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Research Article

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Clinical impact of an angiotensin I-converting enzyme insertion/deletion and kinin B2 receptor +9/-9 polymorphisms in the prognosis of renal transplantation

Abstract: There is a consensus in the scientific literature that supports the importance of the kallikrein kinin and renin angiotensin systems in renal physiology, but few studies have investigated their importance after renal transplantation. The aim of this study was to investigate the clinical effects of the insertion/deletion polymorphism in the angiotensin I-converting enzyme (ACE) gene and the +9/-9 polymorphism in the kinin B2 receptor (B2R) gene in kidney-transplanted patients (n=215 ACE, n=203 B2R) compared with 443 healthy individuals. Demographic results showed that there is a higher frequency of the D allele (high plasma ACE activity) and +9 allele (lower B2R expression) in transplant patients compared with control individuals. We also observed a higher frequency of these alleles in patients who had an elevated level of plasma creatinine. At day 7 post-transplantation, we found a higher prevalence of individuals with the DD genotype with elevated plasma creatinine level. Furthermore, individuals with the DD genotype had a higher chronic allograft dysfunction and graft loss compared with the II patient genotype, which showed no loss of graft. Taken together, our data suggest that the DD genotype is an indicator of an unfavorable prognosis following renal transplantation and could be related to kinin modulation.

Keywords: angiotensin I-converting enzyme (ACE); B2 receptor; renal transplantation.

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Introduction

In the mid-1930s, a group of substances essential for proper human physiology was first identified that is now referred to as the kallikrein-kinin system (KKS) (Regoli and Barabe, 1980). Eighty years later, our understanding of the KKS is still growing. Currently, it is known that the biological actions of the KKS are promoted by kinins, which are generated from the cleavage of kininogen by kallikrein (Kayashima et al., 2012). It is also widely accepted that bradykinin (BK) is the central component of the KKS, and the effects of BK are mediated by binding to the BDKRB2 receptor (B2R) (Kakoki and Smithies, 2009). The expression of B2R is regulated by a common repeat sequence variation of 9 bp (+9/-9 alleles) in exon 1 of the B2R gene (Braun et al., 1996). More specifically, the +9 allele is associated with a lower expression level of B2R (Fischer et al., 2004).

The main factor influencing the interaction between BK and B2R is angiotensin I-converting enzyme (ACE). Kinins appear to be better ACE substrates than angiotensin I (Jaspard et al., 1993). The expression of ACE and its activity are determined by an insertion (I)/deletion (D)

polymorphism of a 287-bp *Alu* repeat sequence in intron 16 of the ACE gene (Martinez-Rios et al., 2008). More specifically, individuals with the DD genotype tend to have elevated ACE plasma activity, and individuals with the II genotype often have lower activity (Rigat et al., 1990; Danser and Schunkert, 2000). Furthermore, a study investigating the interaction between ACE and kinins has shown that the ACE I/D polymorphism does not change the angiotensin II plasma level but that the presence of the D allele increases BK degradation (Bryant and Shariat-Madar, 2009). Previously, we demonstrated that subjects with the DD genotype have a higher level of plasma kallikrein activity (Almeida et al., 2010), which increases the complexity of this system.

The importance of the interaction between the ACE and kinins in maintaining the proper physiological state and preventing pathological conditions [e.g., hypertension, type 2 diabetes, cardiovascular disease, cancers and chronic kidney disease (CKD)], which is primarily mediated through the integration of ACE in the two systems, has been investigated in several studies (Gard 2010; Putnam et al., 2012; Vejakama et al., 2012; Vinson et al., 2012). In the case of CKD, studies have focused on the mechanisms underlying the dysregulation of the renin–angiotensin system (RAS) (Chawla et al., 2010; Adarkwah et al., 2011; Mooyaart et al., 2011; Almeida et al., 2012), the KKS (Buleon et al., 2007; Riad et al., 2007; Tang et al., 2011) or both systems (Buleon et al., 2008; Maric 2008) in animal models of diabetic nephropathy. The effects of ACE inhibitors on CKD have also been investigated in clinical studies (Nakayama et al., 2009; Burrell et al., 2012; Santos et al., 2012; Su et al., 2012; Turner et al., 2012).

The importance of the RAS and KKS in the prognosis of kidney allograft rejection is poorly understood and deserves further investigation because the RAS is important in the regulation of systemic blood flow, arterial blood pressure, renal hemodynamics and proteinuria after renal transplantation. Thus, our study aimed to identify the effects of ACE I/D and/or B2R +9/-9 polymorphisms on clinical and graft outcomes in a cohort of renal transplant patients.

Results

Demographic data

First, we investigated whether there was an association between the polymorphisms and the demographic characteristics of the patients, as shown in Table 1.

Table 1 Demographic data.

