Proprotein Convertase Subtilisin/Kexin 9 level is independently associated with 10-year cardiovascular risk in blood donors in Kinshasa:

A cross-sectional study based on Framingham predictive equation

***Le taux de Proprotein Convertase Subtilisin/Kexin 9 est indépendamment associé au risque cardiovasculaire à 10 ans chez les donneurs de sang à Kinshasa* :** Etude transversale basée sur l'équation prédictive de Framingham

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Résumé

Contexte et objectif. La Proprotéine Convertase Subtilisine Kexin type 9 (PCSK9) est importante dans l'homéostasie des lipides. Cette étude visait à établir le rôle potentiel de PCSK9 comme facteur de risque cardiovasculaire (RCV). *Méthodes*. L'enquête transversale couvrant la période d'août 2016 à juillet 2020 a été conduite dans la ville de Kinshasa (RD Congo), sur des donneurs de sang volontaires et réguliers au sein du réseau médical catholique (BDOM). La technique Elisa a permis l'analyse de PCSK9 sérique et le taux des lipides était dosé par la méthode enzymatique colorimétrique. L'équation de prédiction des événements CV a recourru à la méthode Framingham. La corrélation entre le taux des lipides sériques et le PCSK-9 a été faite à l'aide de corrélation linéraire de Pearson. La régression logistique binaire multivariée a déterminé le niveau du risque futur des événements CV. *Résultats*. 264/296 sujets (89,1 %) avaient un RCV faible, 32 (10,8 %) un RCV élevé. Les principaux déterminants du RCV étaient : âge ≥ 50 ans (ORa 5), taux bas de HDL-c (ORa 4), taux élevé de LDL-c (ORa 6) et/ou de triglycéride (ORa 4) et l'appartenance au 3ème tertile de PCSK9 (ORa 4). *Conclusion*. Le taux plasmatique élevé de PCSK9 constitue un facteur de risque un RCV élevé dans cette population en bonne santé apparente. L'extension de l'étude dans la population générale est nécessaire pour la validation de ces résultats.

Mots-clés : Framingham, Haut risque cardiovasculaire, Proprotein convertase subtilisin/kexin type 9, Population africaine subsaharienne

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Summary

Context and objective. Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) plays an important role in lipid homeostasis. The present study aimed to determine whether PCSK9 is a potential cardiovascular risk (CVR) factor among apparently healthy people. *Methods*. A cross-sectional study was conducted between August 2016 and July 2020 in the City of Kinshasa, Democratic Republic of the Congo. Volunteer and regular blood donors from the Catholic medical network (Bureau Diocésain des Œuvres Médicales [BDOM]/Kinshasa) were enrolled in this study. Serum PCSK9 and lipid levels were measured by ELISA and enzymatic colorimetric method, respectively. Framingham's predictive equation was used for predicting cardiac events. Pearson's correlation coefficients (r) were calculated to assess the association between the different lipid fractions and PCSK-9. The search for the determinants of 10 year-risk of a high cardiovascular event was carried out using the multivariate binary logistic regression model. *Results*. Of 296 subjects included in the present study, 264 (89.1 %) had low and 32 (10.8 %) high CVR. Age ≥ 50 years (aOR 5), low HDL-c (aOR 5), high LDL-c (aOR 6), hypertriglyceridemia (aOR 4), and belonging to the 3rd tertile of PCSK9 ((aOR 4.4) emerged as independent determinants of high CVR. *Conclusion.* High plasma levels of PCSK9 are associated with high CVR in apparently healthy people. Prospective studies in the general population to confirm this Framingham cardiovascular prediction are needed.

Keywords: Framingham, High cardiovascular risk, Proprotein convertase subtilisin/kexin type 9, Sub-Saharan African population

Abbreviations: Ankle-brachial systolic pressure index, ABI; Body mass index, BMI; Cardiovascular risk, CVR; Enzyme-linked immunoassays, ELISA; High-density lipoprotein, HDL-c ; Intima-media wall thickness, IMT; Low-density lipoprotein cholesterol, LDL-c ; Proprotein Convertase Subtilisin Kexin type 9, PCSK9 ; Total cholesterol, TC; Triglyceridemia, TG; Very low-density lipoprotein cholesterol, VLDL-c

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Introduction

Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) is a proteolytic enzyme (protease) whose catalytic action is supported by an amino acid triad of serine, histidine and aspartate. PCSK9 is the ninth member of subtilisin-like serine convertase superfamily, with a key role in lipid metabolism and glucose homeostasis regulation. It is related to the bacterial subtilase family (subtilisin) and belongs to the yeast kexin subfamily (1). Discovered in 2003 in the context of familial hypercholesterolemia (2),

PCSK9 is mainly synthesized in the liver cells as an inactive, 692-amino acid, 75-kDa protein containing 4 domains: a signal peptide, a prodomain, a catalytic domain and a cysteine and histidine-rich C-terminal domain. PCSK9 undergoes an intramolecular self-cleavage of the prodomain from the catalytic domain in the endoplasmic reticulum. However, the prodomain remains bound to the catalytic domain in a non-covalent fashion, thus inhibiting the PCSK9 proteolytic activity which will be restored upon a second cleavage in the prodomain (3).

