Gender specific association of aldosterone synthase gene polymorphism with renal survival in patients with IgA nephropathy

J Song, I Narita, S Goto, N Saito, K Omori, F Sato, J Ajiro, D Saga, D Kondo, M Sakatsume, F Gejyo

I mmunoglobulin A nephropathy (IgAN), which is the most prevalent form of primary glomerulonephritis and one of the major causes of end stage renal disease (ESRD), has a variable clinical course. Poor prognostic factors for the progression of renal dysfunction in IgAN have been identified as high blood pressure, heavy proteinuria, and a severe histopathological appearance of the renal biopsy. In addition to these prognostic factors, it has been proposed that several genetic backgrounds are associated with a susceptibility to ESRD in patients with IgAN.

The renin-angiotensin-aldosterone system is an important regulator of blood pressure and plays a central role in the development and progression of end organ damage. Polymorphisms in genes that encode components of this system have been reported to be associated with physiological risk factors for progressive renal dysfunction in IgAN. The most consistent of these is the angiotensinogen (AGT) gene, which is associated with essential hypertension and with an increased risk of cardiovascular diseases and renal failure. A deletion polymorphism in the angiotensin converting enzyme (ACE) gene influences the circulating ACE levels, and although it has little effect on blood pressure, it has been associated with an increased risk of cardiovascular diseases in some but not all studies.

Aldosterone is one of the main effectors of the renin-angiotensin system and has classically been thought to act as a regulator for the absorption of Na and water, as well as the excretion of K in normal physiology, and as a mediator of oedema in numerous disease states. However, it is now well recognised that the actions of aldosterone are not limited to effects on ion transport in epithelial tissue, and its important role in cardiovascular disease involves non-epithelial tissues. Recently, it has been shown that aldosterone has a number of deleterious effects on the cardiovascular system, including necrosis and fibrosis, vascular stiffening and injury, reduced fibrinolysis, endothelial dysfunction, and catecholamine release.

Aldosterone secretion is regulated largely by the expression level of the final enzyme required for its biosynthesis, aldosterone synthase (CYP11B2). Expression of CYP11B2 is regulated by angiotensin II through cAMP dependent modulator of the gene promoter region, which contains a variety of control factors. Therefore, genetic variants in CYP11B2, which may be associated with the biosynthesis of aldosterone in local tissue, may also affect the progression of renal dysfunction in primary glomerulonephritis.

One potentially interesting variant of CYP11B2 is located in the 5’ flanking region of the gene, with a C or T at 344 nucleotides upstream from the start of translation (C-344T) within a binding site for the transcription factor steroidogenic factor-1 (SF-1). This genetic polymorphism has been reported to be associated with the serum level of aldosterone, as well as the left ventricular size, its function, and myocardial infarction independently of blood pressure. In contrast, a previous study in patients with kidney disease, in which a variety of primary kidney diseases for ESRD were included in the analysis, did not show any significant effect of the CYP11B2 polymorphism on the progression of renal dysfunction. Thus, the possible role of the CYP11B2 polymorphism on renal survival in patients with IgAN remains to be analysed fully.

In this study, we investigated the possible role of the CYP11B2 C-344T polymorphism in renal prognosis in Japanese patients with IgAN.
METHODS

Study subjects

Written informed consent was obtained from all patients. The ethics committee of the institution approved the protocol for the genetic study. The study included 271 patients with biopsy proven IgAN. In all cases, the diagnosis of IgAN was based on the immunofluorescent microscopy of a biopsy specimen, which showed dominant or co-dominant deposition of IgA in the glomerular mesangium. Patients with Schönlein-Henoch purpura and secondary IgAN such as hepatic glomerulosclerosis or rheumatoid arthritis were excluded from the study. Clinical characteristics of the patients at the time of diagnosis including age, gender, urinary protein excretion (g/day), level of serum creatinine (sCr, mg/dl), and 24 hour creatinine clearance (ml/min) were investigated. Hypertension was defined by the use of one or more antihypertensive medications and/or a blood pressure greater than or equal to 140 mm Hg systolic or 90 mm Hg diastolic. The primary end point (PRD, progressive renal disease) was defined as the date when the sCr level was double that at the time of diagnosis, or when patients underwent their first renal replacement therapy. The mean duration of observation was 92.0 (SD 66.8) months. The administration of glucocorticoids, antihypertensive agents, angiotensin converting enzyme inhibitors (ACEI), and angiotensin II receptor blocker (ARB) was also recorded for each patient.

DNA preparation and genotype determination

Genomic DNA was extracted from the peripheral blood cells of patients by an automatic DNA isolation system (NA-1000, Kurabo, Osaka, Japan). The genotype of \( CYP11B2 \) C-344T was determined by the PCR-RFLP method using restriction endonuclease \( Hae\ II \) (Takara, Kyoto, Japan) as described previously. Primers used for the PCR reaction were 5′-CAG GAG GAG ACC CCA TGT GAC-3′ (sense) and 5′-CCT CCA CCC TGT TCA GCC C-3′ (antisense). The reaction mixture contained 1× PCR buffer, 1.5 mmol/l MgCl\(_2\), 200 mmol/l deoxynucleotide triphosphates (dNTPs), 1 unit Taq DNA polymerase (Takara, Kyoto, Japan), 10 pmol of each primer, and 50-100 ng genomic DNA. The PCR amplification reaction consisted of a cycle at 95°C for five minutes, followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing at 67°C for 15 seconds, and extension at 72°C for 30 seconds. A final extension was performed at 72°C for five minutes. The 537 bp PCR products were digested with restriction endonuclease \( Hae\ II \) (Takara), and electrophoresed on a 2.5% agarose gel.

