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Proprotein convertase subtilisin–kexin type 9 is elevated in proteinuric subjects: Relationship with lipoprotein response to antiproteinuric treatment

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ABSTRACT

Objective: LDL-receptor deficiency may provide a mechanism which contributes to atherogenic lipoprotein abnormalities in experimental nephrosis and in humans with glomerular proteinuria. The proprotein convertase subtilisin–kexin type 9 (PCSK9) pathway plays a key role in lipoprotein metabolism by promoting LDL-receptor degradation. We tested whether plasma PCSK9 is elevated in proteinuric states, and determined relationships of PCSK9 with lipoprotein responses to proteinuria reduction.
Methods: Thirty-nine kidney patients (e-GFR 61 ± 29 mL/min/1.73 m^2, proteinuria 1.9 [0.9–3.3] g/day; 19 on statin treatment) were studied during 2 randomized double-blind 6-week periods on either lisinopril (40 mg/day) and a regular sodium diet (194 ± 49 mmol Na+/day; baseline treatment) or lisinopril plus valsartan (320 mg/day) and a low sodium diet (102 ± 52 mmol Na^+/day; maximal treatment), and compared to age- and sex-matched controls. Maximal treatment decreased proteinuria to 0.5 [0.3–1.1] g/day ($P < 0.001$).
Results: Plasma PCSK9 was increased at baseline in proteinuric subjects (213 [161–314] vs. 143 [113–190] ug/L in controls, $P \leq 0.001$), irrespective of statin use, e-GFR and BMI. PCSK9 correlated with proteinuria at baseline ($R = 0.399$, $P = 0.018$) and at maximal antiproteinuric treatment ($R = 0.525$, $P = 0.001$), but did not decrease during proteinuria reduction ($P = 0.84$). Individual changes in total cholesterol ($R = 0.365$, $P = 0.024$), non-HDL cholesterol ($R = 0.333$, $P = 0.041$), and LDL cholesterol ($R = 0.346$, $P = 0.033$) were correlated positively with individual PCSK9 responses. PCSK9 at baseline independently predicted the total/HDL cholesterol ratio response to treatment ($P = 0.04$).
Conclusion: Plasma PCSK9 was elevated in proteinuria, predicted lipoprotein responses to proteinuria reduction but remained unchanged after proteinuria reduction. Inhibition of the PCSK9 pathway may provide a novel treatment strategy in proteinuric subjects.

1. Introduction

Dyslipidemia is regarded an inherent feature of overt glomerular proteinuria [1–3]. Accordingly, higher total cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol (collectively designated as non-HDL cholesterol) and triglycerides are common in proteinuric subjects [1–3], whereas decreased high density lipoprotein (HDL) cholesterol levels have been reported as well [2–4]. Hence, the total

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cholesterol/HDL cholesterol ratio, which is considered a main lipid risk factor in the general population [5], is elevated in proteinuric conditions [6]. Moreover, it is likely that such elevations in apolipoprotein B (apoB)-containing lipoproteins are pathogenetically involved in the increased cardiovascular risk encountered in proteinuric subjects [6,7]. In this regard it is relevant that antiproteinuric therapy improves at least in part the increases in apoB-containing lipoproteins attributable to proteinuria, even irrespective of treatment modality [2,3,6].

Despite intensive research, the complex mechanisms underlying the changes in lipoprotein metabolism in proteinuric states remain incompletely understood. Hypercholesterolemia consequent to hepatic LDL-receptor deficiency has been documented in experimental nephrosis [8]. In humans with non-diabetic nephrotic-range proteinuria, defective LDL apoB catabolism has been proposed to represent the primary metabolic abnormality [9,10]. However, using stable isotope techniques impaired VLDL apoB catabolism in combination with increased LDL apoB synthesis has been identified as the main metabolic abnormality in another study [11].

In the past few years it has increasingly been recognized that the proprotein convertase subtilisin–kexin type 9 (PCSK9) pathway plays a pivotal role in LDL-metabolism by modulating hepatic LDL-receptor expression [12,13]. PCSK9 is a secreted protease which binds to the extracellular domain of the LDL-receptor, where it acts as a chaperone that is able to target the LDL-receptor towards intracellular degradation, thereby preventing LDL-receptor recycling to the cell surface [13]. Indeed, a lower LDL apoB fractional catabolic rate is predicted by higher plasma PCSK9 levels in humans, suggesting that between-subject differences in circulating PCSK9 levels are clinically important [14]. Accordingly, apoB-containing lipoproteins levels are associated positively with the PCSK9 concentration [14–17]. Furthermore, gain-of-function mutations in PCSK9 are associated with hypercholesterolemia, whereas loss-of-function mutations relate to lower apoB levels and cardioprotection [13]. In view of diminished hepatic LDL-receptor expression in experimental nephrosis [8], and impaired LDL catabolism in humans with nephrotic range proteinuria [9,10], it is important to test whether the plasma PCSK9 level, as a measure of activation of the PCSK9 pathway, is increased in proteinuric states.