PCSK9 is involved in the maturation of serum proteins, cell receptors, neuropeptides, various hormones, growth factors and cytokines (4). It has a tropism for low-density lipoprotein cholesterol (LDL-c) receptors located on the outer surface of the cell membrane (5). By binding to these receptors and by endocytosis, PCSK9 induces degradation of these receptors (6). This mechanism underlies the role of PCSK9 as a central regulator of cholesterol metabolism. Indeed, PCSK9 by degrading LDL-c receptors facilitates excessive plasma accumulation of LDL-c and consequently the deleterious effects of circulating cholesterol are amplified (7). Gene encoding PCSK9 located on chromosome 1 at 1p32.3 has either gain-of-function or loss-of-function variants. Loss of function implies a decrease in LDL-c levels and associated cardiovascular risk [CVR] (8). Conversely, gain of function underlies severe hypercholesterolaemia (9) and hypertrigly-ceridaemia (2). The latter involves increased synthesis of the highly atherogenic lipid particles apo B48, very low-density lipoprotein cholesterol [VLDL-c] (10). Several studies have shown that elevated plasma LDL-c levels correlate with the atherosclerosis process, CVR and patient mortality (11). CVR can be estimated using the Framingham equation adopted by the World Health Organization (WHO) and the Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES) in 2004 (12). This equation predicts the risk of occurrence at 10 years of a major cardiovascular event. This model includes the following parameters: sex, age, systolic blood pressure, total cholesterol (TC), high-density lipoprotein (HDL-c), smoking, antihypertensive treatment, diabetes mellitus and family history of hypertension. Score < 20 %, 20-30 % and ≥ 30 % was considered as low risk, high risk and very high risk, respectively.

By promoting LDL-c receptor degradation, PCSK9 plays an important role in cholesterol metabolism and predicts CVR (13). PCSK9 is associated with subclinical atherosclerosis as assessed by carotid intima-media wall thickness [IMT] (14). This subclinical atherosclerosis can be demonstrated by the ankle-brachial systolic pressure index (ABI) which is a non-invasive method used to detect peripheral arterial occlusive disease with a sensitivity of 90 % and a specificity of 95 % (15). Knowledge of the mechanisms of action of PCSK9 has become an important therapeutic focus. The majority of studies focusing on PCSK9 are conducted in patients with various cardio-metabolic diseases. In addition, the prevalence of cardio-metabolic diseases, including obesity and overweight among adults, is increasing alarmingly (16). The association between obesity and chronic diseases has been demonstrated in some studies. This association would explain the high mortality in adults (17). Thus, certain methods, including bioimpedencemetry, are recommended by consensus for the evaluation of obesity and its effects on the vital prognosis of patients (18). Several of these studies are from the northern hemisphere. Given the geographical and racial differences in the prevalence and relative weight of the different CVR factors, it is necessary for sub-Saharan countries to determine the association between plasma PCSK9 levels and

CVR in apparently healthy people. Therefore, the present study investigates the association between PCSK9 and CVR in blood donors in the city of Kinshasa.

Methods

Study design and population

A cross-sectional study was conducted between August 2016 and July 2020 in the city of Kinshasa in the Democratic Republic of Congo (DRC). Volunteer and regular blood donors from the Catholic medical network under the responsibility of the Bureau Diocésain des Œuvres Médicales (BDOM)/Kinshasa were consecutively enrolled in the present study. Inclusion criteria were as follows: aged 16 years or older; blood donor whose last blood donation was more than 3 months before inclusion in the study; no history of a recent infection (< 3 months) or an acute disease (hepatic and/or thyroid); no current hypolipidemic treatment; voluntary agreement to participate in the study. Pregnant women and patients with CV events prior to enrolment in the present study were excluded.

Study design and population

A cross-sectional study was conducted between August 2016 and July 2020 in the city of Kinshasa in the Democratic Republic of Congo (DRC). Volunteer and regular blood donors from the Catholic medical network under the responsibility of the Bureau Diocésain des Œuvres Médicales (BDOM)/Kinshasa were consecutively enrolled in the present study. Inclusion criteria were as follows: aged 16 years or older; blood donor whose last blood donation was more than 3 months before inclusion in the study; no history of a recent infection (< 3 months) or an acute disease (hepatic and/or thyroid); no current hypolipidemic treatment; voluntary agreement to participate in the study. Pregnant women and patients with CV events prior to enrolment in the present study were excluded.

Clinical parameters

Parameters of interest included: age, sex, family history of diabetes mellitus and hypertension, diabetes mellitus, hypertension, smoking, alcohol consumption, and physical inactivity.

