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Polymorphism of RAS in Patients with AT1-AA Mediated Steroid Refractory Acute Rejection

Geng Zhang and Jianlin Yuan
*Department of Urology, Xijing Hospital
 Fourth Military Medical University, Xi'an
 China*

1. Introduction

Acute rejection is one major reason of the graft loss in renal transplantation. Most of the allograft rejections are due to the existence of the antibodies. The importance of the series tests of HLA and HLA antibodies associated with transplantation has been widely recognized. Although considerable rejection episodes can be explained by the appearance of the donor specific anti-HLA antibodies (DSA), it is reported that even in the case of 'ideal' HLA identical donor grafts transplanted into 'ideal' recipients, 23% are rejected within 10 years [1]. So effect of HLA antibodies accounts for only some of the immunological failures of allograft and there must be large fraction of undetected antibodies against non-HLA mismatches.

Despite various ways were used in the prevention of acute rejection, pulse steroid treatment remains the first-line therapy of all immunosuppression projects and has a 60 to 70% success rate [2]. There are still recipients suffered from steroid refractory acute rejection (SRAR) and get worse. These rejections typically have an aggressive clinical course and are associated with graft loss [3]. Some of the patients would have to return to blood dialysis immediately and part of them die from complications. We detected antibodies in these patients and found that some of them do not have HLA antibodies and one of their marked characteristics is malignant hypertension. We presumed this is a special type of rejection. Thus, both immunologic and non-immunologic mechanisms for this specific type of rapid renal allograft dysfunction have to be considered.

In case of the SRAR is appeared in a small part of the renal transplantation recipients, we presume that the genetic factors are involved it. It is reported that single nucleotide polymorphism (SNP) of some immune response related genes is involved in the development the acute rejection. The SNP of some genes can affect their functions by the changing either cytokine production or amino acid sequence. And the structure and function of some critical components would be altered concomitantly. The characteristic change of related proteins would finally induce the immune response and then trigger the rejection process.

Hypertension has been found to be an independent contributor in the progression of renal allograft failure in some studies [4, 5]. The presence of genetic abnormalities in blood pressure regulation could promote post-transplant hypertension and by that contribute to a more rapid loss of renal allograft function. The recipients' blood pressure levels may depend on several factors, such as graft function, type, and dosage of immunosuppressive agents applied, previous rejection episodes, recurrence of the underlying nephropathy, and a previous history of hypertension [6]. The renin-angiotensin-aldosterone system (RAS) is a proteolytic cascade with an important role in blood pressure regulation and maintenance of fluid and electrolyte balance [7]. It was also reported that over activation of the RAS is linked to poor long-term renal transplant function and decreased graft survival times [8]. RAS polymorphisms associated with hypertension in certain groups have been identified [9]. We hypothesize that the occurrence of SRAR may be due to the over activating of RAS in a special group of recipients. And therefore, genes of the RAS deserve consideration. Among these the angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensin type 1 receptor (AT1R), and aldosterone synthase (CYP11B2) genes are of particular interest. Yet the prevalence and distribution, alone or combined, of the M235T-AGT, I/D-ACE, A1166C-AT1R, or -344C/T-CYP11B2 polymorphisms and their relation with SRAR has not been well studied.

In the studies of immunologic mechanism of acute vascular rejection, some data suggested that agonistic angiotensin type 1 (AT1) receptor autoantibodies (AT1-AA) may contribute to the pathogenesis of the rejection [10]. Some experiments implicated the role of renin-angiotensin system in the regulation of specific immune responses and immune-mediated renal injuries [11]. And AT1 receptor blockade appeared to effectively attenuate the agonistic activity of the antibodies.

So the SRAR may be a kind of specific rejection, whose origin, mechanism and results and treatment are all different from other types of rejections. Therefore, this study tried to determine whether or not gene polymorphisms of RAS are associated with steroid refractory acute rejection. Further more, by studying the gene-gene interaction, we searched the association of the occurrence of SRAR with the appearance of AT1-AA.

2. Subjects and methods

2.1 Study patients and biopsies

A total 206 renal transplant recipients were included: 116 males and 90 females, mean age 29.6 ± 10.2 years range 22–55 years transplanted at Department of Urology of Xi Jing hospital, 4th Military Medical University from January 1st, 2001, through December 31st, 2005. The transplants were first grafts in all cases. Serum samples obtained during rejection episodes were screened for donor specific anti-HLA antibodies and also agonistic antibodies targeting the AT1 receptor. This study was approved by the Ethics Committee of the Hospital, and all patients gave their oral informed consent while he or she was awaiting transplantation.

