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Proprotein Convertase Subtilisin/ Kexin type 9 affects insulin but not lipid metabolism in cystic fibrosis

Abstract

Purpose: Cystic Fibrosis (CF) is the most common genetic disorder and, with improved survival, glucose abnormalities have emerged as a major comorbidity. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a regulator of plasma LDL-cholesterol homeostasis, is associated with lipid and glucose metabolism in healthy individuals. Here we report on the link between PCSK9 and markers of metabolism in CF.

Methods: Cross-sectional analysis was performed on CF patients (≥ 18 years, N=94) from the Montreal Cohort, without known diabetes, and on healthy individuals (N=19). The levels of PCSK9 and lipid markers were quantified and all subjects underwent a 2 h oral glucose tolerance test.

Results: No significant differences in PCSK9 levels were found between healthy individuals and patients with CF, or between the groups with different degrees of glucose tolerance. No association was found between PCSK9 and markers of lipid metabolism; however, a positive correlation was found between PCSK9 and total insulin secretion and a negative one with insulin sensitivity in CF patients who had normal glucose tolerance.

Conclusion: Circulating levels of PCSK9 in the CF population are comparable to those in the healthy population. There are no associations between PCSK9 levels and either glucose or lipid homeostasis parameters. Nevertheless, a statistically significant link was observed between PCSK9 and markers of insulin homeostasis, solely in CF patients who presented normal glucose tolerance. Further exploration of the relationship between PCSK9 and insulin homeostasis in CF patients with normal glucose tolerance is warranted.

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Cystic fibrosis (CF) is one of the most common autosomal recessive disorders in Caucasians. CF is a multisystemic disease that affects the gastrointestinal, pulmonary and reproductive systems. CF chronic lung disease is associated with respiratory infection and inflammation, which, in turn, lead to severe lung damage and eventually to respiratory failure [1]. In the past 20 years, the life expectancy of CF patients has improved to such an extent that they now display new challenging complications. CF-related diabetes (CFRD), caused by a significant decrease in insulin levels, is one of the most common complications [2,3] and is associated with accelerated clinical deterioration (loss of weight and lung function) [4]. Numerous CF clinics now report an important prevalence of overweight and obesity in their populations (23% in the United-States and 18% in Canada) [5-7]. Long-term studies are important to determine if this excess weight eventually leads to harmful health complications in these patients.

Predominantly synthesized in the liver, the proprotein convertase subtilisin/kexin type 9 (PCSK9) is a crucial player in the regulation of plasma cholesterol homeostasis [8,9]. In addition, numerous studies have associated increased circulating PCSK9 levels with higher concentrations of metabolic factors related to glucose and insulin homeostasis in healthy individuals [10]. Furthermore, the presence of metabolic syndrome or obesity in non-CF patients has been found to be associated with an increase in circulating PCSK9 levels [11].

No studies have yet explored PCSK9 levels in an adult population of CF patients, especially in the diabetic sub-population [12,13]. We hypothesized that patients with CF may display higher PCSK9 levels than the normal population, possibly due to increased inflammation [9]. An association exists between the levels of circulating PCSK9 and the markers of lipid, glucose and insulin homeostasis [14,10]; hence, we assessed plasma levels of PCSK9 at baseline in patients with CF and then compared these values with those of healthy subjects as well as between glucose tolerance categories. Finally, we studied the association between PCSK9 and biomarkers of lipid or glucose metabolism, such as total insulin secretion and sensitivity.

Methods

Subjects

This cross-sectional study includes patients with CF (≥ 18 years old) from the Montreal Cystic Fibrosis Cohort (MCFC): an ongoing systematic program that screens for glucose abnormalities, including CFRD. Age and body mass

index (BMI)-matched healthy controls were recruited at the Institut de Recherches Cliniques de Montréal (IRCM). Inclusion and exclusion criteria of the MCFC were described previously [15]. None of the participants included in this study (healthy and CF patients) was taking insulin, antihyperglycemic medications or statins. Patients with *de novo* CFRD all underwent a second Oral Glucose Tolerance Test (OGTT) to confirm the diagnosis and, if positive, were excluded from follow-up. The Centre Hospitalier de l'Université de Montréal (CHUM) as well as the IRCM approved the protocol and informed consent was obtained from all participants included in the study.

Clinical data

On the day of the OGTT, lung function was measured by spirometer using predicted forced expiratory volume in one second (FEV₁%) (Medgraphic 1870, St. Paul, MN, USA) as a variable. Presence or absence of pancreatic insufficiency was defined by current enzyme supplementation. Body weight was measured using an electronic scale (Tanita Corporation, Arlington Heights, IL, USA) and standing height by a wall stadiometer. These values were necessary to determine the BMI, which is the weight in kilograms divided by height in square meter (kg/m²). Medical files were consulted to obtain genotype status.

Biochemical dosages

From fasting blood samples, high-density lipoprotein (HDL)-cholesterol, triglyceride and total cholesterol were measured by enzymatic reaction (ADVIA1650, Bayer Health Care Diagnostics). Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedewald equation [16].

