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Short Communication

Plasma proprotein convertase subtilisin–kexin type 9 is predominantly related to intermediate density lipoproteins

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ABSTRACT

Objectives: Proprotein convertase subtilisin–kexin type 9 (PCSK9) is a key regulator of low density lipoprotein (LDL) receptor processing, but the PCSK9 pathway may also be implicated in the metabolism of triglyceride-rich lipoproteins. Here we determined the relationship of plasma PCSK9 with very low density lipoprotein (VLDL) and LDL subfractions.

Design and methods: The relationship of plasma PCSK9 (sandwich enzyme-linked immunosorbent assay) with 3 very low density lipoprotein (VLDL) and 3 low density lipoprotein (LDL) subfractions (nuclear magnetic resonance spectroscopy) was determined in 52 subjects (30 women).

Results: In age- and sex-adjusted analysis plasma PCSK9 was correlated positively with total cholesterol, non-high density lipoprotein cholesterol and LDL cholesterol ($r = 0.516$ to 0.547, all $p < 0.001$), as well as with triglycerides ($r = 0.286$, $p = 0.044$). PCSK9 was correlated with the VLDL particle concentration ($r = 0.336$, $p = 0.017$) and with the LDL particle concentration ($r = 0.362$, $p = 0.010$), but only the relationship with the LDL particle concentration remained significant in multivariable linear regression analysis. In an analysis which included the 3 LDL subfractions, PCSK9 was independently related to intermediate density lipoproteins (IDL) ($p < 0.001$), but not to other LDL subfractions.

Conclusions: This study suggests that plasma PCSK9 predominantly relates to IDL, a triglyceride-rich LDL subfraction. The PCSK9 pathway may affect plasma triglycerides via effects on the metabolism of triglyceride-rich LDL particles.

Introduction

Proprotein convertase subtilisin–kexin type 9 (PCSK9) is a liver-derived secreted protease which has been identified as a key regulator of low density lipoprotein (LDL) receptor processing [1]. PCSK9 binds to the extracellular domain of the LDL receptor (LDLR), a process which directs the LDLR towards intracellular degradation, and results in increased LDL cholesterol [1]. Inter-individual differences in plasma PCSK9 are probably physiologically relevant in view of the observation that higher plasma PCSK9 levels confer a lower fractional catabolic rate of apolipoprotein B (apoB), an abundant protein constituent of LDL and very low density lipoproteins (VLDL) [2]. Furthermore, genetically determined lower PCSK9 levels confer decreased cardiovascular risk [1]. As a consequence, intense effort is currently being paid to the development of PCSK9 inhibiting drugs aimed at reducing cardiovascular risk by lowering apoB-containing lipoprotein levels [3].

In agreement with the importance of the PCSK9 system for LDL metabolism and cholesterol homeostasis, a positive relationship of plasma total cholesterol, LDL cholesterol and apoB levels with plasma PCSK9 has been repeatedly demonstrated [2,4–6]. Interestingly, the PCSK9 pathway may also be involved in the metabolism of triglyceride-rich lipoproteins. Postprandial hypertriglyceridemia is blunted in *Pcsk9* knockout mice, whereas *Pcsk9* overexpression stimulates hepatic VLDL output [7,8]. In humans, plasma triglycerides have been shown to be correlated positively with PCSK9 levels [4–6], whereas the drop in the VLDL particle concentration in response to fenofibrate administration is related to a decrease in plasma PCSK9 [9]. Of further relevance, recent data support the possibility that not all circulating PCSK9 is present in its free form, but is in part complexed to apoB-containing lipoproteins [10]. These particles are highly heterogeneous in size, structure and composition [11,12], making it relevant to determine the relationship of PCSK9 with VLDL and LDL subfractions. In view of the possible role of the PCSK9 pathway in triglyceride metabolism [7–9], we questioned whether plasma PCSK9 would be particularly related to triglyceride-rich apoB-containing lipoproteins.

The present study was, therefore, set out to disclose the extent to which plasma PCSK9 relates to various VLDL and LDL subfractions, measured by nuclear magnetic resonance (NMR) spectroscopy.

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Materials and methods

Subjects

The study was approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands. Caucasian participants (aged > 18 years) were recruited by advertisement, and provided written informed consent. Previously diagnosed diabetes mellitus, hypertension, clinically manifest cardiovascular disease, renal disease, thyroid disorders, liver disease, current smoking, pregnancy were exclusion criteria. Current smokers, subjects who used >3 alcoholic drinks daily and subjects who used any medication on a regular basis (except for oral contraceptives) were also excluded, as were subjects with a positive family history of dyslipidemia or premature cardiovascular disease.

Physical examination did not reveal pulmonary or cardiac abnormalities. Body mass index was calculated as weight (kg) divided by height (m) squared. The study subjects were evaluated between 0800 and 1000 h after an overnight fast.

Laboratory analyses

EDTA-anticoagulated plasma samples were stored at −80 °C until analysis. Glucose was measured shortly after blood collection. PCSK9 was measured with a sandwich enzyme-linked immunosorbent assay using a non-tagged recombinant PCSK9 produced in HEK-293 cells as internal standard [3]. Plasma lipids and lipoproteins were assayed by routine enzymatic methods. LDL cholesterol was calculated using the Friedewald formula. ApoB was assayed by immunoturbidimetry. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particle profiles were measured by nuclear magnetic resonance (NMR) spectroscopy with the LipoProfile-3 algorithm (LipoScience Inc., Raleigh, North Carolina, USA) [11]. Lipoprotein particle subclasses (expressed in nmol/L) were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Diameter range estimates were: large VLDL (>60 nm (including chylomicrons, if present)), medium VLDL (35 to 60 nm) and small VLDL (27 to 35 nm) for VLDL, and IDL (23 to 27 nm), large LDL (21.2 to 23 nm) and small LDL (18 to 21.2 nm) for LDL. The VLDL and LDL particle concentrations were calculated as the sum of these VLDL and LDL subclasses, respectively. The analyses of lipids, lipoproteins, apoB, PCSK9, and VLDL and LDL subfractions were each performed in one run. The intra-assay coefficients of variation (CVs) of lipids, lipoproteins and apoB were <4%; the intra-assay CV of PCSK9 was <6%; and the intra-assay CV of VLDL and LDL subfraction measurement was < 3%.

