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Mannose-binding lectin-2 and ficolin-2 gene polymorphisms and clinical risk factors for acute rejection in kidney transplantation

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ABSTRACT

Introduction: There is growing evidence that the lectin pathway is significantly associated with acute rejection. Rare studies associated both gene polymorphisms of MBL2 and FCN2 with acute rejection after kidney transplantation. The aim of the present study was to investigate the role of the lectin gene profile and clinical risk factors such as PRA level on acute rejection in kidney transplant recipients.
Methods: We prospectively analyzed 157 kidney transplant recipients with and without acute rejection. A total of 6 well-known functional single-nucleotide polymorphisms in the MBL2 gene and 5 in the FCN2 gene of the recipients were determined by gene sequencing. MBL2 and FCN2 genotypic variants were analyzed for association with the incidence of acute rejection within the first year after kidney transplantation.
Results: After adjusting for variables of $P < 0.2$, we found the differences in the incidence of acute rejection were only according to panel-reactive antibodies (odds ratios (OR) = 6.468, 95% confidence intervals (CI) = 2.017–20.740, $P = 0.002$) and the HH genotypes of MBL2 promoter −550 (OR = 2.448, 95%CI = 1.026–5.839, $P = 0.044$).
Conclusion: Panel-reactive antibodies and the HH genotypes of MBL2 promoter −550 have significant impacts on the risk of developing acute rejection after kidney transplantation.

Despite advances in immunosuppressive therapy and better donor-recipient match for renal transplantation, acute renal graft rejection still represents a major clinical problem accounting for most graft failures. Acute rejection (AR) can induce complement activation [1]. Activation of the complement system is initiated by three known pathways: the alternative, the classical, or the lectin pathway. The lectin pathway of the complement is activated via a set of serine proteases named mannose-binding lectin (MBL)/ficolin (FCN)-associated serine proteases when MBL and/or FCN interact with carbohydrate structures on microbial surfaces and altered self-surfaces [2,3]. After ligand binding, MBL2 and FCN2 lead to generation of a C3 convertase by cleaving C4 and C2, resulting in rapid cell injury [4,5]. More functional MBL2 and FCN2 are theoretically expected to disturb graft tolerance and facilitate AR processes.

Single nucleotide polymorphisms (SNPs) in the genes encoding the lectin pathway proteins determine their functional activity and serum levels [6]. MBL function and levels are significantly affected by three missense mutations at codons 52, 54, and 57 of exon 1 (collectively called O variant whereas the wild-type haplotype referred to as A) within the coding region of MBL2 gene [7,8]. Three polymorphisms at positions −551 (H/L), −221 (X/Y) within promoter region, and +4 (P/Q) in the untranslated region (UTR) also affect MBL function and serum concentrations. These polymorphisms impair the assembly of a monomeric MBL into functional multimeric proteins, resulting in the low serum level of MBL, which might prevent AR by interrupting activation of the lectin pathway. Also FCN2 serum concentrations are significantly associated with polymorphisms in the FCN2 gene. Three gene polymorphisms at positions −986 A/G, −602 G/A, and −4 A/G in the promoter region and two at positions +6359C > T and +6424G > T in exon 8 of FCN2 gene are associated with different serum levels of FCN2 or different binding capacity towards *N*-acetylglucosamine [9].

There is growing evidence of the genetic association between the lectin gene profile and the AR or graft survival after solid organ transplantation [10–20], in contrast with other studies [21]. Rare studies investigated the association of lectin complement pathway gene profile [12,15,21], especial FCN2 gene polymorphisms [19,21], with the incidence of acute renal graft rejection. Thus, no uniform message of the role of the lectin gene profile in acute renal graft rejection has emerged as yet to date. Panel-reactive antibodies (PRA) are pre-existing antibodies targeting the human leukocyte antigen (HLA). The complement system has an essential role in antibody-mediated kidney rejection by the classical C1q-dependent pathway [22]. Furthermore, recent research showed that the antibody-independent involvement of complement was through the MBL-dependent pathway of complement activation in graft loss [23]. Both PRA and lectin pathway proteins caused AR via the complement system, although it has been established that elevated

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PRA can induce severe rejection of organ transplant [24–27]. No study had investigated the association of both lectin gene profile and PRA with the incidence of acute renal graft rejection. The aim of the present study was to investigate the role of the lectin gene profile and clinical risk factors such as PRA level on AR in kidney transplant recipients.

