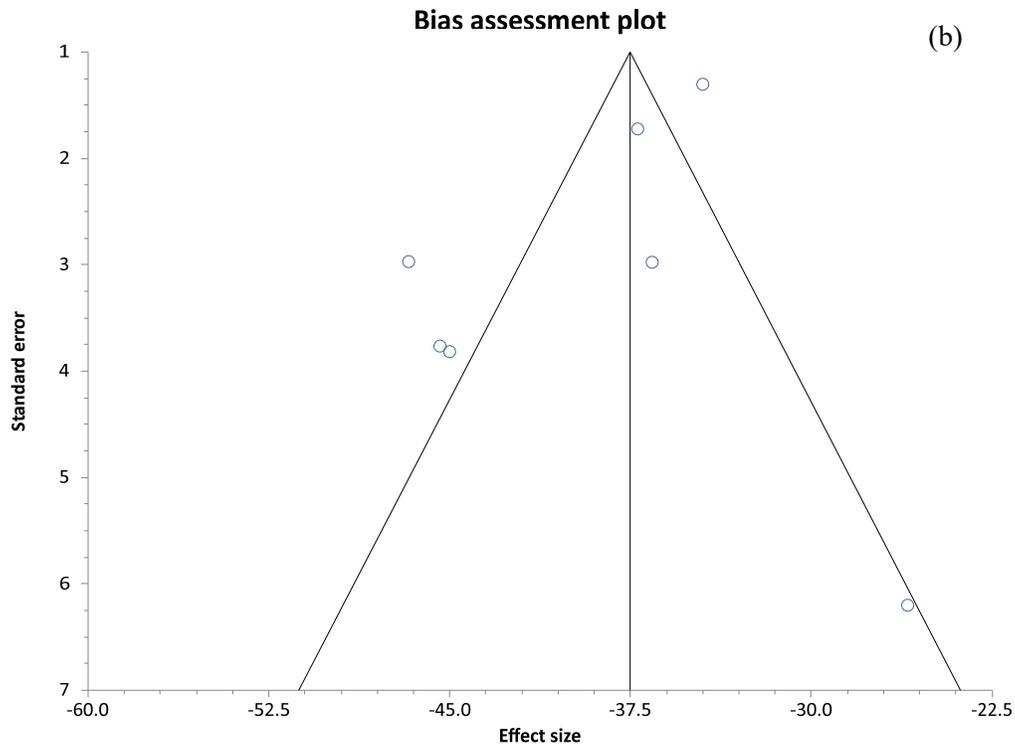


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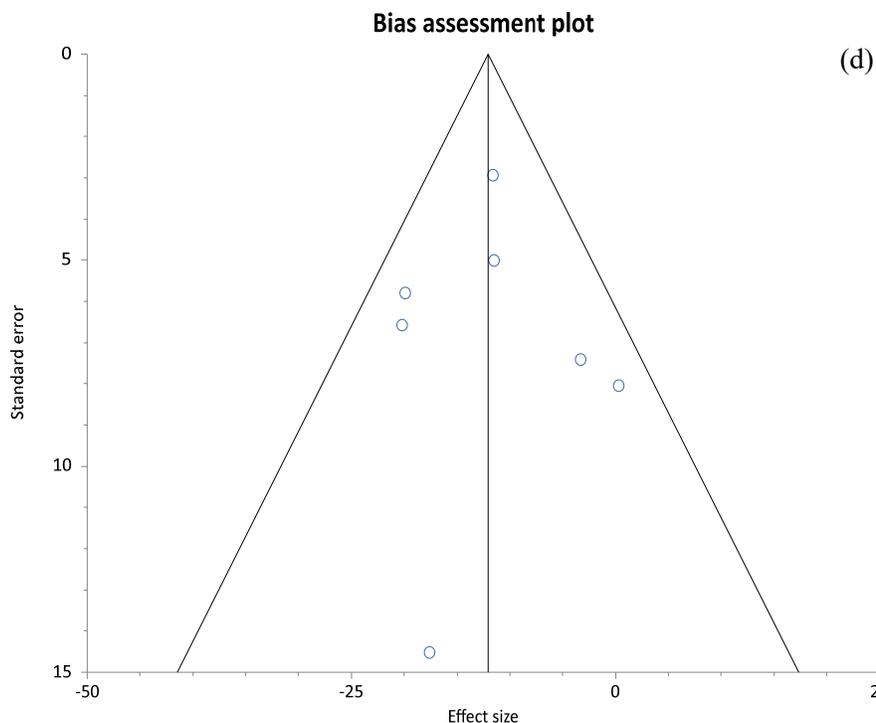


Figure 4. (a) Individual and pooled effect size for the outcome of “ Δ Ratio of TC to HDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients (Heterogeneous Cochrane Q test for $P=0.0002$ and $I^2=77\%$ with 95% CI of 41% to 87.4%). (b) Publication bias indicators for the outcome of “ Δ Ratio of TC to HDL-C” in the studies considering evolocumab comparing to placebo therapy in FH patients. (c) Individual and pooled effect size for the outcome of “ Δ TG” in the studies considering evolocumab comparing to placebo therapy in FH patients (Heterogeneous Cochrane Q test for $P=0.29$ and $I^2=18.9\%$ with 95% CI of 0% to 65.9%). (d) Publication bias indicators for the outcome of “ Δ TG” in the studies considering evolocumab comparing to placebo therapy in FH patients.

Different doses of evolocumab monotherapy comprising of 70 mg, 105 mg, or 140 mg every 2 weeks; and 280 mg, 350 mg, or 420 mg every 4 weeks were scheduled in studies. Through the literatures, comparing the two high doses (140 mg every 2 weeks; and 420 mg every 4 weeks) illustrated no difference in LDL-C reduction (32). Through these, we analyzed data for evolocumab 420 mg every 4 weeks due to its more compliance and less injection frequency.

