Atherosclerosis 325 (2021) 46–56

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

A meta-analysis of medications directed against PCSK9 in familial hypercholesterolemia

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ARTICLE INFO

Keywords:
Familial hypercholesterolemia
LDL-Cholesterol
PCSK9 lowering medication
Genotype
Meta-analysis

ABSTRACT

Background and aims: Several medications targeting PCSK9 reduce LDL-cholesterol (LDL-C) in heterozygous familial hypercholesterolemia (HeFH). We aimed to assess in patients diagnosed clinically as HeFH, whether LDL-C reduction varied by different therapeutic approaches to PCSK9-targeting or by the underlying genetic variant.
Methods: We conducted a random-effects meta-analysis of randomised clinical trials assessing PCSK9-targeting therapies, namely alirocumab, evolocumab and inclisiran, in patients with clinically diagnosed HeFH and restricted analyses to those patients in whom genotypic data were available. A search of MEDLINE and Embase identified eligible trials published between inception and June 29, 2020. We included trials of sufficient duration to allow for a stable treatment effect: ~12 weeks for monoclonal antibodies (mAbs) (alirocumab, evolocumab) and ~1 year for small interfering RNA (siRNA) (inclisiran). Single-moderator meta-regression comparing mean percentage LDL-C reduction between mAbs and siRNA as well as PCSK9-targeting therapies between different genotypes was used to assess heterogeneity.
Results: Eight trials of HeFH met our inclusion criteria, including 1887 genotyped patients. Among monogenic HeFH cases (N = 1347) the LDL-C reduction from baseline was 46.12% (95%CI 48.4-43.9) for siRNA and 50.4% (59.3-41.4) for mAbs compared to control, without evidence of significant heterogeneity between treatment (Q_M = 0.32, df = 1, p = 0.57). Irrespective of therapeutic approach to PCSK9-targeting, reductions in LDL-C were generally consistent across genetic variants (LDL-Receptor variants, LDL-Receptor variants of unknown significance, Apolipoprotein B variants, two variants and no variant) (Q_M = 8.3, df = 4, p = 0.08).
Conclusions: Among patients with HeFH, the LDL-C-lowering effect of PCSK9-targeting medications did not show statistical heterogeneity across different drug-classes and across genetic variants.

1. Introduction

Heterozygous familial hypercholesterolemia (HeFH) is an inherited disorder of the low-density lipoprotein cholesterol (LDL-C) metabolism with a prevalence of ~1:311 in the general population and 1:17 among those with atherosclerotic cardiovascular disease [1]. The majority of cases of HeFH result from abnormalities in genes regulating the structure or function of the LDL-receptor, the function of apolipoprotein B100 (ApoB) or which result in a gain-of-function in proprotein convertase subtilisin-like/kexin type 9 (PCSK9) [2]. Irrespective of the specific genetic abnormality, the net result is a diminished capacity among affected individuals to remove LDL particles from the circulation. A clinical phenotype of exposure to elevated LDL cholesterol (LDL-C) levels from birth leads to an increased risk of atherosclerotic cardiovascular disease, particularly premature coronary artery disease [2]. Statins reduce LDL-C levels among those with HeFH; but even the use of high-intensity statins as monotherapy may be insufficient to achieve desirable LDL-C levels for many subjects with HeFH, because starting levels of LDL-C are so high, necessitating the use of additional LDL-C-lowering medications [3]. Being an asymptomatic condition,

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https://doi.org/10.1016/j.atherosclerosis.2021.03.042
Received 5 February 2021; Received in revised form 21 March 2021; Accepted 31 March 2021
Available online 20 April 2021
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cases of HeFH are diagnosed on average in the fifth decade of life when they present with atherosclerotic cardiovascular disease (unpublished: Vallejo-Vaz, AJ ESC 2019) and median age of treatment initiation is around 39 (interquartile range 25–50) in the United States [4]. As exposure to LDL-C is both causal and cumulative for the development of atherosclerotic cardiovascular disease [5], those diagnosed late in life may require more intensive reductions in LDL-C than those diagnosed earlier, to compensate for missed years of elevated LDL-C exposure and a potentially greater burden of atherosclerosis [6].

Ezetimibe is safe and effective when added to statins and reduces LDL-C by a further 20–25%. Yet this may still be insufficient for many individuals with HeFH [3]. Recent updates to clinical guidelines recommend even lower LDL-C targets, hence necessitating the use of more potent lipid-lowering adjunctive therapies [7]. Therapies directed against PCSK9, whether they bind circulating PCSK9 such as monoclonal antibodies (mAbs) or which inhibit hepatic PCSK9 synthesis such as small interfering RNA (siRNA) based approaches are both safe and effective at reducing LDL-C [8–10]. These trials have included individuals either meeting established clinical criteria for HeFH or in whom a genetic diagnosis was confirmed. Even though, HeFH is an autosomal dominant, monogenic disorder, it has been shown that its clinical phenotype overlaps with other genetic aetiology ranging from double and compound heterozygous to milder homozygous variants [11] although the latter are rare. Additionally, many patients meeting the clinical criteria of HeFH may have a polygenic rather than a monogenic basis for their hypercholesterolemia [12,13]. As some monogenic variants are very rare, uncertainty persists around the implications of certain genetic variants on treatment response to medication directed against PCSK9. We tested two hypotheses. First, that irrespective of the therapeutic approach, medications targeting PCSK9 would result in similar reductions of LDL-C. Secondly, if the first hypothesis were demonstrated, then these therapies would result in similar reductions in LDL-C irrespective of genetic background.

2. Materials and methods

2.1. Search strategy and selection criteria

For this systematic review and meta-analysis, we used the methods proposed in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement (PRISMA). We included double-blind, randomised controlled trials (RCTs) assessing LDL-C reduction by medications directed against PCSK9 in patients with a clinical diagnosis of HeFH and available genetic data on any of the three main genes related to FH on a maximally tolerated background medication (statin with or without ezetimibe), after a minimum treatment duration of 12 weeks for mAbs and one year for siRNA to allow a stable treatment effect. Relevant studies on Medline and Embase were searched from inception to June 29, 2020, using terms related to FH and the different therapies targeting PCSK9, restricted to RCTs, but without any language restrictions (Supplementary Tables 1 and 2). Conference abstracts were excluded due to the limitations in the data provided and insufficient information to assess study quality. No additional studies meeting our eligibility criteria were identified by searching ClinicalTrials.gov. Study authors and trial sponsors were contacted for missing information on baseline characteristics where appropriate (Supplementary Table 6). Eligibility was assessed by two investigators (KID and JB) independently through title and abstract and full-text screening and disagreements resolved by a third author (AJVV) through consensus. We excluded studies assessing bococizumab, which was discontinued without plans for further development.

