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Atherosclerosis 309 (2020) 39–46

Contents lists available at ScienceDirect

Atherosclerosis

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

Sex-specific predictors of PCSK9 levels in a European population: The IMPROVE study

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HIGHLIGHTS

- PCSK9 plasma levels are higher in women than in men.
- The major determinants of PCSK9 levels are lipid-lowering therapies and latitude.
- Hypercholesterolemia and physical activity are independent predictors of PCSK9 only in men.

ARTICLE INFO

Keywords:
Atherosclerosis
Cardiovascular risk factors
Sex differences
PCSK9 predictors

ABSTRACT

Background and aims: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is one of the key regulators of low-density lipoprotein cholesterol plasma levels. Circulating PCSK9, which differs between genders, represents a valid pharmacological target for preventing cardiovascular (CV) events. We aimed to investigate sex-related associations between PCSK9 plasma levels and biochemical and anthropomorphic factors, and familial and personal morbidities, in a large European cohort (n = 3673) of men (47.9%) and women (52.1%).
Methods: Individuals (aged 54–79 years) free of CV diseases were enrolled in seven centers of five European countries: Finland, France, Italy, the Netherlands, and Sweden. PCSK9 plasma levels were measured by ELISA.
Results: PCSK9 was higher in women than in men. Multiple linear regression analysis showed that latitude, sex, and treatments with statins and fibrates were the strongest predictors of PCSK9 in the whole group. These variables, together with triglycerides and high-density lipoprotein cholesterol, were also associated with PCSK9 in men or women. Mean corpuscular hemoglobin concentration and pack-years were PCSK9 independent predictors in women, whereas hypercholesterolemia and physical activity were independent predictors in men. The associations between PCSK9 and latitude, uric acid, diabetes, hypercholesterolemia and physical activity were

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https://doi.org/10.1016/j.atherosclerosis.2020.07.014
Received 10 April 2020; Received in revised form 13 June 2020; Accepted 15 July 2020
Available online 30 July 2020

significantly different in men and women ($p_{interaction} < 0.05$ for all).
Conclusions: Besides confirming the association with lipids in the whole group, our study revealed previously unknown differences in PCSK9 predictors in men and women. These might be taken into account when defining individual risk for CV events and/or for refining PCSK9 lowering treatments.

1. Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a soluble member of the mammalian proprotein convertase family of secretory serine endoproteases, mainly synthesized and secreted by the liver with a lower contribution of the intestine, kidney, brain, and other tissues [1].

PCSK9 post-transcriptionally regulates the levels of low-density lipoprotein receptor (LDLR) by fostering its degradation [2]. By regulating hepatic LDLR, PCSK9 limits the LDL uptake maintaining high plasma levels of LDL-cholesterol (LDL-C) [3]. PCSK9 also mediates the inflammatory response [4] and is associated with the arterial plaques [5].

While PCSK9 represents the major regulator of LDL-C levels and, for this reason, an effective pharmacological target for preventing atherosclerotic cardiovascular (CV) diseases [6], the physiological predictors of its plasma concentrations still need to be elucidated. It is known that the synthesis of PCSK9 is dependent either on genetic polymorphisms [7], on transcriptional regulation by diverse nuclear factors [8], and on epigenetic mechanisms [9,10].

An important issue that needs further investigation is the role of sex. In the vast majority of CV diseases, there are differences between women and men in epidemiology, pathophysiology, clinical manifestations, effects of therapy and outcomes [11]. On this matter, many studies in humans described PCSK9 plasma levels to be significantly higher in females than in males [7,12]. In addition, differences have been observed in postmenopausal compared to premenopausal women, as well as in pregnant compared to non-pregnant women, thus allowing to hypothesize a role of sex hormones in PCSK9 synthesis and/or metabolism [7,13].

Considering the relevant role of PCSK9 on cholesterol homeostasis, on one hand, and the differences in CV risk factors [14] and in subclinical atherosclerosis [15] between women and men, on the other, the aim of this cross-sectional cohort study was to investigate, in a large European cohort, the variables with a sex-specific association with PCSK9 plasma levels.

2. Materials and methods

2.1. Study subjects

The database and the biobank of the IMPROVE study formed the basis of the present investigation. Such cohort consists of 3703 Caucasian patients (1774 men, 1929 women, aged 54–79 years, with ≥3 vascular risk factors) asymptomatic for cardio- and cerebrovascular diseases at the time of recruitment. Participants were enrolled in seven centers of five European countries: Finland (two centers), Sweden, the Netherlands, France, and Italy (two centers). Eligibility criteria for enrollment, objectives, methods, and patients' baseline characteristics have been previously reported [16].

