**Human SA gene Pst1 polymorphism and chronic renal failure: Results of the family-based study**

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**Abstract**

**Background.** Because of the heterogeneous aetiology of kidney diseases, interactions between multiple genetic and environmental factors are thought to be involved in the process of progression to end-stage renal disease (ESRD). Raised blood pressure remains a well-established, independent risk factor for a more rapid decline of renal function in various kidney diseases. The aim of the study was to investigate the role of the human SA gene Pst1 polymorphism in the development and/or progression of chronic renal failure (CRF).

**Methods.** This polymorphism was genotyped in a group of 247 family trios (offspring affected with end-stage renal disease, and both parents): 120 with primary chronic glomerulonephritis, 80 with interstitial nephritis, and 47 with diabetic nephropathy. Transmission/disequilibrium test (TDT) was used to evaluate allele transmission from heterozygous parents to affected offspring.

**Results.** SA gene Pst1 allele transmission did not differ from random proportion of 50 : 50, with no significant variation in the slope of reciprocal serum creatinine over time between patients with SA Pst1 A1A1, A1A2, and A2A2 genotypes. In addition, no impact of this marker on the rate of progression of CRF in the course of diabetes mellitus, interstitial nephritis, and chronic glomerular nephritis was shown.

**Conclusion.** Results of the study suggest no major role of SA gene polymorphism in promoting renal damage. However, the limited numbers of patients having both parents alive included in the analysis might have resulted in insufficient power to detect a minor impact of this polymorphism, especially if such effect is confined to a certain aetiology of CRF.

**Keywords:** chronic renal failure; end-stage renal disease; family-based study; gene; polymorphism; SA protein

**Introduction**

The SA gene was initially identified as likely candidate for a causative gene of hypertension in some animal models of hereditary hypertension, and then with a possible relationship to essential hypertension in humans [1–6]. However, the physiological role of its product in blood pressure regulation remains unclear. The human SA gene was mapped to chromosome 16p13.11 region [3,6]. The expression of this gene was detected in the liver, but also in the kidney and central nervous system—organs involved in blood pressure regulation [1,6]. Thus, it is of interest to examine the possible relationship of the SA gene to progression of renal failure in humans, since raised blood pressure remains a well-established, independent risk factor for a more rapid decline of renal function in various kidney diseases.

According to epidemiological data, only a minority of patients with kidney diseases develop the complication of progressive renal failure leading to end-stage renal disease (ESRD). Thus, interactions between multiple genetic and environmental factors are thought to be involved in the process of development and/or progression to ESRD [7–10].

We report below the results of a family-based study that investigated the role of the human SA gene Pst1 polymorphism in the development and/or progression of chronic renal failure (CRF). This polymorphism
was genotyped in a group of 247 family trios: offspring affected with ESRD, and both alive parents. The transmission/disequilibrium test (TDT), first introduced by Spielman et al. [11], was then applied to examine the transmission of Pst1 alleles from heterozygous parents to affected offspring, and the observed transmission was compared with the transmission expected for no association (i.e. random transmission of 50 : 50%).

**Subjects and methods**

**Selection of family trios**

The method of collecting families for the study is presented in more detail elsewhere [12,13]. Briefly, from 1657 Caucasian patients with a history of end-stage renal disease (creatinine clearance <30 ml/min) or undergoing chronic haemodialysis or peritoneal dialysis we selected 247 patients having both parents alive: 120 with primary glomerulonephritis, 80 with interstitial nephritis (including chronic pyelonephritis), and 47 patients with ESRD due to diabetic nephropathy in type 1 diabetes. We excluded family trios with probands with ESRD attributed to polycystic kidney disease or other heritable conditions (e.g. Alport’s syndrome), and unknown or uncommon aetiologies. A detailed history of kidney disease was collected from all patients, and all parents provided basic epidemiological data, according to our standardized questionnaire. All patients and parents gave their written informed consent, and the study protocol was approved by the Ethics Committee of the Silesian School of Medicine.