Allograft-biopsy specimens were processed by standard techniques and graded according to the Banff97 criteria. C4d staining on paraffin-embedded sections was made according to a protocol described previously [12, 13].

2.2 Immunosuppressive treatment

Initial immunosuppressive therapy consisted of a calcineurin inhibitor, mycophenolate mofetil, methylprednisolone, and antibody against interleukin- 2 receptor for induction. All the recipients were treated with high dose pulse of methylprednisolone for 3 days with the dose of 0.5g, 0.5g, 0.25g in each day after the operation and a dose of 0.75g methylprednisolone was used on the operation day. Cyclosporine A or tacrolimus were introduced when serum creatinine levels were below 300 μ mol/L. Anti-proliferative agents, mycophenolate mofetil (1.5 g/day) or azathioprine (1-2 mg/kg/ day) were started on the day of transplantation. Prednisolone was reduced to 20mg daily after 7 days. Daily doses of CsA and tacrolimus were adjusted according to peak and trough blood levels respectively. High dose of methylprednisolone was utilized when the subject developed acute rejection after renal transplantation.

Patients resistant to steroid were experienced further treatments. The further therapy includes protein A immunoadsorption (IA) and intravenous immune globulin. Patients who were positive for AT1-AA were added with angiotensin II Receptor Blockers (ARB). Patients initially treated with cyclosporine were switched to high dose tacrolimus when refractory rejection was detected.

2.3 DNA extraction, PCR analysis and genotyping

Genomic DNA prepared from blood cells using a standard column extraction technique. ACE I/D polymorphism in intron 16 of the ACE gene was determined as described previously [14]. In order to exclude mistyping of heterozygotes, all DD individuals were also examined using primers specific for the insertion variant. PCR products were resolved on a 2% agarose gel by ethidium bromide staining.

AGT M 235 T was determined by PCR amplification according to a method described previously [15]. The 165 bp PCR product was digested with TthIII at 37°C for 3h and resolved on a 2.5% agarose gel by ethidium bromide staining.

For the analysis of the AT1R (A1166C) polymorphism [16], a 410bp PCR product was digested with DdeI enzyme at 37°C for 3h and separated by electrophoresis in 2% agarose gel.

CYP11B2 gene polymorphism was performed according to method described by Brand *et al* [17]. The PCR product was restricted with 5 U of the restriction endonuclease HaeIII over 2 h at 37°C and final product was electrophoresed in 2% agarose gel and visualized directly by ethidium bromide staining. The -344T allele lacks the HaeIII site that is present in the -344C allele and gives rise to a fragment of 273 bp rather than 202 bp.

2.4 Antibody assays

The detection of AT1-AA The peptides corresponding to the sequence of the second extracellular loop of the human AT1 receptor positions 165aa-191aa (I-H-R-N-V-F-F-I-E-N-T-N-I-T-V-C-A-F-HY- E-S-Q-N-S-T-L) were synthesized by a peptide synthesis system [18]. The peptide was evaluated by HPLC analysis on a Vydac C-18 column, and 95% purity was

achieved. The ELISA assay of antibodies was performed according to the described previously [19].

2.5 Statistics

All statistical analyses were performed with the help of the SPSS 10.0 statistical software package. Categorical variables were assessed by the chi-square test. Survival curves for each group were calculated by the Kaplan-Meier method. Differences in genotype and allele distributions between groups were tested by the χ^2 test. To compare the prevalence of SRAR among the genotypes of each polymorphism, genotypic odds ratios (OR) and 95% confidence intervals (CI) for SRAR were estimated using logistic regression analysis. Data are given as means with SD. In all statistical analysis the significance was considered significant when level (P) values were lower than 0.05. Test of Hardy-Weinberg equilibrium was performed in the subjects.

3. Results

All patients were classified into three groups according to the occurrence of SRAR. Two hundred and six renal transplant recipients were concerned by this study. Posttransplant hypertension was found in 102 cases (49.5%) of all patients. According to the presence of SRAR, patients were classified into two groups: group I (G I), 19 patients (9.2%) underwent SRAR (10 men and 9 women), among them 14 had malignant hypertension. Group II (GII) was the rest 187 patients who did not suffer from SRAR (104 men and 83 women), 16 of them had malignant hypertension, the difference between the two groups is significant. And 150 normal people were set as control group (GIII). Baseline characteristics of the GI and GII were compared. No difference was observed in terms of age and sex in the donors. Moreover, no statistically significant differences were found between the GI and GII in terms of sex distribution, percentage of first kidney transplantation and cause of end-stage renal disease. And there were no significant differences between the GI and GII for total number of HLA-mismatches, ischemia times (Table 1).