2.2. Smoking habits

Subjects were carefully questioned about smoking habits, including duration of smoking calculated according to the year when smoking began (and ended, for former smokers) and average number of cigarettes smoked per day. Patients were classified according to their smoking status as never, former, and current smokers. Never smokers were defined as those who had never smoked in their lifetime. Current smokers were defined as those who were smokers at enrolment and who had smoked at least 10 cigarettes/day for at least thirty months [17]. Former smokers were defined as those who had a pack-years ≥ 0.6 but had not smoked for at least 1 year before their interview. Pack-year (packs per day multiplied by number of years of smoke) was calculated for both current and former smokers.

2.3. Ethical considerations

The study complies with the rules of Good Clinical Practice and with the ethical principles established in the Helsinki Declaration, and was approved by local Ethics Committees in each study center. All patients gave written informed consent.

2.4. Blood sampling and PCSK9 ELISA

Venous blood samples were obtained after an overnight fast. Blood was maintained at 4 °C until the plasma was separated, aliquoted, and stored at −80 °C. Plasma PCSK9 concentrations were measured by a commercial ELISA kit (R&D Systems, MN) able to recognize free and LDLR-bound PCSK9. Plasma samples were diluted 1:20, according to the manufacturer's instructions, and incubated onto a microplate precoated with a monoclonal antibody specific for human PCSK9. Sample concentrations were obtained by generating a four-parameter logistic curve-fit. The minimum detectable concentration was 0.219 ng/mL. Intra- and inter-assay CVs were 5.4 ± 1.2% and 4.8 ± 1.0%, respectively.

2.5. Statistical analysis

All quantitative variables were reported as mean ± SD. Variables with approximately log-normal distributions were presented as median and inter-quartile range (IQR), and log-transformed in parametric analyses. Although distribution was moderately skewed, PCSK9 levels were reported as mean ± SD, in order to allow comparisons with the existing literature. Categorical variables were reported as count and percentage. Trends across PCSK9 quintiles for continuous and categorical variables were assessed by ANCOVA and Mantel-Haenszel $\chi 2$-test, respectively. All variables associated with PCSK9 plasma levels in univariate analysis with $p < 0.05$ were screened as potential independent predictors and included in a multiple linear regression analysis with stepwise selection of variables. In this analysis, the p-value was corrected by the Bonferroni-Holm method, according to the number of variables entered. Relationships with a p-value lower than 0.05 but higher than the Holm-Bonferroni threshold were considered not significant but suggestive of potential association. Covariance analysis (General Linear Models) was used to evaluate interactions between sex and each determinant of PCSK9 plasma levels after adjusting for confounders. Interaction tests were deemed as significant if the *p*-value was below 0.05, taking into account the reduced statistical power of this test. The proportion of PCSK9 variation accounted for each variable was evaluated by using multivariable partial correlations. A sensitivity analysis, aimed at evaluating whether pharmacological treatments modify the effects of predictors, was performed by excluding subjects treated with statins, fibrates or with a combination of both drugs from the original multivariable analysis. Analyses were carried out with the SAS statistical package v. 9.4 (SAS Institute Inc., Cary, NC, USA) and all tests were 2-sided.

3. Results

Plasma PCSK9 was successfully measured in 3673 out of 3703 individuals included in the IMPROVE cohort [16]. PCSK9 measures showed a moderately right-skewed distribution with a mean concentration of 310.8 ± 108.6 ng/mL and a broad range, from 20.1 ng/mL to 1133.9 ng/mL.

The concentrations of PCSK9 were significantly higher ($p < 0.0001$) in women than in men (331 ± 105 ng/mL *vs* 290 ± 109 ng/mL) (Fig. 1) and were positively and significantly correlated with total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and TG in the whole group (Fig. 2).

Sex-specific baseline characteristics of this subsample, stratified by PCSK9 quintiles, are shown in Supplementary Table 1.

3.1. Multiple regression analysis

In a multivariable analysis including all variables significantly associated with PCSK9 in the univariate analysis (Supplementary Table 1), sex remained significantly associated with PCSK9 (Table 1). Moreover, considering the Holm-Bonferroni correction for multiple comparison, latitude, physical activity, and mean corpuscular haemoglobin concentration (MCHC) were negatively associated with PCSK9 in the entire cohort or in one of the two groups; pack-years, HDL-C, TG, personal history of hypercholesterolemia and treatments with statins and fibrates were instead positively associated. Suggestive associations with PCSK9 level were found for estimated glomerular filtration rate (GFR) (positive) and for family history of hypertension or diabetes (negative).

