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Association between plasma PCSK9 and gamma-glutamyl transferase levels in diabetic patients

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ABSTRACT

Background: Proprotein convertase subtilisin kexin type 9 (PCSK9) is a secreted proprotein convertase acting as a natural inhibitor of the low-density lipoprotein (LDL) receptor. Here, we prospectively investigated the relationship between the circulating levels of PCSK9 and metabolic parameters in 117 diabetic patients.
Results: Plasma PCSK9 level was significantly higher in type 2 than in type 1 diabetes ($P = 0.04$), in diabetic patients under statins ($P < 10^{-4}$) and in those with macrovascular complications ($P = 0.002$). Univariable regression analysis revealed that plasma PCSK9 level correlated positively with age ($P = 0.003$), body mass index ($P = 0.04$), systolic blood pressure (SBP) ($P = 0.01$), gamma-glutamyl transferase (GGT) levels ($P = 0.0002$) and statin treatment ($P = 0.001$). In a multivariable linear regression analysis, PCSK9 correlated positively with GGT level ($\beta = 21.91$, $P = 0.0019$) after adjustment for gender, age, type of diabetes, statin treatment, BMI, SBP and HbA1c.
Conclusion: PCSK9 level was independently associated with GGT level in diabetic patients, suggesting potential interaction between PCSK9 and liver function.

1. Introduction

PCSK9 (proprotein convertase subtilisin kexin type 9) is the ninth member of the proprotein convertase family. PCSK9 plays a key role in the regulation of cholesterol homeostasis by inhibiting the low-density lipoprotein (LDL) receptor pathway [1]. Recently, several data have emphasized a possible link between PCSK9 and glucose homeostasis. We and others demonstrated that insulin induces hepatic PCSK9 expression *in vivo* in mice and rats and *in vitro* in human immortalized hepatocytes [2,3]. The impact of PCSK9-deficiency on glucose metabolism in mice remains a controversial issue. Despite an exhaustive characterization both *ex-vivo* on primary cultured pancreatic islets and *in vivo* on whole-body animal, we failed to detect any alteration of glucose homeostasis in PCSK9-deficient mice [4]. In contrast, Mbikay et al. retrieved an altered insulin secretion and an impaired glucose tolerance in PCSK9-deficient mice [5]. Circulating PCSK9 level was found to be positively associated with both the fasting plasma glucose and insulin concentration and the HOMA index [6–8], an indicator of insulin resistance. In addition, plasma levels of PCSK9 were found to be significantly higher in diabetic *vs* non-diabetic subjects in the Dallas Heart Study [8].

Here, we explored the relationship between plasma PCSK9 level and several metabolic parameters in a group of both type 1 and type 2 diabetic patients.

2. Material and methods

2.1. Study population

Overnight fasting blood samples were prospectively obtained from 117 type 1 and type 2 diabetic patients who were routinely hospitalized during a day for their annual medical evaluation in the Department of Endocrinology, University Hospital, Nantes between January 2009 and August 2009. Sixty-seven patients were under statins. The patients were neither on fibrates, ezetimibe nor niacin. The study was approved by the local ethical committee of Nantes University Hospital, and all patients gave their written informed consent according to the French Legislation.

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2.2. Blood sampling and PCSK9 ELISA

Fasting plasma glucose was determined by the glucose oxidase method (Glucose HK, Roche Diagnostics, Meylan, France). HbA1c was measured by high performance liquid chromatography (TOSOH, A1C2.2-G5). Plasma total cholesterol, triglycerides (TG), HDL-cholesterol (HDL-C), ApoB, creatinine and liver enzymes were measured in the accredited biochemical laboratory of Nantes University Hospital using routine clinical methods. LDL-C was calculated using the Friedewald equation. For plasma PCSK9 dosage, the blood was collected in an EDTA tube, maintained at 4 °C until plasma and serum were separated, aliquoted and stored at −80 °C. PCSK9 levels in plasma were assayed in duplicates using a commercially available quantitative sandwich ELISA assay following the manufacturer instructions (Circulex CY-8079, CycLex Co, Nagano, Japan).