Quality assessment was conducted by two investigators (AJVV and JB) using the Cochrane risk-of-bias assessment tool.

2.2. Data extraction and harmonisation

Data was extracted manually by JB using a standardised form. The percentage change in LDL-C from baseline in the treatment and the comparator groups was extracted stratified by genetic variant and dosing regimen. For the mAb trials, we report on the percentage LDL-C reduction from baseline to 12 weeks. This was the timepoint for the primary outcome in trials of evolocumab and the timepoint at which treat-to-target trials assessing bi-weekly 75 mg alirocumab allowed uptitration to bi-weekly 150 mg if the LDL-C treatment target was not achieved. This also allowed comparison with those initiated on bi-weekly 150 mg alirocumab from the outset. For siRNA based therapies, we report on the percentage LDL-C reduction from baseline to week 73 (day 510), which was the timepoint at which the primary endpoint was reported.

Differences in reporting on genetic variants between studies were harmonised to allow data pooling (Supplementary Table 4). Reported variants were grouped as "*LDLR* variants" (pathogenic or likely pathogenic), "*LDLR* variants of unknown significance", "*APOB* variants", "*PCSK9* gain-of-function (GOF) variants", "two variants" (compound and double heterozygous, and homozygous for those subjects with a clinical diagnosis of HeFH but subsequently found to have two variants on genotyping) and "no known variant ". The extracted outcome data were pooled per genetic variant, treatment and dose using the equation recommended in the Cochrane Handbook of Meta-analysis [14].

2.3. Statistical analysis

For the assessment of variation in treatment effect between drug classes and within a class, the subgroup of patients in whom no FH-causing variant could be identified were excluded, but included in the comparison across genotypes as they represent a significant proportion of all individuals phenotypically considered as HeFH. For the same reason, patients with two variants and *LDLR* variants of unknown significance have been included in the analysis. The effect estimates, raw mean differences in LDL-C percentage change from baseline compared to control, were then combined using random-effects models based on the assumption that the treatment effect may vary among the different therapies and genotypes [15]. Summary estimates are reported as the mean and 95% confidence interval (95%CI). Heterogeneity of raw mean differences was assessed using I^2- and Q-statistics [16].

For comparisons between subgroups, we used single-moderator random-effects meta-regression to assess whether LDL-C reduction varied between categories of the following moderators: mAb dose and type, PCSK9-targeting medication, mode of action, and genotype. We tested whether the moderator was associated with variations in the treatment effect by calculating Q_M-statistics. In the case of a significant association ($p < 0.05$ for Q_M), we assumed the LDL-C reduction to be different between subgroups. Q_E-statistics were used to identify residual heterogeneity that was not explained by the model and the included moderator. A significant Q_E-statistic would suggest that other factors, not accounted for in the model, cause variation in LDL-C reduction.

We used R version 3.5.1 for analyses ("metafor" package for meta-analyses).

3. Results

Our search retrieved 582 different reports, of which 30 were selected for full-text assessment (Fig. 1). Through full-text review of those 30 reports, 24 were excluded because they did not separately report on participant's genotype. Additionally, three reports cover trial NCT01604824, which assessed treatment with alirocumab in genetically defined patients with HeFH [17–19]; however, this trial was excluded