The effects on plasma PCSK9 levels of latitude, physical activity, personal history of diabetes or hypercholesterolemia were different in men and women ($p_{interaction} < 0.05$ for all). As an example of such interactions, Fig. 3 shows PCSK9 plasma levels according to latitude in men and women. The Beta values of changes in PCSK9 associated with a standard deviation increment of latitude were −0.334 in men and −0.224 in women ($p_{interaction} < 0.0001$). Personal history of diabetes and uric acid differently affected the plasma levels of PCSK9 in women and in men, even if their association was not significant in either group.

The partial R^2 obtained by multivariable partial correlations, which highlight the strength of the associations between PCSK9 and variables included in Table 1, are reported in Fig. 4. Notably, latitude and treatment with statins or fibrates were the strongest independent predictors of PCSK9, altogether accounting for 15% and 13% of PCSK9 variation in women and men, respectively.

Due to the strong associations of statins and fibrates with PCSK9 levels, we ran a sensitivity analysis by excluding subjects treated with statins (40.1% of the total population), fibrates or a combination of both drugs from the multivariable analysis (Supplementary Tables 2, 3, and 4, respectively). The results clearly show that the exclusion of treated subjects did not substantially modify the β coefficients of all variables.

4. Discussion

Our study revealed unknown sex-specific predictors of PCSK9 plasma levels. In particular, we found that MCHC and pack-years were independent predictors only in women. Conversely, unique independent predictors in men were personal history of hypercholesterolemia and physical activity. A personal history of diabetes and uric acid had non-significant associations with PCSK9 in either gender, but the significant interactions indicated that their relations with PCSK9 are potentially different in the two genders. Common PCSK9 predictors in both sexes were latitude, HDL-C, TG, and treatments with statins or fibrates. These variables, together with sex, were the most significant predictors of PCSK9 plasma levels in the whole cohort. Interestingly, PCSK9 decreased with latitude in both men and women, but with a significant difference between the two groups ($p_{interaction} < 0.0001$).

The present study is one of the largest analyses on PCSK9 plasma levels conducted in a European cohort (n = 3673). Two similarly large observational studies were performed by Lakoski et al. on the Dallas Heart Study (DHS) population (n = 3138) [7], and by Chernogubova et al. in 4 cohorts of healthy, middle-aged white (predominantly Swedish) subjects (n = 5722) [18]. Comparing our results with those of the DHS, a difference in the mean PCSK9 levels (*i.e.*, 310.8 ng/mL *vs* 517 ng/mL in the IMPROVE and DHS, respectively) clearly emerges. An analogous wide range variation in circulating PCSK9 levels (about 50-fold range) was found in the 4 cohorts included in the study by Chernogubova et al. [18] . The use of different ELISA assays may account for this, considering the difference in the maximum plasma concentration detected in the IMPROVE and DHS studies (1133.9 ng/mL *vs* 2988 ng/mL, respectively). A confirmation that between-study differences can be due to methodological issues (*e.g.*, assays used or other confounders) is provided by the study of Leander et al. [19] and by the study of Chernogubova et al. [18], who documented mean levels of PCSK9 (~100 ng/mL) much lower than those observed in the present study. Consistently, Ridker et al. in the Women's Health Study [20], and Gencer et al. in 2030 patients with acute coronary syndrome (ACS) [21] obtained comparable mean values of PCSK9 using the same commercially available kit as in our study (302.1 ng/mL and 323 ng/mL, respectively).

Regardless of the absolute plasma concentrations of PCSK9, our findings are in line with most previous reports showing that PCSK9 varies with sex, with the exception of two studies that find no gender difference in small cohorts [22,23]. Indeed, we found that PCSK9 plasma concentration was higher in women than in men, in both the univariate and the multivariable analysis, and this result is in agreement with studies in large, ethnically diverse, general populations [7,18,24], in a large population-based sample of children and adolescents [25], and in diverse cohorts of patients with stable coronary artery disease (CAD) or ACS [26,27].

The main hypothesis of this work is that sex might affect the relationship between PCSK9 and CV risk factors, of which a key factor is age. Plasma PCSK9 was found to be negatively correlated with age in adult and young males and positively in females [23,25]. In the present study, we observed a negative correlation between PCSK9 and age in both men and women at univariate analysis, which however lost its statistical significance when evaluated with a multivariable approach. Many studies assessed the relationship between PCSK9 levels and age and most of them found a positive correlation [28–30], one found no correlation even in univariate analysis [18], while only one found a negative correlation in human immunodeficiency virus-infected

