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Plasma PCSK9 Is Associated with Age, Sex, and Multiple Metabolic Markers in a Population-Based Sample of Children and Adolescents

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BACKGROUND: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protein convertase that posttranslationally promotes the degradation of the low-density lipoprotein receptor (LDLR) in hepatocytes and increases plasma LDL cholesterol (LDL-C). Heterozygote gain-of-function mutations of PCSK9 are associated with the familial hypercholesterolemia phenotype, whereas loss-of-function variants are associated with reduced LDL-C concentrations and lower coronary risk. Plasma PCSK9 correlates with body mass index, triglyceridemia, total cholesterol, and LDL-C in adults, but no data are available in youth.

METHODS: We studied 1739 French Canadian youth ages 9, 13, and 16 years who participated in the Quebec Child and Adolescent Health and Social Survey, a province-wide school-based survey conducted in 1999. An ELISA assay was used to measure plasma PSCK9.

RESULTS: The mean (SD) plasma PCSK9 concentration was 84.7 (24.7) μg/L in the sample. In boys, plasma PCSK9 decreased with age, whereas the inverse was true for girls. There were statistically significant positive associations between PCSK9 and fasting glucose, insulin, and HOMA-IR (homeostasis model assessment of insulin resistance). In multivariable analysis, a 10% higher fasting insulin was associated with a 1%–2% higher PCSK9 in both sexes. There were also positive associations between PCSK9 and total cholesterol, LDL-C, and triglycerides, as well as with HDL-C and apolipoproteins A1 and B.

CONCLUSIONS: PCSK9 is associated with age, sex, and multiple metabolic markers in youth. A novel finding is that PCSK9 is associated with fasting insulinemia, which suggests that PCSK9 could play a role in the development of dyslipidemia associated with the metabolic syndrome.

Proprotein convertase subtilisin kexin type-9 (PCSK9)[6] is a 692–amino acid serine protease of 72 kDa that is encoded by the *PCSK9* gene located on chromosomal region 1p32.3 *(1)*. *PCSK9* is mainly expressed in the liver, intestine, and kidney *(2)*. The only known enzymatic function of PCSK9 is its own intramolecular autocatalytic processing in the endoplasmic reticulum, which is necessary for its activation and function. PCSK9 acts as a molecular chaperone that binds to the epidermal growth factor–like domain A of LDL receptor (LDLR) and promotes LDLR degradation *(3, 4)* through an endosomal/lysosomal pathway *(5)*. In adults, PCSK9 is produced primarily by the liver and the intestine, but only the liver releases it into circulation *(6)*. Circulating PCSK9 may be cleaved in the plasma by furin, another proprotein convertase, generating a residue of 474 amino acids *(7)*.

Mutations of *PCSK9* are associated with either hypercholesterolemia (gain-of-function mutations) *(8–12)* or hypocholesterolemia (loss-of-function mutations) *(13–17)*. PCSK9 gain-of-function mutations lead to the familial hypercholesterolemia (FH) phenotype; they account for approximately 2% of cases of FH *(1)*. Retrospective studies have shown that carriers of PCSK9 loss-of-function mutation R46L (3.6% of whites), Y142X, and C679X (2.6% of African Americans) are at a significantly reduced risk of cardiovascular atherosclerotic disease. The Y142X and C679X mu-

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[6] Nonstandard abbreviations: PCSK9, proprotein convertase subtilisin/kexin type 9; LDLR, LDL receptor; FH, familial hypercholesterolemia; LDL-C, LDL cholesterol; BMI, body mass index; HDL-C, HDL cholesterol; TG, triglycerides; QCAHSS, Quebec Child and Adolescent Health and Social Survey; apo, apolipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; SREBP-1c, sterol regulatory element–binding protein 1c.

tations are associated with a 28% reduction in plasma LDL cholesterol (LDL-C) and an 88% reduction in the rate of coronary events *(18)*.

