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Association of genetic polymorphisms of angiopoietin-like 4 with severity of posttransplant proteinuria in kidney allograft recipients☆

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ABSTRACT

Background: Proteinuria is a hallmark of glomerular injury, and persistent proteinuria is associated with graft failure in kidney transplant patients. Recently, it is known that the level of circulating angiopoietin-like 4 (ANGPTL4) is elevated in the patients with human nephrotic syndrome, in which ANGPTL4 is responsible for relieving proteinuria.
Purpose: The purpose of this study is to determine effects of clinical factors and genetic polymorphism of ANGPTL4 on proteinuria after kidney transplantation.
Methods: A total of 282 patients out of 400 renal transplant patients between 2008 and 2012 at St. Vincent Medical Center, CA were studied in a retrospective study design. The level of proteinuria was measured by random urine protein to creatinine ratio, and divided into two groups (Group 1: UPC < 500 mg/day, Group 2: ≥500 mg/day). Single nucleotide polymorphisms of ANGPTL4 genes (rs1044250, rs2278236, rs116843064, rs11672433, rs4076317) were determined by real time PCR with sequence specific primers.
Results: Among various clinical factors, only delayed graft function, mTOR inhibitor use and fish oil use were significantly associated with posttransplant proteinuria. Statistical differences were found in genetic polymorphisms of ANGPTL4 (rs1044250, rs2278236) in regards to proteinuria among tested patients. rs1044250 (C/T, T228M, missense mutation) alleles showed multiple significant differences (Group 1 vs. Group 2: C vs. T: OR = 1.893, CI = 1.322–2.710, $p < 0.001$). Similar trends were found in rs2278236 (A/G) alleles with statistical significances (Group 1 vs. Group 2: A vs. G: OR = 0.620, CI = 0.443–0.867, $p = 0.005$). With multiple logistic regression, rs1044250 was still a significant risk factor of moderate/severe proteinuria ($p = 0.021$).
Conclusions: This study suggests that the presence of C allele of rs1044250 and G allele of rs2278236 in ANGPTL4 gene is associated with higher risk of moderate/severe proteinuria in renal transplant patients.

1. Introduction

Proteinuria is often recognized as a hallmark of allograft damage and poor prognosis in the kidney transplant recipients. The detrimental effect of proteinuria on the course of renal disease have been established in both general population [1,2] and kidney transplant recipients [3,4]. It is known that the persistent proteinuria is significantly associated with cardiovascular disease, lower graft survival rates and mortality in kidney transplant patients [4,5]. Even a low grade proteinuria, if it happens early after transplantation, is associated with inferior graft outcome and patient survival [6]. Moreover, it has been shown that severity of proteinuria can be an independent risk factor of poor allograft outcome [7].

In nephrotic syndrome, which is defined by the presence of excessive proteinuria (>3.5 g/day), with hypoalbuminemia, hyperlipidemia, lipiduria, and edema, proteinuria is considered as the principle driving force [9]. Substantial amount of research has addressed each component of nephrotic syndrome, however the underlying molecular mechanism that connects these components has not been well understood. Until recently, only link that has been clearly elucidated was angiopoietin-like 4 (ANGPTL4) protein which explained the connection between proteinuria and hypertriglyceridemia [9].

Angiopoietin-like (ANGPTL) proteins belong to a superfamily of angiogenic-regulating, secreted proteins such as angiopoietin. Currently,

Abbreviations: ANGPTL, angiopoietin-like protein; DGF, delayed graft function; ESRD, end stage renal disease; FFA, free fatty acid; LPL, lipoprotein lipase; mTOR, mammalian target of rapamycin; SKP, simultaneous kidney and pancreas; UPC, urine protein to creatinine ratio; PRA, panel reactive antibodies; BMI, body mass index.

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seven isoforms of ANGPTL are known in human [10]. Among them, ANGPTL4 is known to be secreted from the peripheral tissues such as skeletal muscle, heart and adipose tissues. The circulating ANGPTL4 inhibits lipoprotein lipase (LPL) which is responsible for the hydrolysis of triglycerides. The inhibition of LPL results in decreased production of free fatty acids (FFA) from triglycerides [11].

ANGPTL4 was shown to be involved in two key feedback mechanisms in proteinuria. With the presence of proteinuria, albumin with low FFA is selectively lost through glomeruli, which increases albumin with high FFA content in the serum. Increase of the serum FFA induces local ANGPTL4 up-regulation, which decreases FFA production by blocking LPL. In addition to that, the circulating ANGPTL4 also binds to glomerular endothelial αvβ5 integrin of the kidney, and reduces proteinuria [9,12,13].

Since the discovery of ANGPTL in 2000 [10], a few genetic polymorphisms of ANGPTL4 genes were investigated mostly in relation to the energy metabolism. Loss of function (E40K) mutation has been associated with plasma triglyceride, high-density lipoprotein and coronary artery disease [14–16]. However, these associations were not detected in other study with pre-diabetic white population [17]. Also, the role of genetic polymorphisms of ANGPTL4 in vascular phenotypes has not been clearly demonstrated yet [18,19].