The DD genotype of ACE and CC genotype of AT1R were risk factors for the development and progression of the SRAR. The genotype distributions of four key genes ACE I/D, AGT M235T, AT1R A1166C and CYP11B2 -344C/T of the RAS polymorphisms exhibited a nonsignificant difference with that predicted by the Hardy-Weinberg equilibrium in all cases. The significant differences of gene frequencies between all patients and control group were not observed.

Differences between GI and GII and controls in genotype distributions were observed for both ACE gene polymorphisms with DD genotype and CC genotype of AT1R polymorphism being more prevalent in GI than in other two groups. Genotype distributions of other polymorphisms investigated in AGT and CYP11B2 genes did not differ between these three groups. The difference in allele distributions was only observed for AT1R A1166C with the C allele being more frequent in G I than G II and controls (42.1% v 29.9% and 30.1%) (Table 2). To test the association of ACE and AT1R gene polymorphisms with SRAR, genotypic ORs were calculated. Taking the II genotype of the ACE I/D polymorphism as a reference, the OR for SRAR associated with the ID genotype was 1.53

	Non-SRAR (n=187)	SRAR (n=19)
Age (years)	36.5±11.6	37.7±12.5
Male (%)	53	47
IgG	9.3±2	10.4±2.8
No. of HLA MM	3.6±1.2	3.2±1.8
PRA positive (%)	7.6±1.2	7.5±1.5
Duration of dialysis before NTx (mon)	20.3±16.2	23.6±18.6
CIT(h)	10.6±7.1	11.6±7.3
Cause of ESRD		
Chronic glomerulonephritis	56.9	50.6
Hereditary	16.1	17.6
High blood pressure	6.5	10.6
IgA nephropathy	3.2	4.5
Unknown	17.1	16.7

CIT = Cold ischemia time; NTx=renal transplantation; HLA MM = HLA mismatch

Table 1. Characteristics of patients involved in the study.

		Genotypes			Alleles
<i>AGT M235T</i>	<i>MM (%)</i>	<i>MT(%)</i>	<i>TT(%)</i>	<i>M(%)</i>	
GI	6 (31.6)	9 (47.3)	4(21.1)	21(55.3)	
GII	61 (32.6)	87(46.5)	39(20.9)	209(55.9)	
GIII	44 (29.3)	70 (46.7)	36(24)	158(52.7)	
<i>ACE I/D +</i>	<i>II (%)</i>	<i>ID (%)</i>	<i>DD (%)</i>	<i>D (%)</i>	
GI	3 (15.8)	9 (47.3)	7 (36.8)	19 (50)	
GII	55 (29.4)	108 (57.8)	24 (12.8)	156 (42.9)	
GIII	43 (28.7)	85 (56.7)	22 (14.6)	129 (43)	
<i>AT1R A1166C*</i>	<i>AA (%)</i>	<i>AC (%)</i>	<i>CC (%)</i>	<i>C (%)</i>	
GI	6 (31.6)	6 (31.6)	7 (36.8)	16 (42.1)	
G II	92 (49.2)	82 (43.9)	13 (8.0)	112 (29.9)	
G III	72 (48.0)	64 (42.7)	14 (9.3)	92 (30.1)	
<i>CYP11B2</i>	<i>CC (%)</i>	<i>TC (%)</i>	<i>TT (%)</i>	<i>C (%)</i>	
GI	3 (15.8)	8 (42.1)	8 (42.1)	14 (36.8)	
GII	25 (13.3)	84 (44.9)	78 (41.8)	134 (35.8)	
GIII	19 (12.7)	67 (44.7)	64 (42.6)	105 (35.0)	

* : Significant difference in genotype and allele frequencies of GIversus both G II and G III (*P*<0.01);

+ : Significant difference in genotype frequencies of G I versus both G II and G III (*P*<0.01).

Table 2. Distributions of genotype and allele frequencies in each group.

(95% confidence interval [CI] 0.40-5.86) and the OR associated with the DD genotype was 5.34 (95% CI 1.27-22.42), indicating a recessive effect of the D allele on risk (Table 3). A similar situation was observed for the A1166C polymorphism in the AT1R gene. Taking AA genotype as a reference, the OR for SRAR associated with the AC genotype was 1.15 (95% CI 0.36-3.70) and that associated with the CC genotype was 8.34 (95% CI 2.43-28.69).