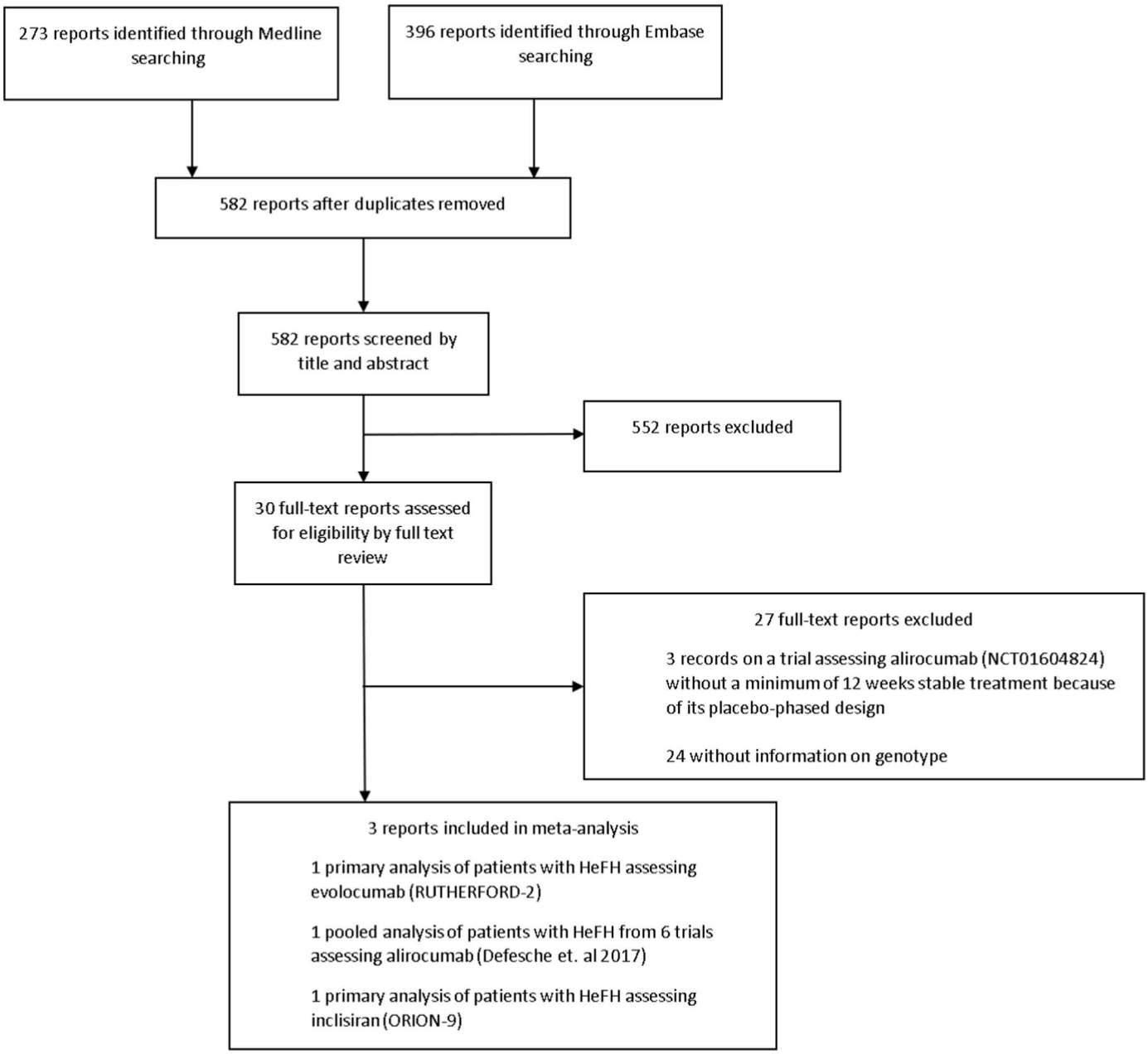

Fig. 1. Prisma flow-chart.
3 reports fulfilled our eligibility criteria. Of those, 2 presented the main results of the RUTHERFORD-2 and ORION-9 trials, respectively. The remaining reported on a pooled analysis of individuals with HeFH, treated with alirocumab among 6 trials. HeFH: heterozygous familial hypercholesterolemia.

because a direct comparison of stable treatment effects *versus* placebo at a minimum of 12 weeks was not available based on study design. Finally, we selected two RCTs (RUTHERFORD-2 assessing evolocumab and ORION-9 assessing inclisiran) [9,10] and one secondary analysis of pooled data from six RCTs assessing alirocumab (R727-CL-1003, ODYSSEY FH I & II, ODYSSEY HIGH FH, ODYSSEY ALTERNATIVE, ODYSSEY LONG TERM) [20] meeting our inclusion/exclusion criteria (Fig. 1). All trials met the criteria for a low risk of bias assessed with the Cochrane risk-of-bias assessment tool (Supplementary Table 5).

3.1. Study characteristics

A total of 1887 participants with available data on genetic sequencing were included in the meta-analysis (Fig. 2). Among those, 177 participants were randomised to evolocumab and 87 to placebo and sequenced for variants in the *LDLR* and *APOB* genes. The remaining were sequenced for *LDLR*, *APOB* and *PCSK9* variants, of those 221 *vs* 211 participants were randomised to inclisiran or placebo, and 758 *vs* 433 to alirocumab or placebo. For the phase II, multiple dosing trial R727-CL-1003 [21] (N = 57) data were only available on baseline characteristics but not on LDL-C lowering by Defesche et al. (therefore they are included only in the assessment of baseline characteristics but not in the analyses of efficacy). Data on percentage change in LDL-C from baseline to week 12 for mAb trials and to day 510 for the siRNA trial were available for 1097 participants on active treatment (including 839 with and 258 without a FH-causing genetic variant), and for 714 participants in the control group (532 with and 182 without a FH-causing genetic variant).

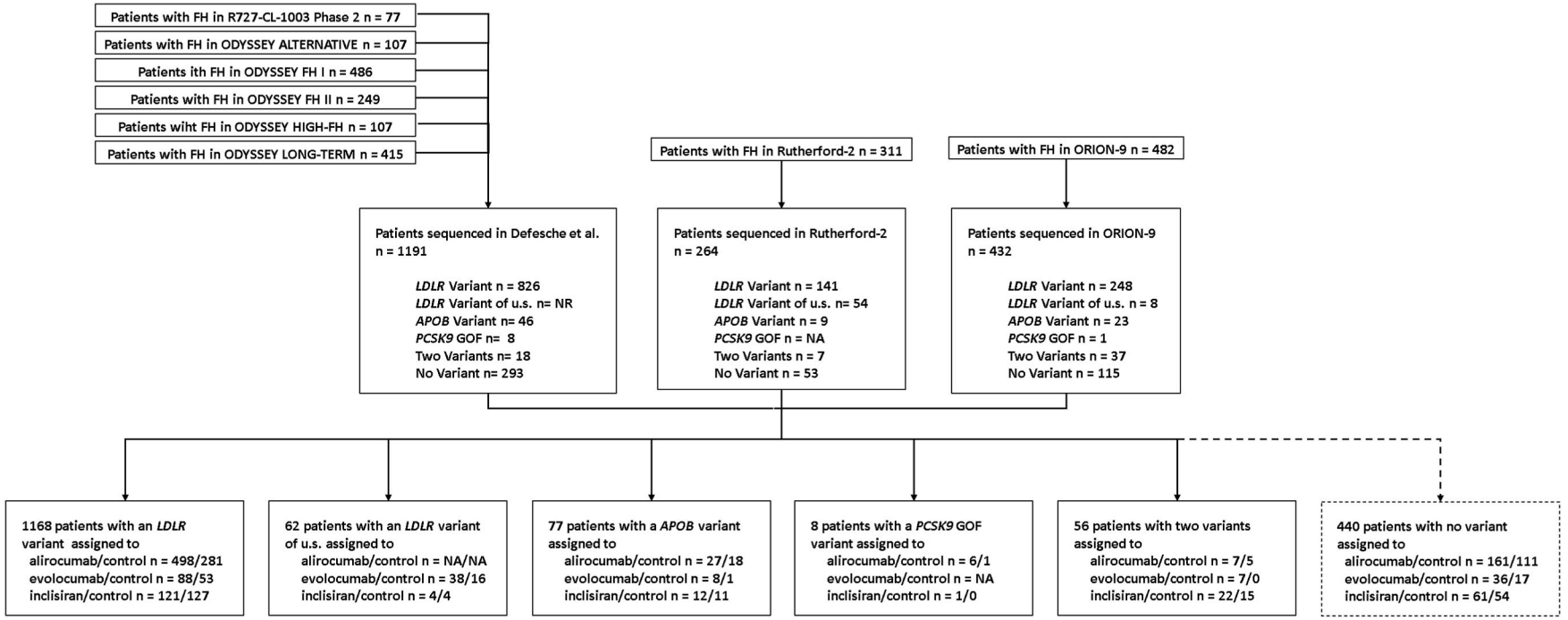

Fig. 2. Source and harmonisation of data used in the meta-analysis.
First row: number of patients identified with clinical HeFH among source studies (n = 2234). Second row: number of patients that underwent genetic sequencing (n = 1887). Last row: Number of patients with available data on percentage LDL-C reduction from baseline grouped by genotype (n = 1811). Data were included in the meta-analysis if the sample size was ≥2 in the treatment and the control group. Data on patients without a known variant (dashed lines) were only included in the comparison of LDL-C reduction between genotypes. APOB: apolipoprotein B, FH: familial hypercholesterolemia, LDLR: LDL receptor, NA: not available, NR: not reported, PCSK9 GOF: proprotein convertase subtilisin-like/kexin type 9 gain of function.