We have recently shown that plasma PCSK9 mass measured by ELISA correlates in adults with lipid and metabolic variables including total cholesterol, LDL-C, triglyceridemia, and fasting glycemia, as well as body mass index (BMI) (unpublished results). No data on plasma PCSK9 have yet been published in children and adolescents. It is known that concentrations of total cholesterol, LDL-C, HDL cholesterol (HDL-C), and triglycerides (TG) vary by age and sex maturity stage in youth *(19, 20)*. Variations in PCSK9 concentrations during puberty may contribute to these changes in lipid profile. Furthermore, PCSK9 concentrations measured during childhood may predict future cardiovascular disease.

The objective of this study was to describe the distribution of plasma PCSK9 in a population-based sample of children and adolescents and to examine the associations between PCSK9 and both metabolic and lipid variables.

Materials and Methods

STUDY POPULATION

The study population comprised children and adolescents who participated in the Quebec Child and Adolescent Health and Social Survey (QCAHSS), a school-based survey conducted between January and May 1999. Details on the survey design and methods have been reported *(21)*. Briefly, the QCAHSS used a cluster sampling design to draw 3 independent, provincially representative samples of youth ages 9, 13, and 16 years. Questionnaire and anthropometric data were available for 83% (1267 of 1520), 79% (1186 of 1498), and 81% (1212 of 1495) of eligible 9-, 13-, and 16-year-olds, respectively. This current analysis was restricted to French Canadians, who comprised 80% (1019 of 1267), 79% (931 of 1186), and 78% (942 of 1212) of the 9-, 13-, and 16-year-old samples. Sixty-three percent (638 of 1019), 69% (640 of 931), and 75% (709 of 942) of 9-, 13-, and 16-year-olds provided a fasting blood sample. Of 1987 blood specimens available for analysis, 248 (12%) were excluded because the parents refused consent for analyses other than glucose and lipids, the samples had thawed on arrival at the laboratory, or the samples were of insufficient quantity for the PCSK9 assay. None of the participants used lipid-lowering medications. There were no differences in sex or BMI *z* scores between youth for whom blood samples were retained or not retained for analysis. The study was approved by the Ethics Review Board of the Centre Hospitalier Universitaire (CHU) Sainte-Justine. Written informed assent and consent were obtained from participants and their legal guardians, respectively.

VARIABLES

Height and weight were measured according to standardized protocols *(21)*. BMI was computed as weight in kilograms divided by height in meters squared. Blood was obtained by venipuncture between 0800 and 1000 after an overnight fast in a 1-g/L EDTA collection tube that was kept on ice until centrifugation. Plasma was separated on site within 45 min of collection, frozen on dry ice, and sent within 24 h to the laboratory, where specimens were stored at −80 °C until analysis. The median duration of the fasting period was 13 h (range 9–17 h). Fasting plasma insulin, glucose, total cholesterol, TG, and HDL-C concentrations were measured as described *(21, 22)*. LDL-C was calculated according to the Friedewald formula *(23)*. Apolipoprotein B (apoB) and apoA-I concentrations were determined by nephelometry (Beckman Array Protein System). Plasma adiponectin was measured by RIA (Linco Research) *(24)*. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as insulin (mU/L) × glucose (mmol/L)/22.5 *(25)*.

PRODUCTION AND PURIFICATION OF ANTI-PCSK9 ANTIBODIES

We produced and purified recombinant truncated human PCSK9 (rPCSK9; Met-amino acids 31–454) as described *(5)*. It was injected into 2 rabbits by a standard protocol to generate a polyclonal antibody (hPCSK9-Ab). The antibodies were first prepurified by precipitation with ammonium sulfate (50% final concentration). After solubilization and dialysis of the precipitate, we purified the antibodies by affinity chromatography using a CNBr-activated Sepharose 4B column (GE Healthcare Bio-Sciences AB), coupled with the purified antigen (rPCSK9). A fraction of this purified antibody was conjugated with horseradish peroxidase (hPCSK9-Ab-HRP) using the EZ-Link™ Plus Activated Peroxidase protocol from Pierce. Finally, we purified the conjugated antibody from excess free horseradish peroxidase using the FreeZyme Conjugate Purification Kit (product 44920; Pierce). We confirmed the specificity of the antibody for PCSK9 by immunoprecipitation and Western blotting.