2.3. Statistical analysis

Continuous variables are expressed as medians, minimum, maximum, quartiles and categorical variables as percent and count. Mann–Whitney's test was used for comparison of clinical parameters between two groups. Categorical variables were compared by chi-square test. Spearman's correlation was used between plasma LDL-C levels and PCSK9 concentrations. Linear univariable and multivariable regression analyses were performed to determine the relationship between metabolic parameters and PCSK9. A P-value ≤ 0.05 was considered statistically significant. Statistical analysis was performed with SAS for Windows version 9.1 software.

3. Results

Clinical and biochemical characteristics of the patients are listed in Supplemental Table 1. Plasma PCSK9 concentrations ranged from 96 to 758 ng/ml (median: 305.5 ng/ml).

We compared plasma PCSK9 levels and clinical characteristics of the diabetes (Supplemental Table 2). Plasma PCSK9 levels were significantly higher in type 2 than in type 1 diabetic patients (+17%; $P = 0.04$). Circulating PCSK9 levels were also significantly increased in patients under statin treatment (+32%; $P < 10^{-4}$), in those with macrovascular complications (+23%; $P = 0.002$) and in those with micro- or macro-albuminuria (+24%, $P = 0.004$). Gender, use of insulin treatment, duration of diabetes and presence of elevated liver enzymes (aspartate [AST], alanine [ALT] aminotransferases and/or gamma-glutamyl transferase [GGT] ≥ 1.5 N) were not related to plasma PCSK9 levels in diabetic patients.

Previous reports demonstrated that plasma PCSK9 concentrations correlate with LDL-C in non-diabetic subjects [6–9]. When considering all patients with or without statins, no correlation was observed using spearman's analysis ($r = -0.011$, $P = 0.9$). However, we observed that PCSK9 was still positively associated with LDL-C levels in diabetic patients without statins ($r = 0.251$, $P = 0.05$). Since statin treatment increased PCSK9 and concomitantly decreased LDL-C levels (median: 78 *vs* 102 mg/dl, $P < 10^{-3}$), this correlation was lost in the subgroup of diabetic subjects under statins ($r = 0.08$, $P = 0.59$).

To investigate the association of PCSK9 with other metabolic parameters, we performed univariable regression analysis (Table 1). Circulating PCSK9 levels were significantly associated with age ($\beta = 2.5$, $P = 0.003$), BMI ($\beta = 3.2$, $P = 0.04$), systolic blood pressure ($\beta = 2.2$, $P = 0.01$) and GGT ($\beta = 25.8$, $P = 0.0002$). There was no significant association between PCSK9 concentrations and both AST and ALT levels. Plasma PCSK9 concentrations were also positively associated with statin treatment ($\beta = 79.1$, $P = 0.001$). Concerning glucose homeostasis, there was a tendency for a positive association between PCSK9 and HbA1c ($\beta = 17.4$, $P = 0.08$). Finally, we found no correlation between plasma PCSK9 and TG levels.

Table 1
Univariable linear regression.

Variable	β	95% CI		P-value
Gender				0.22
Female	1			
Male	−31.0	−80.7	18.8	
Age	2.5	0.8	4.1	**0.003**
Type of diabetes				**0.01**
Type 2	1			
Type 1	−59.4	−106.9	−12.0	
Statin treatment	79.1	33.0	125.2	**0.001**
BMI	3.2	0.10	6.4	**0.04**
HbA1c	17.4	−2.3	37.1	0.08
SBP	2.2	0.5	4.0	**0.01**
DBP	1.9	−0.8	4.6	0.17
TG	0.1	−0.1	0.3	0.25
AST	47.5	−67.4	162.3	0.41
ALT	88.4	−15.4	192.3	0.09
GGT	25.8	12.5	39.1	**0.0002**

Bold values indicate statistical significance to improve the readibility of the table.

In a multiple linear regression model, GGT only remained significantly associated with plasma PCSK9 levels ($\beta = 21.9$, $P = 0.0019$) after adjustment for gender, age, type of diabetes, statin treatment, BMI, systolic blood pressure and HbA1c (Table 2). There was also a tendency for an independent positive association between PCSK9 and statin treatment ($\beta = 51.9$, $P = 0.06$).