Table 1
Study and patient characteristics.

	Study characteristics						Participant baseline characteristics[b]									
Study	Year of publication	FH diagnostic criteria	Type and dose of treatment	Type of comparator	Treatment[a]	Comparator[a]	Age in years	Male	BMI kg/m^2	Statin use	Ezetimibe use	LDL-C mmol/L	Non-HDL-C mmol/L	ApoB mg/dl	Tri-glycerides mmol/L	Lp (a)
Defesche et al. [20]	2017	Genetic confirmation, or Simon Broom criteria (definite), or DLCN >8 points, or LDL-C > 5.0 mmol/l	Alirocumab 75 mg Q2W or 150 mg Q2W	Placebo or ezetimibe or low dose atorvastatin	758	433	53.3 (12.08)	638 (53.6)	29.0 (4.98)	1112 (93.4)	626 (52.6)	4.09 (1.56)	4.79 (1.73)	121.9 (35.2)	1.23 (1.02–1.44)	25.5 mg/dL (21.56–29.35)
RUTHERFORD-2 [9]	2015	Simon Broom criteria (definite, possible)	Evolocumab 140 mg Q2W or 420 mg QM	Placebo	177	87	51.4 (12.74)	153 (58.0)	27.9 (4.7)	264 (100)	164 (62.1)	4.02 (1.21)	4.67 (1.33)	114.6 (27.9)	1.25 (1.14–1.37)	65.0 nmol/L (46–89)[c]
ORION-9 [10]	2020	Genetic confirmation, or Simon Broom criteria (definite, possible)	Inclisiran sodium 300 mg	Placebo	221	211	54.7 (12.19)	210 (48.6)	29.0 (5.40)	393 (91.0)	225 (52.1)	3.98 (1.37)	4.69 (1.55)	124.7 (34.4)	1.35 (0.95, 1.96)	54.0 nmol/L (21,180) ‡

[a] Number of sequenced participants per study arm.
[b] Age, BMI, LDL-C, non-HDL-C, and ApoB are shown as mean (standard deviation). Triglycerides and Lp(a) are shown as median (95% confidence interval for Defesche et al. and RUTHERFORD-2, and IQR for ORION-9). Sex, statin, and ezetimibe use are shown as n (%).
[c] To approximately convert to mg/dL, divide by 2.4 ApoB: apolipoprotein B100, BMI: body mass index, DLCN: Dutch Lipid Clinic Network criteria, LDL-C: low-density lipoprotein cholesterol, Lp(a): Lipoprotein(a), non-HDL-C: non-high-density lipoprotein cholesterol, Q2W: every 2 weeks, QM: once monthly.

3.2. Patient characteristics

Baseline characteristics are shown in Table 1. The mean age was 53 years and similar among the trials. The percentage of men ranged from 48.6% to 58.0%. Baseline LDL-C concentrations ranged from 3.98 mmol/l (SD 1.37) to 4.09 mmol/L (SD 1.56), despite maximally tolerated statin therapy with or without ezetimibe. All but one study used placebo as the comparator; the ODYSSEY-ALTERNATIVE trial (included in the pooled analysis of alirocumab) compared alirocumab with ezetimibe in 37 participants and with atorvastatin 20 mg in 19 participants.

3.3. Comparison of LDL-C reductions within the class of mAbs

The effects of LDL-C-lowering by mAb type and dose across genotypes for patients with an identified FH-causing variant (N = 1031) are shown in Fig. 3. Within the evolocumab group, the treatment effects were consistent between doses (140 mg bi-weekly or 420 mg monthly) and across genotypes, with an overall mean LDL-C reduction of 59.5% (95%CI: 65.0–54.0) compared to placebo. In the alirocumab group, the effect of the 75 mg bi-weekly dose was consistent across genotypes, but there was significant heterogeneity among those treated with the 150 mg bi-weekly dose, with the latter accounting for the overall heterogeneity observed among the alirocumab treated group ($Q_M = 176.55$, df = 5, $p < 0.0001$, $I^2 = 96.0\%$). In part, this was related to more modest LDL-C reductions with alirocumab 150 mg bi-weekly among those with two variants. Residual heterogeneity assessments suggest potential heterogeneity unexplained by the moderators assessed (Supplementary Table 7).

When different alirocumab dosing regimens were compared with evolocumab, there was no significant heterogeneity between alirocumab 150 mg bi-weekly and evolocumab ($Q_M = 3.15$, $p = 0.08$), but the LDL-C reduction with alirocumab 75 mg bi-weekly was ~10% lower (47.8%, 95%CI 51.0–44.6) compared to evolocumab ($Q_M = 13.0$, $p < 0.001$). Thus, among individuals treated with mAbs, the overall reduction in LDL-C in genetically confirmed FH was 50.3% (95%CI 59.3–41.4) with no statistically significant heterogeneity overall between alirocumab and evolocumab ($Q_M = 3.5407$, $p = 0.06$).