PCSK9 ELISA ASSAY

LumiNunc Maxisorp white assay plates were coated with 0.5 μg/well of hPCSK9-Ab by incubation at 37 °C for 3 h in PBS (Na_2HPO_4 10 mmol/L, NaCl 0.15 mol/L, pH 7.4) containing sodium azide (1 g/L), then stored at 4 °C. Before use, the plates were washed 6 times with PBS containing Tween 20 (0.5 mL/L) and incubated for 1 h at room temperature with blocking buffer (PBS, casein 0.1%, merthiolate 0.01%). We prepared calibra-

Table 1. Plasma concentrations of lipids, apolipoproteins, and selected metabolic variables in participants.[a]

	Boys			Girls		
Variable[a]	Age 9 years	Age 13 years	Age 16 years	Age 9 years	Age 13 years	Age 16 years
n	282	290	302	271	248	346
BMI, kg/m^2	17.2 (2.7)	20.4 (3.2)	22.5 (3.8)	17.4 (2.8)	20.9 (3.7)	22.2 (3.7)
Lipids, mmol/L						
Total cholesterol	4.03 (0.61)	3.87 (0.54)	3.65 (0.67)	4.18 (0.64)	4.04 (0.58)	4.16 (0.76)
LDL-C	2.31 (0.51)	2.20 (0.45)	2.11 (0.56)	2.45 (0.56)	2.34 (0.51)	2.38 (0.66)
HDL-C	1.40 (0.25)	1.27 (0.19)	1.14 (0.19)	1.36 (0.21)	1.29 (0.19)	1.32 (0.23)
TG	0.69 (0.31)	0.86 (0.33)	0.89 (0.50)	0.82 (0.35)	0.89 (0.27)	1.0 (0.38)
ApoA-I, g/L	1.25 (0.16)	1.17 (0.13)	1.09 (0.13)	1.21 (0.14)	1.18 (0.13)	1.23 (0.17)
ApoB, g/L	0.64 (0.13)	0.63 (0.13)	0.63 (0.16)	0.69 (0.15)	0.66 (0.13)	0.71 (0.20)
Fasting glucose, mmol/L	5.2 (0.3)	5.3 (0.3)	5.3 (0.4)	5.0 (0.3)	5.2 (0.3)	5.0 (0.3)
Fasting insulin, pmol/L	28.4 (18.7)	49.7 (32.3)	45.0 (35.1)	31.4 (14.7)	56.4 (21.5)	49.6 (22.0)
HOMA-IR	0.92 (0.63)	1.65 (1.12)	1.49 (1.12)	0.98 (0.48)	1.83 (0.75)	1.56 (0.73)
Adiponectin, mg/L	11.2 (3.7)	9.1 (2.9)	8.2 (2.8)	12.5 (4.0)	11.0 (3.1)	10.2 (3.1)

[a] Data are mean (SD).

tors using serial dilutions of recombinant PCSK9 in dilution buffer (PBS, urea 1.8 mol/L, BSA 0.25%, Tween 20 0.5 mL/L, and merthiolate 0.01%). Samples were diluted 1:20 in dilution buffer without BSA. Calibrators and samples were incubated for 30 min in a water bath at 46 °C before application (100 μL) in duplicate. The plates were incubated overnight at 37 °C with shaking. After washing, we added 100 μL hPCSK9-Ab-HRP diluted 1:750 for 3 h at 37 °C with shaking. Finally, after washing, we applied 100 μL substrate (SuperSignal™ ELISA Femto Substrate; Pierce) to each well. Chemiluminescence was quantitated on a Pherastar luminometer (BMG Labtech). We established a standard curve using a conditioned medium containing recombinant human PCSK9. This standard medium was calibrated by comparison with a full-length secreted and purified PCSK9 from a Baculovirus system in *HiFive* cells (donated by Rex Parker, Bristol-Myers Squibb). We determined peptide purity and concentration by quantitative amino acid analysis after 18–24 h of hydrolysis in the presence of 5.7N HCl under decreased pressure at 110 °C on a Beckman autoanalyzer (model 6300) with a postcolumn ninhydrin detection system coupled to a Varian DS604 data station (performed by Dr C. Lazure, Clinical Research Institute of Montreal, Quebec, Canada). We calculated plasma PCSK9 concentrations by comparing sample luminescence with that of the standard luminescence curve. The interassay CV for the measurement of plasma PCSK9 was 7.2% (n = 48) at a concentration of 115 μg/L.

