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ORIGINAL ARTICLE

LDL lowering effect of PCSK9 inhibition is reduced in women

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Aims Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of plasma low-density lipoprotein cholesterol (LDL-C) concentration, and its inhibition reduces the risk of atherosclerotic cardiovascular disease (ASCVD). We aimed to assess the sex-differential effect of either pharmacological or genetic inhibition of PCSK9 on LDL-C levels.

Methods and results We meta-analyzed six real-life studies (1216 men and 641 women) that investigated the effects of PCSK9 monoclonal antibodies (mAbs) on LDL-C reduction in men and women. Despite higher LDL-C levels in women at baseline [mean difference (MD) = 17.4 mg/dL, $P < 0.0001$, women = 175 mg/dL vs. men = 152 mg/dL], the LDL-C reduction under PCSK9 mAb treatment was significantly greater in men (MD = 7.6 mg/dL, 95% confidence interval: 2.7–12.4, $P = 0.002$) than in women.

We tested the sex-related association of the *loss-of-function* variant *PCSK9*-R46L with LDL-C plasma levels in 382 813 individuals (219 301 women and 163 512 men) free of lipid-lowering drugs from the UK Biobank general population cohort. The magnitude of LDL-C reduction was larger in men than in women (mean LDL-C difference: –35 mg/dL vs. –26 mg/dL, when comparing homozygous carriers with non-carriers in men and women, respectively). The relationship between *PCSK9*-R46L and LDL-C was significantly dependent on sex (*P* for interaction = 7.2e–04).

Conclusion These results demonstrate by complementary approaches that the decrease in LDL-C mediated by PCSK9 inhibition is slightly, but significantly, less marked in women than in men. These data reinforce the need for specific studies to develop sex-specific recommendations for the management of ASCVD in women.

Keywords PCSK9 inhibitors • Sex difference • LDL reduction • Genetics

Introduction

High concentrations of low-density lipoprotein cholesterol (LDL-C) are recognized as the main driving factors behind the development of atherosclerotic cardiovascular disease (ASCVD) and its major clinical consequences.[1] The proprotein convertase subtilisin/kexin type 9 (PCSK9) is a natural inhibitor of the LDL receptor (LDLR) and a key regulator of LDL-C plasma levels.[2] Briefly, after an intracellular autocleavage, the mature form of PCSK9 is secreted by the liver in the circulation, where it binds to the extracellular domain of the LDLR at the cell surface of hepatocytes, promoting its endocytosis and lysosomal degradation.

Several studies have suggested a potential sex difference in plasma PCSK9 concentrations. Plasma PCSK9 levels were found to be slightly higher in pre-menopausal women than in men.[3,4] Menopausal status alters plasma PCSK9 concentrations with post-menopausal women exhibiting higher values than pre-menopausal ones.[3] High endogenous estrogen treatment has been shown to decrease hepatic PCSK9 expression in rats[5] and plasma PCSK9 levels in women,[6] while the latter observation was not consistently retrieved across studies.[4] Interestingly, a recent study in a large European cohort demonstrated that the independent association between plasma PCSK9 and cholesterol concentrations is only observed in men.[7] Altogether, these data suggest a sex-specific effect on the regulation of LDL-C metabolism by PCSK9.

Nevertheless, PCSK9 inhibition with pharmacological agents [i.e. specific monoclonal antibodies (mAbs)] or 'natural' genetic factors decreases circulating lipid levels (mostly LDL-C) and reduces the risk of ASCVD in both sexes.[8,9]