Recipients and methods

Between January 2008 and December 2009, 178 recipients underwent kidney transplantation the Third Xiangya Hospital of Central South University (Changsha, China). From this original group, 21 recipients were excluded because of two or more kidney transplantations, simultaneous transplantations, lost to follow-up or technical problems. Of the 157 available transplants, 34 recipients suffered at least one rejection episode, and 18 of these were biopsy proven, with the remaining 16 clinically proven. Twelve of those 18 biopsy-proven ARs were present of humoral rejection. Nine of those 16 clinically proven ARs were antithymocyte globulin-requiring rejection ARs. This left 123 patients with no rejection. There were three sources of renal allografts harvested for transplantation: cadaveric donors, donors after cardiac death and living related donors. All patients were tested for pre-transplant levels of PRA by using a commercial enzyme-linked immunosorbent assay kitμ(LAT Mixed, One Lambda Inc, Canago Park, USA) according to the manufacturer's instructions. All recipients enrolled were treated with triple drug immunosuppression consisting of mycophenolate mofetil, cyclosporine or tacrolimus and corticosteroids. Recipients were rapidly tapered to 20 mg of prednisone per day 7 days after surgery. All patients had a negative complement-dependent cytotoxicity cross-match result before transplantation. We collected demographic and clinical characteristics of the recipients (age, gender, primary disease, PRA level, initial immunosuppression (cyclosporine/tacrolimus) and use of antilymphocytic agents before AR), donor information (age, gender and donor type) and transplant characteristics (cold ischemia time, HLA no. of 0 mismatches). Recipients with and without AR were compared with regarding genotyping and other demographic and clinical variables. The study was performed with informed consent and approved by the ethics review board of our hospital. Follow-up time for all recipients was 1 year after operation. AR was confirmed based on clinical or biopsy findings according to Banff criteria [28]. Clinical rejection was identified by increased creatinine levels in the lack of the evidence of infection, obstruction or drug toxicity. Patients included in the “non-AR” group were defined as having no rejection episodes within the first year after transplantation.

1. MBL2 and FCN2 Genotyping

Genomic DNA was extracted from 2 mL ethylene diamine tetraacetic acid-treated peripheral blood samples. As previously reported, a total of 11 functional SNPs in the MBL2 and FCN2 genes of 157 recipients were determined by using PCR technique [16,29]. Lectin genotypic variants were analyzed for relation to AR. In brief, a DNA fragment (969 bp) including promoter region and exon 1 of MBL2 gene and two DNA fragments including promoter region (1563 bp) and exon 8 (384 bp) of FCN2 gene were obtained by PCR amplification using primes 5′-GGGG AATTCCTGCCAGAAAGT-3′, 5′-CATATCCCCAGGCAGTTTCC TC-3′; 5′-AGCATGCAGTAAAGGAACCTG-3′, 5′-TGCCAGCTTTCAGGGACGAG-3′; and 5′-CTGTCTGTAATGATGTTACTGC-3′, 5′-TACAAACCGTAGGGCCAA GC-3′, respectively. The cycling conditions were 94 °C for 4 min, 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s and finally 72 °C for 5 min. The PCR products were tested by agarose gel electrophoresis and then subjected to DNA sequencing.

2. Genetic groups

These genotypes were divided into high and low levels of MBL based on previous studies [10,30,31]. Recipients were divided into sufficient (A/A, HH, QQ, YY or YX) or insufficient (A/O, O/O, HL or LL, PP or PQ, XX) MBL concentrations regarding the polymorphic positions at exon 1, promoter and UTR of the MBL2 gene [31,32].