STATISTICAL ANALYSES

We used the nonparametric method developed by Hutson *(26)* to estimate age- and sex-specific percentiles and confidence intervals for PCSK9. To take the complex study design into account, sampling weights and clustering effects by school were estimated and incorporated into computations of means, percentile values, and 95% CIs. We used hierarchical maximum likelihood linear regression to estimate regression coefficients for univariate and multivariate associations. Explanatory variables (correlates) were treated as fixed effects, and clustering between participants in the same school was treated as a random effect. All continuous variables (BMI, lipids, apolipoproteins, glucose, insulin, HOMA-IR, adiponectin, PCSK9) were $\log_e$-transformed. The regression coefficient for the 100 $\log_e$-transformed–dependent variable represents the mean percentage change in the dependent variable per unit change in the explanatory variable *(27)*. Because all explanatory variables (with the exception of age) were also $\log_e$-transformed, their units are also percentages. We performed statistical analyses with SAS software (SAS Institute) and SUDAAN (Research Triangle Institute).

Results

Table 1 presents the characteristics of participants. Plasma PCSK9 ranged from 17.6 to 211.7 μg/L, with a CV of 29% across all age and sex groups. Mean PCSK9 concentrations were higher in girls than boys (86.6 vs

Table 2. Distribution of PCSK9 concentrations (μg/L) by sex and age.

	Boys			Girls		
	Age 9 years	Age 13 years	Age 16 years	Age 9 years	Age 13 years	Age 16 years
n	282	290	302	271	248	346
Percentile (95th CI)						
5th	54.7 (51.1–57.8)	48.3 (44.9–50.9)	50.1 (46.5–52.9)	48.8 (43.6–52.8)	55.6 (51.1–58.8)	52.3 (45.7–57.4)
10th	58.3 (56.3–61.4)	52.8 (49.5–57.3)	54.7 (51.7–57.7)	56.6 (50.9–58.0)	60.9 (56.5–62.9)	59.3 (54.6–63.4)
25th	71.4 (67.0–75.3)	63.9 (61.3–65.7)	62.1 (60.8–64.2)	66.3 (62.4–68.8)	70.9 (67.9–74.5)	69.9 (66.5–73.3)
50th	85.1 (82.3–87.1)	77.5 (74.8–81.6)	74.8 (71.3–78.2)	79.2 (75.9–82.2)	86.4 (83.7–90.1)	85.8 (83.2–90.1)
75th	101.3 (98.1–105.3)	96.2 (92.9–100.9)	92.1 (87.9–98.1)	95.4 (92.4–98.9)	103.9 (100.8–108)	103.5 (99.2–107.6)
90th	116.9 (111.2–125.3)	116.1 (109.9–123.5)	115.7 (109.2–126.3)	108.1 (105.8–118.6)	117.7 (113.6–125.8)	124.2 (116.3–132.2)
95th	129.4 (123.3–149.7)	131.0 (120.6–135.9)	130.7 (122.4–140.2)	120.2 (114.8–132.4)	137.3 (124.5–143.9)	135 (128.8–141.4)
Mean (95th CI)	87.8 (84.9–90.8)	81.9 (78.9–85.0)	80.4 (77.4–83.3)	81.7 (78.8–84.5)	89.3 (86.2–92.4)	88.6 (85.9–91.3)

Table 3. Univariate association between PCSK9 and age and selected metabolic variables by sex.

