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Involvement of adropin and adropin-associated genes in metabolic abnormalities of hemodialysis patients

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ABSTRACT

Aims: We examined the involvement of plasma adropin and adropin-associated genes (*ENHO* and *RXRA*) in metabolic abnormalities of hemodialysis (HD) patients.
Main methods: Among 50 HD patients (27 males and 23 females, aged 65.2 ± 12.6 years, HD vintage 29.0, 3.9–157.0 months), there were 26 dyslipidemics and 25 type 2 diabetics. Age-matched healthy subjects ($n = 26$) served as controls. Adropin levels were determined using ELISA. Insulin resistance/sensitivity was assessed using the Homeostasis Model Assessment for Insulin Resistance and Quantitative Insulin Sensitivity Check Index. *ENHO* (rs2281997, rs72735260) and *RXRA* (rs10881578, rs10776909) were genotyped by HRM, *RXRA* rs749759 by PCR-RFLP. Circulating adropin, serum lipids, and insulin indices were compared between bearers of the minor allele of tested polymorphisms and major homozygotes (the dominant model of inheritance).
Key findings: HD patients showed lower circulating adropin concentration compared with controls. In dyslipidemic patients, plasma adropin was lower than that in non-dyslipidemics, but it was not significantly different in diabetics vs. non-diabetics or in patients with or without metabolic syndrome. Major homozygotes of *ENHO* rs2281997 seemed to have higher circulating adropin, whereas major homozygotes of *RXRA* (rs749759, rs10776909) showed lower levels. Major homozygotes of *ENHO* rs2281997 showed borderline lower insulin resistance compared with bearers of the minor allele.
Significance: In HD patients, lower plasma adropin concentration is associated with dyslipidemia. Major homozygosity of *RXRA* seems to have an opposite effect on plasma adropin compared with that of *ENHO* rs2281997.

1. Introduction

Adropin, a peptide hormone discovered in 2008, is involved in energy homeostasis and insulin resistance [1]. Obese mice showed decreased expression of the adropin transcript in the liver and lower plasma adropin concentrations [1,2]. Additionally, adropin knockout mice exhibited increased adiposity, fasting triglyceride (TG) levels, insulin resistance, liver steatosis, and a propensity for impaired glucose tolerance during diet-induced obesity [2]. In volunteers showing a wide range of body mass index (BMI), circulating adropin levels were also low in obesity, and markers of insulin resistance and dyslipidemia were associated with low plasma adropin [3].

The energy homeostasis-associated gene (*ENHO*) encodes adropin [1]. The adropin transcript is abundant in the liver [1]. The liver X receptor alpha gene (*LXRA*) regulates liver *ENHO* mRNA expression, and stimulation of *LXRA* suppresses hepatic *ENHO* expression [1]. The distribution of *LXRA* mRNA showed an expression pattern similar to that of the mRNA of the retinoid X receptor alpha gene (*RXRA*) [4]. LXRA and RXRA form a functional heterodimer in which RXRA is the active ligand-binding subunit. *LXRA* retinoid response requires the presence of RXRA, and *RXRA* synergistically increases *LXRA* responsiveness

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to retinoids [5]. Therefore, *RXRA* polymorphism may also be involved in adropin synthesis.

Diabetes mellitus (DM) is a frequent cause of end-stage renal disease (ESRD) requiring regular dialysis treatment. Among hemodialysis (HD) patients, up to 45% of subjects may suffer from DM [6]. Insulin resistance and dyslipidemia occur in HD patients concomitantly with DM [7] or independent of DM [8]. Metabolic disturbances in HD patients are significant contributors to morbidity and mortality due to close association with cardiovascular diseases [9]. An exploration of the novel potential regulators of these abnormalities is challenging because they may be targets for treatment of ESRD-associated pathology.

We evaluated whether the plasma adropin level and polymorphisms of *ENHO* and/or *RXRA* are associated with metabolic abnormalities (type 2 DM, insulin resistance, dyslipidemia and metabolic syndrome [MeS]) in HD subjects.

2. Patients and methods

2.1. Patients

Fifty HD patients were included in the present study (27 males and 23 females, aged 65.2 ± 12.6 years, HD vintage 29.0, 3.9–157.0 months). Detailed characteristics of all patients are shown in Table S1. Intentionally, in the HD group there were 25 type 2 DM patients with ESRD due to this disease and 25 patients without DM. Inclusion criteria were HD duration for at least 3 months, age over 18 years, and a stable general condition for at least one month before enrolment. Exclusion criteria covered pregnancy, neoplasm, protein-losing enteropathies, missing more dialysis sessions than one per month, acute illness within 3 months before enrolment, acute coronary syndrome and/or cerebral stroke in 6 months before the study commencement, decompensated DM, and treatment with statins or fibrates 6 weeks before commencement into the study.

