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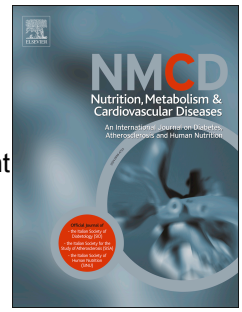

Plasma PCSK9 levels in children and adolescents: evidence from an Italian outpatient paediatric clinic cohort at cardio-metabolic risk.

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Title: Plasma PCSK9 levels in children and adolescents: evidence from an Italian outpatient paediatric clinic cohort at cardio-metabolic risk.

Short title: PCSK9 levels in youth

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Abstract

BACKGROUND AND AIM. Whether measuring circulating PCSK9 plasma levels in cardiovascular contexts may be of use in the clinical practice remains uncertain. The aim of the present study was to investigate PCSK9 levels in a cohort of children and adolescents referred to a paediatric outpatient clinic for cardio-metabolic risk assessment.

METHODS AND RESULTS. 332 children and adolescents were recruited between December 2014 and June 2024. Plasma PCSK9 enzyme-linked immunosorbent assay, glucose, insulin, fasting lipids, and uric acid were assessed, with calculation of non-HDL cholesterol, remnant cholesterol, and homeostasis model assessment index**.** The median age was 11.2 years, with 44.1% having initiated pubertal development, 81.9% were classified as excess weight and 16.3% as hypertensive. The median PCSK9 levels were 187.2 ng/mL. After adjustment for age and BMI z-score, the presence of lipid values above the clinical threshold was significantly associated with higher PCSK9 levels ($p<0.01$) : total cholesterol ≥200 mg/dL (+51.7 ng/mL), non-HDL-C ≥ 145 mg/dL (+43.6 ng/mL), remnant-cholesterol ≥30 mg/dL (+49.6 ng/mL), LDL-C ≥ 130 mg/dL (+34.1 ng/mL), and triglycerides above cut-offs (+49.2 ng/mL). Similarly, a positive parental history of dyslipidaemia was significantly associated with higher PCSK9 values (+39.9 ng/mL) ($p<0.001$).

CONCLUSIONS. In a cohort of children and adolescents at cardio-metabolic risk, circulating PCSK9 levels were significantly associated with atherogenic lipid fractions, including LDL-C, non-HDL-C, triglycerides, and remnant cholesterol. These findings suggest that PCSK9 may play a broader role in lipid metabolism beyond LDL-C regulation, particularly in relation to triglyceride-rich lipoproteins.

Keywords: adolescent; atherogenic lipoproteins; childhood; PCSK9; youth

Introduction

Proprotein convertase subtilisin/Kexin type 9 (PCSK9) is the ninth member of the proprotein convertase family [1]. Identified in 2003 [2], PCSK9 is predominantly secreted by the liver (≈80%) and plays a central role in regulating circulating low-density lipoprotein cholesterol (LDL-C) levels by promoting hepatic LDL receptor (LDLR) degradation, either through direct interaction with LDLR or via an intracellular Golgi–lysosome pathway [3]. PCSK9 was recognized as the third gene linked to familial autosomal dominant hypercholesterolemia, with an estimated prevalence of about 2.3% [4]. Subsequent large-scale epidemiological and genetic studies, including the identification of individuals carrying loss-of-function PCSK9 variants (e.g. R46L) associated with low LDL-C levels, have validated PCSK9 as a therapeutic target for LDL-C lowering [5, 6]. This evidence provided the rationale for evaluating pharmacological PCSK9 inhibition, which subsequently demonstrated clear cardiovascular benefits in both primary and secondary prevention settings [7-9]. Despite these findings, whether measuring circulating PCSK9 plasma levels in cardiovascular contexts may be of use in the clinical practice remains uncertain [10, 11]. A key limitation lies in the complexity of PCSK9 biology at the transcriptional and translational levels, resulting in concentrations that can vary by up to 100-fold. Data from the Dallas Heart Study (n= 3138) and the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study (n= 3673) indicate that age and sex were strong predictors of PCSK9 levels, yet information on prepuberal youth is scarce [12, 13]. In a cohort of 1739 French Canadian youth, age, sex and several metabolic markers (e.g. insulin) were major determinants of PCSK9 levels [14]. This is clinically relevant, since plasma lipid concentrations in infancy (2–12 months of age) correlate with levels in later childhood (1–5 years of age) and adolescence, and these track into adulthood over decades [15]. On this matter, obesity, hypertension, hyperlipidemia, and hyperglycemia at a young age can accelerate atherogenesis, so children and adolescents with obesity have a relative risk of roughly 2.5 for cardiovascular (CVD) mortality later in life, compared to peers with a normal weight [16]. Thus, the aim of the present study was to investigate PCSK9 levels in a cohort of children and adolescents with excess weight referred to a paediatric outpatient clinic for cardio-metabolic risk evaluation.

Patients & Methods

This is a cross-sectional cohort study. A cohort of children and adolescents was recruited between December 2014 and June 2024. Participants were referred by their primary care pediatricians to the Cardio-metabolic Risk Assessment Unit for Children at the Italian Auxological Institute (Milan, Italy). Referrals were based on evidence of excess weight and/or elevated blood pressure and/or altered lipid, glycemic and/or uric acid profiles. Exclusion criteria included impaired glucose tolerance, diabetes, any form of secondary

hypertension, and current treatment with antihypertensive and/or lipid-lowering drugs. The study protocol was approved by the local institutional ethics committee and adhered to the ethical guidelines of the 1975 Declaration of Helsinki (RICARPE study, 2015102002). Written informed consent was obtained from parents or legal guardians prior to enrolment.

Anthropometric and blood pressure measurement:

The height, weight and waist circumference of all participants were measured. Body mass index (BMI) was calculated by dividing weight (kg) by height (m^2). The waist-to-height ratio (WtHR) was calculated by dividing waist circumference by height and expressing the result as a percentage. The BMI z-score (i.e. the BMI value indexed by sex and age) was calculated by the equation (BMI value/M)^L-1)/(L*S) where L, M, S parameters specific for age and sex are available at this link [17]. Weight status was defined according to the International Obesity Task Force classification [18], distinguishing between normal weight (NW), overweight (OW) and obese (OB) categories. Pubertal status was assessed according to the Tanner classification based on a medical examination, and children were classified as prepubertal or pubertal [19, 20]; prepubertal status was defined as gonadal stage 1 in boys and breast stage 1 in girls. Blood pressure was measured using a validated oscillometric device (Omron 705IT, Omron Corporation, Japan) with an appropriately sized cuff. Measurements were obtained after the child had rested for at least five minutes in a seated position. Three blood pressure readings were recorded at intervals of a few minutes, and the average of the last two measurements was used for analysis. Percentiles and z-scores for systolic and diastolic blood pressure were calculated according to the nomograms of the National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Children and Adolescents, as recommended by the European Society of Hypertension [21]. Children were classified as follows: normotensive (when both systolic and diastolic blood pressure percentiles were $<90^{th}$); high-normal (when systolic and/or diastolic blood pressure percentiles were *≥90th but <95th*); hypertensive (when systolic and/or diastolic blood pressure percentiles were $\geq 95^{th}$).