	Boys (n = 874)			Girls (n = 865)		
Explanatory variable	β, % Δ[a]	SE	P	β, % Δ	SE	P
Age, years						
9	Ref			Ref		
13	−8.3	2.7	0.002	9.4	2.6	0.0004
16	−10.0	2.7	0.0002	8.7	2.5	0.0005
BMI, 10% Δ	−0.2	0.5	0.60	0.3	0.5	0.47
Glucose, 10% Δ	7.9	1.4	<0.0001	3.6	1.3	0.008
Insulin, 10% Δ	1.1	0.2	<0.0001	1.1	0.2	<0.0001
HOMA-IR, 10% Δ	1.1	0.2	<0.0001	1.0	1.0	<0.0001
Adiponectin, 10% Δ	0.7	0.3	0.005	0.5	0.3	0.05

[a] For all explanatory variables except age, β refers to the percentage difference in mean PCSK9 concentrations for a 1-unit increment (10% change) in explanatory variable. For age, β refers to the percentage difference in mean PCSK9 concentrations for 13- or 16-year-olds compared with the 9-year-old reference group. % Δ, percentage change; *P*, probability value for the regression coefficient; Ref, reference group.

82.2 μg/L; $P = 0.004$). The association of age with plasma PCSK9 varied significantly as a function of sex ($P_{interaction} < 0.0001$). Therefore, all analyses were stratified by sex. Sex-specific distributions of PCSK9 were skewed positively (see Supplemental Fig. 1, which accompanies the online version of this article at www.clinchem.org/content/vol55/issue9).

Age- and sex-specific means and percentile values of PCSK9 concentrations are presented in Table 2. Nine-year-old boys had higher mean PCSK9 concentrations than 13- and 16-year-old boys ($P = 0.002$ and 0.0002, respectively). In contrast, 9-year-old girls had lower mean concentrations than 13- and 16-year-old girls ($P = 0.0004$ and 0.0005, respectively). For all percentiles reported (5th to 95th), 9-year old boys had higher values of PCSK9 concentrations than 9-year-old girls, whereas the reverse was true for 13- and 16-year olds. In fact, although 9-year-old boys started with higher PCSK9 concentrations than same-age girls, 16-year-old boys ended up with lower values than same-age girls.

Table 3 shows the sex-specific univariate associations between PCSK9 and selected metabolic variables. Fasting plasma glucose and insulin, HOMA-IR, and adiponectin showed significant positive associations with PCSK9 concentration in both sexes. We detected no significant univariable association between BMI and PCSK9. After adjustment for age, glucose, insulin,

Table 4. Multivariable association between PCSK9 and age and selected metabolic variables by sex.

	Boys (n = 874)			Girls (n = 865)		
Explanatory variable	β, % Δ[a]	SE	P	β, % Δ	SE	P
Age, years						
9	Ref			Ref		
13	−14.0	2.7	<0.0001	5.2	2.9	0.08
16	−12.5	2.9	<0.0001	8.2	2.8	0.003
BMI, 10% Δ	−1.5	0.7	0.02	−1.8	0.6	0.003
Glucose, 10% Δ	5.5	1.4	0.0001	0.8	0.3	0.57
Insulin, 10% Δ	1.7	0.2	<0.0001	1.3	0.2	<0.0001
Adiponectin, 10% Δ	0.6	0.3	0.02	0.8	0.3	0.004

[a] For all explanatory variables except age, β refers to the percentage difference of mean PCSK9 concentration for a 1-unit increment (10% change) in the explanatory variable after adjustment for all other variables in the model. For age, β refers to the percentage difference in mean PCSK9 concentration in 13- and 16-year-olds compared with the 9-year-old reference group after adjustment for all other variables in the model. % Δ, percentage change; *P*, probability value for the regression coefficient; Ref: reference group.