Group	ACE			B2R		
Genotype	**II**	**ID**	**DD**	**+9/+9**	**+9/-9**	**-9/-9**
Controls	89	211	141	72	167	99
Transplanted	35	92	88	60	100	43
Transplanted data						
Sex male/ female (%)	63/37	57/43	57/43	61/39	58/42	67/33
Mean age (years)	39±12	41±12	39±12	39±12	39±12	39±12
Primary disease	–	–	–	–	–	–
Chronic glomerulonephritis (%)	28	30	31	32	23	51
Diabetes (%)	6	9	8	10	10	5
Hypertensive (%)	6	13	10	13	8	9
Unknown (%)	37	32	39	33	41	21
Others (%)	23	16	12	12	18	14
Creatinine:						
7 days	1.6±0.6	2.1±1.5	2.3±1.4	2.3±1.4	2.2±1.7	2.2±1.4
1 month	1.4±0.3	1.5±0.5	1.7±0.6	1.7±0.5	1.5±0.5	1.5±0.5
6 months	1.4±0.3	1.4±0.4	1.6±0.4	1.5±0.4	1.5±0.4	1.5±0.5
1 Year	1.4±0.3	1.4±0.4	1.6±0.5	1.6±0.6	1.4±0.3	1.6±0.6
Acute rejection (%)	15	26	59	32	44	24
Chronic rejection (%)	13	30	57	42	32	26
Graft loss (%)	0	25	75	33.33	33.33	33.33
Death (%)	18	64	18	37	36	27

The lines of control and transplanted group refers to the total number of sample; creatinine (mean, mg/dl). ACE, angiotensin I-converting enzyme; B2R, B2 receptor.

The distribution of the ACE I/D polymorphism alleles varied between the kidney transplant patients and controls. Specifically, a lower I allele frequency and a higher D allele frequency were observed in transplant individuals (I allele: 38%; D allele: 62%) compared with controls. For the B2R +9/-9 polymorphism, the transplant patients had a higher frequency of the +9 allele and a lower frequency of the -9 allele (+9 allele: 55%; -9 allele: 45%) compared with the controls (Figure 1).

Renal parameters

We observed an association between the high level of serum creatinine and the frequency of the ACE D allele on day 7 post-transplantation (Figure 2A). Higher levels of serum creatinine were associated with a greater prevalence of the D allele in renal transplant recipients. It is widely accepted that a serum creatinine level >1.5 is above the normal standard, a level >3.0 is an indicator of acute kidney disease and a level >5.0 is an indicator of CKD (Mehta et al., 2007). These same associations between higher serum creatinine levels and kidney function were also observed for the data collected at 1 month, 6 months and 1 year post-transplantation (Table 2). The mean serum creatinine for the DD genotype remained above normal levels even 1 year post-transplantation (Figure 2C).

For the B2R +9/-9 polymorphism to the serum creatinine, we observed statistical differences at all points in the allele**s** and at two points in the genotype (which we previously mentioned is associated with a lower expression of the receptor) compared with the controls (Table 3). However, we found no association between this polymorphism and the high mean serum creatinine level (Figure 2D).

Allograft dysfunction and graft survival

The main finding of this study is that survival is significantly reduced for individuals with the DD genotype who develop chronic allograft dysfunction (CAD) compared with individuals with the II and ID genotypes (Figure 3A). We also found an association between the DD genotype and an increased renal graft loss in patients (Figure 3C).

Acute rejection was also measured using multiple linear regressions, and we found that patients with the DD genotype were at a two-fold greater risk for acute graft rejection compared with patients with the II and ID genotypes (data not shown).

The graft survival analysis of the transplant group revealed that the median number of graft survival days of patients who developed CAD support our findings on the polymorphism of the ACE gene. The number of days of graft survival as follows:

- II genotype – 6744 days;
- ID genotype – 4473 days; and
- DD genotype – 3131 days.