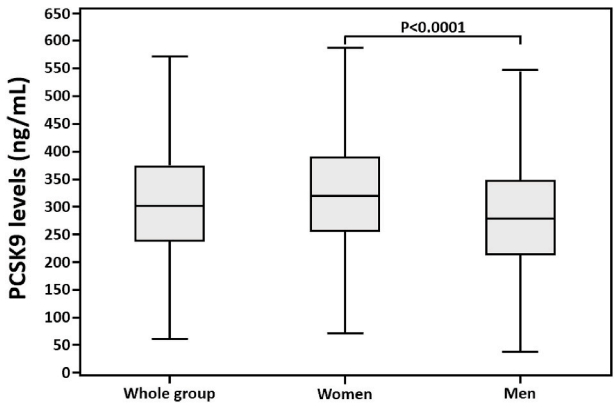

Fig. 1. Boxplot of plasma PCSK9 concentrations in the whole population, in women and in men.
Differences between men and women were significant also after adjustment for age, total cholesterol, and triglycerides ($p < 0.0001$). PCSK9, proprotein convertase subtilisin/kexin type 9.

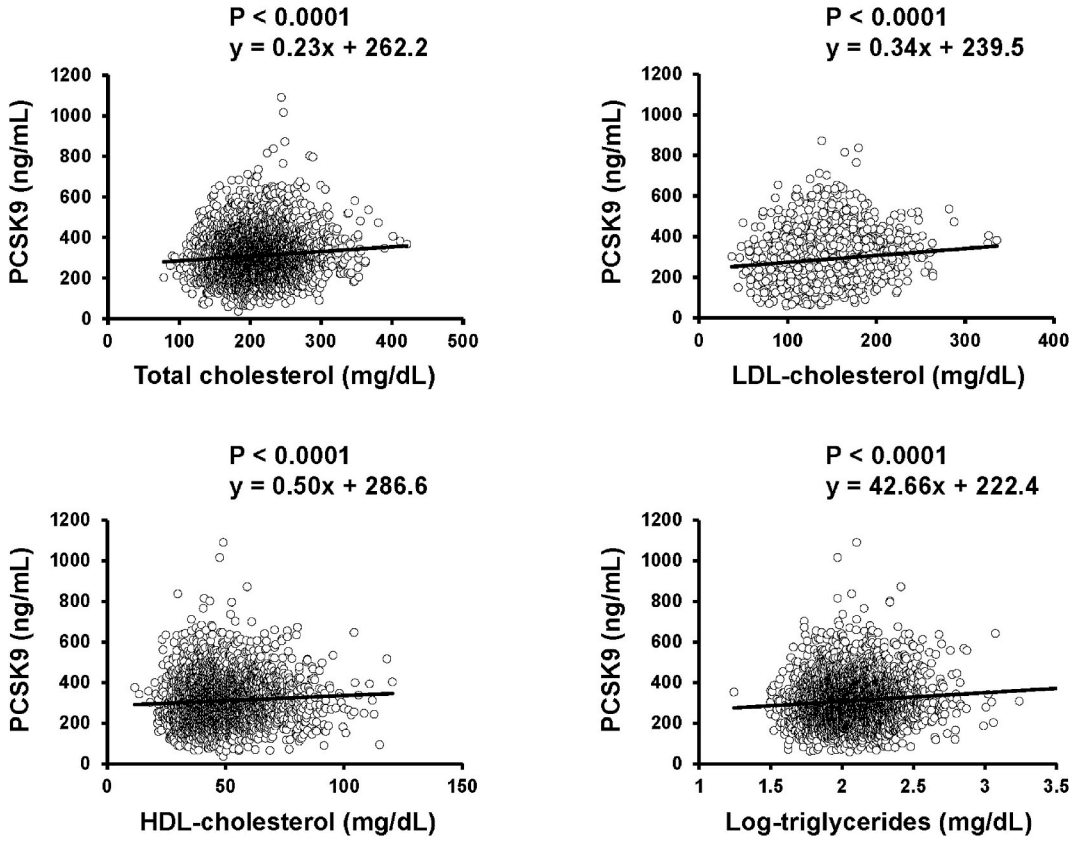

Fig. 2. Correlations between PCSK9 plasma levels and lipid parameters in the whole group.
(A) Total cholesterol; (B) LDL-cholesterol; (C) HDL-cholesterol; (D) triglycerides (log). For the analysis of LDL-cholesterol, statins users were excluded. PCSK9, proprotein convertase subtilisin/kexin type 9; LDL, low-density lipoproteins; HDL, high-density lipoproteins.

patients [31]. Among the studies that used a multivariate analytical approach, two confirmed the positive correlation with age independently of sex [30], whereas one found a positive correlation between PCSK9 levels and age only in women [29]. Overall, the PCSK9-age correlation appears to be scant and potentially influenced by other variables, including the oestrogen status of older vs younger women [29].