and adiponectin, however, BMI was negatively associated with PCSK9: a 10% higher BMI was associated with a 1.5% and 1.8% lower PCSK9 in boys and girls, respectively (Table 4). In contrast, after adjustment for age, BMI, insulin, and adiponectin, glucose was no longer significantly associated with PCSK9 in girls. Glucose remained associated with PCSK9 after adjustment for age and the other metabolic variables in boys: a 10% higher glucose was associated with a 5.5% higher PCSK9. The strength of the associations between PCSK9 and both insulin and adiponectin did not change substantially after adjustment for age and the other metabolic variables. Pearson coefficients of correlation between PCSK9 and, BMI, glucose, insulin, and adiponectin as well as scatter plots are presented in online Supplemental Table 1 and online Supplemental Fig. 2.

Table 5 presents the associations between PCSK9 and lipid variables. In both sexes, a 10% higher plasma PCSK9 concentration was associated with a 1%–2% higher total cholesterol, LDL-C, and apoB and a 2%–3% higher TG. Interestingly, there was a significant association between PCSK9 and HDL-C in both sexes, as well as an association with apoA-I. The

Table 5. Association between PCSK9 and plasma lipids and apolipoproteins by sex.

			Boys (n = 874)			Girls (n = 865)		
Model	Dependent variable	Explanatory variable	β, % Δ[a]	SE	P	β, % Δ	SE	P
1	Total cholesterol	PCSK9, 10% Δ						
Not adjusted			1.5	0.2	<0.0001	1.5	0.2	<0.0001
Adjusted[b]			1.3	0.2	<0.0001	1.5	0.2	<0.0001
2	LDL-C	PCSK9, 10% Δ						
Not adjusted			1.7	0.3	<0.0001	1.6	0.3	<0.0001
Adjusted			1.6	0.3	<0.0001	1.8	0.3	<0.0001
3	ApoB	PCSK9, 10% Δ						
Not adjusted			1.7	0.3	<0.0001	1.5	0.3	<0.0001
Adjusted			1.6	0.3	<0.0001	1.7	0.3	<0.0001
4	HDL-C	PCSK9, 10% Δ						
Not adjusted			0.6	0.2	0.01	0.9	0.2	<0.0001
Adjusted			0.5	0.2	0.02	0.9	0.2	<0.0001
5	ApoA1	PCSK9, 10% Δ						
Not adjusted			0.5	0.2	0.009	0.8	0.2	<0.0001
Adjusted			0.5	0.2	0.002	0.7	0.2	<0.0001
6	Triglycerides	PCSK9, 10% Δ						
Not adjusted			3.1	0.5	<0.0001	2.5	0.5	<0.0001
Adjusted			2.3	0.5	<0.0001	2.0	0.5	<0.0001

[a] β refers to the percentage difference in mean concentration of the dependent variable for a 1-unit increment (10% change) in the explanatory variable (PCSK9). % Δ, percentage change; *P*, probability value for the regression coefficient.
[b] Adjusted for age, BMI, and plasma concentrations of insulin, glucose, and adiponectin.

strength of the associations between PCSK9 and each of the lipid and apolipoprotein variables did not change substantially after adjustment for age, BMI, insulin, adiponectin, and glucose. Pearson coefficients of correlation between PCSK9 and both lipids and apolipoproteins as well as scatter plots are presented in online Supplemental Table 2 and online Supplemental Fig. 3.

Discussion

In this study, we present the first description of the distribution of plasma PCSK9 in a large population-based sample of white children and adolescents. Average plasma PCSK9 concentrations were slightly lower in our pediatric population than in an adult population (unpublished data) in which PCSK9 was measured using the same method [mean (95% CI) 84.8 (83.5–86.0) vs 89.5 (85.6–93.4) μg/L, respectively]. Interestingly, there were no sex differences in PCSK9 concentrations in adults, whereas significant differences were observed in youth. Moreover, sex modified the association between age and PCSK9 in youth.