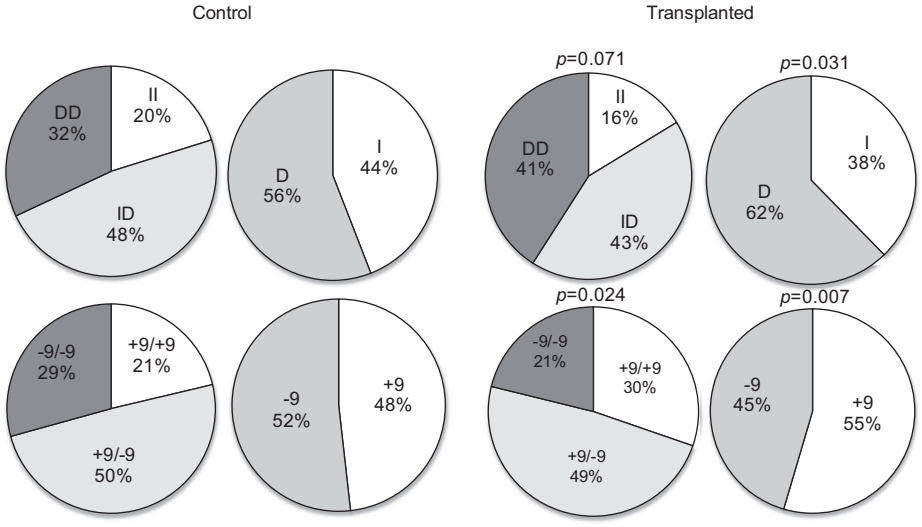

Figure 1 Frequency of genotypes and alleles of the polymorphisms of the ACE and B2R between the transplant and control groups (χ^2-test).

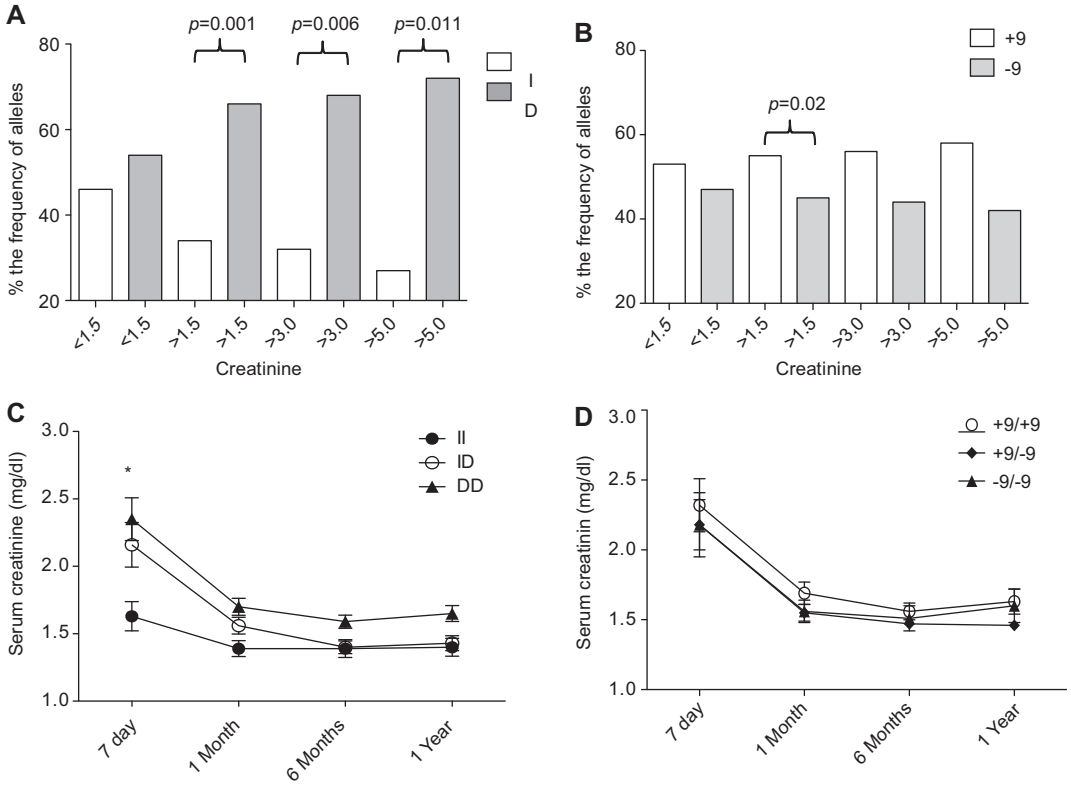

Figure 2 Allele frequency (%) of the ACE (A) and B2R (B) polymorphisms at different levels of creatinine 7 days after renal transplantation. Mean serum creatinine in four different periods in relation to ACE (C) and B2R (D) genotypes compared with the control group distribution. Analyses by χ^2 (A, B) and analysis of variance tests (C, D).

There appears to be an association between the DD genotype and a shorter graft survival time for patients with CAD. For the B2R polymorphism, the median graft survival time for patients who developed CAD between the genotypes +9/+9 and -9/-9 was different, but this difference was not significant. The graft survival times are as follows:

- +9/+9 genotypes – 3941 days;
- +9/-9 genotype – 3681 days; and
- -9/-9 genotype – 6481 days.

Table 2 Statistical χ^2-test analysis between the alleles and genotype compared to the control group in different periods and at different serum creatinine levels.