Familiarity for dyslipidemia was defined as the presence of at least one parent with total cholesterol $\geq$ 200 mg/dL and/or LDL-C $\geq$ 130 mg/dL or taking lipid-lowering drugs. For 42 children data were missing, either because their parents did not provide documentation relating to their lipid profile or because the children were adopted.

Biochemical assessment

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, glucose, insulin, uric acid, and creatinine were evaluated under fasting conditions. Commercially available kits routinely used for clinical testing were employed for all analyses. Low-density lipoprotein cholesterol (LDL-C) was calculated using the

Friedewald formula [LDL-C = total cholesterol − HDL-C − (triglycerides/5)], with triglyceride concentrations expressed in mg/dL. Lipoprotein (a) levels were measured by immunoturbidimetry on a Roche c311 autoanalyzer. The homeostasis model assessment index (HOMA index) was calculated by dividing the product of serum insulin (µU/mL) and serum glucose (mmol/L) by 22.5 [22]. The estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula, based on serum creatinine and height measurements, with a constant k of 0.55 [23]. Dyslipidaemia was defined according to the reference values of the National Cholesterol Education Programme (NCEP) Expert Panel on Cholesterol Levels in Children [24] as the presence of one or more of the following criteria: LDL-C ≥130 mg/dL, HDL-C <40 mg/dL, triglycerides ≥100 mg/dL for children under 10 years of age or ≥130 mg/dL for children aged 10 years and older, non-HDL-C (total cholesterol minus HDL-C) ≥145 mg/dL, or remnant cholesterol (total cholesterol minus LDL-C minus HDL-C) ≥30 mg/dL. Insulin resistance was defined as HOMA index values exceeding the 90th percentile for sex and age in normal-weight children and adolescents [25]. Hyperuricemia was defined as uric acid values greater than the 90th percentile for sex and age [26]. Elevated lipoprotein(a) was defined as levels ≥105 nmol/L [27]. Plasma PCSK9 concentrations were measured using a commercial ELISA kit (R&D Systems). Samples were diluted 1:20 and incubated on microplates pre-coated with a monoclonal human PCSK9-specific antibody [28]. Sample concentrations were determined using a four-parameter logistic curve fit, with a minimum detectable PCSK9 concentration of 0.219 ng/mL. Intra- and inter-assay coefficients of variation were 3.2% and 5.1%, respectively.

Statistical analysis

Descriptive statistics were reported as the mean and standard deviation (SD) or as median, first and third quartiles (Q1, Q3), depending on the distribution of continuous variables. Categorical variables were summarized using absolute and relative frequencies. PCSK9 values were represented in a probability density histogram with an overlaid kernel density estimate. PCSK9 levels were compared across groups using the Mann-Whitney U test or the Kruskal-Wallis test, as appropriated, and represented in boxplots. The association between PCSK9 levels and anthropometric and clinical characteristics was assessed using unadjusted linear regression models. For selected continuous clinical parameters (i.e. total cholesterol, LDL-C, non-HDL-C, remnant cholesterol and triglycerides), the relationship was represented through scatterplots with the fitted linear regression line and its 95% confidence interval. To control for potential confounding, multivariable linear regression models were applied, adjusting each association for age and BMI z-score. All tests were 2-sided, with a significance level of 5%. Statistical analyses were performed with R 4.4.1 (http://www.R-project.org).

Results

Table 1 presents the anthropometric data, clinical characteristics, and blood chemistry results of the 332 children and adolescents enrolled in the study. The median age was 11.2 years (interquartile range, IQR: 9.0–12.7), with 45.8% of participants being female and 44.1% having initiated pubertal development. Overall, 81.9% were classified as excess weight (n=186, 56.0% with obesity) and more than 70% had a WtHr > 50%. Blood pressure above the 90th percentile was identified in 24.4% and 16.3% (n= 54) were hypertensive. The median PCSK9 level was 187.2 ng/mL (IQR: 141.5-236.3) and its distribution is shown in Figure 1. In 21.8% of the sample, total cholesterol was ≥ 200 mg/dL, while 16.8% had LDL-C ≥ 130 mg/dL. Elevated levels of non-HDL-C, remnant-cholesterol and triglycerides were observed in 21.9%, 9.8% and 17.7% of cases, respectively. High lipoprotein(a) levels (i.e. ≥ 105 nmol/L) were found in 15.8% of the children. Additionally, 41.4% had at least one parent with total cholesterol ≥ 200 mg/dL and/or LDL-C ≥ 130 mg/dL or taking lipid-lowering drugs.

Table 2 summarizes the distribution of PCSK9 across anthropometric and clinical variables. With regard to atherogenic lipids, the median PCSK9 level was significantly higher in children and adolescents whose atherogenic lipoprotein values were above the normal threshold: total cholesterol ≥ 200 mg/dL (218.9 vs. 178.3 ng/mL, p< 0.001), LDL-C ≥ 130 mg/dL (204.4 vs. 185.2 ng/mL, p= 0.028), non-HDL-C ≥ 145 mg/dL (223.5 vs 179.7 ng/mL, p<0.001) and triglycerides above age-specific cut-offs (216.6 vs. 184.4 ng/mL, p= 0.004). Additionally, as shown in Figure 2, children with a parental history of dyslipidemia had higher median PCSK9 level than their peers without such a history (206.1 ng/mL vs. 176.2 ng/mL, p< 0.001). The characteristics of the cohort by parental history of dyslipidaemia are reported in Supplemental Table 1. Relative to peers whose parents did not have dyslipidaemia, children and adolescents with a parental history exhibited lower BP and BMI z-scores, lower HOMA-index and serum uric acid concentrations, but a more atherogenic lipoprotein profile. Univariable analyses revealed a significant association between PCSK9 levels and total cholesterol, LDL-C, non-HDL-C, remnant-cholesterol and triglycerides (Table 3, unadjusted models, p < 0.01 for all variables). To strengthen this evidence, Figure 3 shows the scatterplots of PCSK9 levels plotted against total cholesterol (panel A, p < 0.001), LDL-C (panel B, p = 0.005), non-HDL-C (panel C, p < 0.001), remnant-cholesterol (panel D, p = 0.002) and triglycerides (panel E, p = 0.002) values, along with the fitted linear regression line.

