No association between renin–angiotensin system gene polymorphisms and early and long-term allograft dysfunction in kidney transplant recipients

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Abstract

Background. Genes determining the activity of the renin–angiotensin system (RAS) may be alloantigen-independent factors influencing kidney allograft function. We determined if gene polymorphisms of the RAS are associated with early and long-term post-transplantation graft dysfunction in 405 Caucasian kidney recipients with graft survivals of >2 years.

Methods. We calculated the slopes of serum creatinine/C0/year and urinary protein excretion/year to follow graft function over time. Subjects were genotyped for the deletion (D) polymorphism of the gene encoding angiotensin I-converting enzyme, the angiotensin II-receptor type1 gene 1166A-C polymorphism and the M235T polymorphism of the angiotensinogen gene.

Results. The frequencies of factors predicting graft function were similar in patients with different genotypes. None of the polymorphisms influenced need for dialysis in the first week after transplantation, occurrence of at least one rejection episode, the slope of serum creatinine/C0/year or the slope of urinary protein excretion/year. Results were independent of blood pressure or the use of angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers or calcineurin inhibitors. The combination of genotypes did not influence the indicators of early and long-term graft dysfunction.

Conclusions. Neither the investigated gene polymorphisms of the RAS in kidney allograft recipients nor their combinations have an impact on early and long-term graft dysfunction.

Keywords: angiotensin I-converting enzyme; angiotensin II-receptor type 1; angiotensinogen; chronic graft dysfunction; gene polymorphism; kidney transplantation

Introduction

Chronic allograft dysfunction (CAD) is the major cause of transplant loss in kidney graft recipients. The factors leading to CAD are not fully understood, but they consist of a combination of the underlying kidney disease and superimposed environmental and genetic factors. 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 kidney allograft function.

The insertion (I)/deletion (D) polymorphism of intron 16 of the angiotensin-converting enzyme (ACE) gene is associated with the altered activity of this enzyme, determining ~50% of the interindividual variability of plasma ACE activity in humans [1]. Even though blood pressure seems not to be affected by ACE I/D polymorphism [2], the D/D genotype is a risk factor for the progression of chronic renal failure in IgA nephropathy and a predictor of the therapeutic efficacy of ACE inhibition in this disease [3]. Moreover, the ACE I/D polymorphism has been shown to be involved in both the susceptibility to diabetic nephropathy and its progression towards renal failure in insulin-dependent diabetic subjects at risk of renal complications [4]. Another study described homozygosity for the ACE D allele as a risk factor for diabetic nephropathy [5].
for early end-stage renal failure in PKD1 adult polycystic kidney disease [5].

A DNA polymorphism that alters methionine to threonine at position 235 (M235T) within the mature angiotensinogen (AGT) peptide, which is associated with increased circulating AGT levels, showed linkage to essential hypertension [6]. Hypertension is an important risk factor for the progression of CAD, hence one might assume that the variant 235T of the AGT gene is a risk factor for the worsening of CAD; besides which, that allele has been shown to be more frequent in patients with chronic renal failure [7]. In this study, however, the 235T allele was associated only with chronic renal failure when interstitial nephritis was the underlying disease.

Angiotensin II interacts with two pharmacologically distinct subtypes of cell surface receptors, types 1 and 2 (AGTR1 and AGTR2). Type 1 receptors seem to mediate the major cardiovascular effects of angiotensin II. Variants in the AGTR1 gene may affect blood pressure in humans. Bonnardeaux et al. [8] found an association between several AGTR1 gene polymorphisms and essential hypertension. Specifically, an A-to-C variant in the 3' untranslated region at nucleotide 1166 (1166A–C) showed a significantly elevated frequency in 206 Caucasian patients with essential hypertension.

We tried to determine whether or not gene polymorphisms of the RAS are associated with markers of early and long-term graft dysfunction after kidney transplantation. Furthermore, by studying polymorphisms of the ACE, AGT and the AGTR1 genes, we analysed gene–gene interaction with respect to early and long-term graft dysfunction.