By computing interactions analyses, we found that the negative association between PCSK9 and personal history of diabetes is stronger in men than in women. Indeed, even if in our study the negative association between PCSK9 and the personal history of diabetes was not significant in women and only "suggestive" in men, the significant interaction observed indicates that the influence of sex is an additional layer of complexity in the interplay between PCSK9 and glucose metabolism. This finding is in line with the observations that other risk factors for type 2 diabetes mellitus (T2DM) have a sexual dimorphism, such as the hepatokine fetuin A and 25(OH) vitamin D3, which were related to T2DM onset in women but not in men [32,33]. The “suggestive” negative relationship with the personal history of diabetes observed in men is in line with large studies suggesting that loss-of-function PCSK9 genetic variants are associated with a higher risk of T2DM [34]. In addition, although baseline levels of PCSK9 did not predict the onset of diabetes in prediabetic patients, Ramin-Mangata et al. found a positive association with the change of fasting plasma glucose at a 4-year follow-up [35], suggesting that PCSK9 expression may be driven by insulin resistance, a common feature of the metabolic syndrome. Furthermore, the relationship between insulin resistance and PCSK9 levels may be affected by the dysbiosis of the gut microbiota which exacerbates insulin resistance, possibly leading to a rise in the expression of PCSK9 [36].

Another novel observation, stemming from computing the interaction effects, is that a personal history of hypercholesterolemia was positively associated with PCSK9 only in men. A positive association was expected, since it has been reported that LDL-C and total cholesterol directly correlate with PCSK9 and that PCSK9 levels were higher in patients with familial hypercholesterolemia compared to control subjects [37]. Also in our analysis, total cholesterol and HDL-C were significantly correlated with PCSK9, as was LDL-C when excluding statins-treated patients. The Spearman correlation coefficients between PCSK9 and LDL-C were very similar in the IMPROVE study ($\rho = 0.24$) and in the DHS ($\rho = 0.31$). Hypercholesterolemia was one of the most important predictors of PCSK9 levels regardless of sex, but at the same time the association with a personal history of hypercholesterolemia, defined as LDL-C greater than 160 mg/dL, was restricted to men only.

Further, we found a new negative predictor of PCSK9 levels, *i.e.* intensive physical activity in men. It is well known that supervised exercise therapy contributes to significantly lower LDL-C and total cholesterol levels [38], and thus the reduction of PCSK9 levels in response to high physical activity may contribute to the final lipid-lowering effect.

Latitude was one of the strongest independent predictors of PCSK9 variation both in women and men. We also observed a significant interaction with sex, being the decrease with increasing latitude greater in men than in women. A possible explanation of the negative association between PCSK9 and latitude, besides the well-known genetic gradient across Europe [39], could be the circadian variation of PCSK9. Indeed, during the day in healthy subjects PCSK9 levels have a nadir between 3 and 9 p.m. and a peak at 4:30 a.m. [40], and thus different light and dark cycles may affect PCSK9 synthesis.

Regardless of biochemical and anthropomorphic factors, pharmacological treatments with statins and fibrates were the strongest predictors of PCSK9 variation. This effect was not new since the induction

Table 1
Results of multivariable linear regression analysis in the whole group, in women and men.