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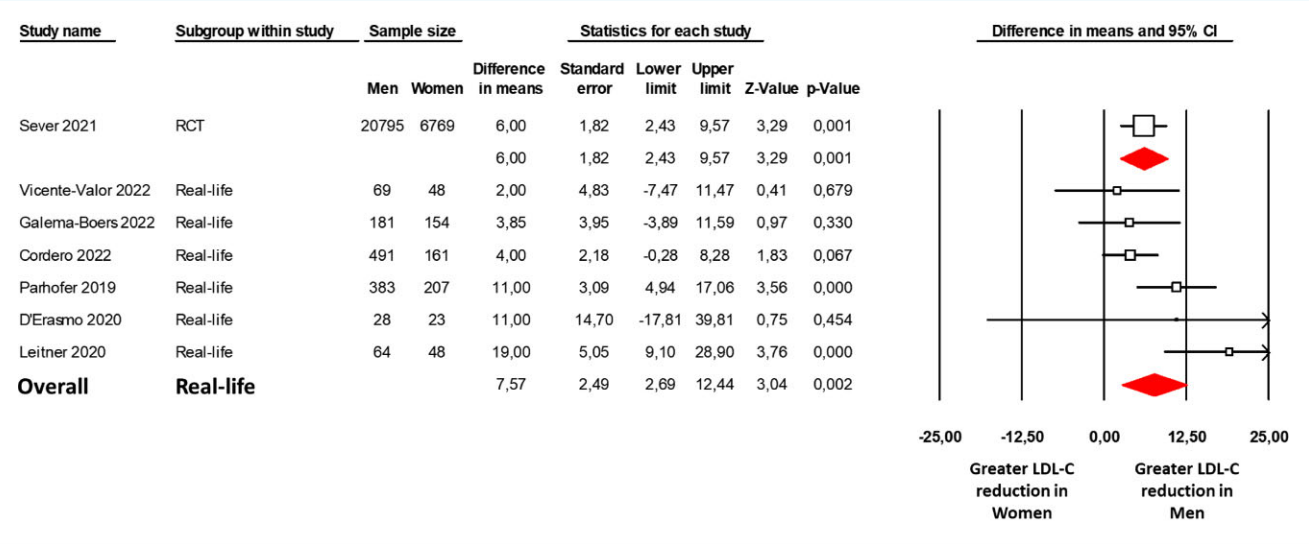

Figure 1 Sex differences in LDL-C reduction induced by PCSK9 monoclonal antibodies in a real-life setting. Forest plot of the difference in LDL-C reduction between men and women. LDL-C reduction was evaluated with a mean difference expressed in mg/dL. The diamond represents the estimated overall effect, while the squares represent each study with 95% CI. LDL-C, low-density lipoprotein cholesterol; RCT, randomized clinical trial; CI, confidence interval.

Notably, the FOURIER randomized clinical trial showed a beneficial effect of evolocumab in both sexes but surprisingly with a stronger LDL-C reduction in men than in women.[10] Also, recent data from a real-life patient registry (the LIPID-REAL Registry) have shown a significant sex-specific difference in the intensity of LDL-C reduction under PCSK9 mAbs.[11]

To get more insights into the sex-specific effects of PCSK9 inhibition on LDL-C reduction, we studied (i) the pharmacological inhibition of PCSK9 on LDL-C by PCSK9 mAbs in real-life studies and (ii) the natural genetic inhibition of *PCSK9* loss-of-function variant (R46L) in the general population.

Methods

Meta-analysis of real-life studies with PCSK9 mAbs

Data sources and searching

We searched for real-life observational studies published until August 2022, reporting data in men and women on LDL-C levels at baseline and during the follow-up. A systematic screening was performed in electronic databases (PubMed, Web of Science, and Scopus) according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[12] The following search string was applied: [(real-world) OR (real-life)] AND [(PCSK9) OR (proprotein convertase subtilisin/kexin type 9)] AND [(LDL-C) OR (low-density lipoprotein cholesterol)]. We retained only studies in which LDL-C concentrations were measured separately in men and women. We also manually consulted the reference lists of all included articles and collected data from published randomized clinical trials. Two authors (V.A.M. and P.P.) analyzed each article and extracted the data separately. In the case of disagreement, a third investigator was consulted (M.C.), and discrepancies were resolved by consensus.