**Subjects and methods**

**Patients**

We studied 405 Caucasian kidney transplant recipients, who were recruited from the nephrology outpatient departments of two clinics (Klinik für Nephrologie, Universitätsklinikum Charité, Humboldt Universität Berlin and Medizinische Klinik IV, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany). To rule out graft loss due to early surgical or early immunological problems, patients with graft survivals of < 2 years were not included. The cohort represents all patients who were seen in the two outpatient departments from January to December 1998. Their transplantations were done between June 1974 and December 1996; the mean duration of their follow-ups was 6.0 years. The following patient characteristics were documented: recipient gender and age, donor gender and age, living or cadaveric donor, recipient and donor CMV status, number of transplantations, duration of dialysis before transplantation, most recent percentage of panel-reactive antibodies at the time of transplantation, cold ischaemia time, HLA mismatch, need for dialysis after transplantation, number of acute rejection episodes, number of steroid-resistant acute rejection episodes, actual immunosuppressive regime and actual medication with ACE inhibitors or angiotensin-receptor (ATR) blockers, for at ≥1 month. Informed consent was obtained in from each patient; thus, the study was in accordance with the declaration of Helsinki 1975 (revised in 1983).

**Methods**

Genomic DNA was extracted from peripheral blood cells from samples drawn at routine visits in the outpatient departments. Patients were genotyped for the D polymorphism of the gene encoding ACE, the M235T polymorphism of the AGT gene and the AGTR1 gene 1166A–C polymorphism. Genotypes were determined as described previously [9].

Early graft function was assessed by the need for dialysis in the first week after transplantation.

Urinary protein excretion was measured on 24h collections using the pyrogallol red reagent in a Hitachi 717 automated analyser.

Serum creatinine, in blood samples drawn at routine visits to the outpatient departments, was measured enzymatically (PAP, Boehringer Ingelheim, Guiblt, Germany). For each patient, using all available measurements since transplantation (with the first post-transplantation year excluded), we calculated by linear regression the slope of serum creatinine−1/year and used it as an indicator of the progression of graft dysfunction over time. The slope of urinary protein excretion/year was calculated analogously. Blood pressure recordings for the time-points of blood samplings for creatinine measurements were retrieved from patients’ files. Means for systolic and diastolic pressures were calculated for each patient from all available blood pressure recordings starting from the year prior to inclusion.

**Statistical analysis**

For statistical analysis we used SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were compared by unpaired t-tests when normal distribution was confirmed by the Kolmogorov–Smirnov (KS) test. If the KS test showed significant (P < 0.05) non-normal distribution, the non-parametric Mann–Whitney U-test was used to compare continuous variables. Categorical data were compared by χ² testing. Genotype-dependent differences in outcome variables in medication subgroups were analysed by analysis of variance (ANOVA). The impact of genotypes on indicators of loss of graft function was subjected to multivariate analysis with systolic and diastolic blood pressures (cut off is median) as covariates. Unless otherwise indicated, means ± SD are given for measured parameters. A P-value of <0.05 was considered significant.

Power calculation provided a sample size of at least 36 per group for a difference between genotype groups of >0.05 dl/mg in the slope of creatinine−1/year and a sample size of at least 165 for a difference of >0.025 dl/mg (for power = 0.9, α = 0.05, SD = 0.07 dl/mg).

**Results**

**Genotype frequencies**

The observed genotypes are shown in Figure 1. The genotype frequencies in our results were not significantly different from the frequencies reported by Pei et al. [10]. The frequencies of polymorphisms
The slopes of creatinine concentration were calculated for each patient from all available serum creatinine measurements, with the first post-transplantation year excluded. There was no significant difference between genotypes. Data are shown as box-and-whisker plots. The boxes show the interquartile ranges between the lower (25th percentile) and the upper (75th percentile) quartiles, representing the middle 50% of the data. The whiskers show the 5th and 95th percentiles. The lines within the boxes represent the medians.