The OR associated with the combination of DD and CC genotypes versus all other combinations was 5.92 (95% CI 2.85-12.37), that is, very similar to the one observed in the separate analyses of these two polymorphisms (Table 3).

		OR	95% CI
ACE I/D	ID versus II	1.53	0.40-5.86
	DD versus II	5.34	1.27-22.42*
AT1R A1166C	CC versus AA	8.34	2.43-28.69*
	AC versus AA	1.15	0.36-3.70
ACE I/D and AT1R A1166C	DD+CC versus all other genotypes on two loci	5.92	2.85-12.37*

CI = confidence interval; OR = odds ratio. *: $P < 0.05$.

Table 3. Odds ratios for the presence of SRAR by ACE I/D and AT1R A1166C.

Malignant hypertension was companied with the AT1-AA mediated SRAR. We performed univariate analysis of the factors affecting SRAR to the overall group. The factors significantly associated with graft transplant failure were HLA mismatch >1 ($P > 0.05$). Among the nineteen recipients suffered SRAR, fourteen patients occurred malignant hypertension. In the detection of AT1R antibodies and DSA, the former were invariably positive in the malignant hypertension recipients and the DSA negative in their peripheral blood. While the rest 4 cases of SRAR recipients have the peripheral DSA and 1 case with neither AT1-AA nor DSA.

The demographic data from the 14 patients who were positive for AT1-receptor antibodies were compared with those for the 4 patients with donor-specific anti-HLA antibodies. As shown in Table 4, patients with anti-HLA antibodies were more likely to have staining for C4d in their renal biopsy specimens and had less rapid allograft loss. Otherwise, there were no significant differences in clinical or demographic characteristics between the two groups.

The biopsy specimens from patients with malignant hypertension and refractory rejection who had no donor-specific anti-HLA antibodies (stained with hematoxylin and eosin) showed the evidence of tubulitis and intravascular inflammatory cells. Interlobular arteries were obstructed by swollen endothelial cells which underwent necrosis and vacuolation. The smooth muscle cells proliferated and the intima thickened greatly. Inflammatory cells can be seen inside the vascular and segmental veinlets. The atrophied glomcrulus were divided into subsections. Tubules were expanded with red cell cast inside it. The peritubular capillaries were aggregated with polymorph nuclear leukocytes. Interstitium was infiltrated with inflammatory cells. The renal shows multiple perfusion defects and cortical infarctions (Figure 1).

Characteristic	AT1-Receptor Antibodies (n=14)	Anti-HLA Antibodies (n=4)
Male sex (n)	8	2
Cold-ischemia (h)		
Median	6.7	6.5
Range	2.4-18.5	2.2-19.1
No. of HLA mismatches		
Median	3	3
Range	0-3	1-5
PRA at transplantation (%)		
Median	5	6
Range	0-7	0-9
Age at transplantation (yr)		
Median	36.5	37.2
Range	19-49	20-48
Time from transplantation to rejections(days)		
Median	3	6
Range	2-360	3-351
C4d-positive (No.)*	3	4

*: P<0.01.

Table 4. Characteristics of patients with AT1-Receptor antibodies and patients with donor-specific anti-HLA antibodies but without AT1R-Receptor antibodies.

AT1-AA mediated SRAR was markedly associated with the prognosis of the renal transplant recipients. The occurrence of SRAR was analyzed for an association with the susceptibility to worsening renal graft function. A stable renal function was more often observed in recipients who did not suffered from SRAR than in GI. There was a striking effect of the SRAR and long-term stable graft function (Figure 2A), with more patients suffering from worsening renal function with the occurrence of SRAR (Kaplan-Meier, $P<0.05$). The analysis also discovered that the occurrence of SRAR is strongly associated with the appearance of the antibodies of AT1R. The χ^2 analysis revealed that the positive of AT1-AA is strongly related to the appearance of SRAR. We also noticed that the frequency of the AT1R-C allele and ACE D-allele is comparable in SRAR group. Patients with anti-HLA antibodies had substantially better graft survival than did those who had malignant hypertension but did not have anti-HLA antibodies (Figure 2B).

Treatment consisting of protein A immunoadsorption (IA), intravenous immune globulin, and 100 mg of losartan daily in nine patients with AT1R antibodies resulted in significantly improved allograft survival, as compared with that in patients receiving standard anti-rejection treatment (Figure 2C). Serum from four patients with the longest rejection-free follow-up (who are still receiving losartan) became negative for AT1-receptor antibodies.