Date	Creatinine	II	ID	DD	*p*-Value	Allele I	Allele D	*p*-Value
Control		89	211	141		389	493	–
Transplanted		35	92	88		162	268	–
7 days	<1.5	16	40	27	0.929	72	94	0.929
7 days	>1.5	16	53	62	0.003[a]	85	177	0.001[a]
7 days	>3.0	3	22	23	0.02[b]	28	69	0.006[a]
7 days	>5.0	0	11	12	0.025[b]	11	35	0.011[b]
1 Month	<1.5	18	45	43	0.139	81	131	0.139
1 Month	>1.5	13	39	50	0.004[a]	65	139	0.004[a]
6 Months	<1.5	17	47	40	0.203	81	127	0.203
6 Months	>1.5	13	26	45	0.001[a]	52	116	0.002[a]
1 Year	<1.5	17	44	33	0.565	78	110	0.565
1 Year	>1.5	11	30	42	0.005[a]	52	114	0.003[a]

The lines of the control and transplanted groups refer to the total number of samples. [a]$p<0.01$, [b]$p<0.05$. Creatinine (mg/dl), normal values <1.5; high levels: 1.5–3.0; acute renal failure: 3.0–5.0; chronic renal failure: >5.0 (Mehta et al., 2007).

Table 3 Statistical χ^2-test analysis of the alleles and genotype of transplant patients compared to the control group at different points in time and the levels of serum creatinine.

Data	Creatinine	+9/+9	+9/-9	-9/-9	*p*-Value	Allele +9	Allele -9	*p*-Value
Control		72	167	99		311	365	
Transplanted		60	100	43		220	186	
7 days	<1.5	18	41	13	0.151	77	67	0.124
7 days	>1.5	38	55	26	0.047[a]	131	107	0.020[a]
7 days	>3.0	14	21	9	0.222	49	39	0.110
7 days	>5.0	6	10	3	0.348	22	16	0.207
1 Month	<1.5	21	57	19	0.142	99	95	0.248
1 Month	>1.5	32	40	22	0.037[a]	104	84	0.029[a]
6 Month	<1.5	22	53	19	0.215	97	91	0.202
6 Month	>1.5	25	35	17	0.095	85	69	0.049[a]
1 Year	<1.5	21	46	18	0.321	88	82	0.208
1 Year	>1.5	23	38	15	0.120	84	68	0.048[a]

The lines of control and transplanted group refer to the total number of sample. [a]$p<0.05$, creatinine (mg/dl), normal values <1.5; high levels: 1.5–3.0; acute renal failure: 3.0–5.0; chronic renal failure: <5.0 (Mehta et al., 2007).

Although there is no statistical difference, the average length of graft survival and the severity of CAD for patients with the II genotype appears to be different compared with patients with the other two ACE genotypes. This observation supports the suggestion that this genotype offers protection against CKD, as shown by the cross-sectional study, and that this genotype is associated with a better prognosis post-renal transplantation.

In the mean graft survival analysis, ACE polymorphism did not appear to affect the post-transplant results in patients with the II genotype. The mean graft survival for patients with the

- ID genotype – 5750 days; and
- DD genotype – 6103 days.

These results are significantly different. For the B2R polymorphism, we found mean graft survivals of