**Patient characteristics**

The frequency of known factors predictive of graft function was similar in patients with different genotypes (Table 1).

**Known risk factors for loss of graft function over time**

A cold ischaemia time (CIT) of >24 h emerged as a risk factor for the progressive loss of graft function. Patients were subdivided genotype-independently into groups with CIT shorter or longer than 24 h. The slopes of creatinine concentration showed a statistically significant difference between the two groups, with a worse slope in patients with CIT > 24 h (CIT < 24 h: 0.0096 ± 0.0014 dl/mg, n = 266; CIT > 24 h: −0.0201 ± 0.0485 dl/mg, n = 139; P = 0.013). Patients with at least one acute rejection episode within the first year after transplantation had a significantly worse slope of creatinine concentration compared with patients without acute rejections within the first year (at least one acute rejection: −0.0184 ± 0.0650 dl/mg, n = 201; no acute rejection: 0.0084 ± 0.0811 dl/mg, n = 204; P = 0.031).

**Acute rejection episodes**

The percentage of patients with at least one acute rejection episode was 48.6%. The distribution of genotypes in patients with at least one rejection was similar to their distribution in patients without acute rejection: for the distribution of the ACE I/D polymorphism, χ² (2 df) = 4.903 (P = 0.179); for the ATR 1166A–C polymorphism, χ² (2 df) = 1.645 (P = 0.649); and for the AGT M235T polymorphism, χ² (2 df) = 0.386 (P = 0.943). The numbers of patients with at least one steroid-resistant acute rejection episode were not significantly different between different genotypes: 11 : 23 : 15 for the ACE I/D polymorphism [DD : DI : II, χ² (2 df) = 1.494, P = 0.474]; 19 : 24 : 6 for the ATR 1166A–C polymorphism [AA : AC : CC, χ² (2 df) = 1.713, P = 0.425]; and 23 : 19 : 7 for the AGT M235T polymorphism [MM : MT : TT, χ² (2 df) = 0.971, P = 0.615].

**Need for dialysis after transplantation**

The need for dialysis within 1 week after transplantation was used to gauge early graft function. The overall percentage of patients who did not need dialysis during the first week after transplantation was 47%. The distribution of the genotypes of the investigated polymorphisms in patients with a need for dialysis was not significantly different from that in patients without a need for dialysis: for the distribution of the ACE I/D polymorphism, χ² (2 df) = 1.9 (P = 0.654); for the ATR 1166A–C polymorphism, χ² (2 df) = 1.345 (P = 0.716); and for the AGT M235T polymorphism, χ² (2 df) = 1.636 (P = 0.651).

**Loss of graft function over time**

As indicators for the progression of graft dysfunction, neither the slope of creatinine concentration nor the annual enhancement of urinary protein excretion were significantly different between the investigated genotypes (Figures 1 and 2). The mean number of creatinine measurements was 7.1 ± 8.45 per patient per year and of urinary protein excretion measurements 2.8 ± 2.7 per patient per year. The mean of the slope of creatinine concentration from all patients was −0.0059 ± 0.069 dl/mg.

**ACE inhibitors and ATR blockers**

In our study population, 155 patients were on medication with ACE inhibitors or ATR blockers for >1 month. In patients on ACE inhibitors or ATR blockers, the slopes of creatinine concentration for the ACE I/D polymorphism were similar (DD: −0.00011 ± 0.04231 dl/mg, n = 36; DI: −0.00684 ± 0.05803 dl/mg, n = 85; II: −0.01125 ± 0.05106 dl/mg, n = 34; P = 0.663 for comparison between groups by ANOVA). Also similar were urinary protein excretion for the ACE I/D polymorphism. Neither for the ATR 1166A–C polymorphism nor for the AGT M235T polymorphism did the slope of creatinine concentration or the annual improvement of urinary protein excretion differ significantly between patients medicated or not medicated with ACE inhibitors or ATR blockers (data not shown).
Immunosuppression

In the cohort, 334 patients were on a calcineurin inhibitor-based immunosuppression protocol. From them, 290 received cyclosporin A in a mean dose of 171.3±61.0 mg/day and 44 received FK 506 in a mean dose of 6.74±0.42 mg/day at the time of inclusion. Another 71 patients were on an immunosuppression regimen (without use of calcineurin inhibitors) with azathioprine at a mean dose of 73.7±27.7 mg/day.