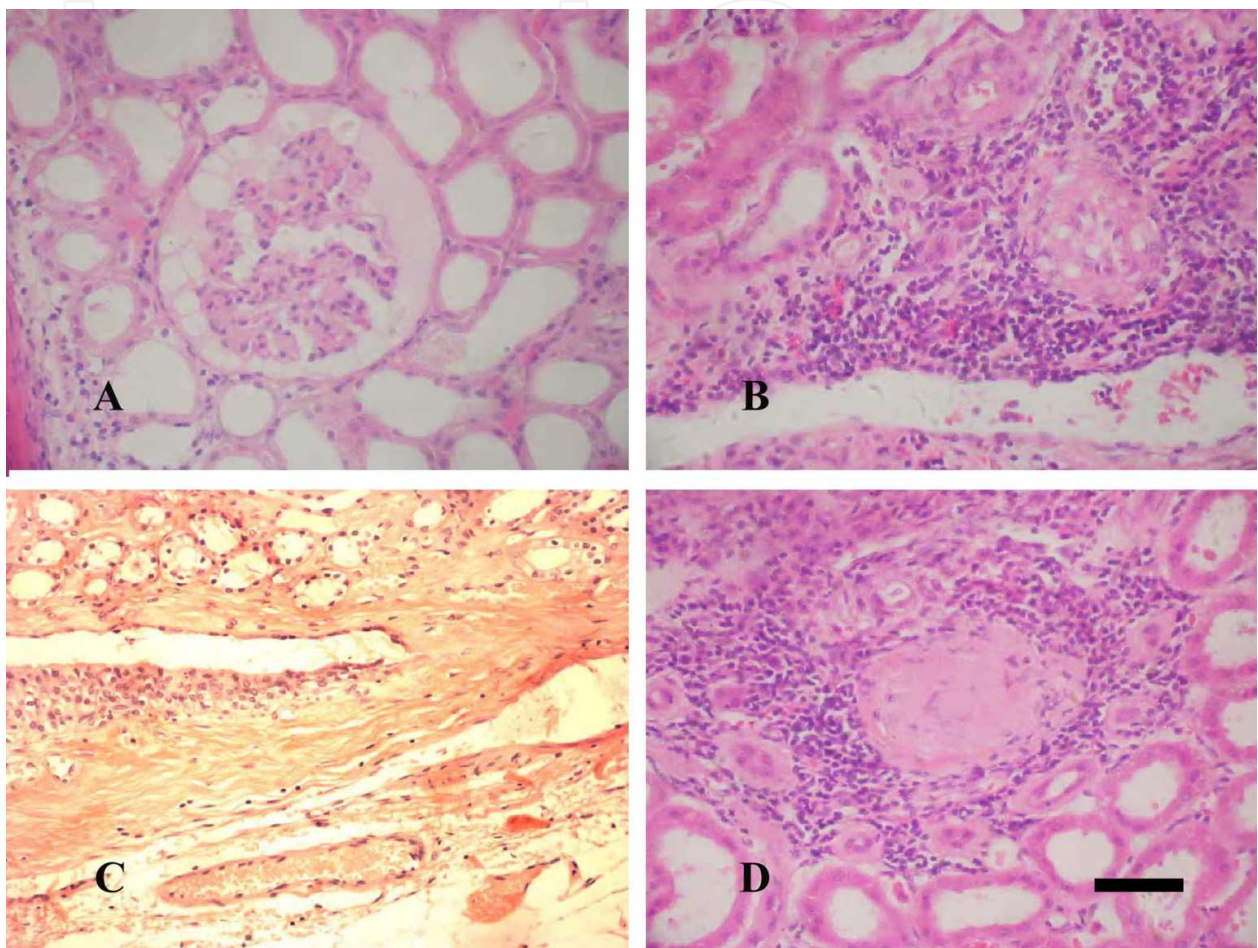


Fig. 1. Features of Refractory Rejection in Patients without Donor-Specific Anti-HLA Antibodies The biopsy specimens from representative patients showed the evidence of tubulitis and intravascular inflammatory cells. The atrophied glomerulus were divided into subsections. Tubules were expanded with red cell cast inside it (Panel A). Inflammatory cells can be seen inside the vascular and segmental veinlets (Panel B). Interlobular arteries were obstructed by swollen endothelial cells which underwent necrosis and vacuolation. The smooth muscle cells proliferated and the intima thickened greatly. The peritubular capillaries were aggregated with polymorph nuclear leukocytes. Interstitium was infiltrated with inflammatory cells (Panel C and D).