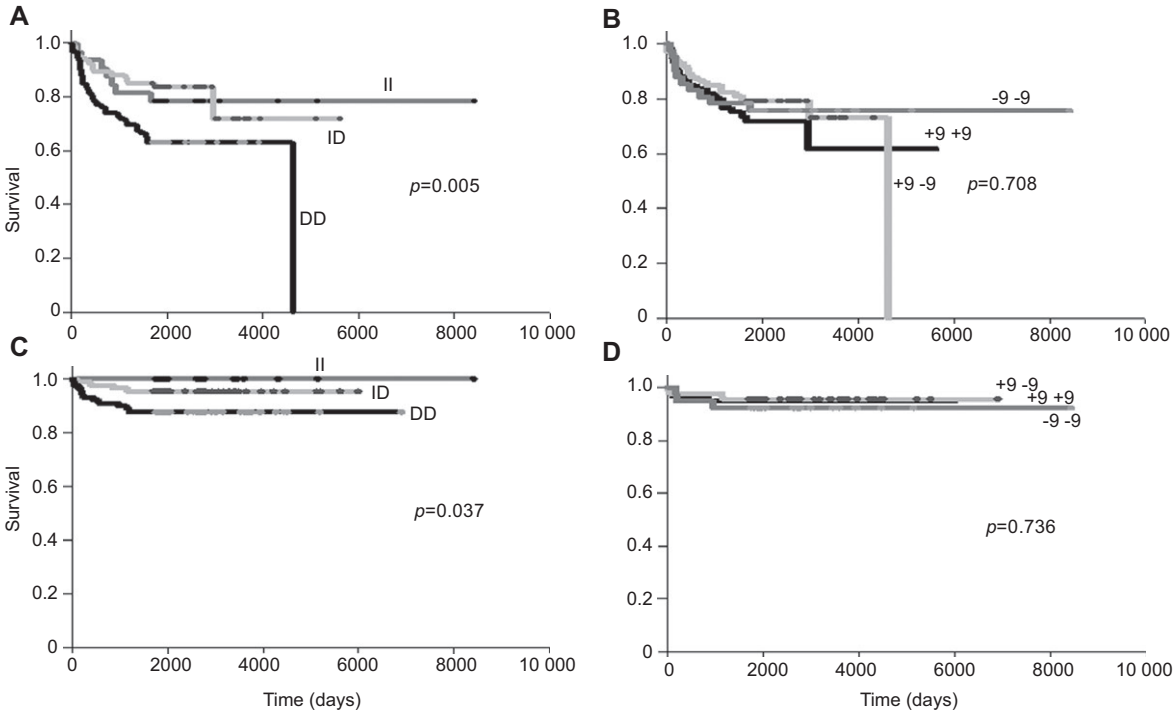

Figure 3 Analysis for (A, B) CAD events and (C, D) graft survival in relation to (A, C) ACE and (B, D) B2R genotype. Survival analysis determined using Kaplan-Meier.

– +9/+9 genotype – 5715 days;
– +9/-9 genotype – 6626 days; and
– -9/-9 genotype – 7812 days.

Despite there not being significant differences between the genotypes, these results suggest a clinical relevance.

Discussion

The main finding of the present study is that the DD genotype of the ACE polymorphism is associated with reduced graft survival and increased graft loss after kidney transplantation. These results are in accordance with observations made by Siekierka-Harreis et al. (2009). Also, results are very important when we analyze both polymorphisms and they suggest that the majority of transplant patients have higher ACE activity and lower B2R expression, highlighting the role of bradykinin in kidney transplantation. These observations are consistent with the finding that individuals homozygous for the D allele are at a greater risk for developing certain pathological disorders (Nicod et al., 2002; Akcay et al., 2004; Sabatini et al., 2008). Lower B2R expression, however, could also be involved in other pathologies (Van Guilder et al., 2008; Alhenc-Gelas et al., 2011; Alvim Rde et al., 2012).

The potentially deleterious effects of ACE I/D polymorphism have been investigated in non-transplant patients, where the DD genotype was associated with lower renal function, increased rates of graft rejection and longer hospitalization (Siekierka-Harreis et al., 2009). Our data support these previous findings. We also demonstrate that high creatinine levels and the B2R polymorphism may influence the prognosis of allograft survival.

CAD is one of the main causes of graft loss. Our results indicate that patients with the DD genotype develop this condition more quickly compared with patients with the other two ACE genotypes. It is important to mention that no patient with the II genotype developed CAD in this study. Despite the significant role the B2R +9/-9 polymorphism plays in creatinine levels, we found no significant difference in the allograft survival curve for this polymorphism.

The higher frequency of CAD in patients with the DD genotype was also associated with a gradual reduction in graft function, as demonstrated by higher serum levels and the median value of creatinine in DD carriers. Huang et al. (2003) also reported an association between RAS, especially high ACE activity, and an increased serum creatinine level. A statement from the Brazilian Society of Organ Transplant reports that by day 7 post-transplantation, patients have generally already been released from the hospital or will be released from hospital, and that creatinine level is used to support the decision to release the transplanted patient. Thus, the influence of the ACE and B2R genotypes on creatinine level could result in additional hospital costs. Furthermore, the higher serum creatinine in patients with the DD genotype during this period suggests that post-transplant recovery is also influenced by the ACE polymorphism, as was previously suggested by Huang et al. (2003).

Further emphasizing the importance of our data is the finding that the same associations between high levels of serum creatinine and the alleles investigated were found throughout the period of data analysis. These findings are especially significant for the DD genotype in which the creatinine level remained higher during the entire 1-year period.

Interestingly, loss of function was also higher in patients with reduced expression of B2R, i.e., the +9 allele carriers. The detrimental increase in BK degradation (resulting from the D allele) associated with the lower B2R expression could be explained by the well-known renoprotective role of BK. The association between the B2R +9/-9 genotype and graft function in transplant patients compared with controls was also observed at all other time points (i.e., 1 month, 6 months and 1 year after transplant) evaluated in this study.

In the context of clinical practice, our findings support the increased prescription of ACE inhibitors to patients with CKD, and the genotyping of ACE and B2R polymorphisms as they can have a clinical impact. This trend in prescribing ACE inhibitors began shortly after the first studies investigating the treatment of diabetic nephropathy (Lewis et al., 1993), proteinuria (Klahr et al., 1994) and renal failure (Maschio et al., 1996), and has continued to the present day (Francois et al., 2011; Lee et al., 2011).