	Whole sample (n = 3,546[a])		Women (n = 1877)		Men (n = 1669)		Interaction × gender
	Beta (95% CI)	*p*-value	Beta (95% CI)	*p* -value	Beta (95% CI)	*p* -value	$p_{interaction}$
Fibrates treatment	0.609 (0.495, 0.723)	< **0.0001**	0.682 (0.524, 0.840)	< **0.0001**	0.471 (0.302, 0.640)	< **0.0001**	0.81
Statins treatment	0.474 (0.404, 0.544)	< **0.0001**	0.494 (0.410, 0.579)	< **0.0001**	0.429 (0.312, 0.546)	< **0.0001**	0.71
P.H. of hypercholesterolemia	0.110 (0.032, 0.188)	**0.006**	0.047 (−0.055, 0.149)	0.36	0.179 (0.058, 0.299)	**0.004**	**0.01**
Log triglycerides	0.097 (0.064, 0.130)	< **0.0001**	0.110 (0.066, 0.155)	< **0.0001**	0.080 (0.031, 0.129)	**0.001**	0.27
HDL-cholesterol	0.077 (0.044, 0.111)	< **0.0001**	0.080 (0.040, 0.120)	< **0.0001**	0.079 (0.020, 0.138)	**0.008**	0.50
Log pack-years	0.056 (0.025, 0.086)	**0.0003**	0.068 (0.027, 0.108)	**0.001**	0.040 (−0.005, 0.086)	0.08	0.38
Log GFR	0.040 (0.010, 0.071)	0.01	0.039 (0.001, 0.076)	0.04	0.036 (−0.014, 0.086)	0.16	0.52
Uric acid	−0.026 (−0.058, 0.006)	0.11	−0.006 (−0.049, 0.037)	0.77	−0.064 (−0.113, −0.016)	0.01	**0.03**
Physical activity	−0.039 (−0.070, −0.009)	0.01	−0.012 (−0.051, 0.026)	0.53	−0.072 (−0.120, −0.023)	**0.004**	**0.005**
MCHC	−0.046 (−0.077, −0.015)	**0.004**	−0.058 (−0.099, −0.017)	**0.006**	−0.042 (−0.090, 0.006)	0.08	0.19
P.H. of diabetes	−0.064 (−0.136, 0.008)	0.08	−0.003 (−0.101, 0.096)	0.96	−0.109 (−0.214, −0.004)	0.04	**0.01**
F.H. of hypertension	−0.072 (−0.132, −0.012)	0.02	−0.053 (−0.131, 0.026)	0.19	−0.099 (−0.190, −0.007)	0.04	0.60
F.H. of diabetes	−0.073 (−0.134, −0.012)	0.02	−0.051 (−0.127, 0.026)	0.2	−0.104 (−0.201, −0.007)	0.04	0.06
Latitude (degrees)	−0.270 (−0.304, −0.235)	< **0.0001**	−0.224 (−0.268, −0.180)	< **0.0001**	−0.334 (−0.388, −0.280)	< **0.0001**	< **0.0001**
Male sex	−0.308 (−0.380, −0.237)	< **0.0001**	//	//	//	//	//
Oestrogen supplementation	//	//	−0.140 (−0.254, −0.026)	0.02	//	//	//

CI, confidence interval; P.H., personal history; HDL, high-density lipoproteins; GFR, glomerular filtration rate; MCHC, mean corpuscular hemoglobin concentration; F.H., family history.
Beta values indicate the change in Log proprotein convertase subtilisin/kexin type 9 (PCSK9) (expressed in standard deviations) associated with a standard deviation increment for all the continuous variables and for the presence *vs.* absence for dichotomic variables (therapies with fibrates or statins, P.H. of hypercholesterolemia and diabetes, F.H. of hypertension and diabetes, gender and oestrogen supplementation in women).
p-values in bold are significant after Holm-Bonferroni correction for multiple testing. The table includes all variables associated with PCSK9 with a *p*-value < 0.05 in the whole group, in women or in men. The following variables, all associated with *p*-values ≥ 0.05 in the whole group, in women and in men were tested but not included in the table: age, body mass index, waist, systolic and diastolic blood pressure, educational level (study years), total cholesterol, high-sensitivity C-reactive protein, blood glucose, creatinine, leucocytes, erythrocytes, P.H. of hypertriglyceridemia, of low HDL-cholesterol, and of hypertension.

[a] Because they had at least one missing covariate, 127 participants were excluded, bringing the sample size from 3.673 to 3.546.