Oral glucose tolerance test

After an overnight fast, CF patients underwent a 2-h OGTT. OGTT was performed according to international guidelines [17] and classified as described in previous studies [18]. The index proposed by Stumvoll *et al.*, as well as insulin area under the curve, were used to evaluate insulin sensitivity and secretion, respectively [19]. The five patients without values for assessing insulin sensitivity were excluded from the PCSK9/insulin sensitivity association analysis.

PCSK9 levels

PCSK9 concentrations were measured as previously described using a homemade ELISA [20]. A standard curve was

established using a conditioned medium containing recombinant human PCSK9, as described previously [20].

Statistical methods

Data are represented as the mean $\pm$ SD. Categorical variables are presented with absolute (n) and relative (%) frequencies. Statistical significance of the demographic and bioclinical data between the controls and patients with CF was assessed by non-parametric Mann-Whitney *U* and Pearson's Chi-Squared tests for genotype, pancreatic and sex status comparison. The software GraphPad Prism for Windows was used for calculating the area under the curve. Statistical significance in PCSK9 levels between glucose tolerance and BMI categories was determined by 1-way ANOVA repeated measure. Simple

linear regression was performed to investigate the relationship between PCSK9 and clinical parameters (age, insulin sensitivity and insulin secretion). Statistical analyses were undertaken with SPSS 20.0 (SPSS Inc., Chicago, IL, USA). A probability value of $P \leq 0.05$ was considered statistically significant.

Results

PCSK9 in patients with CF versus healthy subjects

A total of 19 healthy subjects (42.1% men) and 94 patients with CF (50% men) with a mean age of 26.8 ± 4.4 and 24.4 ± 6.1 years (P value = 0.018), respectively, were included in the study. There was no difference in mean BMI and PCSK9

FIGURE 1. Relationship between PCSK9 levels and the AUC for total insulin secretion in A) NGT (n=38) and C) INDET/IGT/CFRD (n=56) patients with CF. Relationship between PCSK9 levels and the index of insulin sensitivity in B) NGT (n=38) and D) INDET/IGT/CFRD (n=51) patients with CF. NGT, normal glucose tolerance; INDET, indeterminate glucose tolerance; IGT, impaired glucose tolerance; CFRD, cystic fibrosis related diabetes. P values < 0.05 represent the Spearman *r* correlation test.

TABLE 1. Physical and biochemical cohort characteristic of healthy adults (n=19) and adults with CF (n=94).

	Control	CF
N	19	94
Age (years)	26.8 ± 4.4	24.4 ± 6.1*
Sex (% Men)	42.1	50.0
BMI (kg/m ²)	22.6 ± 1.9	21.9 ± 2.8
FEV ₁ (%)	-	76.4 ± 21.0
F508del-Homozygote/Heterozygote/Other	-	43/39/12
Exocrine Pancreatic Insufficiency (% Yes)	-	80.4
PCSK9 Serum levels (ng/mL)	99.4 ± 31.0	93.3 ± 31.3

BMI, body mass index; FEV₁, forced expiratory volume in 1 second; PCSK9, proprotein convertase subtilisin/kexin type 9. Mean and Standard deviations are shown (±). *: P values < 0.05 represent the Mann-Whitney Student's T-Test between control and CF.

TABLE 2. Comparison of metabolic values for glucose tolerance groups in adult patients with CF

	NGT	INDET	IGT	CFRD	P value
N	38	11	32	13	
PCSK9 (ng/mL)	97.1 ± 38.3	103.2 ± 19.7	83.0 ± 25.6	98.7 ± 25.2	0.137
Triglyceride (mmol/L)	0.98 ± 0.39	1.16 ± 0.66	1.23 ± 0.65	1.36 ± 1.27	0.307
Cholesterol (mmol/L)	3.6 ± 1.0	3.3 ± 0.9	3.5 ± 0.8	3.3 ± 0.8	0.760
HDL-Cholesterol (mmol/L)	1.2 ± 0.3	1.1 ± 0.2	1.2 ± 0.3	1.3 ± 0.4	0.734
LDL-Cholesterol (mmol/L)	1.9 ± 0.8	1.7 ± 0.9	1.7 ± 0.6	1.5 ± 0.5	0.230

NGT, normal glucose tolerance (n=38); INDET, indeterminate glucose tolerance (n=11); IGT, impaired glucose tolerance (n=32); CFRD, cystic fibrosis related diabetes (n=13). PCSK9, proprotein convertase subtilisin/kexin type 9. LDL, low-density lipoprotein. HDL, high-density lipoprotein. One way ANOVA to determine the difference in triglyceride, cholesterol, LDL-cholesterol and PCSK9 levels between glucose tolerance groups. Mean and Standard deviations are shown (±).

serum levels between healthy controls and CF subjects (Table 1). Patients were then separately categorized based on their BMI as underweight (<18.5 kg/m²), normal (between 18.5 and 24.9 kg/m²) and overweight/obese (≥ 25 kg/m²). No statistical difference in circulating PCSK9 levels was observed between BMI groups (data not shown).

PCSK9 levels and markers of metabolism in CF

When patients were categorized based on their glucose tolerance status, our statistical analysis showed that there was no significant difference in PCSK9 levels between the groups. Also, the levels in markers of lipid metabolism were comparable among glucose tolerance categories (Table 2).