In conclusion, our data suggest that the higher activity of ACE in patients with the DD genotype is detrimental to graft survival and function in renal transplant patients, and a higher expression of B2R is protective in terms of graft function and preservation. It may be that ACE inhibitor/angiotensin II receptor blocker prescription is beneficial among multiple medications for reducing mortality in kidney transplant recipients, but its use is not associated with longer graft survival as shown in this study.

Materials and methods

Samples

Between January 2010 and December 2011, 265 DNA samples from consecutive kidney transplant patients (113

females and 152 males of varying age) were obtained for this study (all the subjects were selected in accordance with the criteria for donation issued by the Brazilian Ministry of Health, ordinance number 1353, and from the Kidney Transplant and Hypertension Unit, Federal University of Sao Paulo, Brazil). The control group consisted of 443 ACE and 338 B2R healthy blood donors (including both genders ranging between 16 and 68 years of age) that reported to the Blood Center of the Hospital Sao Paulo (Federal University of Sao Paulo). The Ethics Committee of the Universidade Federal de São Paulo Hospital at UNIFESP approved all of the procedures (number 0353/04).

Genetic polymorphism

ACE I/D polymorphism was determined according to the methods described by Almeida et al. (2010), and B2R +9 /-9 kinin polymorphism was determined according to the methods described by Fischer et al. (2004).

Clinical parameters

All of the clinical parameters investigated in this study were collected directly from the medical records of each patient after renal transplantation. Only patients that had at least 1 year of follow-up appointments were included.

The parameters investigated included the following: demographic data, serum creatinine levels at 7 days, 1 month, 6 months and 1 year post-transplantation, episodes of graft rejection, diagnosis of CAD, interstitial fibrosis and tubular atrophy (referred to as chronic allograft nephropathy at the 8th Banff conference), graft loss and death, and CKD.

For renal graft loss analysis, patients whose renal function was lost due to technical problems with the graft or death were excluded.

Analysis of variables and statistical analysis

To verify any deviation from normal in our population, we used the Hardy-Weinberg equilibrium. To compare the demographic categorical covariates between groups, we used the χ^2-test or Fisher's exact test when appropriate. Serum creatinine level was analyzed at 7 days, 1 month, 6 months and 1 year post-transplantation using the χ^2-test or Fisher's exact test according to the genotypes or alleles for the polymorphism. Acute rejection was also measured using multiple linear regressions and graft survivals were calculated using the Kaplan-Meier analysis. The log rank test was used to investigate the significant differences between survival curves. Differences were considered significant when the p-value was <0.05. All analyses were performed using the SPSS 18.0 statistical software.

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References

Adarkwah, C.C., Gandjour, A., Akkerman, M., and Evers, S.M. (2011). Cost-effectiveness of angiotensin-converting enzyme inhibitors for the prevention of diabetic nephropathy in The Netherlands – a Markov model. PLoS One *6*, e26139.

Akcay, A., Sezer, S., Ozdemir, F.N., Arat, Z., Atac, F.B., Verdi, H., Colak, T., and Haberal, M. (2004). Association of the genetic polymorphisms of the renin-angiotensin system and endothelial nitric oxide synthase with chronic renal transplant dysfunction. Transplantation *78*, 892–898.

Alhenc-Gelas, F., Bouby, N., Richer, C., Potier, L., Roussel, R., and Marre, M. (2011). Kinins as therapeutic agents in cardiovascular and renal diseases. Curr. Pharm. Des. *17*, 2654–2662.

Almeida, S.S., Barros, C.C., Moraes, M.R., Russo, F.J., Haro, A.S., Rosa, T.S., Alves, M.F., Pesquero, J.B., Carmona, A.K., Bacurau, R.F., et al. (2010). Plasma kallikrein and angiotensin I-converting enzyme N- and C-terminal domain activities are modulated by the insertion/deletion polymorphism. Neuropeptides *44*, 139–143.

Almeida, S.S., Naffah-Mazzacoratti, M.G., Guimaraes, P.B., Wasinski, F., Pereira, F.E., Canzian, M., Centeno, R.S., Carrete, H., Yacubian, E.M., Carmona, A.K., et al. (2012). Carbamazepine inhibits angiotensin I-converting enzyme, linking it to the pathogenesis of temporal lobe epilepsy. Transl. Psychiatry *2*, e93.

Alvim Rde, O., Santos, P.C., Nascimento, R.M., Coelho, G.L., Mill, J.G., Krieger, J.E., and Pereira, A.C. (2012). BDKRB2 +9/-9 Polymorphism is associated with higher risk for diabetes mellitus in the Brazilian general population. Exp. Diabetes Res. *2012*, 480251.