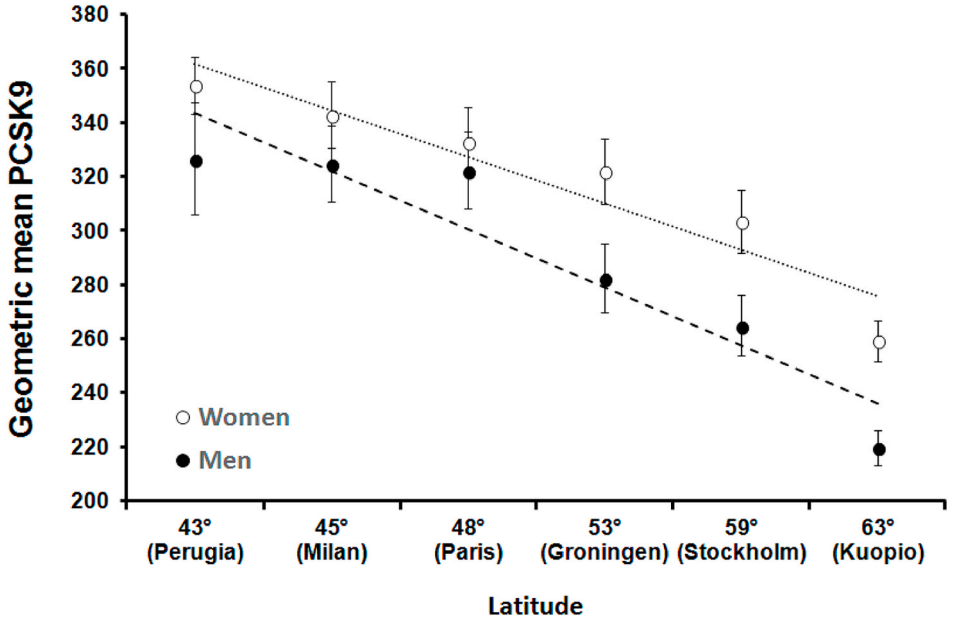

Fig. 3. PCSK9 plasma levels according to latitude.
Geometric means were adjusted for therapies with statins or fibrates, triglycerides (log), personal history of hypercholesterolemia, physical activity, high-density lipoproteins cholesterol, and uric acid. PCSK9, proprotein convertase subtilisin/kexin type 9.

of PCSK9 by statins and fibrates is well known [41]; we confirmed this evidence in both men and women without any sex interaction.

The relationship between PCSK9 and inflammation is still an object of intensive investigation. Indeed, while inflammation raises PCSK9 liver expression and PCSK9 is positively linked to tumor necrosis factor-α [42], in the present study we found no relationship between PCSK9 levels and high-sensitivity C-reactive protein, consistently with previous studies [20]. Our findings are also in line with the observation that PCSK9 antagonists do not exert a systemic anti-inflammatory activity in treated patients [43]. On the contrary, a more intriguing finding is the correlation between plasma PCSK9 and platelet count. We have previously shown that PCSK9 added to platelet-rich plasma samples significantly enhanced platelet aggregation induced by a subthreshold concentration of epinephrine [24]. The absence of PCSK9 reduced arterial thrombus formation and stability and platelet function in mice [24]. Taken together, these findings suggest a possible direct role of PCSK9 on platelets counts and aggregation, which are relevant factors not only in thrombus formation but also in the onset and progression of atherothrombotic disease [44].

A strong relationship between atherosclerosis and various factors linked to nutrition (obesity, blood sugar, saturated fat intake, microbiome, etc.) and smoking has been widely demonstrated [45]. On the other hand, several studies have related PCSK9 levels to obesity, insulin levels and certain dietary characteristics (high fat and protein intake, n-3 polyunsaturated fatty acids, etc.) [46] In our study, however, the univariate relationships with BMI, waist/hip ratio and blood glucose were not confirmed in the multivariate analysis, after correcting for gender, geographical origin and physical activity.

Biomarkers of inflammation are the most sensitive to diet [47] and smoking intensity [48] even at the lowest dose of exposure, and this

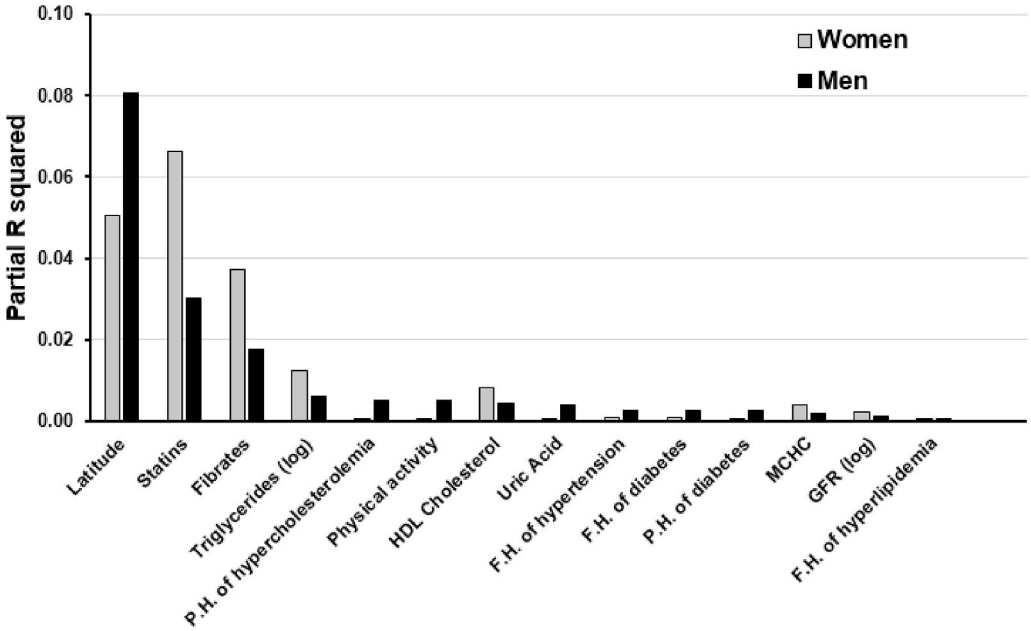

Fig. 4. Strength of the associations between PCSK9 and predictors included in Table 1.
Bars express the partial R^2 of each predictor, *i.e.*, the proportion of PCSK9 variation explained by each variable in the multivariable analysis, for both women and men. PCSK9, proprotein convertase subtilisin/kexin type 9; P.H., personal history; HDL, high-density lipoproteins; F.H., family history; MCHC, mean corpuscular hemoglobin concentration; GFR, glomerular filtration rate.