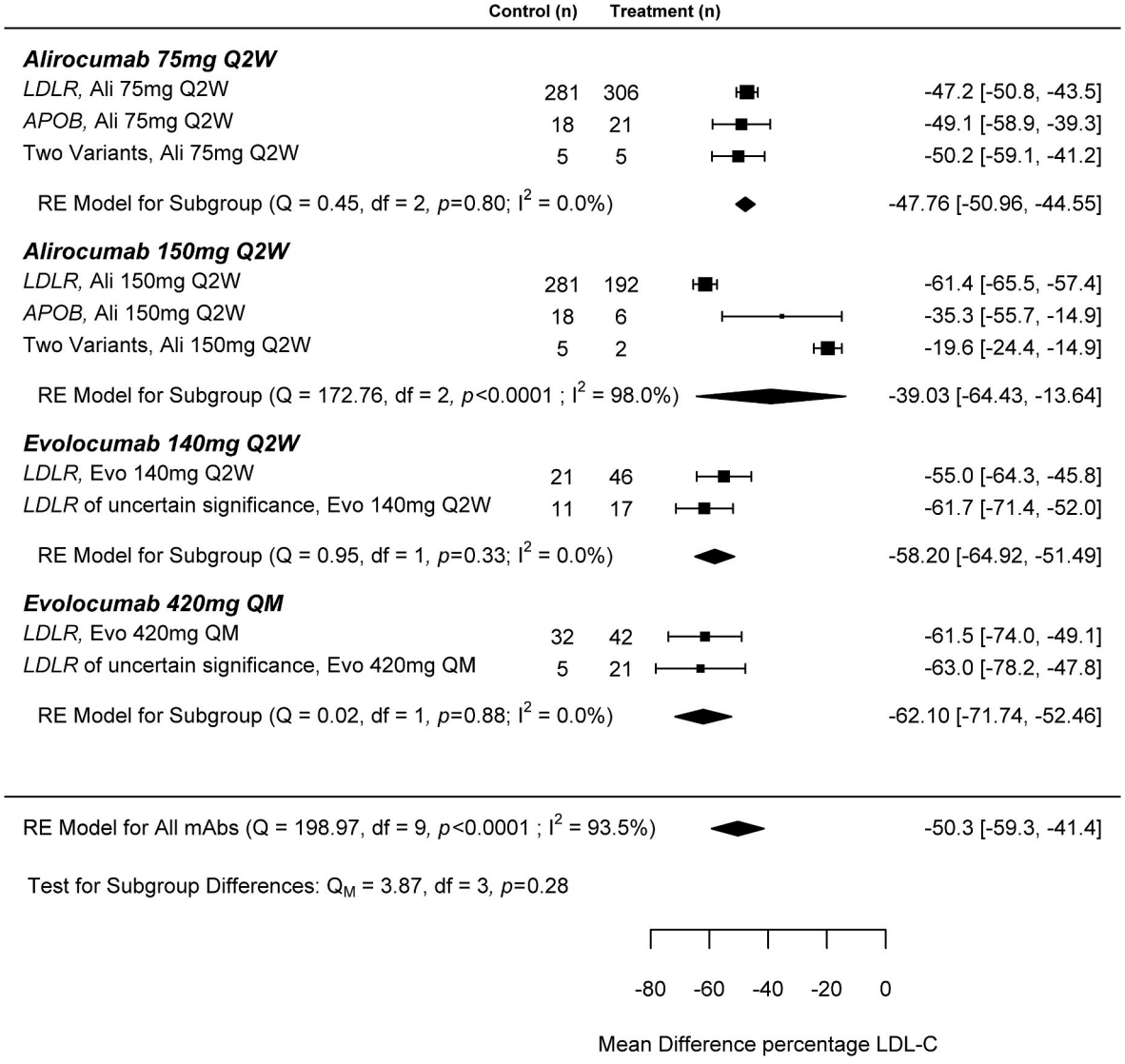

Fig. 3. Reductions in LDL-cholesterol with PCSK9-targeting monoclonal antibodies in patients with genetically confirmed FH (N = 1031), stratified by type of monoclonal antibody and dose.
Bars represent 95% confidence intervals. Patients with no known genetic variant had been excluded from this analysis. Ali: alirocumab, APOB: apolipoprotein B variant, df: degrees of freedom, Evo: evolocumab, LDL-C: low-density lipoprotein cholesterol, LDLR: low-density lipoprotein receptor, mAbs: monoclonal antibodies, Q2W: every 2 weeks, QM: monthly, RE: random effects.

3.4. Comparison of LDL-C reductions between drugs directed against PCSK9 and approaches targeting PCSK9

In patients with identified variants in FH-causing genes (N = 1347), the overall reduction in LDL-C with inclisiran was 46.1% (95%CI 48.4-43.9) without evidence of significant modification of treatment effect across genotypes. A comparison of the efficacy of evolocumab, alirocumab and inclisiran (Fig. 4A) did not show statistically significant variation in the treatment effect between the three therapies ($Q_M = 4.58$, $p = 0.10$). Comparison of the mean effect-size between all mAbs as a class *vs* siRNA (Fig. 4B) did not show significant differences between the classes ($Q_M = 0.32$, $p = 0.57$). After excluding the less potent alirocumab 75 mg bi-weekly regimen from the mAb group, the summary estimate of the mAb class increased slightly to 51.1% LDL-C reduction (95%CI 64.2-38.1) but wasstatistically similar to siRNA ($Q_M = 0.31$, $p = 0.58$). Assessments of heterogeneity among the subgroup of patients with an LDL-receptor variant, which represent the majority of patients with monogenic FH, were consistent with the overall findings (Supplementary Table 7).

3.5. Comparison of LDL-C reductions between genotypes

Pooled LDL-C levels at baseline across the three therapies for each genotype are shown in Table 2 for all patients expressing a HeFH phenotype (N = 1811). LDL-C levels varied by genotype ($Q_M = 16.2$, df = 5, $p = 0.01$), but were generally constant within each genotype across the three therapies, except for the subgroup of patients exhibiting two genetic variants ($Q = 7.67$, df = 2, $p = 0.02$, $I^2 = 71.3\%$). LDL-C reduction through therapies directed against PCSK9 per se appeared generally consistent between the different genetic variants ($Q_M = 8.26$, df = 4, $p = 0.08$) (Fig. 5). Sensitivity analyses excluding patients with two genetic variants, thus assessing PCSK9-targeting in patients with a single variant, attenuated measures of heterogeneity ($Q_M = 3.1$, df = 3, $p = 0.37$).

The small sample size and the uneven distribution between treatment groups of patients with a PCSK9 GOF variant (Fig. 2) did not allow inclusion in our model. In 5 patients treated with 75 mg alirocumab bi-weekly and 1 patient treated with 150 mg alirocumab bi-weekly, LDL-C was reduced by 53.3% and 93.4%, respectively, compared to placebo (20). For the single patient treated with inclisiran the LDL-C reduction compared to placebo was 89.7% (10).

4. Discussion

The findings of the present study suggest that similar reductions in LDL-C can be achieved through the two major classes of medications targeting PCSK9 in patients with FH. Overall, these therapies, when added to maximally tolerated statins, result in reductions in LDL-C by around one half in FH patients. Furthermore, among the different types of genetic variants which result in a HeFH phenotype, there is an absence of statistical heterogeneity on LDL-C reduction with therapies directed against PCSK9.