Braun, A., Kammerer, S., Maier, E., Bohme, E., and Roscher, A.A. (1996). Polymorphisms in the gene for the human B2-bradykinin receptor. New tools in assessing a genetic risk for bradykinin-associated diseases. Immunopharmacology *33*, 32–35.

Bryant, J.W. and Shariat-Madar, Z. (2009). Human plasma kallikrein-kinin system: physiological and biochemical parameters. Cardiovasc Hematol Agents Med. Chem. *7*, 234–250.

Buleon, M., Mehrenberger, M., Pecher, C., Praddaude, F., Couture, R., Tack, I., and Girolami, J.P. (2007). [New perspectives for bradykinin in nephroprotection]. Med. Sci. (Paris) *23*, 1141–1147.

Buleon, M., Allard, J., Jaafar, A., Praddaude, F., Dickson, Z., Ranera, M.T., Pecher, C., Girolami, J.P., and Tack, I. (2008). Pharmacological blockade of B2-kinin receptor reduces renal protective effect of angiotensin-converting enzyme inhibition in db/db mice model. Am. J. Physiol. Renal. Physiol. *294*, F1249–1256.

Burrell, L.M., Burchill, L., Dean, R.G., Griggs, K., Patel, S.K., and Velkoska, E. (2012). Chronic kidney disease: cardiac and renal angiotensin-converting enzyme (ACE) 2 expression in rats after subtotal nephrectomy and the effect of ACE inhibition. Exp. Physiol. *97*, 477–485.

Chawla, T., Sharma, D., and Singh, A. (2010). Role of the renin angiotensin system in diabetic nephropathy. World J. Diabetes *1*, 141–145.

Danser, A.H. and Schunkert, H. (2000). Renin-angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. Eur. J. Pharmacol. *410*, 303–316.

Fischer, M., Lieb, W., Marold, D., Berthold, M., Baessler, A., Lowel, H., Hense, H.W., Hengstenberg, C., Holmer, S., Schunkert, H., et al. (2004). Lack of association of a 9 bp insertion/deletion polymorphism within the bradykinin 2 receptor gene with myocardial infarction. Clin. Sci. (Lond.) *107*, 505–511.

Francois, H., Jacquet, A., Beaudreuil, S., Seidowsky, A., Hebibi, H., Charpentier, B., and Durrbach, A. (2011). Emerging strategies to preserve renal function. J. Nephrol. *24*, 133–141.

Gard, P.R. (2010). Implications of the angiotensin converting enzyme gene insertion/deletion polymorphism in health and disease: a snapshot review. Int. J. Mol. Epidemiol. Genet. *1*, 145–157.

Huang, X.R., Chen, W.Y., Truong, L.D., and Lan, H.Y. (2003). Chymase is upregulated in diabetic nephropathy: implications for an alternative pathway of angiotensin II-mediated diabetic renal and vascular disease. J. Am. Soc. Nephrol. *14*, 1738–1747.

Jaspard, E., Wei, L., and Alhenc-Gelas, F. (1993). Differences in the properties and enzymatic specificities of the two active sites of angiotensin I-converting enzyme (kininase II). Studies with bradykinin and other natural peptides. J. Biol. Chem. *268*, 9496–9503.

Kakoki, M. and Smithies, O. (2009). The kallikrein-kinin system in health and in diseases of the kidney. Kidney Int. *75*, 1019–1030.

Kayashima, Y., Smithies, O., and Kakoki, M. (2012). The kallikrein-kinin system and oxidative stress. Curr. Opin. Nephrol. Hypertens *21*, 92–96.

Klahr, S., Levey, A.S., Beck, G.J., Caggiula, A.W., Hunsicker, L., Kusek, J.W., and Striker, G. (1994). The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. N. Engl. J. Med. *330*, 877–884.

Lee, Y.J., Jang, H.R., Kim, S.G., Chae, D.W., Do, J.Y., Lee, J.E., Huh, W., Kim, D.J., Oh, H.Y., and Kim, Y.G. (2011). Renoprotective efficacy of valsartan in chronic non-diabetic proteinuric nephropathies with renin-angiotensin system gene polymorphisms. Nephrology (Carlton) *16*, 502–510.

Lewis, E.J., Hunsicker, L.G., Bain, R.P., and Rohde, R.D. (1993). The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N. Engl. J. Med. *329*, 1456–1462.

Maric, C. (2008). Vasoactive hormones and the diabetic kidney. ScientificWorldJournal *8*, 470–485.

Martinez-Rios, M.A., Pena-Duque, M.A., Fragoso, J.M., Delgadillo-Rodriguez, H., Perez-Hernandez, N., Miranda-Malpica, E., Rodriguez-Perez, J.M., Gonzalez-Quesada, C., and Vargas-Alarcon, G. (2008). No association found between the insertion/deletion of a 287-bp alu repeat sequence within intron 16 of the angiotensin-I-converting enzyme (ACE) gene in Mexican patients and binary restenosis after coronary stenting. Clin. Chim. Acta. *397*, 65–67.