Study selection, data extraction, and quality assessment

According to the established protocol, all studies reporting data about the association of the PCSK9 inhibitors' effect on LDL-C reduction in men and women were included, while case reports and studies on animal models were excluded. Data related to clinical and demographic characteristics of patients treated with PCSK9 mAbs were extracted in each study. Methodological quality evaluation for each study was performed according to the Newcastle–Ottawa Scale (Supplementary material online, *Table S1*).

Data synthesis and analysis

Statistical analyses of real-life studies with PCSK9 mAbs were performed using Comprehensive Meta-Analysis version 3 (Biostat). The LDL-C levels were collected from the above-reported studies, and the data were transformed into a format needed for analyses, e.g. from the reported median and 95% confidence interval (CI) to mean and standard deviation (SD).[13] The overall effect was tested using Z scores and significance was set at $P < 0.05$. Statistical heterogeneity was assessed with chi-square Cochran's Q test and with the I^2 statistic (I^2 values of 0% indicate no heterogeneity, 25% low, 25–50% moderate, and 50% or more high heterogeneity). To explore the effect of clinically relevant baseline covariates on the association between LDL-C reduction differences in men and women, we performed a study-level random effect model meta-regression analysis. Publication bias was assessed by Egger's test. In the case of significant publication bias, Duval and Tweedie's trim and fill method was used to estimate the adjusted effect size.[14]

Genetic analysis

Study population

The UK Biobank (UKBB) study is a population-based prospective cohort in the United Kingdom in which approximately 500 000 individuals aged between 40 and 69 years were recruited from 2006 through 2010.[15] All participants have given informed consent. The UKBB has ethical approval from the North West–Haydock Research Ethics Committee (REC reference: 16/NW/0274). The present research has been conducted using the UKBB resource under the application number 49823. The records of 77 individuals (last updated on 22 February 2022) who have withdrawn from UKBB were removed from the analyses. LDL-C was measured using an enzymatic selective protection method.

Genetic analysis, data processing, and plotting

Variant filtering (GRCh38) was performed using bgen and vcf files (format VCFv4.2) with plink2[16] and BCFtools (v1.14).[17] Genetic and phenotypic data were combined and processed using RStudio (v.2022.02.1). Plots were generated using RStudio using the ggplot2[18] R library, and statistical comparisons between groups were tested with Wilcoxon and two-way ANOVA tests using the R software.

Results

Effect of pharmacological PCSK9 inhibition on LDL-C reduction in men and women in a real-life setting

We identified six real-life studies,[11,19–23] including 1216 men and 641 women (overall mean age of 60 ± 4 years) with follow-ups ranging from 2 to 47 months (Supplementary material online, *Figure S1*). The presence of cardiovascular risk factors, such as hypertension, occurred in 32–83%, diabetes in 16–28%, previous stroke in 5–12%, and coronary artery disease (CAD) was present in 66–83% of patients (Supplementary material online, *Table S2*). All patients were under lipid-lowering therapy (LLT) with statin and/or ezetimibe. Total statin intolerance was observed from 18 to 50% of the studied subjects, while partial statin intolerance was presented from 10 to 27% of the patients.

The meta-analysis showed that despite higher LDL-C levels in women at baseline [mean difference (MD) = 17.4 mg/dL; $P < 0.0001$, women = 175 mg/dL vs. men = 152 mg/dL; Supplementary material online, *Figure S2* and *Table S3*], the LDL-C reduction was significantly greater in men (MD = 7.6 mg/dL, 95% CI: 2.7–12.4; $P = 0.002$; *Figure 1*) than in women under PCSK9 mAbs treatment. The heterogeneity among the studies was moderate ($I^2 = 54\%$; $P = 0.052$), and no differences between PCSK9 mAbs (evolocumab or alirocumab) or study type (prospective or retrospective) were observed. Moreover, the examination of the funnel plot showed no publication bias (Egger's test $P = 0.511$; Supplementary material online, *Figure S3*).