Trials of PCSK9-targeting have used different study designs. For instance, evolocumab trials tested the efficacy of one or two doses against placebo. Trials of alirocumab included treat-to-target approaches meaning patients were randomised to two alirocumab doses for 12 weeks and some up-titrated from lower doses if the pre-specified treatment LDL-C goal was not achieved at week 12. Trials of inclisiran were of longer duration than mAb trials. Therefore, our meta-analysis provides an opportunity to compare different types of PCSK9-targeting therapies and doses. Differences within the class of mAbs were statistically significant when we compared evolocumab with the lower dose of alirocumab, but not for the comparison of evolocumab to the higher dose of alirocumab. This finding is in line with earlier studies indicating a lower efficacy of alirocumab 75 mg bi-weekly compared to 150 mg bi-weekly, with a 15% difference in percentage LDL-C reduction [22]. For evolocumab, similar efficacy of the two dosing regimens (140 mg bi-weekly or 420 mg monthly) on LDL-C reduction has been demonstrated in the RUTHERFORD-2 trial [9]. That said, there was wide variation in the treatment effect among the patients on higher doses of alirocumab, largely due to an attenuated response among those with two variants.

To date, most studies assessing treatments in patients with HeFH have included individuals based on a clinical rather than a molecular diagnosis. However, among patients with a clinical diagnosis of FH, 20–70% of patients may have a polygenic rather than a monogenic basis [13,23,24]. As the latter are at even higher risk of CVD despite similar LDL-C levels [23], these patients may potentially warrant a different clinical approach, with earlier and more intensive add-on therapies to statins [12]. The present study, therefore, reliably quantifies the effects of add-on therapies directed against PCSK9 in those with a confirmed molecular diagnosis rather than a clinical phenotype alone.

A trend for effect modulation by genotype with PCSK9-targeting was observed in our study, which was not statistically significant. This appeared in part to be attributed to the subgroup of patients with two identified variants. This group exhibited the highest variance in the treatment effect ranging from 17.8% to 55.2%. This may be partly explained by the smaller sample size, but potentially also by the broad spectrum of genotypes included in this group, ranging from compound heterozygotes (two different alleles of the same gene are affected), double heterozygotes (two variants in different genes, e.g. *LDLR* and *APOB*) to homozygous patients (two identical variants in the same gene).

A wide range of phenotypes have been described for those patients carrying two variants, including different treatment responses to lipid-lowering therapies that enhance LDL-C clearance mainly by up-regulation of hepatic LDL-receptor [11]. For instance, in homozygous patients where both alleles are affected by a null mutation, no or very little response to treatment targeting PCSK9 is observed (25) as residual LDL-receptor activity is severely impaired or absent. By comparison, HeFH patients without an *LDLR* negative variant tend to respond well to therapies directed against PCSK9, because upregulation of normal LDL-receptors by overexpression of the healthy allele may compensate for the more dysfunctional allele. Of note, differences in treatment response according to the *LDLR* variant have been observed with statins [26,27]. This is consistent with our observations among the subgroup of *LDLR* variants in the present analysis. It was not possible to harmonise the effects of all therapies targeting PCSK9 further by different types of *LDLR* variants as the reporting on these variants between studies varied and would not yield meaningful results.

The present study may have several implications for clinical practice. The vast majority of cases of HeFH result from variants in the *LDLR* (>90% of cases), followed by *APOB* defective variants (~5% of cases) and *PCSK9* GOF variants (~1% of cases) [28]. However, genotyping is performed globally in <5% of potential cases, with most cases, reliant on a clinical diagnosis [1]. The consistent LDL-C-lowering effect from therapies targeting PCSK9 was observed in those, both with and without, a known FH causing variant. This provides reassurance about the utility of targeting PCSK9 as a therapeutic approach irrespective of genetic background or diagnostic strategy when applying current clinical recommendations for add-on therapies in FH [7,29].

The present findings underscore the importance of the LDL-receptor to the removal of apoB containing LDL particles. Whilst statins increase the quantity of LDL-receptors through transcriptional regulation, they do not increase their survival time, which is reduced by PCSK9. Therefore, therapies which either bind circulating PCSK9 or which reduce circulating PCSK9 will increase the survival time of any LDL-receptors present, which in turn contributes to removing apoB containing particles. Therefore, combination therapy utilising approaches which increase both LDL-receptor production with approaches which increase LDL-receptor survival are ideal combinations for overcoming the inherited molecular defects causing FH. For instance, for those