We have shown that evolocumab therapy significantly decreased TC in FH patients. The RUTHERFORD study (33) showed more reduction of TC in comparison to other studies. The included patients were HeFH with statin therapy. Evolocumab therapy in the TESLA Part B (34) study had least effect on TC probably because of HoFH-included patients which is the severe form of FH and likely less responsive to evolocumab. These results prove that evolocumab is more effective in HeFH rather

than HoFH. To confirm this, the correlation between response to evolocumab and underlying genetic cause of FH has been announced (34). However, another study reported that response to evolocumab is not related to the underlying genetic background (36).

Among these studies, YUKAWA (30) with the Japanese FH patients had more reduction in LDL-C. Maybe that is why in Japan, lower doses and less potent of statins are used and fewer included patients in YUKAWA (30) were on high-intensity statin therapy. Also, it has been shown that evolocumab therapy in patients receiving non-intensive statin therapy had greater reduction of LDL-C compared to group receiving intensive statin therapy. Considering this fact, it could be concluded that evolocumab decreases LDL-C more in non-intensive statin therapy rather than high-intensity statin therapy. Evolocumab in TESLA Part B (34) was less effective to reduce LDL-C, justifying that evolocumab does

not have the same effect on HoFH. However, in the group of genetically authenticated homozygous patients with mutations in LDLRs, there was a variation in LDL-C reduction, proposing that other factors are involved in lowering of LDL-C.

On the other hand, evolocumab therapy increased HDL-C in HF patients. Improvement in HDL-C concentrations was speculated as reduced ability of cholesterol to be transferred from HDL-C to LDL-C, rather than a direct effect on HDL-C production (39). In Japanese FH patients, more increment of HDL-C was reported (30). Maybe this refers to their better responsiveness to evolocumab due to non-intensive statin therapy background.

Combination therapy of evolocumab and statins in FH patients helped most of the patients to achieve the goal LDL-C concentration (39). Interestingly another study reported that patients receiving evolocumab with moderate intensity statins had a slightly greater reduction in PCSK9 compared to those received high-intensity statins, representing the upregulative effect of statins on PCSK9 (40). Furthermore, not only the baseline PCSK9 levels were higher in patients receiving atorvastatin plus evolocumab, but there was also a rapid increase in PCSK9 levels 4 weeks after administration of this combination (29). Due to this phenomenon, it seems that a combination of non-statin medication with evolocumab could be more efficient. Besides, it has not been shown which statin in combination with evolocumab is more beneficial, propelling the design of future studies. Also, addition of ezetimibe to evolocumab was shown to be more efficient compared to evolocumab alone (38, 41).

In a large longer-term evaluation of evolocumab with heterogeneous population which was recruited from 4 phase 2 parent studies of FH patients (31), showed that patients who were receiving evolocumab in their parent study and received it in this study too, had a continuing reduction of LDL-C, implying that no pharmacoresistance was detected during this period. Discontinuation of evolocumab therapy for patients who were using it in parent study and interrupted it in this study, led to rapid return of LDL-C level, representing that evolocumab therapy is somehow a “treatment” rather than “cure”.

In conclusion, our meta-analysis shows that evolocumab as a PCSK9 inhibitor, could ameliorate lipid profile in FH patients. Evolocumab decreased not only LDL-C, but also other lipoprotein markers and increased HDL-C significantly. These findings indicate that evolocumab, with a notable efficacy and

novel approach could be a hopeful agent for challenging patients, such as statin intolerance or patients who fail to attain the target goal of LDL-C despite consumption of maximum doses of statins. However statistical heterogeneity exists, thus random effects have been applied in the current meta-analysis. To provide valid results about its efficacy, long-term adverse effects and assessment of its cost-benefit issues, further studies are required.

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AUTHORS' CONTRIBUTION

Data were collected by SME and MG. SN did meta-analysis and completed methods and results. The manuscript was drafted by SME, SN, MG and edited by MA. The idea of the study was from SN and MA. MA supervised whole study. All authors read and approved the final manuscript.

COMPETING INTEREST

The authors declare no conflict of interest.

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Table 3. Assessment of the quality of evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. RCT: Randomized Controlled Trial.

No of studies	Design	Risk of bias	Quality assessment				Other considerations	No of patients		Effect		Quality
			Inconsistency	Indirectness	Imprecision			Evolocumab therapy	Placebo	Relative (95% CI)	Absolute	
Total Cholesterol (TC)												
6	RCT	not serious	not serious	not serious	not serious	None	1522	873	-	Decreased 35.08% (95% CI= -38.34 to -31.81)	High	
Low-density lipoprotein Cholesterol (LDL-C)												
8	RCT	not serious	not serious	not serious	not serious	None	1785	970	-	Decreased 54.71% (95% CI= -59.39 to -50.03)	High	
High-density lipoprotein Cholesterol (HDL-C)												
8	RCT	not serious	not serious	not serious	not serious	None	1785	970	-	Increased 6.06% (95% CI= 4.7 to 7.43)	High	
Very Low-density Lipoprotein Cholesterol (VLDL-C)												
5	RCT	not serious	not serious	not serious	not serious	None	886	497	-	Decreased 28.37% (95% CI= -36.7 to -20.04)	High	
Ratio of TC to HDL-C												
7	RCT	not serious	not serious	not serious	not serious	None	1632	892	-	Decreased 39.14% (95% CI= -43.34 to -34.95)	High	
Triglycerides (TG)												
7	RCT	not serious	not serious	not serious	not serious	None	1049	602	-	Decreased 12.11% (95% CI= -16.05 to -8.16)	High	