Statistical analysis

Continuous variables are expressed as medians (interquartile ranges). Since triglycerides and several VLDL and LDL subfraction characteristics were not normally distributed, these variables were logarithmically transformed for correlation analyses. Partial correlation coefficients were calculated taking age and sex into account. Multivariable linear regression analyses with a subsequent backward elimination procedure were carried out to disclose the independent relationship of PCSK9 with the VLDL and the LDL particle concentrations, as well as with LDL subfractions. The level of significance was set at two-sided p-values < 0.05.

Results

Fifty two predominantly middle-aged subjects (30 women) participated in this study. Their clinical characteristics, plasma PCSK9 levels, (apo)lipoproteins and lipoprotein subfractions are shown in Table 1. One pre-menopausal and two post-menopausal women used estrogens.

Table 1
Clinical characteristics, proprotein convertase subtilin–kexin type 9, (apo)lipoproteins and lipoprotein subfraction characteristics in 52 subjects.

Age (years)	52 (46–62)
Sex (men/women)	22/30
Systolic blood pressure (mm Hg)	128 (117–142)
Diastolic blood pressure (mm Hg)	81 (72–88)
BMI (kg/m^2)	25.1 (23.5–27.3)
Plasma glucose (mmol/L)	5.6 (5.2–6.1)
PCSK9 (μg/L)	152 (112–190)
Total cholesterol (mmol/L)	5.73 (5.06–6.45)
Non-HDL cholesterol (mmol/L)	4.06 (3.66–5.00)
LDL cholesterol (mmol/L)	3.54 (3.01–4.20)
HDL cholesterol (mmol/L)	1.50 (1.20–1.76)
Triglycerides (mmol/L)	1.37 (0.89–1.88)
ApoB (g/L)	0.91 (0.77–1.14)
VLDL particle concentration (nmol/L)	61.5 (51.6–103.0)
Large VLDL (nmol/L)	3.5 (2.0–8.1)
Medium VLDL (nmol/L)	25.1 (12.9–42.8)
Small VLDL (nmol/L)	35.5 (20.5–45.6)
LDL particle concentration (nmol/L)	1161 (956–1379)
IDL (nmol/L)	198 (168–280)
Large LDL (nmol/L)	522 (423–647)
Small LDL (nmol/L)	363 (247–734)
VLDL particle size (nm)	44.3 (41.8–52.3)
LDL particle size (nm)	21.3 (20.9–21.5)

Data are numbers and median (interquartile ranges). ApoB: apolipoprotein B; BMI: body mass index; HDL: high density lipoproteins; IDL: intermediate density lipoproteins; LDL: low density lipoproteins; PCSK9: proprotein convertase subtilin–kexin type 9; and VLDL: very low density lipoproteins.

Plasma PCSK9 was not only correlated positively with total cholesterol, non-HDL cholesterol, LDL cholesterol and apoB, but also with triglycerides (Table 2). PCSK9 was unrelated to HDL cholesterol ($r = 0.063$, $p = 0.66$). Furthermore, plasma PCSK9 was correlated positively with both the VLDL and the LDL particle concentrations (Table 2). Of the VLDL subfractions the relationship with medium VLDL reached significance in age- and sex-adjusted analysis, whereas of the LDL subfractions, the relationship with IDL was significant (Table 2). Besides strong positive correlations between plasma total cholesterol, LDL cholesterol, non-HDL cholesterol and apoB, these (apo)lipoprotein variables were also correlated positively with the VLDL particle concentration, large VLDL and medium VLDL, as well as with the LDL particle concentration, IDL and small LDL (Table 2). Modest or non-significant relationships of plasma total cholesterol, LDL cholesterol, non-HDL cholesterol and apoB with large VLDL, small VLDL and large LDL were observed. Plasma triglycerides were correlated positively with non-HDL cholesterol, the VLDL particle concentration, large VLDL and medium VLDL as well as with the LDL particle concentration, IDL and small LDL. Plasma triglycerides were correlated inversely with large LDL (Table 2). In an analyses in which 3 women using estrogens were excluded ($n = 49$), PCSK9 was also positively correlated with the VLDL particle concentration ($r = 0.333$, $p = 0.022$) and with medium VLDL ($r = 0.302$, $p = 0.039$), as well as with the LDL particle concentration ($r = 0.373$, $p = 0.010$), IDL ($r = 0.532$, $p < 0.001$) and with small LDL ($r = 0.329$, $p = 0.024$).

We next determined the strength of the relationship of PCSK9 with VLDL and LDL particle characteristics. In age- and sex-adjusted multivariable linear regression analysis, PCSK9 was independently related to the LDL particle concentration ($p = 0.010$), but not to the VLDL particle concentration ($p = 0.25$). Subsequent age- and sex-adjusted analysis now including the 3 LDL subfractions demonstrated that PCSK9 was only independently related to IDL ($p = 0.002$), but not to large LDL ($p = 0.79$) and to small LDL ($p = 0.89$).

Discussion

The present study demonstrates that plasma PCSK9 is correlated positively with the VLDL and the LDL particle concentration in separate