All studied HD women except one were in the postmenopausal age (over 50 years old). One 28-year woman had secondary amenorrhea due to uremic state. HD men compared to HD women had significantly higher body mass and waist to hip ratio as well as lower serum HDL cholesterol concentration. However, both groups did not differ in prevalence of DM, dyslipidemia, and MeS. BMI and insulin resistance also did not differ HD men and HD women (Table S2).

Each patient was diagnosed for dyslipidemia according to the recommendations of the National Kidney Foundation/Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines [10]. In the entire HD group there were 26 dyslipidemic patients.

The HD group included 30 individuals with MeS that were diagnosed according to the International Diabetes Federation Worldwide Definition [11].

2.2. Controls

Controls were searched among subjects who declared good health and were taking no medication. At the first step, each potential candidate for enrolment was matched to one HD patient of the same gender and age ± 5 years. Eleven men and 26 women were initially enrolled. After taking a detailed medical history and performing a physical examination, only 4 men and 23 women could be still regarded as a healthy person. All these 27 subjects underwent laboratory testing designed for HD patients. Analysis of laboratory data revealed type 2 diabetes mellitus in one 66-year old man. Finally, 3 men and 23 women were enrolled as controls for our study. All control women were in the postmenopausal age (the youngest one was 61-year old).

2.3. Clinical and laboratory examinations

Anthropometric measures were taken in all study subjects. Blood samples were collected before midweek dialysis session for adropin, insulin and adropin-associated gene polymorphisms, and for routine biochemistry, including serum lipids.

The low-density lipoprotein (LDL) cholesterol concentration was calculated using the Friedewald formula, such that LDL cholesterol = total cholesterol (TC) – high-density lipoprotein (HDL) cholesterol – TG/5 [12]. Non-HDL cholesterol was the TC minus HDL cholesterol. Homeostasis model assessment–insulin resistance (HOMA-IR) was determined as [fasting plasma insulin (μIU/mL) × fasting plasma glucose (mmol/L)]/22.5 [13]. Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated as 1/[log fasting plasma insulin (nU/mL) + log fasting plasma glucose (mg/dL)] [14].

2.4. Laboratory methods

Adropin was determined by enzyme-linked immunosorbent assay (Cusabio, Wuhan, China). All measurements were performed in duplicate. Within-assay coefficient of variation (CV) was below 8%, CV for inter-assay precision below 10%.

Other parameters were determined using routine laboratory methods.

2.5. Genotyping

Characteristics of the analyzed polymorphisms of *ENHO* and *RXRA* are shown in Table S3.

Genomic DNA for genotype analysis was isolated from blood lymphocytes using the salt-out extraction procedure. Genotyping of *ENHO* (rs2281997, rs72735260) and *RXRA* (rs10881578, rs10776909) was carried out using high-resolution melting curve analysis (HRM) on the Bio-Rad CFX96 Real-Time PCR system (Bio-Rad, Hercules, CA). DNA fragments amplified with the use of specific primers were subjected to HRM with 0.1 °C increments in temperatures ranging from 71 to 92 °C. *RXRA* rs749759 was genotyped using polymerase chain reaction and the restriction fragment length polymorphism (PCR-RFLP) method according to the manufacturer's instructions (Fermentas, Vilnius, Lithuania). Primer sequences and conditions for HRM and PCR-RFLP analyses are presented in Table S4. For quality control, approximately 50% of the randomly chosen samples were re-genotyped. Samples with ambiguous results were excluded from further statistical analyses.

Plasma adropin levels, HOMA-IR, QUICKI, and serum lipids were compared between bearers of the minor allele and major homozygotes of tested polymorphisms (the dominant model of inheritance).

2.6. Statistical analysis

The results are presented as percentages for categorical variables, the mean with standard deviation for normally distributed continuous variables, or the median and range for non-normally distributed continuous variables as tested by the Shapiro–Wilk test. For comparison of continuous variables, the Mann–Whitney *U* test, Student's *t*-test, Cochran Cox test, or Kruskal-Wallis test with post hoc tests were used, as appropriate. Dichotomous variables were compared using Chi-square test with Yates correction. Correlations were evaluated using the Spearman method. Linear regression was used to determine the unadjusted associations among adropin concentrations and patient characteristics. The multivariable analyses were adjusted for characteristics associated with adropin concentrations in unadjusted analyses (at a p-value < 0.1).

Statistical analyses were performed using Graph-Pad InStat 3.10, 32 bit for Windows (GraphPad Software, Inc., San Diego, California, United States), CytelStudio version 10.0, (CytelStudio Software Corporation, Cambridge, Massachusetts, United States), and Statistica version 10 (Stat Soft, Inc., Tulsa, Oklahoma, United States).

A p-value of <0.05 was considered significant; a borderline significance was mentioned at a p-value < 0.10.