We found no influence of the investigated genotypes on the indicators of graft dysfunction in subgroup analyses of patients receiving or those not receiving calcineurin inhibitors (data not shown).

Blood pressure

Blood pressure was similar in different genotype groups (Table 1). Multivariate analysis with systolic and diastolic blood pressures as covariates showed no significant impact of different genotypes on parameters of loss of graft function. Systolic blood pressure was significantly higher in patients immunosuppressed with calcineurin inhibitors (n=334) compared with patients immunosuppressed without calcineurin inhibitors (n=71) (140.2±10.8 vs 135.0±11.0 mmHg; P<0.001), whereas diastolic blood pressure was similar in both groups (88.5±6.7 vs 87.9±7.7 mmHg; P=0.526). Subgroup analysis revealed that the investigated genotypes influenced neither systolic nor diastolic blood pressures in either group (data not shown).

Combination of genotypes

We compared the combinations of genotypes that have been reported to increase the activity of the RAS with those reported to have a lesser impact on the slope of creatinine−1/year as a marker of the progression of graft dysfunction over time. Therefore, patients...
homozgyous for the ACE D allele (DD) and harbouring at least one ATG T allele (TT or MT) were compared with patients homozygous for the ACE I allele (II) and for the ATG M allele (MM) and no significant difference was found between these groups in the slope of creatinine^{-1}/year ($n=62$ and 44, respectively; $P=0.645$). We tested the combinations of genotypes described above for their impacts on the enhancement of daily urinary protein excretion, the occurrence of at least one acute rejection episode and the need for dialysis within the first week after transplantation and we found no differences. We also tested all other possible combinations of genotypes. There was no combination of genotypes that resulted in a difference in the outcome variables described above (data not shown).

**Discussion**

In our study we analysed whether or not gene polymorphisms of the RAS contribute to kidney allograft dysfunction. None of the investigated polymorphisms showed an association with the need for dialysis after transplantation, occurrence of acute rejection episodes or deterioration of graft function over time. The results where independent of blood pressure or the use of ACE inhibitors and ATR blockers or calcineurin inhibitors. Furthermore, the combination of genotypes did not influence the indicators of early and long-term graft dysfunction.

In contrast, the known epigenetic risk factors of chronic graft dysfunction were reproducible in our study population, indicating that our study population is reliable for the analysis of factors influencing graft function.

Beige et al. [11] showed that a donor polymorphism (C825T) of the gene encoding the β3 subunit of heterotrimeric G proteins (GNB3) and associated with enhanced activation of G proteins, but not the recipient’s genotype – leads to a significantly decreased kidney allograft survival.

The histological findings in CAD characteristically show interstitial and vascular fibrosis, with occlusion of renal arteries and ischaemic glomeruli and interstitial infiltration by macrophages and lymphocytes [12]. Regarding these histological findings, it is conceivable that those genetic factors native to the transplant recipient that affect mesangial or vascular proliferation, aspects of inflammatory response, blood pressure regulation or fibrosis may influence the progression of CAD. Therefore, in the RAS there might be candidate genes that could influence the outcome of the renal allograft.

The ACE D allele has been shown to be a risk factor for the development and progression of diabetic nephropathy and the progression of chronic renal failure in immunoglobulin-A nephropathy and in PKD1 adult polycystic kidney disease [3–5]. In our study, ACE polymorphisms were not associated with any of the tested markers of early and long-term graft function. This finding is in line with other studies that examined the effect of the ACE I/D polymorphism on the onset or the progression of CAD in adult kidney allograft recipients [13,14].