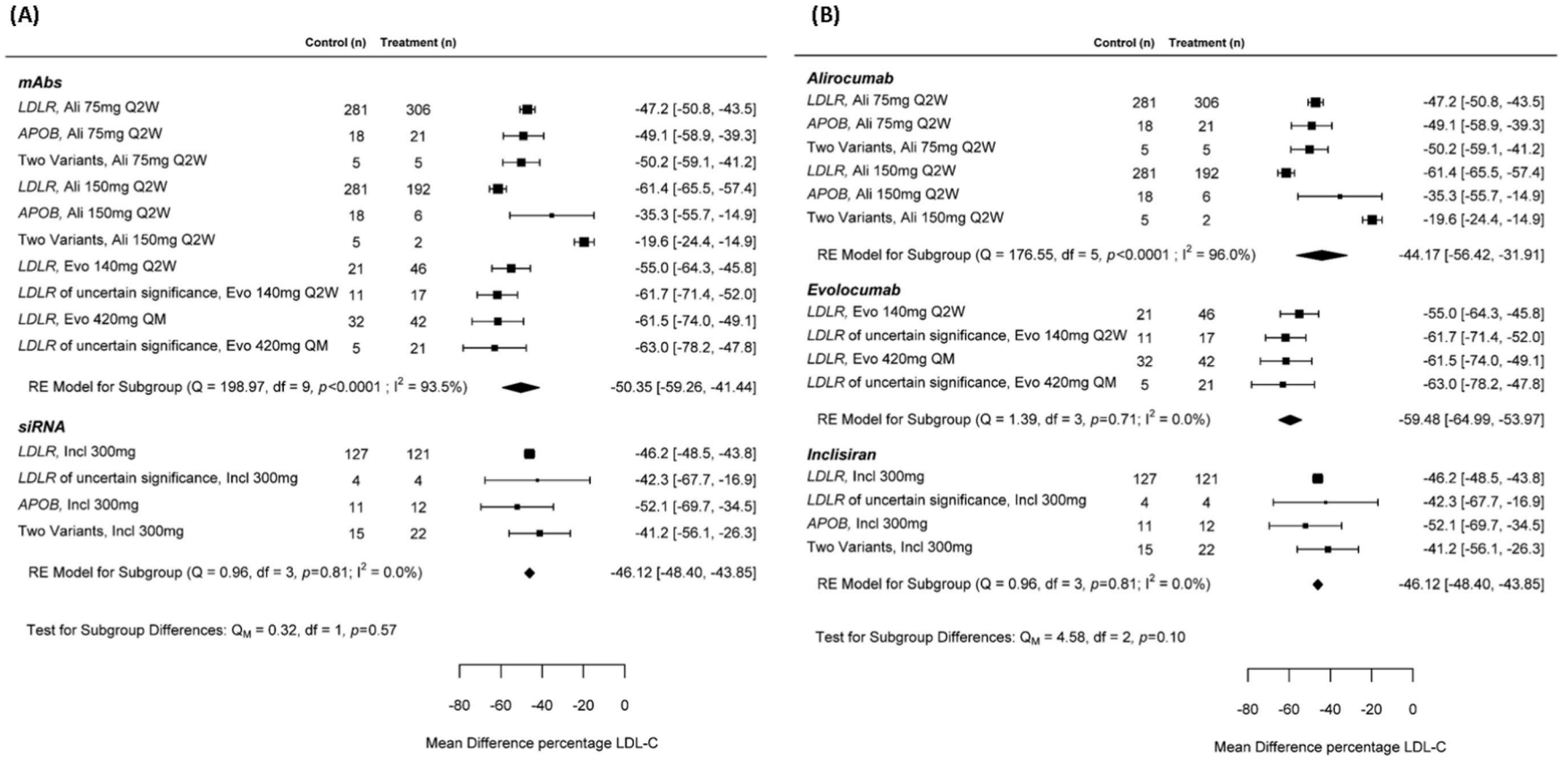

Fig. 4. Reductions in LDL-cholesterol stratified by drug directed against PCSK9 (A) and by mechanism targeting PCSK9 (B) in patients with genetically confirmed FH (N = 1347).
Bars represent 95% CIs. Patients with no known genetic variant had been excluded from this analysis. Incl: inclisiran, other abbreviations as in Fig. 3.

Table 2
Baseline LDL-C by genetic variant.

	LDLR	LDLR of uncertain significance	APOB	PCSK9 GOF	Two variants	No variant
Defesche et al. [18]						
n, (%)	826 (69.4)	NA	46 (3.9)	8 (0.7)	18 (1.5)	293 (24.6)
[a]LDL-C at baseline (95% CI)	4.1 (4.0–4.2)	NA	3.6 (3.2–3.9)	4.8 (3.2–6.5)	5.2 (4.3–6.1)	4.0 (3.8–4.2)
RUTHERFORD-2 [8]						
n, (%)	141 (53.4)	54 (20.5)	9 (3.4)	NA	7 (2.7)	53 (20.0)
[a]LDL-C at baseline (95% CI)	4.1 (3.9–4.3)	4.0 (3.7–4.3)	3.7 (3.0–4.4)	NA	5.3 (3.2–7.4)	3.7 (3.5–3.9)
ORION-9 [9]						
n, (%)	248 (57.4)	8 (1.9)	23 (5.3)	1 (0.2)	37 (8.6)	115 (26.6)
[a]LDL-C at baseline (95% CI)	4.1 (3.9–4.3)	3.5 (2.4–4.6)	3.9 (3.3–4.4)	3.8	3.9 (3.6–4.3)	3.7 (3.5–3.9)
Total						
N, (%)	1215 (64.4)	62 (3.3)	78 (4.1)	9 (0.5)	62 (3.3)	461 (24.4)
Weighted mean LDL-C (95% CI)[a]	4.1 (4.0–4.2)	4.0 (3.7–4.3)	3.7 (3.4–4.0)	4.8 (3.2–6.5)	4.6 (3.6–5.6)	3.8 (3.6–4.0)

[a] In mmol/L.95% CI: 95% confidence interval, APOB: apolipoprotein B100, LDL-C: low-density lipoprotein cholesterol, LDLR: low-density lipoprotein receptor, NA: not available, PCSK9 GOF: proprotein convertase subtilisin-like/kexin type 9 gain of function.