Effect of genetic PCSK9 inhibition on LDL-C reduction in men and women in the general population

To investigate a potential sex effect on the reduction of LDL-C induced by genetic inhibition of *PCSK9*, we made use of the loss-of-function variant *PCSK9*-R46L as a genetic instrument. We first selected participants from the UKBB for whom we had genotyping data available (n = 487 418) (Supplementary material online, *Figure S4*). We excluded individuals under lipid-lowering therapies (LLT, n = 71 805) and participants who retracted consent (n = 77). A total of 382 813 individuals (219 301 women and 163 512 men) with genetic information for *PCSK9*-R46L (rs11591147) and plasma LDL-C levels were included in the final analysis.

We compared non-carriers of *PCSK9*-R46L (0/0) with heterozygous carriers (0/1) and homozygous carriers (1/1) (*Figure 2*). We first observed that the magnitude of the LDL-C difference between homozygous R46L carriers, when compared with controls, is larger in men than in women {mean LDL-C reduction: 35 mg/dL [143(±30)–108(±27)] vs. 26 mg/dL [145(±33)–119(±32)], respectively ($P = 7.0E^{-18}$ vs. $4.6E^{-10}$, respectively) (Table *1* and *Figure 2*)}. This difference was even more significant when correcting LDL-C for age ($P = 1.2E^{-18}$ vs. $4.8E^{-11}$, respectively).

We further tested the interaction of *PCSK9*-R46L and sex using a two-way ANOVA. We found that the relationship between *PCSK9*-R46L and LDL-C concentrations was significantly associated with sex (P for interaction = 3.3e–3). This interaction was even more significant when adjusted for age (P for interaction = 7.2e–4).

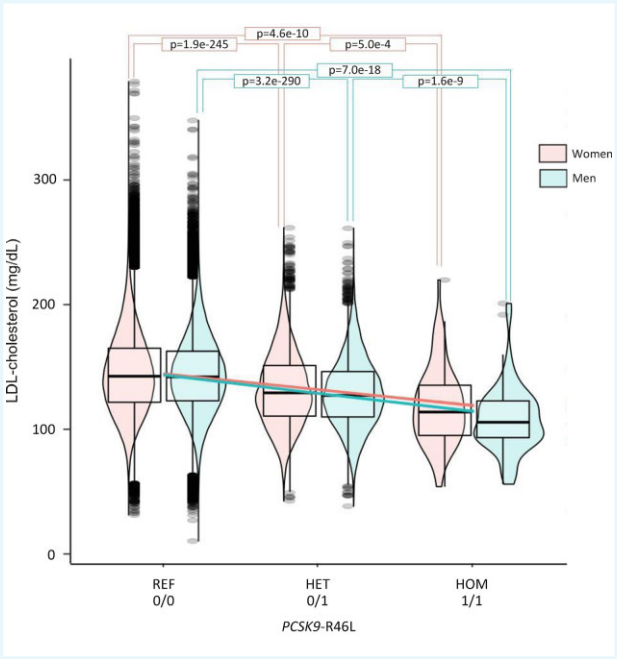

Figure 2 Plasma LDL-cholesterol levels according to the genetic inhibition of PCSK9 (as a function of *PCSK9*-R46L variant) in the UK Biobank. The plot depicts data from 382 813 individuals from the UK Biobank [219 301 women (pink symbols), 163 512 men (blue symbols)] free for lipid-lowering therapies. The *y*-axis showed LDL-C plasma levels (mmol/L) of individuals and the *x*-axis show groups of individuals based on their sex and genetic status. PCSK9, proprotein convertase subtilisin/kexin type 9; REF (0/0), non-carriers of the *PCSK9*-R46L variant; HET (0/1), heterozygous carriers of the *PCSK9*-R46L variant; HOM (1/1), homozygous carriers of the *PCSK9*-R46L variant; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation to the mean.