These patterns mirror the results adjusted for age and BMI z-score, shown in Table 3 (adjusted model), where an increase of 10 mg/dL in atherogenic lipids corresponded to an approximate increase in PCSK9 of 5.4 ng/mL (95%CI 2.6 to 8.1) in the case of total cholesterol (p < 0.001), of 5.2 ng/mL (95%CI 2.4 to 8.0) in the case of non-HDL-C (p < 0.001), of 4.2 ng/mL (95%CI 1.1 to 7.3) in the case of LDL-C (p = 0.015), of 3.7 ng/mL (95%CI 1.6 to 5.7) in the case of triglycerides (p < 0.001), and 18.6 ng/mL (95%CI 8.4 to 28.8) in the case of remnant cholesterol (p = 0.002), underscoring a tight link between PCSK9 and atherogenic lipid fractions.

After adjusting for age and BMI z-score values, a trend to a correlation emerged also for continuous values of insulin and HOMA index (p= 0.052 and p= 0.058, respectively) (Table 3, multivariable model). Conversely, a weak inverse correlation was observed between PCSK9 levels and both uric acid values (p = 0.047) and age (p = 0.040) only in the unadjusted model (Table 3).

Additionally, the adjusted models showed that children with a positive family history of dyslipidemia had substantially higher PCSK9 values compared to those without (mean difference: +39.9 ng/mL; p < 0.001). Similarly, PCSK9 levels were significantly higher among participants whose lipid values were above the clinical threshold: total cholesterol ≥200 mg/dL (+51.7 ng/mL; p < 0.001), non-HDL-C ≥ 145 mg/dL (+43.6 ng/mL; p < 0.001), remnant-cholesterol ≥30 mg/dL (+49.6 ng/mL; p = 0.002), LDL-C ≥ 130 mg/dL (+34.1 ng/mL; p = 0.015), and triglycerides above cut-offs (+49.2 ng/mL; p < 0.001) (Table 3).

Discussion

The present study is among the few evaluating the relationship between PCSK9 levels and metabolic profile in children and adolescents at increased cardio-metabolic risk. Considering that replication is the backbone of science, our findings should be interpreted within this framework [14].

The prevalence of paediatric obesity has increased worldwide over the past five decades, and childhood represents a critical 'window of opportunity' for preventing atherosclerotic CVD [29]. Thus, assessing atherogenic risk early may help identify youth at higher cardiovascular risk, including those with familial hypercholesterolaemia. As atherosclerotic processes begins as early as the first decade of life the identification of reliable biomarkers reflecting cardiometabolic dysfunction in youth is essential for timely risk stratification and prevention strategies—particularly in populations with overweight and obesity, where metabolic derangements are already established. Laboratory tests can complement clinical assessment, emphasizing the need for biomarkers (e.g. lipid profile, fasting glucose, glycated haemoglobin and insulin values) that can monitor this trajectory over time [29]. While LDL-C plays a well-established causal role in atherogenesis, PCSK9 also contributes to lipid metabolism and cardiovascular risk [4]. PCSK9 promotes LDL receptor degradation, thereby influencing LDL-C clearance, and preclinical evidence supports its involvement in atherosclerosis. However, clinical studies have reported conflicting results regarding circulating PCSK9 levels and cardiovascular events [28, 30, 31]. In our cohort, PCSK9 levels were significantly associated with atherogenic lipid fractions, including LDL-C, non-HDL-C, triglycerides, and remnant cholesterol. This association extends beyond LDL-C regulation, highlighting the link of PCSK9 and non-HDL-C, which reflects the total burden of apoB-containing particles. Non-HDL-C is now incorporated into adult cardiovascular risk algorithms such as SCORE2, guiding personalized lipid-lowering strategies [27, 32].

We observed that PCSK9 levels increased most markedly with changes in remnant cholesterol, with a ≈3.5–4.4-fold stronger slope than for total/non-HDL-C/LDL-C. In the present study, remnant cholesterol was

calculated as 'total cholesterol–LDL-C–HDL-C'. Subtracting LDL-C and HDL-C from total cholesterol provides a rough approximation of remnant cholesterol content that includes cholesterol in triglyceride-rich lipoprotein not yet fully processed to remnants [33]. Remnant cholesterol includes both intestinally derived chylomicrons and hepatic very low-density lipoproteins (VLDL), which are atherogenic and contribute to foam cell formation and plaque progression [34]. In the intima, remnants are taken up by macrophages without any need for previous modification, and thereby converting such cells into foam cells, which are the hallmark of atherosclerotic lesions [35]. In keeping with this line of evidence, data from 767 youths (594, overweight/obese; 173, normal weight) showed that in youths, elevated levels of remnant cholesterol might represent a marker of early atherosclerotic damage. Notably, among youths of normal weight as well as those with overweight/obesity, children in the highest remnant cholesterol category had, respectively, 3.8-fold and 2.3-fold greater odds of increased carotid intima media thickness [36]. Translating these findings in our setting, although we were unable to provide data on subclinical atherosclerosis, we can speculate that PCSK9 could contribute indirectly to atherogenesis via triglyceride-rich lipoproteins. While recognizing that association does not prove causality, two non-mutually exclusive mechanisms have been suggested for how PCSK9 may influence triglyceride-rich lipoproteins metabolism, i.e. (i) the positive association between plasma PCSK9 and TG levels could be indirect, reflecting shared upstream drivers such as insulin resistance; and (ii) circulating PCSK9 may dircetly modulate LDLR-dependent reuptake of circulating triglyceride-rich lipoproteins [37, 38].

