Angiotensinogen-M235T genotype and post-transplant hypertension

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Abstract

Background. The angiotensinogen gene has been linked to the development of essential hypertension, and a M235T variant of this gene, associated with increased plasma levels of angiotensinogen, is more common in hypertensives than in normotensive controls in various populations. The present study was conducted to examine whether the M235T variant of the angiotensinogen gene may be a risk factor for the development of hypertension in patients undergoing renal transplantation.

Methods. DNA for genetic analysis was prospectively collected from 269 consecutive patients undergoing kidney transplantation between 1988 and 1993 and their corresponding donors. Presence of hypertension and graft survival was analysed by blinded review of all case records over a follow-up period up to 30 months. Angiotensinogen genotype was determined by a mutagenically separated allele-specific polymerase-chain-reaction technique.

Results. While post-transplant hypertension was present in 78% of all patients, no relationship was found between either donor or recipient genotype and the presence or severity of post-transplant hypertension. Furthermore, there was no relationship between angiotensinogen genotype and graft survival during the course of the study.

Conclusions. These findings do not support the hypothesis that the M235T variant of the angiotensinogen gene is a risk factor for the development of post-transplant hypertension.

Key words: angiotensinogen; genotype; hypertension; post-transplant; M235T variant

Introduction

The development of hypertension remains a severe problem in the management of a large proportion of patients undergoing renal transplantation. Hypertensive renal-transplant recipients have a markedly lower transplant survival, as well as a higher risk for the development of cardiovascular complications such as myocardial infarction and stroke [1-3]. According to current understanding, multiple factors including native kidney disease, renal-artery stenosis, chronic rejection, recurrent disease, and the use of steroids and cyclosporin appear to play a role in the aetiology of post-transplant hypertension [2,3]. However, in most patients the possible contribution of each of these factors as well as their interrelationship remains unclear.

Several lines of evidence suggest a role for the renin–angiotensin system in the development of post-transplant hypertension [1-3]. Thus, hypertrophy of the juxtaglomerular apparatus is a common histological feature of chronic rejection, presumably indicating increased renin secretion [4], and higher plasma renin activity has been reported in patients with worsening of graft function [5]. Stimulation of renal and extrarenal synthesis of angiotensinogen by steroids has also been suggested as a possible factor contributing to the development of post-transplant hypertension [1].

Several family and sib-pair studies have recently demonstrated linkage between the angiotensinogen-gene locus and essential hypertension [6-8], and a so-called M235T variant of this gene, associated with increased circulating angiotensinogen levels [6], has been linked to an increased risk for the development of hypertension both in Caucasian [6,9,10] and in Japanese subjects [11-13]. We have recently also reported that the M235T variant is associated with an early onset of hypertension in German Caucasian populations both in Berlin and Heidelberg [14]. Since a variant of the angiotensinogen gene associated with essential hypertension may also increase the risk for the development of post-transplant hypertension, the aim of the present study was to determine the relationship between the angiotensinogen M235T genotype and the existence of post-transplant hypertension in patients undergoing cadaveric renal transplantation for end-stage renal failure. Due to the possible significance of the intrarenal renin–angiotensin...
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system, both donor and recipient genotypes were examined.

Subjects and methods

Patient population and clinical characterisation

DNA samples were collected prospectively between 1988 and 1993 from 269 consecutive Caucasian individuals receiving first-time cadaveric kidney transplants, and from their respective donors. The clinical course for the first 30 months following transplantation was retrospectively analysed by blinded review of all case records with regard to graft loss and prevalence of hypertension, defined by the repeated recording of blood pressure levels above 150/95 mmHg and/or prescription of drugs used for lowering blood pressure, excluding diuretics. The number of antihypertensive drugs administered to a single patient was used as a surrogate measure for the severity of hypertension.