Discussion

The present work aimed to determine the existence of a sex-dependent regulation of LDL-C metabolism by PCSK9 in humans. As a first approach, a meta-analysis of real-life studies showed a slight, but significantly smaller decrease in LDL-C, under PCSK9 mAbs in women than in men. As a next step, in order to eliminate potential environmental or psychosocial confounding factors, we compared the intensity of LDL-C decrease in relation to genetic inhibition of PCSK9 in both sexes. By using the *PCSK9*-R46L loss-of-function variant as a tool to study the 'genetic' inhibition of PCSK9 in the UKBB population cohort, we confirmed that the decrease in LDL-C, associated with *PCSK9*-R46L, is less marked in women than in men with a significant interaction with sex. Taken together, these data support the hypothesis of differential regulation of LDL-C metabolism by PCSK9 according to sex, in agreement with some pre-clinical studies.[24,25]

The FOURIER and ODYSSEY clinical trials demonstrated the powerful effect of PCSK9 mAbs (evolocumab and alirocumab, respectively) on LDL-C reduction in both sexes.[10,26] However, in the former trial, a significantly greater LDL-C reduction was observed in men compared with women (−58% vs. −52%, respectively; $P < 0.001$) in the evolocumab-treated group. Similarly, in the pooled analysis of 10 ODYSSEY phase three clinical trials, women had

Table 1 Plasma LDL-cholesterol levels according to the PCSK9-R46L variant in the UKBB

	PCSK9-R46L	UKBB carriers (*n*)	LDL-C (mg/dL)(mean ± SD)
Women	REF (0/0)	211 742	145 (±33)
	HET (0/1)	7494	132 (±30)
	HOM (1/1)	65	119 (±32)
Men	REF (0/0)	157 610	143 (±30)
	HET (0/1)	5839	129 (±28)
	HOM (1/1)	63	108 (±27)

PCSK9, proprotein convertase subtilisin/kexin type 9; REF (0/0), non-carriers of the *PCSK9*-R46L variant; HET (0/1), heterozygous carriers of the *PCSK9*-R46L variant; HOM (1/1), homozygous carriers of the *PCSK9*-R46L variant; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation to the mean; UKBB, UK Biobank.

lower LDL-C reduction compared with men (−48.3% and −60.0%, respectively) under alirocumab. Of note, in the ODYSSEY trials, fewer women reached the optimal LDL-C goal <50 mg/dL than men (36.5% vs. 58.7%, respectively; $P < 0.0001$).[26] In addition, several real-life studies shed light on differences between men and women regarding the magnitude of LDL-C reduction in patients treated with PCSK9 inhibitors.[11,19]

Overall, our meta-analysis of real-life cohorts confirmed significant differences in LDL-C reduction between men and women under treatment with evolocumab or alirocumab. Furthermore, the results of two[11,23] out of six real-life studies confirmed the data on the number of patients that achieved the on-treatment target. In particular, only 25% of women and 50% of men reached the treatment goal (<55 mg/dL as per 2019 ESC/EAS guidelines).[27]

It is known that women are less likely to be prescribed LLT and are more susceptible to discontinuing treatment; thus, they could have greater difficulty reaching LDL-C targets.[28,29] The possible reasons for such non-adherence to LLT in women could lie in gender-specific factors, such as satisfaction, health beliefs, naïve illness theories, preferences for health care, and fear of side effects.[29] Nevertheless, recent results from ODYSSEY APPRISE demonstrated a high patient adherence to both background statin therapy and a new LLT administration (i.e. alirocumab) in real-life settings.[30] This may suggest that, in this particular study, physicians paid specific attention to sex-related adherence or that patients followed in clinical trials are more adherent than those in real-life cohorts.

Another potential explanation for the smaller decrease in LDL-C in response to PCSK9 mAbs in women could be related to differences in background LLT. Indeed, Cordero *et al.*[11] suggested that LDL-C reduction was lower in women than in men who were not taking high-dose statins but were on ezetimibe treatment. Interestingly, the same authors also highlighted the potential involvement of body weight in the sex difference in LDL-C reduction; in particular, women with body mass index (BMI) > 25 kg/m^2 showed significantly lower LDL-C reduction than men.