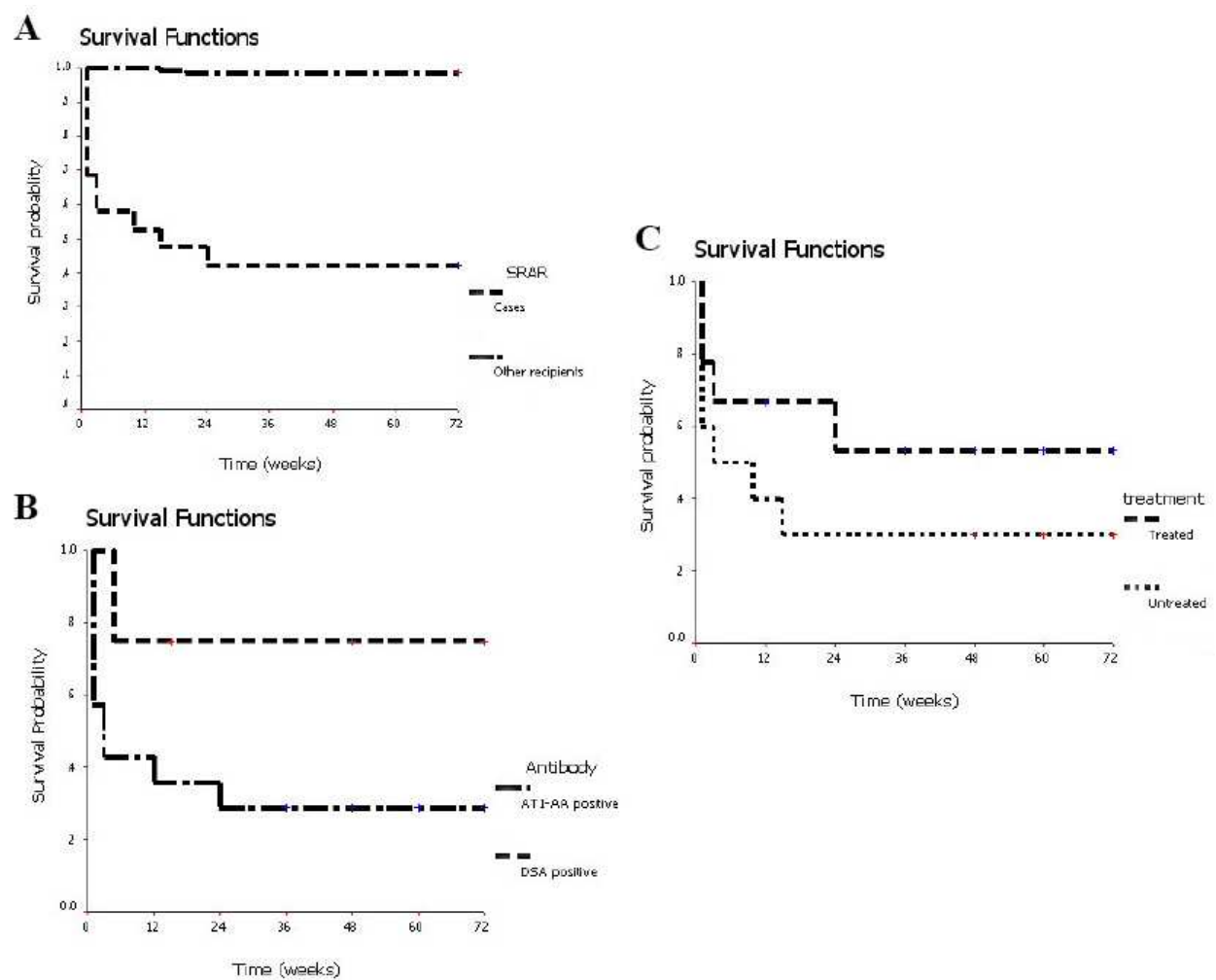


Fig. 2. Graft survival in the Kaplan-Meier analysis was significantly decreased in the patients with SRAR compared with those who did not suffered from it (Panel A). The graph in Panel B demonstrates accelerated allograft loss in patients who had refractory vascular rejection with non-HLA antibodies, compared with patients who had vascular rejection and donor-specific anti-HLA antibodies. Graft survival in the Kaplan-Meier analysis was significantly increased in the patients with AT1-receptor antibodies who received losartan and IA, as compared with those who received nonspecific treatment (Panel C).