Smoking was defined as consumption of at least 1 cigarette/d for 5 years or more (active smoking) or cessation for less than 5 years [past smoking] and alcohol as consumption of at least 20 g alcohol/d or > 2 glasses of beer/d for at least one year. Physical inactivity in adults was defined as less than 60 minutes of moderate-intensity aerobic activity per day. All participants were subjected to a physical examination by a trained clinician, and clinical parameters such as weight (Kg), height (cm), blood pressure (mm Hg), waist circumference (cm), pulse, and heart rate (beat/min) were recorded. Overweight and obesity were defined by BMI ≥ 25 kg/m² and BMI ≥ 30kg/m², respectively.

Furthermore, the ABI was measured as previously described elsewhere (19). Briefly, the blood pressure was collected at the ankle and arm to the subjects, and a ratio of the systolic blood pressure (SBP) measured at the ankle to that measured at the arm was established. The body composition (weight, body fat, muscle mass, and body mass index [BMI]) were also determined using a bio-impedance meter analyzer: Electronic scale OM-BF 214, Brand Omron, Type Body fat monitor, EAN 4015672107045, 2015 with 4 sensor accuracy technology, large LCD panel, 4 user's memory with guest mode.

Laboratory measurements

Venous blood samples were collected from included participants to measure the levels of hemoglobin, hematocrit, serum urea, serum creatinine, glycemia, uric acid, TC, LDL-c, HDL-c, triglycerides (TG), non-HDL-c, calcium, phosphorus, intact parathyroid hormone (PTHi), Vit D, and PCSK9. Blood was taken in the fasting state (six hours after the last meal) for the determination of lipid fractions. PCSK9 measurement was performed by the competitive inhibition enzyme-linked immunoassays according to the ELISA MBS 920252 kit. PCSK9 levels were divided into three tertiles (PCSK9 < 9.58 ng/ml; 9.58 - 23.0 ng/ml and >

23.0 ng/ml). Lipid determination was automated (Cobas C 311/version 2010) using an enzymatic colorimetric method. Atherogenic dyslipidemia was defined as TG ≥ 150 mg/dl and HDL-c <50 mg/dl (female) or < 40 (male). Isolated dyslipidemia was defined as TC ≥ 200 mg/dl; HDL-c <50 mg/dl (in female) and <40 mg/dl (in male); LDL-c ≥ 100 mg/dl or TG ≥ 150 mg/dl (14). Combined dyslipidemia met Frederickson's international classification (20).

Outcome measures

The primary endpoint was an estimated 10-year CVR according to the Framingham equation (20). Secondary outcomes were subclinical atherosclerosis assessed by ABI < 0.9 and a high level of PCSK9.

Statistical analysis

All data were analyzed using SPSS for Windows version 24 software. The descriptive data were presented as mean and standard deviation for quantitative variables with Gaussian distribution; the median and interquartile range (IQR) for variables not normally distributed; and the relative (%) and absolute (n) proportions for categorical data. The student's t-test was performed to compare two means. The ANOVA test was used for multiple comparisons. ANOVA tests that were significant at the p <0.05 level were supplemented by a post hoc Scheffé Test. The Mann–Whitney U test was used for comparison of the medians of the two groups, and the Kruskal–Wallis H test for more than 2 medians.

The linear regression test was applied to determine the correlation between the different lipid fractions and PCSK-9. Pearson's correlation coefficients (r) were calculated to assess this association. The search for the determinants of future risk of 10-year high cardiovascular events was carried out using the multivariate binary logistic regression model. The adjusted ORs and their 95 % CIs were calculated to determine the degree of association between 10-year CVR and the independent variables. The significance level retained was then p-value < 0.05. Written informed consent was obtained from all participants before enrollment. This study protocol was submitted to the ethics committee of the Kinshasa School of Public Health for analysis and approval (ESP/CE /053/2016).

Results

Demographic characteristics

In the general population, 296 candidates (249 men and 47 women) with a mean age of 36.0 ± 10.4 years were enrolled in the present study. Table 1 shows the general characteristics of this population including physical inactivity (49.3 %), alcohol consumption (31.1%), smoking (29.4 %), and overweight (19.9 %). This population had a frequency of abnormal ABI, hypercholesterolemia, elevated LDL-c of 54.7 %, 16.9 %, and 14.5 %, respectively. The elevated 10-year hazard ratio was 10.8 % in this population.