We observed that plasma PCSK9 concentrations were significantly higher in 9-year-old boys than in 13- and 16-year-old boys. In contrast, plasma PCSK9 concentrations were significantly lower in 9-year-old girls than in 13- and 16-year-old girls. We suggest that these differences contribute to the differences in total cholesterol and LDL-C concentrations observed between boys and girls during childhood and adolescence. It is well established that plasma total cholesterol and LDL-C peak at 9–10 years of age, decrease during pubertal development, and then increase thereafter *(28)*. It is also known that there is a significant effect of sex on the concentrations of both total cholesterol and LDL-C in youth. Specifically, compared to girls, more important decreases in total cholesterol are observed in boys between 9 and 16 years of age (approximately 18% and 10%, respectively) *(28)*. Our data indicated that decreases in total cholesterol with age in boys were consistent with decreases in plasma PCSK9. In girls, higher concentrations of total cholesterol observed at 13–14 years coincided with the peak in plasma PCSK9 concentrations. The sex differences in PCSK9 concentrations during puberty may in part explain differences in total cholesterol concentrations between boys and girls during this period (Fig. 1).

In contrast, there was no evidence for age-by-genotype interactions in the Bogalusa Heart Study, which focused on loss-of-function mutations in PCSK9 in children and adolescents *(29)*. However, plasma PCSK9 concentrations were not measured and only sex-adjusted lipid profiles were shown, so the effect of sex on the phenotypic expression of the PCSK9 mutation remained unknown.

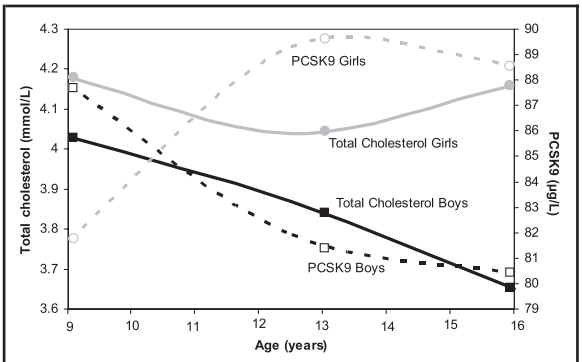

Fig. 1. Mean plasma total cholesterol and PCSK9 concentrations in boys and girls ages 9, 13, and 16 years and interpolation between these ages.

In boys, mean total cholesterol (black squares) is higher in 9-year-olds than in 13-year-olds and higher in 13-year-olds than in 16-year-olds. There is a similar age effect on mean plasma PCSK9 concentrations (open squares). In girls, mean total cholesterol (gray circles) is higher in 9-year-olds than in 13-year-olds, whereas 16-year-old girls have a higher mean plasma total cholesterol concentration than do 13-year-old girls. There is an inverse age effect on mean plasma PCSK9 concentrations (open circles) in girls.

Differences between sexes and during pubertal development suggest an influence of sex hormones on plasma PCSK9 concentrations. Very little is known about this topic. Studies in rodents show that treatment with high doses of ethinylestradiol result in a 50% decrease in PCSK9 gene expression and protein *(30)*. It is interesting that in girls, plasma PCSK9 concentrations level off at approximately 13 years, an age by which the majority of girls have begun puberty and estradiol production is greatly augmented *(31)*. There are to our knowledge no studies that explore the relationship between testosterone and PCSK9. It is also very likely that other factors play a role in the regulation of PCSK9 during puberty. Indeed, studies in rodents show that growth hormone stimulation increases the level of intracellular PCSK9 mRNA *(32)*.