Statistical analysis

Statview 5.0 statistical software (Abacus Concepts Inc, Berkeley, CA, USA) was used for statistical analyses on a Macintosh G4 computer. Chi-square analysis was used when comparing allele frequencies and categorical variables between the groups. Continuous variables were compared using the Mann-Whitney U test or Kruskal-Wallis analysis of variance.
The Hardy-Weinberg equilibrium was tested by a chi-square test with 1 df. The time from renal biopsy to end point (initiation of dialysis or a doubling of the sCr level after the time of diagnosis) was analysed by the Kaplan-Meier method and the Cox proportional hazards regression model. Covariates were selected by a stepwise backward method and their effects were summarised as a hazard ratio. A value of p<0.05 was considered statistically significant.

**RESULTS**

In total, 271 patients with IgAN were genotyped for the CYP11B2 C-344T polymorphism. The frequencies of the genotypes TT, TC, and CC were as follows: males, 40.6%, 50.0%, and 9.4%, respectively; females 44.8%, 44.1%, and 11.2%, respectively, and overall, 42.8%, 46.9%, and 10.3%, respectively. These results are consistent with a previous report on a large population-based sample of Japanese. The observed genotype frequencies were in agreement with those expected under the assumption of Hardy-Weinberg equilibrium. Table 1 shows the clinical characteristics of the patients and their comparisons by each genotype in women (n=143) and men (n=128). There were no statistically significant differences among the three genotypes with regard to any clinical characteristics. Blood pressures both at the time of diagnosis and during observation, as well as the incidence of hypertension at the baseline, tended to be numerically higher in patients with the CC genotype both in men and women, but the differences were not statistically significant. Duration of observation was significantly shorter in female patients with the CC genotype than those with other genotypes, but not in males.

Table 2 shows the allele frequencies of CYP11B2 C-344T in hypertensive and normotensive subjects. In both female and male patients, there was no difference in the allele frequencies between hypertensives and normotensives.

Of 271 patients, 84 (31.0%) progressed to the primary end point (PRD). The incidence of PRD was also numerically, but not significantly, higher in patients with the CC genotype. Figure 1 shows the length of time of renal survival in each genotype. The renal survival rate was significantly worse in the CC genotype than that in other genotypes (Kaplan-Meier, log rank test, \( \chi^2=5.208, p=0.0225 \)).

The Cox proportional hazard regression model was used to test further the significance of variates for progressive renal dysfunction. Although in univariate analysis the CC genotype of CYP11B2 was a significant risk factor for PRD with a hazard ratio (HR) of 2.099 (p=0.0249, 95% confidence interval (CI) 1.098 to 4.013), after adjusting for other clinical risk factors, it was not recognised as an independent risk factor in the multivariate analysis. In this multivariate analysis, urinary protein excretion >1.0 g/day (HR=3.362, p<0.0001, 95% CI 1.891 to 5.977), hypertension (HR=2.301, p=0.0007, 95% CI 1.423 to 3.721), and no ACEI/ARB therapy (HR=2.779, p=0.0002, 95% CI 1.629 to 4.740) were identified as significant and independent risk factors for PRD (table 3). These covariates were selected by stepwise backward analysis. No other clinical variables, such as gender, age, glucocorticoids, antihypertensives other than ACEI/ARB, were selected as a significant prognostic factor by this analysis.

We next investigated the significance of these risk factors and the CYP11B2 genotype within groups for each gender (table 4). In both female and male patients, urinary protein, hypertension, and no ACEI/ARB administration were significant risk factors. In female patients, the CC genotype of the CYP11B2 C-344T polymorphism was also recognised as an independent risk factor (HR=4.284, p=0.0022, 95% CI 1.686

---

**Figure 1** The renal survival rate in IgAN patients with each genotype of the CYP11B2 C-344T polymorphism. The renal survival rate of patients with the CC genotype (solid line, n=28) was significantly worse than that in other genotypes (dotted line, CT, n=127 and broken line, TT, n=116). Kaplan-Meier log rank test, \( \chi^2=5.208, p=0.0225 \).

---

**Table 3** Cox proportional hazard model to test the significance of clinical covariates and genotypes of the CYP11B2 polymorphism as predictors of renal survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary protein excretion &gt;1.0 g/day</td>
<td>&lt;0.0001</td>
<td>3.362</td>
<td>1.891 to 5.977</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.0007</td>
<td>2.301</td>
<td>1.423 to 3.721</td>
</tr>
<tr>
<td>No ACEI/ARB administration</td>
<td>0.0002</td>
<td>2.779</td>
<td>1.629 to 4.740</td>
</tr>
<tr>
<td>CC genotype of CYP11B2</td>
<td>0.1667</td>
<td>1.584</td>
<td>0.825 to 3.042</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; ACEI/ARB, angiotensin-I converting enzyme inhibitor and/or angiotensin receptor blocker.