3. Statistical analysis

SPSS (version 17.0, SPSS, Inc., Chicago, IL) was used to perform statistical analyses, and two-sided P values under 0.05 were considered to indicate statistical significance. Continuous variables were analyzed using the Student's t-test. The chi-square test or the Fisher exact test was performed for categorical variables. For multivariate logistic regression models, we included all variables with $P < 0.20$ in the univariate analysis to identify independent risk factors for AR. Associations were given as odds ratios (OR) with a confidence interval (CI) established at 95%.

Results

During the 2-year period, 178 kidney transplants were performed in our center, and 34 recipients with AR (age range, 23–58 years; mean age, 36.50 ± 8.84 years; 23 males and 11 females) and 123 recipients without AR were finally analyzed. Table 1 shows the traditional risk factors for AR including demographic and clinical characteristics of both donors and recipients, and transplant characteristics. Table 2 shows the frequencies of all MBL2 and FCN2 variants in all recipients. Tables 1 and 2 also show that in univariate analysis, higher AR incidence was found to be statistically significant to PRA ($P = 0.001$), and nearly significant to MBL2 promoter −550 HH genotypes ($P = 0.056$). There were no significant differences for gene polymorphisms of FCN2 or the rest of MBL2 and other traditional risk factors for AR among the recipients with and without AR. In multivariate analysis, after adjusting for all variables of $P < 0.2$, we found the differences in the incidence of AR were only according to PRA (OR = 6.412, 95% CI = 2.000–20.557, $P = 0.002$) and MBL2 promoter −550 HH genotypes (OR = 2.424, CI = 1.016–5.783, $P = 0.046$) (Table 3). In order to except the effect of FCN2 gene polymorphisms, we tested the MBL2 association by analyzing the combination of demographic and clinical variables of donor and recipient and MBL2 genotype. The result showed that the presence of MBL2 promoter −550 HH genotypes still predicted incidence of AR independently of demographic and clinical risk factors (OR = 2.448, 95% CI = 1.026–5.839, $P = 0.044$), and PRA still showed the strongest association with AR (OR = 6.468, 95% CI = 2.017–20.740, $P = 0.002$), which were shown in Table 4.

Discussion

Acute renal graft rejection is mediated by complex immunologic mechanisms and still represents a major clinical problem following kidney transplantation. Since localized complement deposition can orchestrate several immune processes including graft neutrophil infiltration, vascular endothelial disruption and ultimately graft cell death, lectin complement pathway, comprising MBL, FCN and MBL-associated serine protease(MASP), may play a significant role in AR [10,11,33]. The amount of MBL protein can result from a direct consequence of genetic variations in both the promoter- and protein-encoding regions of the MBL2 gene [34]. However, the exact role of MBL2 and FCN2 gene polymorphisms in AR after transplantation remains controversial and inconclusive [10,11,14–21]. Our main finding was that lower AR rates were noted among recipients with HL/LL genotype, compared with HH genotype (18.2% vs. 33.3%), in line with the previous findings of a lower AR incidence in recipients with MBL deficiency [14], and in contrast with others [17,20]. Our result that HH genotypes of MBL2 associated with more AR did not seem surprising since more functional MBL2 was theoretically expected to disturb graft tolerance and to facilitate rejection processes [10]. This finding showed that the genetic reason for the high MBL protein level associated with AR could be traced back to the mutation of a single nucleotide. FCN deposition was previously demonstrated during AR episodes, which indicated that FCN was also related to AR [15]. However, Damman et al. [21] did not observe an association of donor and recipient MBL2, FCN2 and MASP2 genotype with biopsy-proven AR after kidney transplantation, in contrast with the study by Eikmans et al. [19], who demonstrated that the presence of the FCN-2 Ala258Ser variant in the donor predicted lower incidence of severe rejection and of graft loss independently of clinical risk factors. Berger et al. [11] found a correlation between higher serum MBL levels and more severe rejections of renal transplants. In the present study, no