This study was initiated to determine whether plasma PCSK9 levels are elevated in humans with glomerular proteinuria compared to healthy subjects. We furthermore assessed relationships between lipoprotein responses during maximal antiproteinuric treatment and PCSK9 levels in proteinuric subjects.

2. Material and methods

2.1. Study subjects

The study population consisted of 39 proteinuric subjects and 39 healthy controls.

Inclusion criteria for proteinuric subjects were proteinuria >1 g/day during high dose angiotensin converting enzyme inhibition (ACEi), blood pressure >125/75 mmHg, creatinine clearance ≥30 mL/min, and age ≥18 years. All proteinuric subjects were of Caucasian race. Exclusion criteria were systolic blood pressure ≥180 mmHg, diastolic blood pressure ≥110 mmHg, diabetes mellitus (using World Health Organization criteria [18]), renovascular hypertension, decrease of creatinine clearance ≥6 mL/min in the preceding year, cardiovascular event in the previous 6 months, immunosuppressive treatment, regular use (>1 day/week) of non-steroidal anti-inflammatory drugs, and pregnancy. Renal diagnosis was focal segmental glomerulosclerosis ($n = 13$), immunoglobulin A nephropathy ($n = 13$), membranous nephropathy ($n = 7$), hypertensive nephropathy ($n = 2$) and other/inconclusive ($n = 4$).

Caucasian healthy subjects (aged ≥18 years) were recruited by advertisement and served as controls. Diabetes mellitus, hypertension, proteinuria, renal function or thyroid impairment, and pregnancy were exclusion criteria. None of these subjects used any medication except for oral contraceptives. Healthy subjects were individually matched with proteinuric subjects with respect to age (within 5 years) and sex.

2.2. Study protocol

The current study was a secondary analysis among participants from a previously published study [19]. This study was a prospective randomized double-blind, placebo-controlled cross-over trial in which the effects of angiotensin receptor blockade (ARB) and dietary sodium restriction on proteinuria and blood pressure were evaluated in subjects with stable proteinuric states.

All proteinuric subjects were enrolled in a run-in period of at least 6-weeks in which subjects received background treatment with an ACEi at maximum dose (lisinopril 40 mg/day) while stopping other renin-angiotensin-aldosterone-system blockers. Subjects were subsequently treated with combinations of placebo, ARB (valsartan 320 mg/day), regular sodium diet (mean intake 194 ± 49 mmol Na^+/day) and a low sodium diet (mean intake 102 ± 52 mmol Na^+/day) during four random 6-week study periods. Additional antihypertensive medication was allowed but kept stable during the study. To test the hypothesis of the present study we used ACEi combined with regular sodium diet as the baseline treatment period, and ACEi combined with ARB and low sodium diet, which was documented to result in maximal antiproteinuric treatment [19], as the intervention period. Plasma samples for measurement of PCSK9 and (apo)lipoproteins from both study periods were available in 39 out of the original 52 study subjects. Age, sex, proteinuria, e-GFR, blood pressure and BMI were not different between the 39 subjects included in the present report compared to the 13 subjects not currently studied (data not shown).

The study protocol was approved by the local ethical committee of the University Medical Center Groningen, The Netherlands, and conducted according to guideline for good clinical practice and declaration of Helsinki. Written informed consent was obtained from each subject.

2.3. Measurements and calculations

Proteinuric subjects visited the outpatient nephrology clinic at end of each 6-week treatment period for clinical assessment. Blood pressure was measured for 15 min at 1-min intervals by an automatic device (Dinamap, G.E. Medical Systems, Milwaukee, WI, USA), with subjects in a supine position. We used the mean of the last thee readings. Body mass index (BMI) was calculated by dividing body weight by height squared (kg/m^2). Subjects collected 24-h urine one day prior to their visit to the outpatient clinic. To correct for sampling errors urinary protein/creatinine excretion was calculated as the ratio of urinary protein excretion and creatinine excretion. Estimated glomerular filtration rate (e-GFR) was calculated using CKD-EPI formula [20]. Absolute treatment responses were calculated by subtracting baseline treatment values from values at maximal antiproteinuric treatment.

2.4. Laboratory analyses

Blood was obtained after an overnight fast, collected in EDTA-containing tubes (1.5 mg/mL) and placed immediately on ice. Plasma was obtained by centrifugation at 3000 G for 10 min at 4 °C. Plasma glucose was measured shortly after blood sampling (APEC