---

**Table 4** Cox proportional hazard model to test the significance of clinical covariates and genotypes of the CYP11B2 polymorphism as predictors of renal survival within groups for each gender

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female patients (n=143)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary protein excretion &gt;1.0 g/day</td>
<td>0.0141</td>
<td>2.505</td>
<td>1.203 to 5.217</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.0073</td>
<td>1.918</td>
<td>1.259 to 3.942</td>
</tr>
<tr>
<td>No ACEI/ARB administration</td>
<td>0.0074</td>
<td>3.225</td>
<td>1.668 to 7.599</td>
</tr>
<tr>
<td>CC genotype of CYP11B2</td>
<td>0.0022</td>
<td>4.284</td>
<td>1.686 to 10.881</td>
</tr>
<tr>
<td>Male patients (n=128)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary protein excretion &gt;1.0 g/day</td>
<td>0.0036</td>
<td>4.768</td>
<td>1.644 to 13.666</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.0007</td>
<td>3.649</td>
<td>1.725 to 7.718</td>
</tr>
<tr>
<td>No ACEI/ARB administration</td>
<td>0.0214</td>
<td>2.299</td>
<td>1.131 to 4.672</td>
</tr>
<tr>
<td>CC genotype of CYP11B2</td>
<td>0.6269</td>
<td>0.788</td>
<td>0.301 to 2.061</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; ACEI/ARB, angiotensin-I converting enzyme inhibitor and/or angiotensin receptor blocker.
The role of aldosterone in the progression of renal injury has been explored, with a focus on cardiovascular disease outcomes. The tissue that mediates these actions including fibrogenesis/sclerosis in the glomerular mesangium.

Aldosterone receptors have been proven in non-epithelial tissue including vascular smooth muscle cells and endothelial cells both in experimental animals and in human.

Recent studies have shown that the aldosterone receptor antagonist, spironolactone, was reported to have a protective effect on cardiovascular diseases including glomerulonephritis. The Randomized Aldactone Evaluation Study (RALES), in which a low dose of spironolactone was used as an adjuvant to conventional therapy with an ACEI, loop diuretic with or without digitalis, showed a 30% reduction in the overall risk of mortality that could not be accounted for by a blood pressure reduction or fluid loss. The result of this study may suggest that the therapeutic efficacy of anti-aldosterone agents can be enhanced by more selective and active usage in female patients with the particular genotype of CYP11B2 C-344T.

Although genetic polymorphism of CYP11B2 C-344T has been reported to be associated with the serum level of aldosterone, as well as the left ventricular size, its function, and myocardial infarction independently of blood pressure, the limitation of this study is that the association between the level of aldosterone and disease progression could not be.
tested, because we have no data on the level of circulating or local tissue activity of aldosterone in each genotype group.

It also remains to be seen if the effect of the genetic variant investigated in the present study is observed in other ethnic groups. The allele frequency of CYP11B2 C-344T in a white population was reported as 0.47 to 0.52. This is higher than that in the Japanese population, which was 0.34 in this study. As it is well known that a polymorphism with a higher allele frequency has more statistical power in an association study, investigations in other ethnic groups may provide further evidence for the role of this genetic polymorphism in the progression of renal dysfunction.

In conclusion, the present study provides the first evidence for a gender specific association between the CYP11B2 C-344T polymorphism and the prognosis of renal function in Japanese patients with IgAN. Although the genotype has no influence on renal survival in men, the CC genotype of CYP11B2 is a possible predictive genetic marker for progression of renal dysfunction in women.

ACKNOWLEDGEMENTS

This work was supported in part by a Health and Labour Science Research Grant for Research on Specific Diseases from the Ministry of Health, Labour and Welfare, and by a Grant-in-Aid for Scientific Research (C, No 11671012) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors wish to thank Narufumi Imai, Keiko Yamagishi, Noriko Ikeda, and Kumiko Furui for excellent technical assistance.

Authors’ affiliations


REFERENCES


Gender specific association of aldosterone synthase gene polymorphism with renal survival in patients with IgA nephropathy

J Song, I Narita, S Goto, N Saito, K Omori, F Sato, J Ajiro, D Saga, D Kondo, M Sakatsume and F Gejyo

doi: 10.1136/jmg.40.5.372

Updated information and services can be found at:
http://jmg.bmj.com/content/40/5/372

These include:

**References**
This article cites 36 articles, 8 of which you can access for free at:
http://jmg.bmj.com/content/40/5/372#BIBL

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**
Articles on similar topics can be found in the following collections

- Hypertension (60)
- Immunology (including allergy) (603)
- Clinical diagnostic tests (356)
- Ischaemic heart disease (43)
- Surgery (105)
- Surgical diagnostic tests (105)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/