Table1. General characteristics of the study population according to gender

Variables	Over all (n=296)	Male(n249)	Female(n=47)	P value
Age	36.0 ±10.4	35.1±10.6	40.6±8.1	0.111
Physical inactivity	146 (49.3)	113 (45.4)	33 (70.2)	0.001
Smoking	87 (29.4)	35 (34.1)	2 (4.3)	<0.001
Alcohol	92 (31.1)	84 (33.7)	8 (17.0)	0.015
Medicinal plants	24 (8.1)	19 (7.6)	5 (10.6)	0.327
Obesity	31 (10.5)	12 (4.8)	19 (40.4)	<0.001
Overweight	59 (19.9)	41 (16.5)	18 (38.3)	0.001
Hypercholesterolemia	50 (16.9)	29 (11.6)	21 (44.7)	<0.001
Low HDL-c	80 (27.0)	64 (25.7)	16 (34.0)	0.158
High LDL-c	43 (14.5)	20 (8.0)	23 (48.9)	<0.001
Hypertriglyceridemia	35 (11.8)	30 (12.0)	50 (10.6)	0.506
Abnormal ABI	162 (54.7)	141 (56.6)	21 (44.7)	0.089

Variables	Over all (n=296)	Male(n249)	Female(n=47)	P value
CVR				0.016
Low	264 (89.2)	227 (91.2)	37 (78.7)	
High	32(10.8)	22 (8.8)	10 (21.3)	
SBP (mmHg)	121.3±12.7	121.2±12.5	121.3±14.7	0.957
DBP (mmHg)	76.2±10.1	75.6±9.9	79.1±10.7	0.030
MBP (mmHg)	91.2±9.9	90.8±9.9	93.2±10.6	0.138
PP (mmHg)	45.1±10.3	45.6±9.7	42.2±13.0	0.040
Radial pulse (bpm)	78.9±12.6	78.0±12.9	84.0±9.5	0.003
BMI (Kg/m^2)	23.6±4.6	22.6±3.8	28.9±5.0	<0.001
Fat mass (%)	20.9±10.5	17.9±7.3	36.8±10.7	<0.001
Muscular mass (%)	33.9±9.2	35.6±8.8	24.6±4.9	<0.001
Hb (mg/dL)	12.7±1.5	12.8±1.5	12.6±1.2	0.428
TC (mg/dL)	164.0±43.4	157.7±40.3	197.5±44.7	<0.001
HDL-c (mg/dL)	54.3±20.6	53.9±21.5	56.0±14.6	0.524
LDL-c (mg/dL)	97.3±40.2	90.9±35.7	130.8±45.8	<0.001
TG (mg/dL)	76.3 (71.4-80.9)	76.8 (71.9-83.7)	67.3 (62.5-93.0)	0.253
PCSK9 (ng/ml)	19.6±1.7	20.0±1.9	17.8±6.1	0.434
Creatinine (mg/dL)	0.90 (0.90-1.0)	1.0 (0.9-1.0)	0.8 (0.7-0.8)	<0.001
eGFR ($ml/min/1.73m^2$)	115.5 (113.0-118.9)	116.4 (114.6-119.8)	105.8 (99.0-116.0)	0.084
Urea (mg/dL)	19.0 (18.3-19.9)	18.9 (18.3-20.0)	19.1 (18.0-21.6)	0.600
tCa (mmol/L)	2.3 (2.2-2.4)	2.3 (2.2-2.4)	2.4 (2.2-2.44)	0.248
iCa (mmol/L)	0.98 (0.96-0.99)	0.96 (0.95-0.98)	1.04 (0.99-1.08)	0.033
Ph (mmol/L)	1.48 (1.4-1.53)	1.5 (1.4-1.52)	1.55 (1.42-1.67)	0.441
PTHi (mmol/L)	25.8 (23.7-27.8)	26.1 (24.5-28.3)	23.1 (20.8-28.5)	0.418
VitD (ng/L)	84.9 (78.9-92.9)	88.2 (80.5-96.7)	74.1 (58.7-83.7)	0.147

Data are expressed as mean ± standard deviation, absolute (n) proportions or relative frequency (%), median and interquartile range (IQR).ABI ankle-brachial index; BMI, body mass index; CVR, cardiovascular risk; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-c, high-density lipoprotein cholesterol; iCa, ionized calcium; LDL-c, low-density lipoprotein cholesterol; MBP, mean blood pressure; PCSK9, proprotein convertase subtilisin/kexin type 9; Ph, phosphorus; PP, pulse pressure; PTHi, intact parathormone; SBP, systolic blood pressure; tCa, total calcium; TC, total cholesterol; TG, triglycerides; VitD, vitamin D.

Characteristics of the study population according to PCSK 9 level

Detailed baseline characteristics are shown in Table 2.

Table 2. General characteristics of the study population according to tertile of PCSK9