Maschio, G., Alberti, D., Janin, G., Locatelli, F., Mann, J.F., Motolese, M., Ponticelli, C., Ritz, E., and Zucchelli, P. (1996). Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. N. Engl. J. Med. *334*, 939–945.

Mehta, R.L., Kellum, J.A., Shah, S.V., Molitoris, B.A., Ronco, C., Warnock, D.G., and Levin, A. (2007). Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit. Care. *11*, R31.

Mooyaart, A.L., Valk, E.J., van Es, L.A., Bruijn, J.A., de Heer, E., Freedman, B.I., Dekkers, O.M., and Baelde, H.J. (2011). Genetic associations in diabetic nephropathy: a meta-analysis. Diabetologia *54*, 544–553.

Nakayama, Y., Nonoguchi, H., Kohda, Y., Inoue, H., Memetimin, H., Izumi, Y., and Tomita, K. (2009). Different mechanisms for the progression of CKD with ACE gene polymorphisms. Nephron. Clin. Pract. *111*, c240–246.

Nicod, J., Richard, A., Frey, F.J., and Ferrari, P. (2002). Recipient RAS gene variants and renal allograft function. Transplantation *73*, 960–965.

Putnam, K., Shoemaker, R., Yiannikouris, F., and Cassis, L.A. (2012). The renin-angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. Am. J. Physiol. Heart. Circ. Physiol. *302*, H1219–1230.

Regoli, D. and Barabe, J. (1980). Pharmacology of bradykinin and related kinins. Pharmacol. Rev. *32*, 1–46.

Riad, A., Zhuo, J.L., Schultheiss, H.P., and Tschope, C. (2007). The role of the renal kallikrein-kinin system in diabetic nephropathy. Curr. Opin. Nephrol. Hypertens *16*, 22–26.

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., and Soubrier, F. (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J. Clin. Invest. *86*, 1343–1346.

Sabatini, R.A., Guimaraes, P.B., Fernandes, L., Reis, F.C., Bersanetti, P.A., Mori, M.A., Navarro, A., Hilzendeger, A.M., Santos, E.L., Andrade, M.C., et al. (2008). ACE activity is modulated by kinin B2 receptor. Hypertension *51*, 689–695.

Santos, P.C., Krieger, J.E., and Pereira, A.C. (2012). Renin-angiotensin system, hypertension, and chronic kidney disease: pharmacogenetic implications. J. Pharmacol. Sci. *120*, 77–88.

Siekierka-Harreis, M., Kuhr, N., Willers, R., Ivens, K., Grabensee, B., Mondry, A., Loh, M.C., Rump, L.C., and Blume, C. (2009). Impact of genetic polymorphisms of the renin-angiotensin system and of non-genetic factors on kidney transplant function – a single-center experience. Clin. Transplant. *23*, 606–615.

Su, S.L., Lu, K.C., Lin, Y.F., Hsu, Y.J., Lee, P.Y., Yang, H.Y., and Kao, S.Y. (2012). Gene polymorphisms of angiotensin-converting enzyme and angiotensin II type 1 receptor among chronic kidney disease patients in a Chinese population. J. Renin Angiotensin Aldosterone Syst. *13*, 148–154.

Tang, S.C., Chan, L.Y., Leung, J.C., Cheng, A.S., Lan, H.Y., and Lai, K.N. (2011). Additive renoprotective effects of B2-kinin receptor blocker and PPAR-gamma agonist in uninephrectomized db/db mice. Lab. Invest.; A J. Tech. Methods and Pathology *91*, 1351–1362.

Turner, J.M., Bauer, C., Abramowitz, M.K., Melamed, M.L., and Hostetter, T.H. (2012). Treatment of chronic kidney disease. Kidney Int. *81*, 351–362.

Van Guilder, G.P., Pretorius, M., Luther, J.M., Byrd, J.B., Hill, K., Gainer, J.V., and Brown, N.J. (2008). Bradykinin type 2 receptor BE1 genotype influences bradykinin-dependent vasodilation during angiotensin-converting enzyme inhibition. Hypertension *51*, 454–459.

Vejakama, P., Thakkinstian, A., Lertrattananon, D., Ingsathit, A., Ngarmukos, C., and Attia, J. (2012). Reno-protective effects of renin-angiotensin system blockade in type 2 diabetic patients: a systematic review and network meta-analysis. Diabetologia *55*, 566–578.

Vinson, G.P., Barker, S., and Puddefoot, J.R. (2012). The renin-angiotensin system in the breast and breast cancer. Endocr. Relat. Cancer *19*, R1–19.