Furthermore, no association was found between these lipid markers and PCSK9 levels.

We next determined if there is a potential correlation between PCSK9 serum levels and insulin secretion as well as insulin sensitivity in patients with CF. The area under the curve (AUC) for total insulin secretion during the OGTT was positively associated with PCSK9 levels in patients who were NGT (Spearman $r=0.550$, P value ≤ 0.001, Figure 1A) but not in those who presented with abnormal glucose tolerance (INDET/IGT/CFRD, Figure 1C). Moreover, a negative association was also observed between the insulin sensitivity index and PCSK9 levels solely in patients who were NGT (Spearman $r=-0.492$, P value = 0.002, Figure 1B). Once more, no correlation was found between the insulin sensitivity index

and PCSK9 levels in the group of patients that have INDET/IGT/CFRD (Figure 1D).

Discussion

This is the first study that analyzes the relationship among circulating levels of PCSK9 and parameters of lipid and glucose metabolism in an adult population of CF patients. Patients with CF who display high levels of triglycerides have increased levels of total cholesterol, higher insulin secretion and a better pulmonary function than patients with normal triglycerides levels [21]. We observed that circulating levels of PCSK9, a metabolic protein that enhances the degradation of the LDL receptor and regulates LDL-cholesterol levels, were comparable between healthy individuals and CF patients. We did not find any association between PCSK9 levels and markers of lipid metabolism, such as cholesterol, triglycerides and LDL. Finally, our analysis showed that PCSK9 level is not a clinical indicator of nutritional status since we failed to detect a difference between underweight/normal patients and overweight/obese patients. Our observation suggests that PCSK9 is not responsible for changes in lipid metabolism in CF.

Besides its role in lipid metabolism, numerous studies have suggested that PCSK9 is a potential regulator of glucose metabolism. Studies in non-CF animal models showed that insulin increases the expression of PCSK9 [22,23]. In addition, mice deficient in PCSK9 had reduced insulin plasma levels primarily because of increased apoptotic pancreatic islets, thus reducing cell functions and content [24]. Previous non-CF studies in adult populations demonstrated that insulin sensitivity was negatively correlated with PCSK9 plasma levels [25,23]. It was plausible that PCSK9 might be involved in the regulation of glucose metabolism in CF. Our data show that PCSK9 levels were positively correlated with total insulin secretion, but only in patients with CF who presented NGT. Furthermore, we also detected a negative relationship between PCSK9 and insulin sensitivity but solely in CF patients with NGT. Categorization of the CF cohort according to the glucose tolerance status showed that PCSK9 levels were stable across the different groups, suggesting that, in adult CF patients, PCSK9 does not play a major role in glucose metabolism. This observation might not be surprising since recent epidemiological studies did not find an effect of the R46L loss-of-function mutation of human PCSK9 on glucose and insulin homeostasis, including type 2 diabetes risk [26]. Although it is possible that the observed relationship between PCSK9 and insulin sensitivity in CF patients with NGT is fortuitous, it is also possible that, in these CF patients, the link

between insulin resistance, hepatic steatosis and the circulating levels of PCSK9 is preserved [27,28] whereas this relationship might be lost in patients categorized as dysglycemic (INDET/IGT/CFRD). Further studies are necessary to identify the mechanism by which PCSK9 might influence insulin sensitivity in CF.

In the CF population, patients with and without CFRD all display significantly reduced insulin secretion [29]. There is mounting evidence that mutant CF Transmembrane Conductance regulator (CFTR), the CF disease-causing gene, is expressed in islet cells and is associated with abnormal cell function [30,31]. Although, it is one of the critical factors in the development of CFRD, whether this defect, by itself, is sufficient for the development of CFRD is unlikely. Since PCSK9 is expressed and modulates cell function [8,9,27], it is possible that it might modulate the impact of the mutant CFTR on islet function. This is a challenging question that will be difficult to answer by monitoring the circulating level of PCSK9 in patients.

Our study has several limitations. First, our study was a cross-sectional study in a homogenous French-Canadian CF patient population, so it is difficult to extrapolate the results to a less homogenous population. Second, we measured PCSK9 levels in patients with either pre-diabetes (IGT and INDET) or with newly diagnosed CFRD, but not with long term diabetes, so we cannot presume that circulating levels of PCSK9 would be identical in CFRD patients under treatment with insulin or other hypoglycemic medication. Finally, we have measured PCSK9 level only at the time of the OGTT, so we cannot assume that this level remains stable as the pulmonary and metabolic conditions of the patient evolve.

Our adult CF population, which includes newly diagnosed CFRD, displays levels of PCSK9 comparable to those of healthy individuals regardless of their glucose tolerance and nutritional status. There was no link between clinical markers of lipid or glucose metabolism and circulating levels of PCSK9. Interestingly, plasma PCSK9 is related to insulin (secretion and sensitivity) only in patients with CF that have NGT. Future mechanistic studies in animal models of CF are essential to better understand the potential role of PCSK9 in insulin homeostasis.

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