4. Discussion

The major finding of our study is that circulating PCSK9 concentrations was positively and independently associated with plasma GGT levels in diabetic patients. This association could not be explained by the hepatotoxicity of statins, since it persisted after adjustment for statin treatment. GGT is a sensitive indicator of liver dysfunction, as seen in insulin resistance and non-alcoholic fatty liver disease [10]. A weak but statistically significant correlation between plasma PCSK9 levels and hepatic TG content determined by proton magnetic spectroscopy was previously found in 2027 participants of the Dallas Heart Study [8]. GGT has emerged as an independent predictor of cardiovascular disease and mortality [11,12], suggesting that GGT itself can promote the development of atherosclerosis. Although the underlying molecular mechanisms sustaining this potential proatherogenic activity of GGT remain unclear, it has been suggested that some GGT protein are carried by LDL particles [13]. The association between PCSK9 and GGT remained statistically significant even when the LDL-C was used as a covariable, instead of statin treatment, in the multivariable regression analysis ($\beta = 22.56$, $P = 0.002$), demonstrating that the link between PCSK9 and GGT occurs independently of the LDL-C.

Table 2
Multivariable linear regression.

Variable	β	95% CI		P-value
Gender	−38.4	−87.8	11.0	0.13
Age	0.4	−1.6	2.5	0.69
Type of diabetes (DT1)	−11.5	−71.4	48.3	0.70
Statin treatment	51.9	−1.5	105.4	0.06
BMI	−0.1	−4.0	3.8	0.97
SBP	1.2	−0.7	3.1	0.22
HbA1c	9.7	−8.8	28.2	0.30
GGT	21.9	8.3	35.5	**0.0019**

As observed in the cohorts of non-diabetic patients [6–9], we confirmed that plasma PCSK9 levels remain positively associated with LDL-C in diabetic patients without statins [14]. The increase of PCSK9 levels by statins probably sustains the increase of PCSK9 concentrations in type 2 compared to type 1 diabetics, and in those with macrovascular complications. The association between PCSK9 and LDL-C is abolished in patients under statin therapy, thereby limiting the scope of PCSK9 as a biomarker of lipid metabolism in clinical practice. No significant association between plasma PCSK9 and TG was found in diabetic patients in both univariable and multivariable regression analysis, in contrast of what was observed in larger cohorts of non-diabetic patients [6–8].

An intriguing hypothesis is that PCSK9 may affect glucose homeostasis, since plasma PCSK9 concentrations have been found to be associated with both fasting plasma glucose and HOMA in non-diabetic subjects [6–8]. A non-significant tendency ($P = 0.08$) for a positive association between HbA1c and PCSK9 levels was found in univariable regression analysis, but it did not persist in multivariable analysis.

A significant positive association between systolic, but not diastolic, blood pressure and PCSK9 concentrations in univariable regression analysis was also found. Previous correlations between both systolic and diastolic blood pressure and circulating PCSK9 levels were described in non-diabetic subjects [8]. In addition, there were fewer hypertensive subjects among Afro-Americans carriers of non-secreted loss-of-function C679X and Y142X than among non-carriers in the Atherosclerosis Risk in Communities Study [15]. Circulating PCSK9 levels were found to be significantly higher in diabetic patients with micro- or macro-albuminuria compared to normoalbuminuric subjects. PCSK9 is highly expressed in the kidney and further studies are warranted to unravel the functional role of PCSK9 in this organ.

Finally, our study had certain limitations. The small number of the subjects limited the ability to detect weak correlations in both univariable and multivariable analysis. Accurate assessments of the hepatic TG content and the histological grade of fatty liver are lacking to investigate the association between PCSK9 and liver steatosis.

In summary, we observed that the plasma GGT level is independently associated with PCSK9 concentration in diabetic patients using multivariable analysis. This interesting new finding needs to be confirmed in larger cohorts.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.04.015.

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