Accordingly, in interpreting our findings, it is important to factor in the potential influence of obesity-related insulin resistance, also in light of evidence that insulin can directly induce the expression of PCSK9 [39]. In children and adolescents with type 1 diabetes mellitus serum PCSK9 are elevated and significantly associated with dyslipidaemia and microvascular complications [40] . Prior reports have also described correlations between PCSK9 and markers of glucose metabolism, including insulin, HOMA-IR and HbA1c [41, 42]. In this scenario, it is worth recalling that insulin resistance confers an elevated CVD risk even in the absence of type 2 diabetes [43]. Consistent with this, data from CARDIA (Coronary Artery Risk Development in Young Adults) study show higher HOMA-IR in young adulthood is associated with a greater likelihood of coronary artery calcification in midlife, underscoring the need to identify subclinical cardiovascular risk factors early and to implement primary prevention strategies [44]. Although, in our sample, diabetes was an exclusion criteria, the analysis showed a trend toward an association between PCSK9 and insulin level or HOMA-IR after adjustment for body weight. This finding confirms that the link between PCSK9 and insulin resistance is direct, rather than being mediated by obesity.

Another key finding was higher PCSK9 levels in children with a positive parental history of dyslipidaemia. Our data indicate that individuals with a positive family history of dyslipidaemia have higher PCSK9 levels than those without such a history ($\Delta \approx +40$ ng/mL). Children whose parents had a documented history of dyslipidaemia were less likely to be obese and/or hypertensive, and tended to have a healthier overall

metabolic profile, with fewer cases of high uric acid and insulin resistance. Even so, this group showed higher levels of all atherogenic lipoproteins than children whose parents had a normal lipid profile. This was not unexpected, as children referred for high cholesterol levels only were often of normal weight and had normal blood pressure values, but had a documented history of dyslipidaemia in their parents. Even in the absence of monogenic defects, polygenic contributions likely upregulate PCSK9. These elevated PCSK9 concentrations in childhood suggest early inherited dysregulation of lipid homeostasis, reinforcing the importance of timely intervention. Recognizing elevated PCSK9 levels in children with a familial predisposition may support more precise screening strategies, targeted genetic testing (e.g. *LDLR*, *PCSK9*, *APOB*), and the early implementation of interventions aimed at reducing cardiovascular risk [45]. Our findings highlight the importance of genetic predisposition. Indeed, in our cohort, neither weight (BMI z-score) nor central obesity (WtHr) were associated with higher PCSK9 levels. While the majority of participants were overweight or obese, only a proportion of these children had abnormal lipid profiles. In fact, it has been shown that excess weight in childhood is not always associated with metabolic abnormalities [46].

Relative to PCSK9 levels, in our cohort we found higher values than those reported in other paediatric studies (188 ng/mL vs 88 ng/mL or 65 ng/mL). While differences between assays may partially explain this discrepancy, it is important to note that there are no reference intervals for PCSK9 levels to indicate normal values underscoring the need of personalized reference values [47]. Although this protein is tightly regulated at both transcriptional and translational levels - yielding concentration differences of roughly 100-fold [48] - the ELISA assay we used is rigorously validated, with low intra- and inter-assay coefficients of variation, demonstrating excellent reproducibility [49, 50]. Thus, the possibility that these discrepancy in PCSK9 levels could be driven by the high percentage of children with familial predisposition to dyslipidaemia, rather than to measurement methods, can not be overlooked.

Another difference compared to previous studies in children and adolescents [14] was the lack of variations according to sex or pubertal status. This suggests that PCSK9 expression in youth may be largely independent of the hormonal changes associated with sexual maturation. Although delayed or accelerated puberty (e.g. the acquisition of reproductive capacity and sexual maturity) is known to influence lipid fractions [51], our data indicate that PCSK9 levels remain relatively stable across developmental stages, supporting its potential utility as a reliable marker for cardiovascular risk assessment in paediatric populations. Notably, despite in adults higher PCSK9 levels being reported in adult women compared with men, no definite mechanistic explanations have yet been identified regarding the role of sexual hormones at transcriptional or post-transcriptional levels [52].

Limitations of the study. These findings should be interpreted in light of several limitations. First, this was a mono-centric study without a control group. Second, there is currently no universally accepted gold standard for PCSK9 quantification and the assay used does not allow for a "functional" assessment of PCSK9. Third,

since genetic testing for hypercholesterolemia was not performed, this limits the ability to identify individuals with familial hypercholesterolemia, a drawback considering that plasma PCSK9 concentrations are known to correlate positively with LDL-C levels in carriers of *LDLR* or *APOB* pathogenic variants [53]. Conversely, although we miss to identify carriers of *PCSK9* loss-of-function variants (e.g. R46L), which are associated with lower PCSK9 levels, being these variants relatively uncommon in Caucasian populations [4], the exclusion of these subjects from the analysis is predicted to have a minimal and not significant impact on the statistical results [13]. Fourth, considering that evidence from candidate-gene studies supports the presence of gene-obesity interactions in lipid profiles, as the effects of lipid-associated loci are modified by obesity traits such as BMI [54, 55], the current results cannot be generalized.

Conclusions. This study demonstrates that circulating PCSK9 levels in children and adolescents at cardio-metabolic risk are significantly associated with atherogenic lipid fractions, including LDL-C, non-HDL-C, triglycerides, and remnant cholesterol. These findings suggest that PCSK9 may play a broader role in lipid metabolism beyond LDL-C regulation, particularly in relation to triglyceride-rich lipoproteins reinforcing the importance of timely cardiovascular risk assessment. Finally, although the clinical relevance of PCSK9 measurement in childhood requires further validation, measurement of PCSK9 levels has been proposed as a tool to identify “hypo-responders” to lipid-lowering therapies, a strategy that may have clinical relevance in paediatric populations [11]. Future studies should explore longitudinal trajectories of PCSK9, its genetic determinants, and its predictive value for CVD cardiovascular outcomes from early life into adulthood.