4. Discussion

The factors leading to SRAR are not fully understood, but they consist of humoral immune response and genetic factors [20]. We studied the SNP of RAS and AT1R antibodies and DSA in patients, and tested C4d on their biopsy specimens. The findings of this study suggest an association of RAS polymorphism and the occurrence of SRAR in renal transplantation recipients. The single nucleotide polymorphism of RAS may be one of the

non-immunologic mechanisms of the SRAR. Yet the appearance of autoantibody of AT1R may be one of the immunologic mechanisms of the occurrence of this type rejection. Our results support the hypothesis that there is an association between the occurrence of the SRAR and the appearance of the AT1-AA in recipients whose genotype of RAS performed certain polymorphism. The recipients with the DD genotype of ACE and CC genotype of AT1R may trend to suffer the AT1-AA mediated rejection. The process of the rejection may be improved by the application of ARB, coupled with protein A immunoadsorption and high dose intravenous immunoglobulin.

The outcome of the renal transplantation was improved obviously with the use of CsA and FK506. Methylprednisolone was widely used in preventing and treatment of acute rejection and most recipients benefit from it. But there are still some patients who are refractory to the steroid treatment. Most of them have the similar characteristics including anuria after transplantation, malignant hypertension and graft function loss. It has been proved that many diseases have their own genotype polymorphism backgrounds. Genes that affect blood pressure regulation, mesangial or vascular proliferation or aspects of inflammatory response may play important roles in this complex syndrome. Genes determining the activity of a recipient's renin-angiotensin system (RAS) may be alloantigen-independent factors that influence the kidney allograft function.

Investigation of the genetics, physiology, and pharmacotherapeutics of RAS and its regulators has made it one of the most intensely studied molecular pathways of human diseases. The RAS is implicated in the development of a variety of human disorders, including cardiovascular diseases [21]. Inhibition of this system has had proven benefits in reducing the risk of cardiovascular endpoints [22], diabetes, and end stage renal disease (ESRD) [23]. Genetic variability in the RAS may modify renal responses to injury and disease progression. It seems some differential group of recipients is destined to lose their grafts faster. Our study was designed to elucidate the relationship between genetic RAS polymorphisms namely ACE I/ D, AGT M235T, AT1R A1166C and CYP11B2 and SRAR in Chinese renal transplant recipients.

The angiotensin converting enzyme (ACE) gene displays an insertion/deletion polymorphism in intron 16, and homozygosity of the deletion allele is known to be associated with higher serum ACE levels [24]. The DD genotype of ACE gene is a risk factor for the progression of chronic renal failure in IgA nephropathy [25]. It has also been reported that patients with ACE (DD) and angiotensinogen AGT (TT) genotypes are linked to poorer long-term renal transplant function [26, 27]. Demonstrated survival benefits may be a result, in large measure, of the salutary effects of angiotensin converting enzyme (ACE) inhibition on the endothelium [28] and vascular smooth muscle cells [29]. A beneficial association of CAD progression in Caucasian pediatric renal transplant recipients with the II genotype and/or the presence of the I allele is also found [26, 30, 31]. Current evidence on the nature of the renal risk is associated with the D-allele, as also apparent from a large meta-analysis that addressed cardiovascular risk, indicates that the D-allele acts as a course-modifying gene rather than as a disease-inducing gene [32].

In the present study, the D allele of ACE is a risk factor for the development and progression of the SRAR while no significant relationship was found between ACE I/D polymorphism and CAD [33]. The result is in line with other studies that examined the effect of the ACE

I/D polymorphism on the progression of diabetic nephropathy [34], and the progression of chronic renal failure in immunoglobulin A nephropathy [35] and in adult polycystic kidney disease [36]. This effect of the D allele of ACE is also similar with the findings reported by Abdi *et al* [30] and Akcay *et al.* [37] which found that ACE DD genotype was associated with poorer chronic transplant function and more rapid chronic progression. Some related results also have been reported by Barocci *et al.* [38] in pediatric transplant recipients and Gaciong *et al.* [39] in adult transplant recipients. Our data are consistent with these views. Thus, the presence of a D-allele does not appear to enhance renal risk in itself, but once a sequence of events leading to progressive renal function loss is initiated by whatever cause, its course is more rapid in presence of the D allele [40].