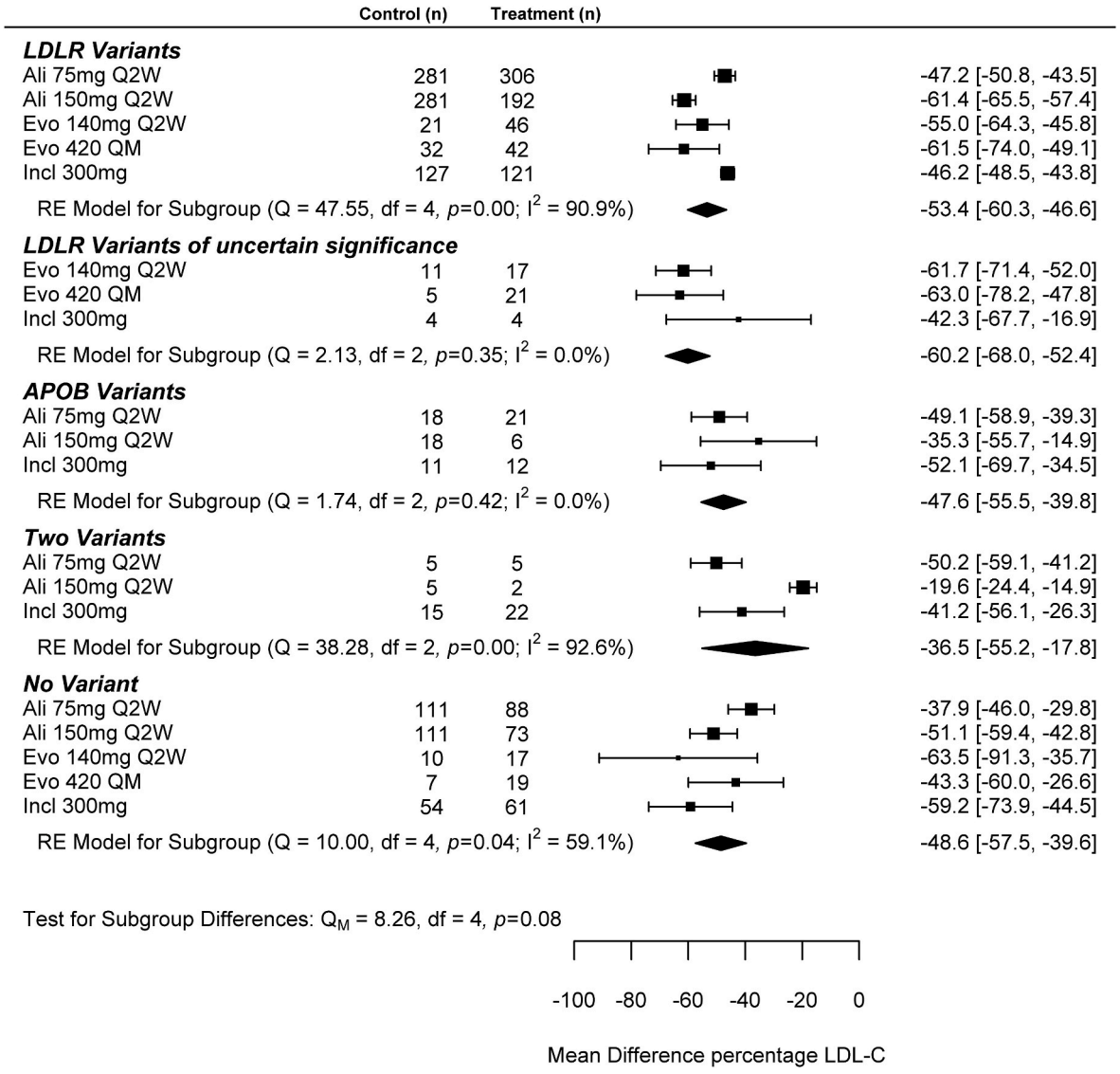

Fig. 5. Reductions in LDL cholesterol based on the type of genetic variants in patients with FH (N = 1811).
Bars represent 95% Confidence Intervals. Abbreviations as in Figs. 3 and 4.

inheriting a defect in one *LDLR* allele, the normal allele is able to increase LDL-receptor expression with statin treatment, and the durability of these receptors can be enhanced through any PCSK9-directed therapy producing significant reductions in LDL-C. Even when apoB is defective, increasing expression and durability of LDL-receptors though statins and PCSK9-directed therapies can produce meaningful reductions in LDL-C.

As well as improving early case detection, our observation of a consistent, meaningful LDL-C reduction irrespective of the approach to

target PCSK9 and genotype may allow clinicians to consider other factors like patient preference, adherence, costs and availability of specific treatments to guide choice, and improve cholesterol control for the estimated 30 million FH patients globally of whom less than 5% have been identified [2]. As effects were consistent by genotype, these data provide reassurance around potential efficacy where genetic testing is not available or accessible. Whilst the effect of targeting PCSK9 per se among those with two genetic variants was attenuated, these patients accounted for only 3% of the present study, and this is consistent with observed efficacy in homozygous FH [25,30].

4.1. Study limitations

The strengths and limitations of the present study merit consideration. Limitations include the relatively small sample size (despite pooling of data from 3 studies reporting on 8 RCTs) within the subgroups of certain genetic variants and uneven group sizes, which is dependent upon the prevalence of rarer variants such as *APOB, PCSK9* and two genetic variants and may underestimate true between-group differences. Furthermore, none of the evolocumab treated patients were screened for *PCSK9* GOF variants, and the uneven distribution of this variant between treatment groups did not allow inclusion in our model. Also, not all LDL-C reductions are placebo-corrected because results of 107 patients from ODYSSEY-Alternative that compared alirocumab to an active comparator were included in the pooled alirocumab data and thus included in our analysis as individual data without these patients could not be obtained. This potentially introduced additional heterogeneity within the alirocumab treatment group and underestimated its LDL-C-lowering efficacy. However, the pooled results reported by Defesche et al. ranging from 48 to 61% LDL-C reduction are in line with the results of placebo-controlled studies of alirocumab in FH populations (FH I 49%; FH II 49%; LONG TERM 61%; and HIGH FH 46%) [8.20]. Lastly, most of the included RCTs assessed the medication directed against PCSK9 as an adjunct therapy in patients with high LDL-C levels despite maximally tolerated statins with or without ezetimibe, thus patients with FH and genetic variants associated with lower LDL-C were not included. In addition, the response to treatments targeting PCSK9 as monotherapy (without background oral lipid-lowering) could not be assessed. However, the eligibility criteria of the present study closely reflect the current practice and approval pathways for reimbursement of medications directed against PCSK9, allowing inferences about clinical use. The strengths of this study include standardised analyses, the numbers of patients with FH allowing comparisons of different approaches to reduce LDL-C through PCSK9-targeting across genetic variants, including increasing the efficacy data among rare FH-causing genetic variants by accessing all currently available data.

4.2. Conclusions

In summary, both approaches directed against PCSK9 yield similar, substantial reductions in LDL-C among patients with FH and among the types of FH-causing genetic variants.

CRediT authorship contribution statement

Julia Brandts: Methodology, Writing – original draft, Writing – review & editing, Formal analysis, Validation, Visualization. **Kanika I. Dharmayat:** Methodology, Writing – original draft. **Antonio J. Vallejo-Vaz:** Methodology, Writing – original draft. **Mansour Taghavi Azar Sharabiani:** Methodology, Writing – review & editing, Formal analysis, Validation. **Rebecca Jones:** Methodology, Writing – review & editing. **John J.P. Kastelein:** Writing – original draft, Resources. **Frederick J. Raal:** Writing – original draft, Resources. **Kausik K. Ray:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

KKR reports personal fees for consultancy from AbbVie, Amgen, AstraZeneca, Sanofi, Regeneron, Merck Sharp & Dohme, Pfizer, Resverlogix, Akcea, Boehringer Ingelheim, Novo Nordisk, Takeda, Kowa, Algorithm, Cipla, Cerenis, Dr. Reddys, Lilly, Zuellig Pharma, Bayer, Daiichi Sankyo, The Medicines Company, and Esperion, as well as research grant support from Pfizer, Amgen, Sanofi, Regeneron, and Merck Sharp & Dohme. JB reports participation in research grants from AstraZeneca. AJVV reports honoraria for lectures from Amgen, Mylan, and Akcea; personal fees for consultancy from Bayer; and participation in research grants from Amgen, Sanofi, MSD, Pfizer, Regeneron and Daiichi Sankyo to Imperial College London/European Atherosclerosis Society; all outside the submitted work. JK reports personal fees for consultancy from AstraZeneca, CSL Behring, Daiichi-Sankyo, Esperion, Genentech, Menarini, Novartis, Novo-Nordisk, Pfizer, and Regeneron. FJR has received research grants, honoraria, or consulting fees for professional input and/or delivered lectures from Sanofi, Regeneron, Amgen, Novartis and The Medicines Company. The other authors report no conflicts.

Acknowledgements

This report is independent research supported by the National Institute for Health Research Applied Research Collaboration Northwest London (KD, KKR). The views expressed in this publication are those of the author(s) and not necessarily those of the National Institute for Health Research or the Department of Health and Social Care.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.03.042.

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