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Declaration of interest. All the authors have nothing to declare

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Table 1. Characteristics of the study population according to sex

	Overall (N=332)	**Females (N=152, 45.8%)**	**Males (N=180, 54.2%)**
Age (years), median (Q1-Q3)	11.2 (9.0-12.7)	10.9 (8.5-12.8)	11.2 (9.4-12.7)
Puberty, N (%)	145 (44.1)	82 (53.9)	63 (35.6)
Weight at birth (g), median (Q1-Q3)	3280.0 (2900.0-3600.0)	3250.0 (2825.0-3570.0)	3295.0 (3000.5-3683.8)
Parental dyslipidaemia (n=290) N (%)	120 (41.4)	49 (37.1)	71 (44.9)
BMI, median (Q1-Q3)	25.2 (21.9-27.7)	25.1 (21.6-27.6)	25.3 (22.2-27.7)
BMI z-score, median (Q1-Q3)	1.9 (1.4-2.2)	1.9 (1.3-2.1)	1.9 (1.4-2.2)
Weight class, N (%)			
NW	60 (18.1)	28 (18.4)	32 (17.8)
OW	86 (25.9)	35 (23.0)	51 (28.3)
OB	186 (56.0)	89 (58.6)	97 (53.9)
WtHr, median (Q1-Q3)	53.7 (49.3-58.2)	53.0 (48.8-57.3)	55.4 (50.6-58.8)
WtHr, N (%)			
≤50	91 (27.4)	47 (30.9)	44 (24.4)
>50	241 (72.6)	105 (69.1)	136 (75.6)
SBP (mmHg), median (Q1-Q3)	110.0 (102.0-119.0)	110.0 (101.0-118.0)	110.0 (102.8-120.0)
SBP z-score, median (Q1-Q3)	0.6 (-0.2-1.2)	0.6 (-0.1-1.1)	0.5 (-0.2-1.2)
DBP (mmHg), median (Q1-Q3)	64.0 (58.8-70.0)	64.0 (58.0-70.0)	63.5 (59.0-70.0)
DBP z-score, median (Q1-Q3)	0.2 (-0.2-0.7)	0.2 (-0.2-0.7)	0.2 (-0.3-0.7)
BP category, N (%)			
NT	251 (75.6)	117 (77.0)	134 (74.4)
HN	27 (8.1)	11 (7.2)	16 (8.9)
HT	54 (16.3)	24 (15.8)	30 (16.7)
Total cholesterol (mg/dL), median (Q1-Q3)	167.0 (144.0-195.0)	164.0 (144.0-190.5)	173.0 (143.2-198.8)
Total cholesterol ≥200 mg/dL, N (%)	72 (21.8)	29 (19.1)	43 (24.2)
Non-HDL cholesterol (mg/dL), median (Q1-Q3)	116.0 (93.0-142.0)	115.0 (92.0-138.0)	120.5 (94.2-143.0)
Non-HDL cholesterol ≥145 mg/dL	72 (21.9)	31 (20.5)	41 (23.0)
Remnant cholesterol (mg/dL), median (Q1-Q3)	15.4 (11.3-20.6)	15.6 (11.4-20.4)	15.2 (11.2-20.6)

Remnant cholesterol ≥ 30 mg/dL	32 (9.8)	18 (12.0)	14 (7.9)
HDL cholesterol (mg/dL), median (Q1-Q3)	50.0 (43.0-58.0)	50.0 (43.0-59.0)	51.0 (44.0-58.0)
HDL cholesterol < 40 mg/dL, N (%)	41 (12.5)	19 (12.6)	22 (12.4)
LDL cholesterol (mg/dL), median (Q1-Q3)	97.8 (78.5-118.6)	95.2 (78.2-114.8)	101.8 (78.6-124.0)
LDL cholesterol ≥ 130 mg/dL, N (%)	55 (16.8)	17 (11.3)	38 (21.5)
Triglycerides (mg/dL), median (Q1-Q3)	77.0 (56.5-103.0)	78.0 (57.0-101.8)	76.0 (56.0-103.0)
Triglycerides ≥ 100 mg/dL or ≥ 130 mg/dL*, N (%)	58 (17.7)	31 (20.7)	27 (15.3)
Lipoprotein(a) (nmol/L), median (Q1-Q3)	24.6 (7.7-61.9)	24.3 (7.2-54.5)	25.0 (8.7-67.3)
Lipoprotein(a) ≥105 nmol/L, N (%)	31 (15.8)	15 (15.8)	16 (15.8)
Glucose (mg/dL), median (Q1-Q3)	86.0 (82.0-89.8)	85.5 (81.0-89.0)	86.0 (82.2-90.0)
Insulin (mol/L), median (Q1-Q3)	13.6 (9.5-19.7)	14.8 (10.6-21.1)	12.6 (8.6-18.1)
HOMA index, median (Q1-Q3)	3.0 (2.0-4.2)	3.1 (2.2-4.3)	2.8 (1.8-4.0)
HOMA index ≥ 90th percentile, N (%)	226 (68.5)	104 (68.4)	122 (68.5)
Uric acid (mg/dL), median (Q1-Q3)	4.4 (3.7-5.2)	4.3 (3.7-4.9)	4.4 (3.7-5.5)
Uric acid ≥ 90th percentile, N (%)	61 (18.7)	25 (16.6)	36 (20.5)
eGFR (mL/min), median (Q1-Q3)	148.0 (131.8-163.8)	147.7 (131.9-165.2)	148.0 (132.1-163.1)
PCSK9 (ng/mL), median (Q1-Q3)	187.2 (141.5-236.3)	186.1 (141.5-229.4)	190.1 (142.0-238.2)

* ≥ 100 mg/dL if children < 10 years or ≥ 130 mg/dL if children ≥ 10 years

BMI, body mass index; BMI z-score, BMI adjusted for a child's age and sex; BP, blood pressure; DBP, diastolic blood pressure; DBP z-score, DBP adjusted for age, sex and height; eGFR, estimated Glomerular Filtration Rate; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/Kexin type 9; SBP, systolic blood pressure; SBP adjusted for age, sex, and height; WtHr, Waist-to-Height Ratio.
Q1, 25th interquartile; Q3, 75th interquartile.

Table 2. Anthropometric and clinical characteristics of the study population according to plasma PCSK9 values (ng/mL).