Genotyping

Donor and recipient genomic DNA was prepared from peripheral white blood cells using a DNA-selective preparation method (Quiagen, Hilden, Germany). Subsequently the angiotensinogen M235T genotype was determined using the mutagenetically-separated polymerase chain reaction technique using allele-specific primers as described previously [14]. In brief, amplification with primer P1 (5'CCA GGG TGC TGT CCA CAC TGC CTC CGG 3') and primer P3 (5'TGT GGT CCT CCC ACG CTC TCT GG 3') yielded a 134 bp product representing the T allele, whereas amplification with primer P2 (5'AAG TGG ACG TAG GTG TGT A 3') and primer P3 yielded a 156 bp product representing the M allele. Reactions were carried out using 100 ng genomic DNA in a total volume of 20 μL containing 50 mM KCl, 100 mM Tris-HCl, 1% Triton X-100, 1.5 mM MgCl2, 0.125 mM dNTP, 0.3 μM primer P1, 0.4 μM primer P2, 0.1 μM primer P3, and 1 U Taq DNA polymerase. The amplification parameters were as follows: initial denaturation for 4 min at 94°C followed by 35 cycles with 45 s at 94°C, 50 s at 62°C and 1 min at 72°C. Amplicons were visualized on 3.5% ethidium-bromide-stained agarose gels. In a series of samples comparing this method to that involving allelic-specific primers as described previously [14], in brief, amplification with primer P1 (5'CAC GAG GGT CCT CCC ACG CTC TCT GG 3') and primer P2 (5'AGG GTG CAC TGC TCT GGT 3') yielded a 134 bp product representing the T allele, whereas amplification with primer P1 (5'CCA GGG TGC TGT CCA CAC TGC CTC CGG 3') and primer P3 (5'TGT GGT CCT CCC ACG CTC TCT GG 3') yielded a 156 bp product representing the M allele, whereas amplification with primer P2 (5'AAG TGG ACG TAG GTG TGT A 3') and primer P3 yielded a 156 bp product, representing the M allele. Reactions were carried out using 100 ng genomic DNA in a total volume of 20 μL containing 50 mM KCl, 100 mM Tris-HCl, 1% Triton X-100, 1.5 mM MgCl2, 0.125 mM dNTP, 0.3 μM primer P1, 0.4 μM primer P2, 0.1 μM primer P3, and 1 U Taq DNA polymerase. The amplification parameters were as follows: initial denaturation for 4 min at 94°C followed by 35 cycles with 45 s at 94°C, 50 s at 62°C and 1 min at 72°C. Amplicons were visualized on 3.5% ethidium bromide-stained agarose gels. In a series of samples comparing this method to that involving allelic-specific oligonucleotide hybridization as described by Jeunemaitre et al. [6], sensitivity and specificity of the above method was 100% (unpublished observation).

Statistical analysis

Presence of post-transplant hypertension and other variables were compared between the genotypic subgroups by χ² statistics. Graft survival was analysed by Kaplan–Meier life-table analysis and two-tailed global log rank testing.

Results

Allelic frequencies for the angiotensinogen M235T variants were comparable between the recipient (qT = 0.47) and the donor groups (qT = 0.43), and were similar to those described for Caucasian populations by us (qT = 0.41) [14] and other investigators [6,7,9,10]. Allelic distribution was in Hardy–Weinberg equilibrium in all groups.

Both the proportion of patients diagnosed as having post-transplant hypertension during the observation period, as well as the average number of antihypertensive drugs (excluding diuretics) prescribed to these patients were similar between the genotypic groups (Table 1). There was likewise no significant differences in gender distribution, body-mass index, or recipient and donor age between the groups. Life-table analysis revealed a virtually identical allograft survival in all groups regardless of recipient or donor genotype (Figure 1).

Discussion

Post-transplant hypertension was present in around 78% of the patients during the observation period, and was unrelated to both recipient and donor M235T genotype. Overall graft survival was likewise comparable between all genotypic groups. Therefore our findings do not support the hypothesis that the M235T variant of the angiotensinogen gene is a risk factor for the development of post-transplant hypertension.