relationship persists regardless of sex and race/ethnicity [48]. In our cohort, in a univariate analysis, we observed a positive association between pack-years and PCSK9 levels, both in men and women (Supplementary Table 1). Such relationship remained highly significant in the multivariate analysis, indeed pack-years was selected among the independent predictors of serum PCSK9. This finding may indicate a direct role of inflammatory mediators, induced by tobacco smoke, such as xanthine oxidoreductase [49], on PCSK9 levels [50].

4.1. Strengths and limitations

The main strengths of the present study are related with the remarkably large sample size (unique in this kind of studies), with the multicenter design, encompassing several European countries with different social and environmental characteristics, with the centralized measurement of PCSK9 and of most of the clinical variables, and with the standard of the data analysis, based on multivariable models controlling for a wide array of factors. The study has also some potential limitations. The first arises from the peculiarity of the IMPROVE study population, including only subjects with multiple cardiovascular risk factors. The associations of PCSK9 levels with hypercholesterolemia, diabetes, treatment with statins and fibrates may be detectable only for this subpopulation. Therefore, the present results should be extrapolated to the general population with great caution. Second, the positive association detected between PCSK9 and treatment with statins and fibrates may have been amplified by reverse causality. In fact, subjects at higher CV risk are more likely to have higher PCSK9 levels and to receive lipid-lowering treatment. However, the impact of this bias on our results is probably not substantial, because on one side the analyses were thoroughly corrected for many CV risk factors, and on the other the association of PCSK9 with lipid-lowering drugs has been widely documented in the literature. Third, the potential bias generated by unknown, unmeasured confounders and effect modifiers could not be addressed, although we carefully selected potential predictors of PCSK9 by running complex multivariable models and sensitivity analyses. A fourth limitation is the absence of a genetic analysis for the identification of PCSK9 mutations, although loss of function and particularly gain of function mutations are relatively rare in the Caucasian population [51]. Thus, the exclusion of these subjects from the analysis is predicted to have a minimal and not significant impact on the statistical results. Finally, this was a retrospective cohort study with a single determination of PCSK9 plasma levels and, thus, possible fluctuations and different exposures to lipid-lowering drugs could not be considered.

The clinical implications of the current findings are uncertain. Although evidence from this study clearly shows that PCSK9 is associated with many independent markers and that some of these associations (*i.e.* personal history of hypercholesterolemia and diabetes, uric acid and physical activity) are stronger in men than in women, whether such relationships are causal is still unknown. Clarifying the link between multiple risk factors and the blood level of PCSK9 and whether such link is different in men and women may, however, be important to help in understanding the characteristics of patients who may best benefit of specific treatments, including those with PCSK9 inhibitors, in a first step towards a “precision medicine”.

4.2. Conclusions

In our study, conducted on 3673 patients, we found several independent predictors of PCSK9 levels. Although some associations found were confirmatory, the novel finding of the present retrospective analysis is the influence of sex on the relationships between PCSK9 plasma levels and several CV riskfactors and comorbidities. To our opinion, the observation that only in men PCSK9 levels are associated with pathological conditions, such as a personal history of diabetes or hypercholesterolemia, deserves a further investigation with prospective clinical studies.

Financial support

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The IMPROVE study was supported by the European Commission [Contract number: QLG1- CT- 2002- 00896] (to ET, DB, PG, SK, MP); Ministero della Salute Ricerca Corrente, Italy [RC2017 BIO30 ID:2631169; RC2018 MMP4.9 ID:2634520; RC2019 MPP 4D ID:2755475] (to DB); UKRI Innovation- HDR-UK Fellowship [MR/S003061/1] (to RJS).

Author contributions

NF, MR, DB contributed to the conception and design of the work and drafted the manuscript. DC, KS, AS, SK, PG, MP, RJS, ET, BG and GIC contributed to the interpretation of data. AB, NC, and FV contributed to the analysis of data. AM, BF, DS, AR, SC, LMG, and CM contributed to the acquisition of data. Except for NF, MR, and DB, all critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of competing interest

The authors declared that they do not have anything to disclose regarding conflicts of interest with respect to this manuscript.

Acknowledgements

The authors wish to express their deep and sincere appreciation to all members of the IMPROVE group for their time and their extraordinary commitment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2020.07.014.

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