Until now, influence of genetic polymorphisms of ANGPTL4 on proteinuria has not been studied on both general population and renal transplant recipients to our knowledge. With the recent progress in our understanding on the molecular mechanism of proteinuria and the role of ANGPTL4, we investigated the impact of genetic polymorphisms of ANGPTL4 on the severity of proteinuria in kidney transplant recipients.

2. Objective

The objective of this study was to investigate the impact of genetic polymorphisms of ANGPTL4 and clinical factors on the severity of proteinuria in kidney transplant recipients.

3. Materials and methods

This is a retrospective study of 282 kidney transplant patients as a part of the overall genetic study which was approved by the Institutional Review Board for Human Subjects at Saint Vincent Medical Center, Los Angeles, California.

3.1. Study subject

A total of 400 renal transplant patients between 2008 and 2012 at St. Vincent Medical Center were reviewed in a retrospective study design. For the determination of genetic polymorphisms and proteinuria, patients with both genomic DNA samples and reported random urine to protein (UPC) ratio information were included, which result in 282 kidney recipients for the analysis. All patients' clinical data were collected from the patients' chart records. The patient data were followed until 2014.

3.2. Proteinuria classification

The study subjects were grouped according to the level of peak UPC ratio for comparison. According to Cantarovich et al. [20], urine protein excretion >500 mg/day appears to be a prevalent prognostic factor for patient and graft survivals after kidney transplant, so the patients were divided into two groups based on the severity of proteinuria. A total of 136 patients were assigned to normal to mild whose peak UPC ratio were <500 mg/day (Group 1). A total of 146 patients were assigned to moderate to severe to nephrotic range proteinuria group who had peak UPC ratio equal to or >500 mg/day (Group 2).

3.3. Genotyping

Patient blood samples were collected and genomic DNA was extracted using an extraction kit (QIAmp DNA Blood Mini Kit, Qiagen, Mississauga, Ontario, Canada). Genotyping was performed by using TaqMan SNP Genotyping (ThermoFisher Scientific) based on the 5′ nuclease allelic discrimination assay using ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, California). The SNP probes used for genotyping include rs1044250 (C/T, T228M), rs2278236 (A/G, Intron), rs116843064 (A/G, K40E), rs11672433 (A/G, P351P) and rs4076317 (C/G, intron).

The PCR was performed with MicroAmp® Optical 384-Well Reaction Plate (Applied Biosystems®). 1–10 ng of the DNA was mixed with 1 μL of gene-specific primers and probes. 1.5 μL of PCR universal master mix was added for the final volume of 5 μL. Thermal cycler parameters were as follows: 10 min denaturing at 95 °C, followed by 50 cycles involving denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. As a negative control, a sample without DNA was included in every tray.

3.4. Statistics

The Chi-square test and Fisher tests were used for categorical variables and the *t*-test for continuous variables. Logistic regression was conducted to generate adjusted odds ratios and 95% confidential intervals to validate the significance of trends. In all cases, p values of <0.05 were considered to be statistically significant. All analyses were performed using SPSS statistical program version 14.0.

4. Results

4.1. Patient classification by the severity of proteinuria

The study cohort consisted of 400 kidney transplant patients from St. Vincent Medical Center, Los Angeles, CA between 2008 and 2012. Out of 400 patients, 282 patients had both genomic DNA samples and random UPC ratio data. The patients were followed up until 2014, which resulted in minimum of 2 year and maximum of 6 year follow-up.

Fig. 1(A) shows the distribution (histogram) of the peak UPC ratio without data transformation, which follows Poisson-like distribution. Fig. 1(B) shows the distribution of square root transformed UPC ratio. The square root transformed distribution showed initial fast increase peak followed by slowly decaying later phase, which could be divided at UPC ratio 500 mg. For the comparison, the patients were grouped by normal-to-mild (Group 1) (48.2%, n = 136) and moderate-to-severe proteinuria groups (Group 2) (51.8%, n = 146).

4.2. Patient characteristics

A total of 282 patients out of 400 kidney transplant patients had both DSA samples and UPC ratio data available, and the characteristics are summarized in Table 1. The average age was 47.5 years which were not significantly different between the two comparison groups ($p = 0.598$). The percentage of female was 35.8% on average, which showed marginal differences in two comparison groups (41.2% for normal/moderate proteinuria, 30.8% for severe proteinuria, $p = 0.07$). Most of the patients reported them as Hispanic (n = 173, 61.3% on average) followed by Asian (n = 41, 14.5% on average). Other ethnic groups (African Americans, White non-Hispanic and mixed ethnicity) including unknown white (n = 1) were <10% of the study population. Overall, there was no statistically significant difference in ethnicity between the two study populations ($p = 0.778$).

A total of 12.4% (n = 35) patients had previous kidney transplantation(s), and the Group 2 proteinuria group showed marginally higher number (n = 23, 15.8%) compared to Group 1 proteinuria group (n = 12, 8.8%, $p = 0.078$). There were 35 patients (12.4%) who had