**DNA analysis**

From all patients and parents, genomic DNA was isolated from peripheral blood leukocytes. Human SA Pst1 polymorphism was genotyped with a method similar to that described by Zee et al. [3]; 315 bp fragment of the SA gene was amplified with specific primers: 5'-GTC ACA CAT TAG GGC AGC TGC ACAC-3', 5'-GCC AGG CAT GGT GAT GCA ATC CTG-3'; 5 min of initial denaturation at 95°C were followed by 30 cycles of 1 min of 94°C, 1 min of 62°C, and 1 min of 72°C. Ten microlitres of the PCR product were digested with 15 U of Pst1 (New England Biolabs, Beverly, MA, USA). A2 allele was presented on 2% agarose as 230 and 85 bp bands, whereas A2 allele remained uncut. PCR was performed from 200 ng of genomic DNA in 50 µl volume, with 10 pmol of the specific primers, 20 U/ml of PrimeZyme DNA polymerase (Biometra, Göttingen, Germany), 1.5 mmol/l MgCl2, 2.5 mmol/l of each dNTP, in one of the following cyclers: UNO II (Biometra) or Mastercycler Gradient (Eppendorf, Hamburg, Germany).

**Statistical analysis**

Basic epidemiological data describing the study patients are presented as mean and standard deviation for normally distributed continuous variables, while for variables not distributed normally, we presented median and quartiles. In the TDT, we used McNemar’s test to compare the observed transmission of alleles from heterozygous parents to affected offspring with an expected ratio of 50 : 50 transmission for alleles not associated with the phenotype. For 136 patients, a retrospective history of repeated measurements of serum creatinine from the onset of CRF with serum creatinine below 1.5 mg/dl, who were followed for a period of at least 1 year, with at least five consecutive serum creatinine measurements, was available. In these patients, the reciprocal serum creatinine concentration was plotted vs time between measurements by means of the least-squares regression method. For each case, such plot fitted the model of linear regression, with correlation coefficients varying from −0.884 to −0.997 (P<0.05), and the slope was used to define the rate of progression of renal function loss. Mean regression coefficients were compared between subgroups of patients carrying different genotypes in the examined loci using the parallelity test.

All calculations were performed in Excel 97 spreadsheet (Microsoft) and Statistica rel 5.0 for Windows (StatSoft Inc., Tulsa, OK, USA).

**Results**

Among 247 cases included in the study, 144 patients were undergoing haemodialysis, while 13 were on peritoneal dialysis, with median duration of dialysis of 2.0 years (0.0–8.0). Ninety patients were conservatively treated. Mean age, BMI, median serum creatinine, and median creatinine clearance (calculated according to the Cockcroft-Gault formula) of the examined patients, as well as the proportion of genders and hypertensive patients in our study group are presented in Table 1. SA gene Pst1 allele transmission from

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**Table 1.** Selected clinical data of the affected offspring analysed in TDT

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>GN</th>
<th>IN</th>
<th>DN</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>247</td>
<td>120</td>
<td>80</td>
<td>47</td>
</tr>
<tr>
<td>Female/male</td>
<td>111/136</td>
<td>55.67</td>
<td>36.44</td>
<td>22.25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.7±12.1</td>
<td>27.5±10.9</td>
<td>19.3±9.8</td>
<td>37.2±9.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.9±3.9</td>
<td>21.1±3.6</td>
<td>19.3±4.1</td>
<td>23.4±3.3</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>8.2 (5.5–9.9)</td>
<td>9.0 (7.4–10.1)</td>
<td>7.1 (5.9–9.1)</td>
<td>7.7 (4.0–9.2)</td>
</tr>
<tr>
<td>Creatinine clearance (conservatively treated patients) (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14.5 (8.4–27.5)</td>
<td>8.1 (6.4–9.5)</td>
<td>8.4 (4.1–30.1)</td>
<td>13.3 (7.7–29.9)</td>
</tr>
<tr>
<td>Male</td>
<td>17.3 (12.6–27.7)</td>
<td>10.5 (9.0–12.9)</td>
<td>10.6 (8.0–14.9)</td>
<td>11.0 (10.0–17.7)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>196 (79.3%)</td>
<td>108 (90%)</td>
<td>45 (56.2%)</td>
<td>43 (91.5%)</td>
</tr>
</tbody>
</table>

GN, glomerulonephritis; IN, interstitial nephritis; DN, diabetic nephropathy.
Table 2. SA gene PstI allele transmission of the examined polymorphism from heterozygous parents to offspring affected with CRF; genotype distributions and allele frequencies in the study patients