Variables	Tertile 1 (n=98)	Tertile 2 (n=99)	Tertile3 (n=99)	P
Age	33.7±10.0	35.4±10.1	38.9±10.4	0.001
Sex				0.780
Male	84(85.7)	84(84.8)	81(81.8)	
Female	14(14.3)	15(15.2)	18(18.2)	
Physical inactivity	49(50.0)	46(46.5)	51(51.5)	0.775
Smoking	34(34.7)	27(27.3)	26(26.3)	0.367
Alcohol	37(37.8)	26(26.3)	29(29.3)	0.208
Medicinal plants	10(10.2)	9(9.1)	5(5.1)	0.344
Obesity	7(7.1)	14(14.1)	10(10.1)	0.288
Overweight	15(15.3)	21(21.2)	23(23.2)	0.027
SBP (mmHg)	119.6±11.9	121.9±14.1	122.1±12.6	0.302
DBP (mmHg)	74.8±8.9	77.0±10.1	76.8±11.1	0.235
MBP (mmHg)	89.7±8.9	91.9±10.3	91.9±10.6	0.194
PP (mmHg)	44.8±9.5	44.9±11.2	45.4±10.1	0.931
Radial pulse (bpm)	79.7±11.8	78.6±14.4	78.4±11.7	0.750
BMI (Kg/m^2)	22.5±4.1	24.3±4.7	24.1±4.8	0.010

Variables	Tertile 1 (n=98)	Tertile 2 (n=99)	Tertile3 (n=99)	P
Hypercholesterolemia	12(12.2)	16(16.2)	22(22.2)	0.038
Low HDL-c	23(23.5)	35(35.4)	22(22.2)	0.074
High LDL-c	14(14.3)	13(13.1)	16(16.2)	0.075
Hypertriglyceridemia	8(8.2)	16(16.2)	11(11.1)	0.231
Abnormal ABI	57(58.2)	53(53.5)	52(52.5)	0.707
Fat mass (%)	18.9±8.4	21.8±11.1	22.0±11.5	0.076
Muscular mass (%)	31.9±10.4	34.8±8.5	34.9±8.4	0.034
High CVR	6(6.1)	9(9.1)	17(17.2)	0.027
Hb (mg/dL)	12.9±1.5	12.7±1.6	12.7±1.5	0.571
TC(mg/dL)	152.5±43.9	167.8±43.4	171.6±41.0	0.005
HDL-c(mg/dL)	56.1±21.7	51.1±17.9	55.7±21.6	0.165
LDL-c(mg/dL)	89.9±36.1	101.0±45.9	100.7±37.1	0.039
TG (mg/dL)	70.0 (66.4-78.9)	81.8 (72.4-100.9)	76.8 (68.9-89.0)	0.277
Creatinine (mg/dL)	0.9 (0.86-1.2)	0.95 (0.90-1.00)	1.0 (0.9-1.1)	0.277
eGFR(ml/min/1.73m^2)	120.4 (117.3-126.9)	115.0 (107.2-119.3)	112.9 (106.1-115.5)	0.697
Urea(mg/dL)	18.4 (17.7-19.8)	20.0 (18.2-21.0)	18.9 (18.1-20.0)	0.911
tCa (mmol/L)	2.21 (2.04-2.31)	2.4 (2.3-2.4)	2.4 (2.3-2.4)	0.001
iCa (mmol/L)	0.97 (0.95-1.00)	0.98 (0.95-0.99)	0.98 (0.95-1.0)	0.558
Ph (mmol/L)	1.58 (1.47-1.74)	1.3 (1.2-1.5)	1.5 (1.4-1.6)	0.048
PTHi (mmol/L)	22.6 (19.5-26.2)	26.2 (24.0-30.5)	27.1 (23.5-29.0)	0.105
Vit D (ng/L)	64.8 (56.9-78.6)	89.7 (75.5-99.3)	99.3 (85.2-108.0)	0.001

Data are expressed as mean ± standard deviation, absolute (n) proportions or relative frequency (%), median and interquartile range (IQR).ABI ankle-brachial index; BMI, body mass index; CVR, cardiovascular risk; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-c, high-density lipoprotein cholesterol; iCa, ionized calcium; LDL-c, low-density lipoprotein cholesterol; MBP, mean blood pressure; PCSK9, proprotein convertase subtilisin/kexin type 9; Ph, phosphorus; PP, pulse pressure; PTHi, intact parathormone; SBP, systolic blood pressure; tCa, total calcium; TC, total cholesterol; TG, triglycerides; VitD, vitamin D.

Plasma PCSK9 level distributed in tertile increases significantly with age, BMI, and muscle mass. Similarly, lipid fractions (TC, LDL-c) and high CVR increase significantly with PCSK9 level from 1st to 3rd tertile (Table 3).

Table 3. General characteristics of the study population according to CVR (Framingham)