Angiotensin II has been proven to have growth factor-like and angiogenic activities [41] and these activities are mediated through the activation of the angiotensin type 1 receptor [42]. The AGT1R A1166C polymorphism was found to predict the systemic and renal response to angiotensin receptor antagonism and angiotensin II infusion in healthy subjects [43]. In the present study patients with the AGT1R C allele tended to have a worse preservation of graft function than patient homozygous for the A allele. Yet patients with the C allele exhibited a stronger response to losartan, showed a faster serum creatinine degression, and had a better response of the decent of the mean blood pressure compared with subjects homozygous for the AA allele, suggesting an increase in intrarenal angiotensin II activity with the AC/CC genotype. However, the treatment was neither randomly assigned nor blinded, and the numbers of patients are too small to permit us to draw firm conclusions. This would indicate that recipient AGT1R C allele may adversely affect renal outcome after kidney transplantation. Moreover, our data are in contrast with another report in which the C allele of AT1R dose not adversely affect renal response after transplantation [44]. However, we do not know the genotype of the donors because they were not genotyped. As the frequency of the A allele in the general population is high (71% in our patients), the probability of a recipient with the C allele receiving a kidney with the C allele is low. In the analysis of the results, the incidence of SRAR is very low (9.2%), the recipients with AT1-AA is even lower (6.8%). We suppose that the occurrence of SRAR may be based on the condition when the C recipient meets the C donor coincidentally, and then the autoantibody of AT1-AA would be created in this group. Of course this is only an imagination need to be tested.

There are many polymorphisms in the AGT gene located on chromosome 1q42-q43. In exon 2, a nonsynonymous substitution of T by C in codon 235 of the AGT gene, leads to a change from Methionine to Threonine. In Caucasians, African and Japanese populations, the T235 variant of this M235T polymorphism of this gene has been consistently associated with higher levels of angiotensinogen in plasma and an increased risk for hypertension [45, 46, 47].

The primary regulation of aldosterone synthesis is via the renin-angiotensin system, which is responsive to the state of the electrolyte balance and plasma volume. The -344T/C polymorphism in the CYP11B2 promoter region, the steroidogenic factor-1 binding site, was reported to be associated with blood pressure or aldosterone secretion. Some investigators found a positive association between the -344T polymorphism in the CYP11B2 gene and essential hypertension. Our study suggests that the CYP11B2 gene promoter region polymorphism and AGT M235T is not directly associated with SRAR. Therefore, this

polymorphism may not be a risk factor for the rejection or the malignant hypertension, at least not in the Chinese population. The lack of relationship between these two genotypes and early graft function in our study may indicate that other factors have major impacts in the setting of kidney transplantation.

We also examine the relationship between the given RAS polymorphism and the emergency of peripheral AT1-AA of the recipients. Draun *et al* reported that AT1-receptor-mediated pathway may contribute to refractory vascular rejection [48]. Our results revealed that the recipients with D allele of the ACE and the C allele of the AT1R are inclined to have the AT1-AA. This phenomenon can partly explain the fact that only small percent of recipients have SRAR.

Our report suggests that a SNP of the RAS component may contribute to the development of the steroid refractory acute rejection of kidney grafts. Individuals bearing D allele of ACE and C allele of AT1R may be sensitive to suffer steroid resistant acute rejection after renal transplantation. And the patients subjected to SRAR always produce AT1-AA. There is some statistical association between the appearance of AT1-AA and the D allele of ACE and C allele of AT1R. Patients with these types of antibodies have a less favorable prognosis, greater graft loss, and poorer long-term function as compared with patients with DSA alone. The treatment against such antibody mediated immune response is necessary and effective. We speculate the AT1-AA may have been produced when the subjects experienced preeclampsia or renal transplantation before or for other reasons. The importance of the detection of the genotype of ACE and AT1R and AT1-receptor antibodies in patients on a waiting list for a transplant might be comparable with the detection of PRA in identifying risk factors for refractory rejection.

5. Conflict of interest

The authors have declared that no conflict of interest exists.

6. Acknowledgments

We would like to give special thanks to Professor Yanfang Liu for his insightful pathological observations.

7. References

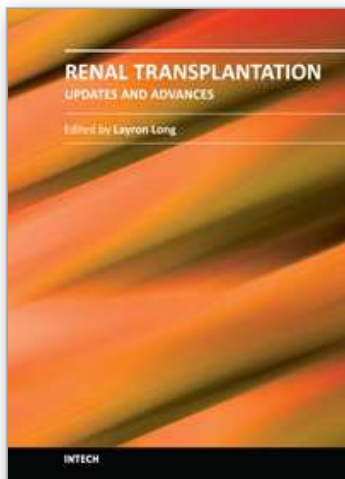
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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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