<table>
<thead>
<tr>
<th>Informative trios (n)</th>
<th>Total n (%)</th>
<th>GN n (%)</th>
<th>IN n (%)</th>
<th>DN n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 allele transmitted</td>
<td>76 (54)</td>
<td>48 (51)</td>
<td>20 (52)</td>
<td>15 (44)</td>
</tr>
<tr>
<td>A2 allele transmitted</td>
<td>40 (46)</td>
<td>19 (48)</td>
<td>11 (48)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>A1A1 (%)</td>
<td>82.7</td>
<td>83.3</td>
<td>81.3</td>
<td>82.9</td>
</tr>
<tr>
<td>A1A2 (%)</td>
<td>16.5</td>
<td>15.0</td>
<td>18.7</td>
<td>17.1</td>
</tr>
<tr>
<td>A2A2 (%)</td>
<td>0.8</td>
<td>1.7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>A1 allele (%)</td>
<td>90.9</td>
<td>90.8</td>
<td>90.6</td>
<td>91.5</td>
</tr>
<tr>
<td>A2 allele (%)</td>
<td>9.1</td>
<td>9.2</td>
<td>9.4</td>
<td>8.5</td>
</tr>
</tbody>
</table>

GN, glomerulonephritis; IN, interstitial nephritis; DN, diabetic nephropathy.

heterozygous parents to affected offspring, numbers of informative trios, genotype distributions, and allele frequencies among the study patients are shown in Table 2.

For the human SA gene PstI polymorphism, allele transmission did not differ from the expected random proportion of 50:50 (Table 2), with no significant variation in the slope of reciprocal serum creatinine over time between patients with SA PstI A1A1, A1A2 and A2A2 genotypes (data not shown).

Discussion

The SA gene was initially identified by Iwai and Inagami [1] because of its markedly higher expression in the kidneys of the spontaneously hypertensive rats (SHR) compared with those of normotensive Wistar-Kyoto rats. A later case-control study performed in Japan by Iwai et al. [2] comparing 89 hypertensive individuals with 81 healthy normotensive subjects provided evidence for an association between a restriction fragment length polymorphism (RFLP) of the human SA gene and hypertension. However, comparing several phenotypic variants among the hypertensive patients with different genotypes of the SA gene, no significant correlation between those phenotypes and genotypes of the SA gene polymorphism was observed [2].

Although these results suggested a certain role of the SA gene locus in essential hypertension in the Japanese population, they differ from those obtained in sib-pair and case-control studies of Nabika et al. [4]. These authors revealed no evidence supporting either linkage or association of SA gene with hypertension in French population. While the SA gene RFLP was also observed in this population of Caucasians, allele frequencies were not significantly different between hypertensive subjects and normotensive ones [4]. Preliminary data from Ji et al. [14] have also failed to show association between SA gene PstI polymorphism allele and diabetic nephropathy.

In the present study we revealed no significant deviation from random transmission of the examined human SA gene PstI alleles to offspring affected with CRF. Also, no impact of this marker on the rate of progression of chronic renal failure in the course of diabetes mellitus, interstitial nephritis, and chronic glomerular nephritis was shown, thus suggesting no major role of the SA gene in promoting renal damage.

However, it may be that the effect of the SA gene on blood pressure, and by extension, on the progression of kidney disease, is present in SHR rats, but not in humans. The fact that positive association was obtained in Japanese subjects, but was not confirmed in Caucasian hypertensive patients may suggest that such effect is confined to certain ethnic subgroups. On the other hand, the SA gene might be only a neutral marker that is linked to another gene which affects blood pressure regulation and influences the progression rate of kidney diseases, with different pattern of linkage disequilibrium in various ethnic groups.

Interestingly, recent reports from animal studies provided evidence against the SA gene being responsible for the effect of a major blood pressure quantitative trait locus (QTL) on rat chromosome 1. Firstly, it was demonstrated that transfer of a sub-region of chromosome 1 that did not include the SA locus from normotensive Brown Norway rats onto the spontaneously hypertensive rats (SHR) was associated with a significant reduction in blood pressure [15]. Secondly, congeneric substitution mapping approaches suggest that the blood pressure QTL on rat chromosome 1 is located about 3.8 cM centromeric to the SA gene locus [16].

Taken together, further investigations and clarification of physiological functions of the human SA locus products will be necessary to point definitive conclusions about the role of this gene in blood pressure regulation. Moreover, identification of a region centromeric to the SA locus as a narrowed blood pressure QTL on rat chromosome 1, with its syntenic human chromosome 11p15.4–p15.3 region should provide rationale for further positional candidate approaches in human hypertension and other blood-pressure-related conditions.

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References


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