The rationale for the study was based on the assumption that a genetic variant predisposing to the develop-

Table 1. Characteristics of subgroups of patients undergoing renal transplantation based on recipient and donor angiotensinogen M235T genotypes

<table>
<thead>
<tr>
<th>Recipient genotype</th>
<th>Donor genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM (n=77)</td>
</tr>
<tr>
<td>Recipient gender (m/f)</td>
<td>53/24</td>
</tr>
<tr>
<td>Recipient BMI (kg/m²)</td>
<td>22.7±4.2</td>
</tr>
<tr>
<td>Recipient age (years)</td>
<td>40.9±13.4</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>43.8±16.0</td>
</tr>
<tr>
<td>Patients on antihypertensive drugs (%)</td>
<td>75</td>
</tr>
<tr>
<td>Average number of antihypertensive drugs</td>
<td>2.0±0.9</td>
</tr>
</tbody>
</table>

Mean±SD.
Graft Survival

Recipient Genotype

Donor Genotype

Fig. 1. Relationship between graft survival and recipient and donor angiotensinogen M235T genotype.

ment of essential hypertension may also increase the risk for the development of hypertension following renal transplantation. Not only has the M235T variant of the angiotensinogen gene been linked to essential hypertension [6,9–14], but individuals harbouring this variant also have higher circulating levels of angiotensinogen [6]. In rats exogenous angiotensinogen increases blood pressure under a low-salt diet, demonstrating that with high renin levels, availability of angiotensinogen determines angiotensin I generation [15]. As higher levels of renin activity are found in a large proportion of renal-allograft recipients [2,3,5], one may expect that individuals harbouring the M235T variant would be more prone to developing post-transplant hypertension. This is clearly not the case.

Several factors could explain why, despite being a risk factor for the development of essential hypertension, the M235T variant does not predispose to the development of post-transplant hypertension. Firstly, factors other than the renin–angiotensin system may be primarily responsible for the development of post-transplant hypertension. The most likely factor may well be the administration of cyclosporin, whose hypertensive effect is believed to be primarily due to increased vascular resistance secondary to enhanced activity of the sympathetic nervous system and marked renal vasoconstriction [2,3]. Both the finding that renin levels tend to be lower in patients receiving cyclosporin than in patients receiving azathio-prine [16], and that cyclosporin-induced changes in systemic and renal haemodynamics are reversed more effectively by calcium-channel blockers than by angiotensin-converting enzyme inhibitors [17], argue against a predominant role of the renin–angiotensin system in the aetiology of cyclosporin-associated hypertension. As all but three subjects in our study were treated with cyclosporin, this may have masked any genetic predisposition for the development of hypertension attributable to the angiotensinogen genotype. Apart from that, recent results from cross-transplantation studies with stroke-prone spontaneously hypertensive rats and normotensive Wistar–Kyoto rats, suggest that even without the use of cyclosporin, neither the systemic nor the renal renin–angiotensin system appears to play a primary role in the development of post-transplant hypertension, at least in this model [18].

A second factor may be that basal levels of angiotensinogen expression in the liver as well as other tissues including the kidney is markedly increased by the administration of steroids [19]. It is therefore very likely that angiotensinogen levels were markedly elevated due to the administration of steroids in virtually all subjects, so that the comparatively minor variations in angiotensinogen levels attributable to the angiotensinogen genotype may well fail to significantly affect blood pressure in this setting.

Another explanation for our failure to find a relationship between angiotensinogen genotype and the development of post-transplant hypertension may be that primary hypertension accounts for only a relatively small proportion of individuals developing end-stage renal failure [20]. Thus only a minority of individuals in whom the M235T variant may have contributed to the development of primary hypertension is likely to be represented in our study population.

In conclusion, our study does not support the hypothesis that the M235T variant of the angiotensinogen gene, associated with a genetic predisposition for the development of essential hypertension, is a potent risk factor for the development of post-transplant hypertension.

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References


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