The angiotensinogen M235T polymorphism has been shown to be associated with increased amounts of circulating angiotensinogen and development of essential hypertension [6]. As many as 70% of all kidney transplant recipients develop arterial hypertension, which is an important risk factor for the progression of CAD. However, no impact of the AGT M235T polymorphism on blood pressure and on early and long-term graft function was detectable in our analysis of 405 patients. Our data are in good agreement with another recent study of kidney allograft recipients, which likewise showed no association between the AGT M235T polymorphism and CAD. Furthermore, no influence of the AGT M235T polymorphism on post-transplantation hypertension was detectable in that study [15]. However, it is important to note in this context that another study, of 210 renal allograft recipients younger than 36 years, found an association between the AGT 235T/T genotype and a shorter time to sustained doubling of serum creatinine [16]. The differences between our data and those of the above-mentioned study [16] might be due to the selection of completely different study populations (age, for example, is totally different between the two studies).

In humans, variance in the AGTR1 gene may affect blood pressure [8]. AGTR1a knocked-out mice show a decrease of the infiltration of inflammatory mononuclear cells in ischaemic tissue and decreased expressions of monocyte chemoattractant protein-1 and vascular endothelial growth factor [17]. Regarding this phenotype, polymorphisms regulating the activity of AGTR1 may influence the development and progression of CAD, as well as of acute rejections and delayed graft dysfunction. In our study population, we were unable to find any association between the AGTR1 1166A–C polymorphism and blood pressure and early and long-term graft function. Two other studies in patients with chronic renal failure reported similar results, as did one study in paediatric kidney allograft recipients [5,10].

The lack of relationship between the investigated genotypes and early and long-term graft function in our study may indicate that other factors have major impacts in the setting of kidney transplantation. Immunological factors, for instance, HLA mismatches or cytokine release, have strong impacts on the development of early and late graft dysfunction and so a comparatively weaker influence of elevated RAS activity might not be detectable. Non-immunological factors, such as a pro-coagulatory disposition from an activated-protein C resistance caused by the factor V Leiden mutation, have been shown to be associated with early and late kidney allograft dysfunction [18].

In addition, there are at least two epigenetic factors that may have stronger impacts on the activity of the RAS than do the investigated genetic variants of the
RAS by themselves. These are calcineurin inhibitors, widely used immunosuppressants known to modify the activity of the RAS, which might make the role of polymorphism of the RAS less important [1], and blood pressure control, which has been shown to be important in the management of the progression of CAD. Therapy with ACE inhibitors and other blood pressure-lowering drugs is common in kidney transplant recipients. However, in our study population the investigated polymorphisms had no detectable impacts, even in patients not on medications that directly influence the activity of the RAS.

Regarding our negative results, it is also important to note that in a recent review [19] dealing with the ‘problems of reporting genetic associations with complex outcomes’, the authors concluded that the most important of the factors underlying the inability to replicate such associations is publication bias against negative results. There often are found smaller initial studies showing associations between genetic variants and complex outcomes which are not confirmed in larger follow-up studies.

It is also noteworthy that we investigated the impact of gene polymorphisms in allograft recipients, since DNA samples from the donors were not available to us. As shown by Beige et al. [11] for the protein β3 subunit 825TT allele, the genotype of the donor and, therefore, of the kidney allograft might be important in early and long-term graft dysfunction. In particular, the RAS is strongly operant locally. Thus, our data could not fully exclude the possible impact of genetic variances in donors’ native RAS on early and late kidney graft dysfunction.

In conclusion, our study shows that none of the investigated gene polymorphisms of the RAS in recipients of kidney allografts have an impact on early and long-term kidney graft dysfunction, alone or in combination.

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References


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