	PCSK9 (ng/mL), median (Q1-Q3)	p-value
Puberty		0.145
Yes	179.7 (137.3-217.3)	
No	192.4 (144.0-244.7)	
Parental dyslipidaemia (n=290)		<0.001
Yes	206.1 (167.0-251.9)	
No	176.2 (117.4-229.5)	
Weight class		0.986
NW	185.6 (155.4-225.7)	
OW	187.4 (137.5-236.0)	
OB	188.2 (141.5-237.5)	
Waist-to-Height-ratio		0.598
≤50%	180.5 (149.2-222.0)	
>50%	189.3 (141.1-239.9)	
BP category		0.236
NT	187.6 (148.4-241.3)	
HN	199.3 (132.8-241.4)	
HT	180.6 (117.6-215.6)	
Total cholesterol		<0.001
<200 mg/dL	178.3 (132.6-223.5)	
≥200 mg/dL	218.9 (169.3-263.1)	
LDL cholesterol		0.028
<130 mg/dL	185.2 (138.6-228.0)	
≥130 mg/dL	204.4 (157.4-262.1)	
HDL cholesterol		0.615
<40 mg/dL	186.1 (148.1-225.3)	
≥40 mg/dL	187.4 (141.5-237.4)	
Non-HDL cholesterol		<0.001
<145 mg/dL	179.7 (137.3-221.0)	
≥145 mg/dL	223.5 (167.7-269.1)	
Remnant cholesterol		0.091
<30 mg/dL	186.1 (141.1-233.5)	
≥30 mg/dL	204.7 (159.9-291.6)	
Triglycerides*		0.004
<100 or 130 mg/dL	184.4 (138.7-225.0)	
≥100 or 130 mg/dL	216.6 (163.1-287.9)	
Lipoprotein(a)		0.559
<105 nmol/L	176.5 (122.7-218.8)	
≥105 nmol/L	181.8 (141.3-223.5)	
HOMA index		0.515
< 90th percentile	185.6 (153.9-235.9)	
≥ 90th percentile	188.0 (137.3-236.1)	
Uric acid (mg/dL)		0.406
< 90th percentile	187.4 (144.4-237.0)	
≥ 90th percentile	177.0 (123.8-235.0)	

* ≥ 100 mg/dL if children < 10 years or ≥ 130 mg/dL if children ≥ 10 years

BP, blood pressure; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/Kexin type 9.
NW, normal weigh; OW, overweight; OB, obese.
NT, normotensive; HN, high-normal; HT, hypertensive.

Table 3. Unadjusted and adjusted linear regression models assessing the association between each covariate and PCSK9 (ng/mL).

	Unadjusted models			Adjusted models		
	Coeff	95% CI	p-value	Coeff	95% CI	p-value
Males	-2.534	(-21.393; 16.325)	0.792	-1.628	(-20.486; 17.230)	0.865
Age (years)	-3.931	(-7.684; -0.178)	0.040	-	-	-
Weight at birth (g)	-0.008	(-0.025; 0.009)	0.348	-0.009	(-0.026; 0.009)	0.331
Familiarity for dyslipidaemia (n=290)	36.473	(15.897; 57.049)	0.001	39.880	(18.683; 61.077)	<0.001
Puberty (yes)	-4.997	(-24.024; 14.029)	0.606	17.218	(-8.955; 43.391)	0.197
BMI z-score	1.718	(-8.377; 11.813)	0.738	-	-	-
Weight class						
NW	Ref.	-	-	-	-	-
OW	-1.504	(-30.348; 27.340)	0.918	-	-	-
OB	-1.325	(-26.784; 24.134)	0.919	-	-	-
WtHr	-0.165	(-1.461; 1.130)	0.802	-	-	-
WtHr >50 %	3.544	(-17.519; 24.606)	0.741	-	-	-
SBP z-score	-6.363	(-15.001; 2.275)	0.148	-4.053	(-13.672; 5.567)	0.408
DBP z-score	1.469	(-10.920; 13.859)	0.816	3.604	(-9.211; 16.418)	0.580
BP category						
NT	Ref.	-	-	Ref.	-	-
HN	-0.588	(-35.170; 33.994)	0.973	1.604	(-33.132; 36.340)	0.928
HT	-21.807	(-47.421; 3.806)	0.095	-17.472	(-44.238; 9.294)	0.200
Total cholesterol (mg/dL)	0.464	(0.215; 0.712)	<0.001	0.535	(0.258; 0.812)	<0.001
Total cholesterol ≥200 mg/dL	41.509	(19.129; 63.890)	<0.001	51.715	(26.488; 76.942)	<0.001
Non-HDL cholesterol (mg/dL)	0.510	(0.243; 0.776)	<0.001	0.518	(0.237; 0.799)	<0.001
Non-HDL cholesterol ≥ 145 mg/dL	42.888	(20.521; 65.255)	<0.001	43.560	(20.448; 66.672)	<0.001
Remnant cholesterol (mg/dL)	1.523	(0.545; 2.501)	0.002	1.858	(0.841; 2.875)	<0.001
Remnant cholesterol ≥ 30 mg/dL	44.176	(12.589; 75.763)	0.006	49.584	(17.642; 81.526)	0.002
HDL cholesterol (mg/dL)	0.172	(-0.579; 0.923)	0.653	0.164	(-0.702; 1.030)	0.709
HDL cholesterol < 40 mg/dL	-12.509	(-41.079; 16.062)	0.390	-9.920	(-7.484; 0.139)	0.504
LDL cholesterol (mg/dL)	0.407	(0.125; 0.690)	0.005	0.420	(0.111; 0.729)	0.008

LDL cholesterol ≥ 130 mg/dL	31.088	(5.933; 56.242)	0.016	34.083	(6.572; 61.593)	0.015
Triglycerides (mg/dL)	0.304	(0.108; 0.500)	0.002	0.368	(0.163; 0.572)	<0.001
*Triglycerides ≥ 100 mg/dL or ≥ 130 mg/dL**	45.412	(21.051; 69.772)	<0.001	49.219	(24.463; 73.975)	<0.001
Lipoprotein(a) (nmol/L)	0.091	(-0.125; 0.307)	0.406	0.083	(-0.138; 0.304)	0.459
Lipoprotein(a) ≥105 nmol/L	2.321	(-32.133; 36.775)	0.894	0.565	(-34.337; 35.467)	0.975
Glucose (mg/dL)	-0.016	(-1.439; 1.407)	0.983	0.344	(-1.119; 1.806)	0.644
Insulin (mol/L)	0.452	(-0.360; 1.265)	0.274	0.950	(-0.008; 1.909)	0.052
HOMA index	1.566	(-2.064; 5.196)	0.397	4.108	(-0.142; 8.357)	0.058
HOMA index ≥90th percentile	-4.123	(-24.342; 16.096)	0.689	-2.683	(-27.381; 22.015)	0.831
Uric acid (mg/dL)	-8.392	(-16.657; -0.126)	0.047	-7.767	(-17.660; 2.126)	0.123
Uric acid ≥ 90th percentile	-9.732	(-34.115; 14.652)	0.433	-7.594	(-32.727; 17.539)	0.553
eGFR (mL/min)	0.255	(-0.128; 0.638)	0.191	0.151	(-0.252; 0.554)	0.462

* ≥ 100 mg/dL if children < 10 years or ≥ 130 mg/dL if children ≥ 10 years

BMI z-score, body mass index (BMI) adjusted for a child's age and sex; BP, blood pressure; DBP, diastolic blood pressure; DBP z-score, DBP adjusted for age, sex and height; eGFR, estimated Glomerular Filtration Rate; HDL, high-density lipoprotein; HOMA, Homeostatic Model Assessment; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/Kexin type 9; SBP, systolic blood pressure; SBP adjusted for age, sex, and height; WtHr, Waist-to-Height Ratio.