Variables	Whole group (n=296)	Low CVR (n=264)	High CVR (n=32)	P
Sex				0.016
Male	249 (84.1)	227 (86.0)	22 (68.8)	
Female	47 (15.9)	37 (14.0)	10 (31.3)	
Age (years)				<0.001
<50	259 (87.5)	246 (93.2)	13 (40.6)	
≥50	37 (12.5)	18 (6.8)	19 (59.4)	
Physical inactivity	146 (49.3)	127 (48.1)	19 (59.4)	0.155
Smoking	87 (29.4)	79 (29.9)	8 (25.0)	0.363
Alcohol	92 (31.1)	82 (31.1)	10 (31.3)	0.564
Medicinal plants	24 (8.1)	21(8.0)	3 (9.4)	0.494
Obesity	31 (10.5)	22 (8.3)	9 (28.1)	0.002
Overweight	59 (19.9)	49 (18.6)	10 (31.3)	0.076
Hypercholesterolemia	50 (16.9)	35 (13.3)	15 (46.9)	<0.001
Low HDL-c	80 (27.0)	62 (23.5)	18 (56.3)	<0.001
High LDL-c	43 (14.5)	25 (9.5)	18 (56.3)	<0.001
Hypertriglyceridemia	35 (11.8)	23 (8.7)	12 (37.5)	<0.001
PCSK 9				0.008
Tertile 1	98 (33.2)	92 (34.8)	6 (18.8)	
Tertile 2	99 (33.4)	90 (34.1)	9 (28.1)	
Tertile 3	99 (33.4)	82 (31.1)	17 (53.1)	
ABI				0.004

Variables	Whole group (n=296)	Low CVR (n=264)	High CVR (n=32)	P
Normal	134 (45.3)	124 (47.0)	10 (31.3)	
Abnormal	162 (54.7)	140 (53.0)	22 (68.8)	

Data are expressed as absolute (n) proportions or relative frequency (%). ABI ankle-brachial index; CVR, cardiovascular risk; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; PCSK9, Proprotein convertase subtilisin kexin type 9.

Moreover, we addressed whether there was any correlation between the level of PCSK9 with different lipid components. PCSK-9 showed a statistically significant positive correlation with the level of TC, even though weak (r=0.18); and LDL-c (r=0.12). No associations were observed with the other lipid components (Figure 1A-D).

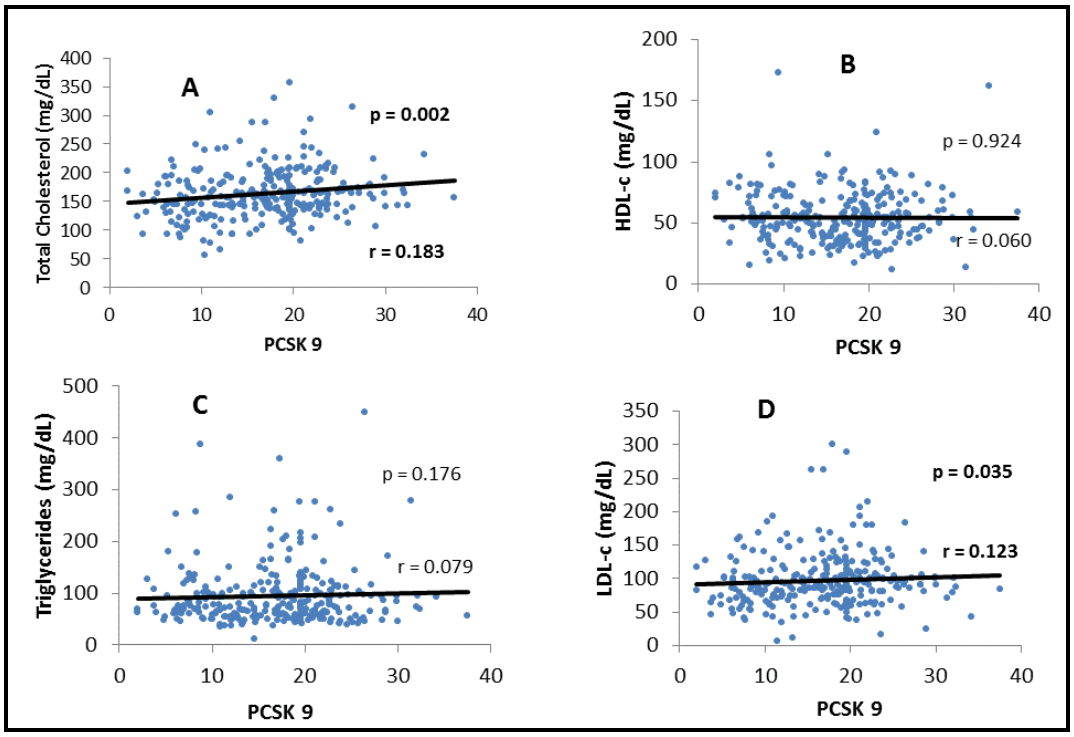

Figure 1. Correlation between PCSK9 and lipid profile in the study population

A: Correlation between PCSK9 and total cholesterol; B: Correlation between PCSK9 and HDL-c; C: Correlation between PCSK9 and triglycerides; D: Correlation between PCSK9 and LDL-c

Characteristics of the study population according to cardiovascular risk

A total of 264 (89.1 %) participants had a low CVR, whereas 32 (10.8 %) had a high CVR. Male, over 50 years, and obese candidates had higher CVR than their respective counterparts. Subjects with abnormal ABI had a high CVR. This risk was also observed in subjects with hypercholesterolemia, hypertriglyceridemia, low HDL-c, and high LDL-c. CVR was higher in subjects at 3 rd tertile of PCSK9 than in those at 1 st or 2 nd tertile (Figure 2).