Based on the slightly lower efficacy of PCSK9 mAbs on LDL-C reduction, one could expect that women are at higher risk of cardiovascular outcomes than men. However, recent clinical trials, in which target LDL-C levels were only partially achieved for both sexes, revealed no sex-specific differences in cardiovascular risk reduction in secondary prevention patients.[10,26,31] As for the real-life context, no studies are available, and therefore we cannot completely rule out any sex differences in PCSK9 inhibition treatments on cardiovascular outcomes.

In addition to a lower efficacy of PCSK9 mAbs in women, our complementary genetic study strongly suggests the existence of sex-specific biological mechanisms of PCSK9, which cannot be only attributed to treatment adherence. Moreover, pre-clinical studies in mice support the hypothesis of a sex-specific effect of PCSK9 on LDL-C metabolism. Indeed, the regulation of hepatic LDLR expression in response to genetic deficiency (using PCSK9 knockout mouse models) or pharmacological inhibition (with PCSK9 mAbs) of PCSK9 differs between male and female mice.[24,25] As a possible molecular mechanism, a sex-specific shedding of excess hepatic LDLR in female mice following PCSK9 inhibition has recently been described.[24] Even though further investigations are needed to get more insights into these putative mechanisms in humans, these findings will have a direct impact on the treatment of hypercholesterolaemia in clinical practice, with a particular focus on the achievement of the LDL-C target in women.

Limitations

The present study has limitations that should be acknowledged. First, the difference in LDL-C reduction between sexes at the individual study level is variable, ranging from 2 to 19 mg/dL, with a moderate heterogeneity among the real-life studies considered. However, the genetic analysis confirmed the effect size (i.e. LDL-C reduction). Thus, we are confident that women taking mAbs have a decreased LDL-C reduction compared with men. Second, it was not possible to consider the rate of non-adherent patients in real-life studies. Indeed, as discussed, women are known to be less adherent to therapy than men, which could have influenced our meta-analysis's results. Nevertheless, the genetic data confirm the sex difference with no bias in adherence since *PCSK9*-R46L is independent of personal behaviour. Third, both the meta-analysis and the genetic data showed a small but significant difference in LDL-C reduction between men and women. Still, recent findings unveil sex-specific shedding of excess hepatic LDLR in an animal model, indicating a potentially relevant biological mechanism.[24] Fourth, some parameters, such as hypertension, BMI, diabetes, presence of CAD, or stroke, were collected in our study, but unfortunately, the number was not sufficient to perform a meta-regression analysis. Finally, we cannot exclude that other confounding factors, such as underlying LLT or other genetic variants, might have influenced our analysis.

Conclusions

High LDL-C is the main modifiable ASCVD risk factor, and PCSK9 inhibitors are the most potent drugs to achieve the LDL-C target. Based on complementary evidence, we showed here that PCSK9 inhibition is slightly less effective on LDL-C reduction in women than in men. These findings reinforce the need for dedicated studies to develop sex-specific recommendations for the management of ASCVD. In addition, the underlying molecular mechanism(s) sustaining this sex difference requires further studies.

Supplementary material

Supplementary material is available at *European Heart Journal—Cardiovascular Pharmacotherapy* online.

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Role of the funder/sponsor

The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Author contributions

Concept and design: V.A.M., B.C., and P.P.

Acquisition, analysis, or interpretation of data: V.A.M., A.R., M.C., C.L.M., R.C., B.C., and P.P.

Drafting of the manuscript: V.A.M., A.R., M.C., and P.P.

Critical revision of the manuscript for important intellectual content: C.L.M., R.C., and B.C.

Statistical analysis: A.R. and P.P.

Obtained funding: V.A.M., A.R., R.C., B.C., and P.P.

Supervision: B.C. and P.P.

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Data availability statement

The meta-analysis data underlying this article will be shared on reasonable request to the corresponding author.

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