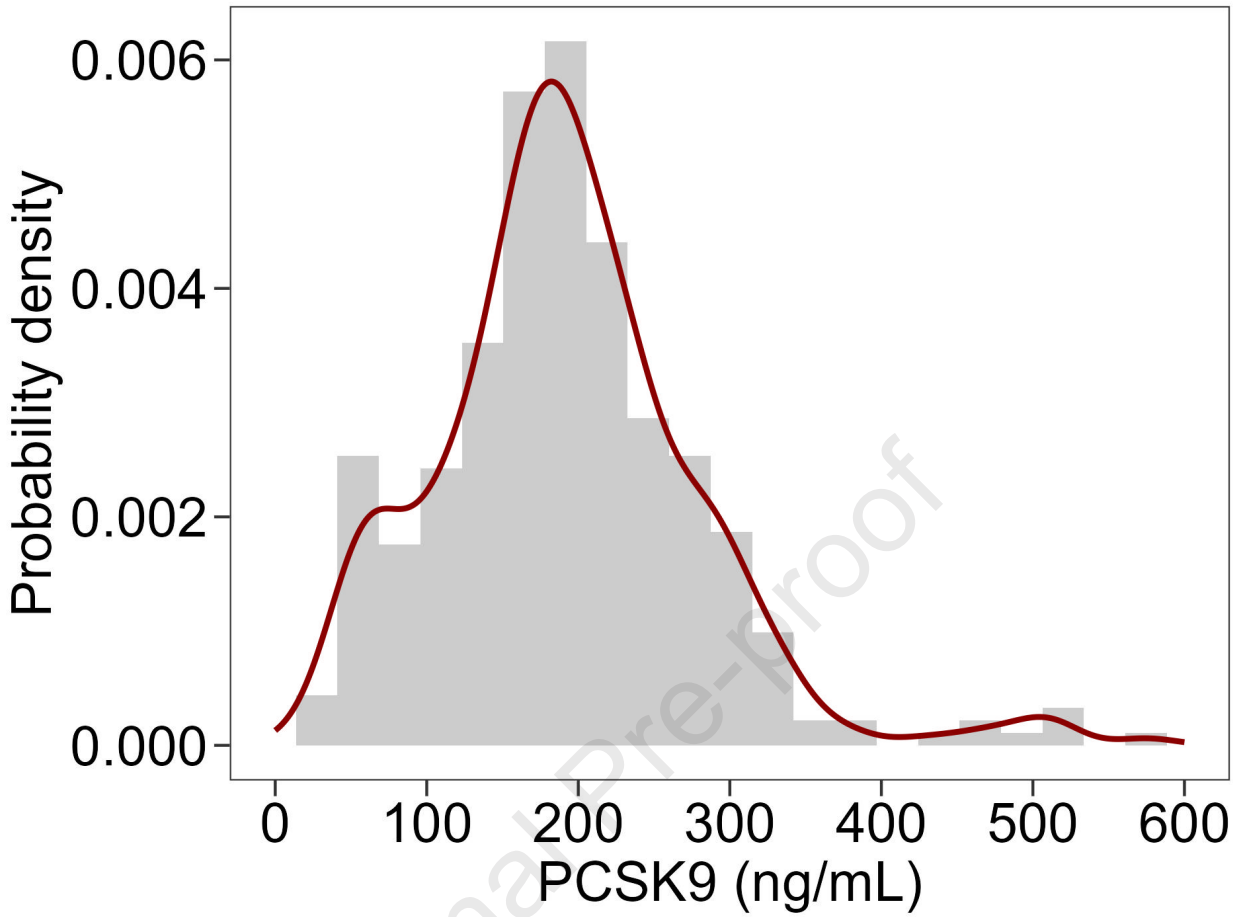
NW, normal weigh; OW, overweight; OB, obese.
NT, normotensive; HN, high-normal; HT, hypertensive.

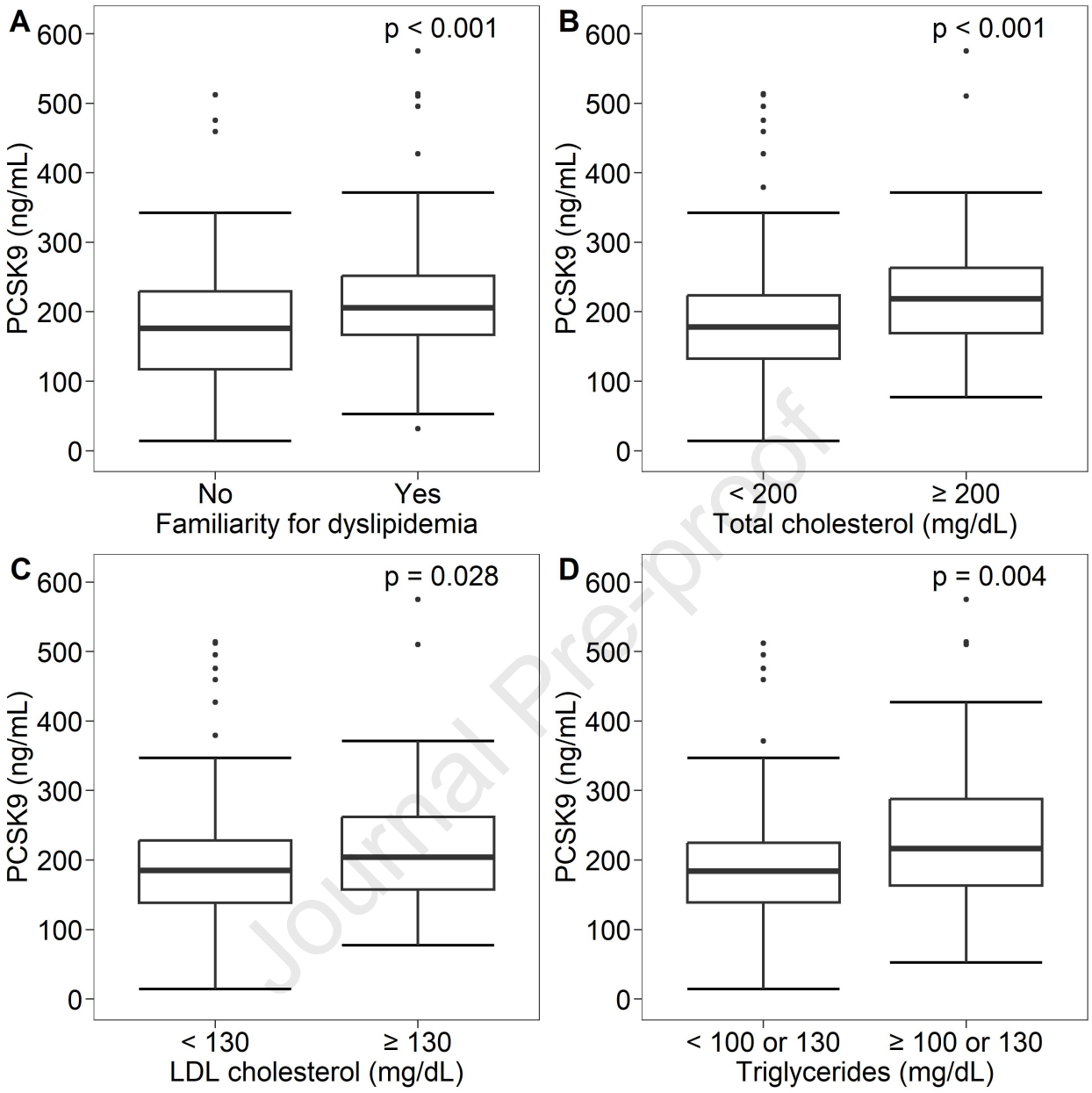
Figure Legend.