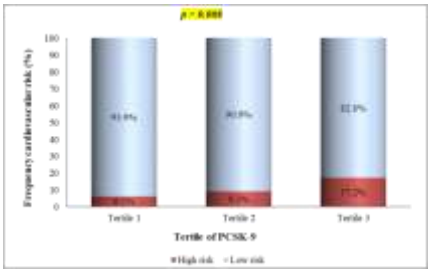

Figure 2. Evolution high-risk cardiovascular according to the tertile of PCSK-9 in the study population

A Correlation was found between PCSK9 level and Framingham cardiovascular event predilection score [r = 0.651] (Figure 3).

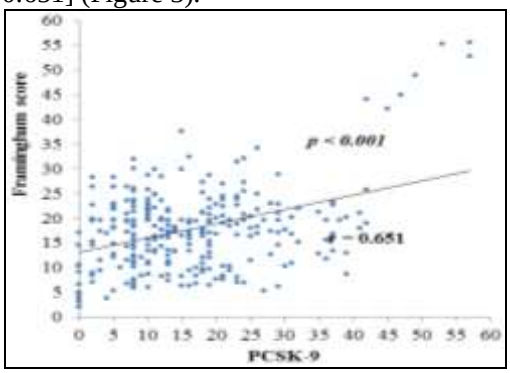

Figure 3. Correlation between PCSK-9 and Framingham score in the study population

In univariate analysis, females, age ≥ 50 years, obesity, hypercholesterolemia, low HDL-c, high LDL-c, hypertriglyceridemia, PCSK9 at 3rd tertile, and abnormal ABI emerged as determinants of high CVR in the study population (Table 4). In the multivariate analysis and after adjustment for all these variables, age ≥ 50 years, low HDL-c, high LDL-c, hypertriglyceridemia, and PCSK9 3rd tertile persisted as independent determinants of high CVR in this study population. The probability of having 10-year high CVR is 6 folds higher in subjects with elevated LDL-c; 5 folds higher in subjects ≥ 50 years. Hypertriglyceridaemia and low HDL-c each increased the likelihood of 10-year high CVR by 4 in the study population. Similarly, being at the 3rd tertile of PCSK9 increased CVR by a 4-fold [(p 0.038; 4.44 (1.55-8.18)].

Table 4. Determinants of high CVR in the study population

Variables	Univariate analysis		Multivariate analysis	
	P	OR (CI 95%)	P	aOR (CI 95%)
Sex				
Male		1		1
Female	0.015	2.79 (1.22-6.36)	0.779	1.23 (0.29-5.26)
Age (years)				
<50		1		1
≥50	<0.001	19.97 (8.52-46.85)	<0.001	4.84 (2.22-8.65)
Obesity				
No		1		1
Yes	0.001	4.30 (1.78-10.44)	0.790	1.22 (0.28-5.39)
Hypercholesterolemia				
No		1		1
Yes	<0.001	5.77 (2.65-12.60)	0.783	1.22 (0.30-4.86)

Variables	Univariate analysis		Multivariate analysis	
	P	OR (CI 95%)	P	aOR (CI 95%)
Low HDL-c				
No		1		1
Yes	<0.001	4.19 (1.97-8.91)	0.002	3.78 (1.97-6.32)
High LDL-c				
No		1		1
Yes	<0.001	12.29 (5.46-27.65)	<0.001	5.92 (2.78-9.56)
Hypertriglyceridemia				
Non		1		1
Oui	<0.001	6.29 (2.73-14.47)	0.024	4.24 (1.21-7.84)
PCSK 9				
Tertile 1		1		1
Tertile 2	0.435	1.53 (0.52-4.48)	0.064	1.27 (0.66-1.78)
Tertile 3	0.020	3.12 (1.20-8.45)	0.038	4.44 (1.55-8.18)
ABI				
Normal		1		1
Abnormal	0.006	2.99 (1.36-6.56)	0.469	1.53 (0.49-4.79)

ABI ankle-brachial index; CI, confidence interval; CVR, cardiovascular risk; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; OR, odds ratio; PCSK9, Proprotein convertase subtilisin kexin type 9

Discussion

The current study investigated the association of the plasma level of PCSK9 with the risk of cardiovascular events among the apparently healthy population of Kinshasa. The resulting data brought to light two major observations. First, there was a positive correlation between the plasma level PCSK9 and high CVR in the general population of Kinshasa, Democratic Republic of Congo. Second, after adjusting for traditional CVR factors, elevated plasma PCSK9 levels were associated with 4 folds the probability of high CVR in next ten years.

The first observation is similar to that made in two separate studies published from a Kenyan and another from a Chinese population. Paquette M *et al.* found an association between plasma PCSK9 levels and metabolic syndrome in the general sub-Saharan population (21). In the black hemodialysis population, PCSK9 levels have previously been shown to predict cardiovascular event risk (22). The Chinese study showed that increased PCSK9 levels were a significant risk factor for atherosclerosis and independently predicted future recurrent cardiovascular events in patients with familial hypercholesterolemia receiving standard lipid-lowering therapy (23).