Independent of age, BMI, glucose, and adiponectin, insulin was positively associated with PCSK9 concentrations in both sexes. Previous studies in rodents demonstrate the importance of insulin in PCSK9 regulation. After induction of type 1 diabetes in rats by streptozotocin injection, Niesen et al. *(33)* observed a significant reduction in LDLR mRNA while LDLR protein was unaffected. This was explained by a marked

decrease in PCSK9 protein in hypoinsulinemic rats, thus reducing LDLR degradation. Further evidence of the importance of insulin in the regulation of PCSK9 concentrations is available in studies of hyperinsulinemia in rodents. Hyperinsulinemic clamps upregulated PCSK9 mRNA in rats *(34)*, and this was shown to be through the action of insulin on sterol regulatory element–binding protein 1c (SREBP-1c), which regulates PCSK9 transcription. Conversely, glucose had no effect on PCSK9 mRNA in rodent primary hepatocytes. However, we observed a positive association between glucose and PCSK9. It is interesting that the strength of the association between fasting glucose and PCSK9 was attenuated after adjustment for other metabolic variables (a 10% higher glucose was associated with an 8% and 6% higher plasma PCSK9 in univariable and multivariable models, respectively, in boys, and a 4% and 1% increase in PCSK9, respectively, in girls). Because insulin was associated with PCSK9 and also accounted for part of the variance in glucose when predicting PCSK9, it may be that insulin plays a mediating role in the glucose and PCSK9 relationship. Taken together, these results indicate an important role for insulin in the regulation of PCSK9 expression. PCSK9 could contribute to the development of the hyperinsulinemic dyslipidemia observed in patients with the metabolic syndrome or diabetes *(35)*.

Adiponectin had a small but significant positive effect on PCSK9 concentrations even after controlling for BMI, glucose, and insulin. No report is available in the literature on the association between adiponectin and PCSK9 in humans or in animal studies.

We observed a positive association between PCSK9 and both total cholesterol and LDL-C, similar to that in adults *(36–38)*. A 10% higher plasma PCSK9 was associated with a 1.7% higher LDL-C. This increase may appear small, but contributes significantly to the interindividual variation in plasma LDL-C. Individuals with a plasma PCSK9 concentration of 180 μg/L (95th percentile) have a mean LDL-C concentration that is 0.3 mmol/L higher than individuals with a plasma PCSK9 concentration of 55 μg/L (5th percentile). Similar variation in PCSK9 contributes to a mean change of 0.5 mmol/L in total cholesterol. There was a strong association between PCSK9 and plasma TG. Recent studies show that a polymorphism in PCSK9 (p.L21 tri) is associated with familial combined hyperlipidemia (hypercholesterolemia and hypertriglyceridemia) *(39)*. There is currently no clear mechanistic explanation for the association between PCSK9 and TG, but VLDL may be less efficiently cleared when the apoB/E receptor (LDLR) is degraded by PCSK9.

In contrast to adults *(36–38)*, there was a positive association between PCSK9 and HDL-C in both boys and girls. Differences in the power to detect an association related to sample size could explain these discrepant results: the larger sample size in the current analysis allowed us to detect small but significant differences. The mechanisms explaining the association between PCSK9 and HDL-C remain to be identified. Mice in which the PCSK9 gene has been knocked out presented a reduction in HDL-C *(6, 40)*. A possible hypothesis is that apo-E–containing HDL particles may be cleared less efficiently when the LDLR is degraded by PCSK9.

A limitation of the study is that PCSK9 measurement is not standardized across laboratories, so concentrations measured in our study are not directly comparable to those measured in another laboratory using a different method. Because we restricted our study to a French Canadian sample, the findings may not be generalizable to other populations. To date, there is no evidence of heterogeneity in the effect of PCSK9 on LDL receptor metabolism between populations. In cross-sectional studies, we cannot infer causality between factors examined.

In conclusion, our study in a large population-based sample of children and adolescents showed that mean plasma concentrations of PCSK9 vary by age in a sex-dependent manner. Similar to those in adults, we found positive associations between PCSK9 and total cholesterol, LDL-C, and TG. We extended these findings by demonstrating a relationship between plasma PCSK9 and each of fasting insulinemia and HDL-C. Our results suggest a role for insulin in the regulation of PCSK9, which may explain in part the dyslipidemia observed in the metabolic syndrome and diabetes. Further, similar to PCSK5 *(1)*, PCSK9 may play a role in HDL-C metabolism. Large prospective human studies, as well as cellular and animal research, are needed to better understand PCSK9 metabolism and actions.

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