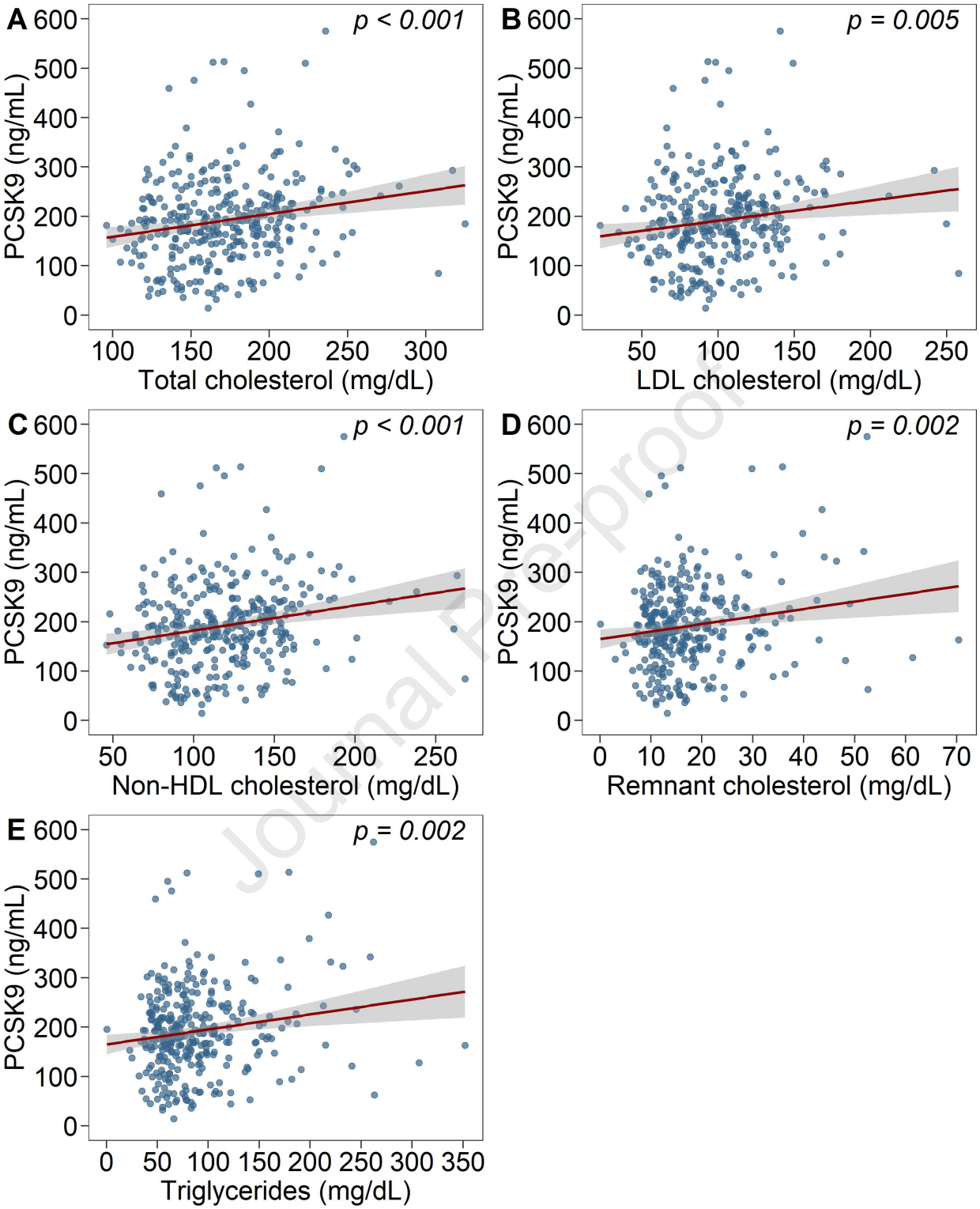
Figure 1. Probability density histogram of PCSK9 values (ng/mL) with an overlaid kernel density estimate (red line). PCSK9, proprotein convertase subtilisin/kexin type 9.

Figure 2-- Boxplots of the distribution of PCSK9 values in groups of patients identified by: A) presence of familiarity for dyslipidaemia, B) levels of total cholesterol, C) levels of LDL cholesterol, D) levels of triglycerides. LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/Kexin type 9.

Figure 3 – Scatterplot of PCSK9 (ng/mL) vs A) total cholesterol (mg/dL), B) LDL cholesterol (mg/dL), C) triglycerides (mg/dL) with linear regression line (solid red). The shaded area represents the 95% confidence interval. LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/Kexin type 9;







Highlights

1) PCSK9 levels in children and adolescents are significantly associated with atherogenic lipid fractions

2) PCSK9 levels were significantly higher in youth whose atherogenic lipoprotein values were above the normal thresholds

3) Children with a positive family history of dyslipidemia had substantially higher PCSK9 values compared to those without