Experimental and clinical studies help to understand the association between PCSK9 and stroke. Indeed, the plasma level of PCSK9 contributes directly to the progression of atherosclerosis by increasing the expression of proinflammatory genes, promoting cell apoptosis, and causing endothelial dysfunction (24). More than half of the subjects included in our study have an abnormal ABI which points to the presence of subclinical atherosclerosis. This observation suggests that elevated PCSK9 levels can accelerate the atherosclerosis process. Indeed, studies have shown that elevated PCSK9 levels are associated with the development of coronary, carotid, and femoral atherosclerosis (25). However, this association was not investigated in the present study.

In addition, CVR may be increased by the influence of known traditional CVR factors including hypertension. Animal studies have shown that PCSK9 slows down the movement of the epithelial sodium channel (ENaC) in the renal proximal tubule. As this channel is the pathway by which sodium is reabsorbed into the epithelial cell at the tubular apical membrane, this reabsorption is responsible for blood pressure regulation (26). By having a modulatory effect on the movement of this channel, PCSK9 would play an important role in the regulation of blood pressure and its repercussions on the cardiovascular outcome of patients. These effects of PCSK9 are, in addition to other known effects of PCSK9 in LDL-c

receptor metabolism, leading to dyslipidemia (7,27). In the present study, hypertriglyceridemia and low HDL-c levels were observed. These findings constitute the atherogenic dyslipidemia that increases CVR (28).

The second observation of the present study is suggestive of potential benefic effects with therapeutic implications. Lowering the level of PCSK9 would reduce lipid disorders and, especially atherogenic dyslipidemia (29). Furthermore, this treatment would induce the reduction of CVR. The benefit of the anti-PCSK9 treatment can be justified by the multiple roles of PCSK9 in cardiometabolic homeostasis by acting on the regulation of blood glucose, insulin, and homeostasis model assessment-estimated insulin resistance (HOMA-IR) index; modulation of HDL-c, ApoA-I, Apo B (29) and Lp (a) (30). Monoclonal inhibitors of PCSK9 (Evolucumab) lower plasma LDL-c and reduce CVR (31). Genetic analysis of PCSK9 shows that loss and gain of function variants are possible. Gain of function has been identified in Caucasians of African descent (4). The patients in our study were all of African descent and would have a gain of function of PCSK9 which would explain the correlation between this protein and the high CVR observed in our study.

The current study has some limitations. First, as a cross-sectional study, the level of PCSK9 and lipids was performed at the beginning of the study. Future investigations are required in this population, with a prospective study following those participants over a period of time or some years to follow up the occurrence of CVR. Second, PCSK9 activity would be more accurate if PCSK9 had been linked to LDL-c receptors.

Conclusion

Elevated plasma PCSK9 levels are associated with high CVR in the blood donor African sub-Saharan population included in the present study. Future studies are needed to determine genetic variants of PCSK9 in the sub-Saharan African population.

Declarations

Ethics approval and consent to participate

Patients were interviewed by trained physicians in ethics for this study. These patients were given the consent form developed in accordance with Helsinki recommendations. All rules of confidentiality and ethics were respected. Patients were recruited on the basis of free and informed consent obtained after careful reading of the consent form. All patients included in the present study gave written consent to participate in the study and to the publication of the study results.

The study protocol was submitted, in accordance with the Declaration of Helsinki, to the Ethics Committee of the Kinshasa School of Public Health for review and approval. After analysis of the protocol, the School of Public Health of Kinshasa in collaboration with the Faculty of Medicine of the University of Kinshasa, in the Democratic Republic of Congo, approved the protocol and gave the authorization for the realization of this study. The approved protocol is registered at N° ESP/CE/ 053/2016 of the registers of the School of Public Health of the University of Kinshasa.

Consent for publication

The authors of the present study used a consent form for publication specific to the Kinshasa University Hospital and approved by the School of Public Health of Kinshasa in collaboration with the Faculty of the Medicine / University of Kinshasa, in the Democratic Republic of Congo.

Availability of data and materials

The data and analyses carried out for this study are available from the corresponding author (francoiskajingulu@gmail.com) upon reasonable request.

Competing interests

The authors declare no conflict of interest.

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Author's contributions

F-P MK participated in protocol elaboration, data collection, and analysis, and wrote the manuscript; E KS designed the study, participated in data analysis, and reviewed the manuscript; A NN contributed to the study design, performed the statistical analysis, and reviewed the manuscript. J-R RM, P ME, and D-J KY designed the study and reviewed the manuscript. V MM, J BB, ALL, Y MN, and N MN reviewed the manuscript.

Contribution for author in memoriam †

François LEPIRA designed this research work passed away prior to the submission of this research paper.

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