Interaction Between ACE and ADD1 Gene Polymorphisms in the Progression of IgA Nephropathy in Japanese Patients

Ichiei Narita, Shin Goto, Noriko Saito, Jin Song, Junya Ajiro, Fuminori Sato, Daisuke Saga, Daisuke Kondo, Kohei Akazawa, Minoru Sakatsume, Fumitake Gejyo

Abstract—An interaction effect between the angiotensin-converting enzyme insertion/deletion (ACE I/D) and α-adducin (ADD1) Gly460Trp polymorphisms (G460W) on blood pressure regulation has recently been suggested, although its significance in the prognosis of renal function in IgA nephropathy (IgAN) has not been fully investigated. Therefore, we evaluated the clinical manifestations and renal prognosis in 276 Japanese patients with histologically proven IgAN with respect to their ACE I/D and ADD1 G460W polymorphisms. The prognosis of renal function was analyzed by Kaplan-Meier survival curves and multivariate Cox proportional-hazards regression models. Baseline data, including blood pressures, proteinuria, renal function, and incidence of hypertension, were similar for the different genotypes of ACE and ADD1. The individual genotypes taken alone were not associated with the progression of renal dysfunction. However, renal survival of patients with the G460WW polymorphism of ADD1 was significantly worse within the group with the II genotype of ACE (Kaplan-Meier, log rank test; χ²=6.062, P=0.0138) but not for those with other ACE genotypes. In the Cox proportional-hazards regression model with adjustment for clinical risk factors, including hypertension, proteinuria, and no administration of an angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, the G460WW variant of ADD1 was a highly significant and independent risk factor only for patients with the ACE II genotype, with a hazard ratio of 3.65 (P=0.0016), but not for those with other ACE genotypes (hazard ratio=0.65, P=0.2902). These findings suggest an interaction between ACE and ADD1 polymorphisms not only on blood pressure regulation but also on the progression of renal dysfunction in patients with IgAN. (Hypertension. 2003; 42:1182–1187)

Key Words: angiotensin-converting enzyme ■ blood pressure ■ hypertension, genetic ■ kidney failure ■ polymorphism ■ renal disease ■ renin-angiotensin system

Immunglobulin A nephropathy (IgAN), the most prevalent form of primary glomerulonephritis, is one of the major causes of end-stage renal disease (ESRD).1 IgAN has a variable clinical course,2 and the mechanisms of interindividual differences in the rate of disease progression are still unclear. Poor prognostic factors for the progression of renal dysfunction in IgAN have been identified as high blood pressure, marked proteinuria, and a severe histopathologic appearance of the renal biopsy specimen.3,4 In addition to these prognostic risk factors, several genetic backgrounds have been proposed to be associated with a susceptibility to ESRD in patients with IgAN.4,5 Recently, interactions among multiple genetic variants of complex traits, including blood pressure regulation as well as the prognosis of kidney disease, have been suggested.

Among the genetic polymorphisms proposed to date, an insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene has attracted much attention and is the most studied, because renin-angiotensin system (RAS) activity is an important regulator of blood pressure and plays a central role in cardiovascular and renal diseases. Many previous studies have suggested a significant association between the D allele and functional deterioration of the kidney.5–10 However, these studies had common limitations, in that they only evaluated small numbers of patients. Larger-scale studies and a meta-analysis have reported rather negative results on the association between ACE I/D and progression of renal disease in both white and Japanese populations.11–13

The α-adducin gene, which encodes an actin accessory and calmodulin-binding protein, is another candidate that has been suggested to affect blood pressure by regulating renal sodium reabsorption.14 In human α-adducin (ADD1), a single-nucleotide polymorphism accompanied by an amino acid substitution of tryptophan (W) in place of glycine (G) at residue 460 (G460W) has been implicated in the pathogenesis of salt-sensitive and low-renin hypertension.15–17 Recently, an epistatic or synergistic interaction of the ADD1 G460W

Received May 30, 2003; first decision June 13, 2003; revision accepted June 24, 2003.
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Hypertension is available at http://www.hypertensionaha.org
DOI: 10.1161/01.HYP.0000085193.25617.78
polymorphism with ACE I/D has been suggested for both blood pressure regulation and renal disease progression in white populations. On the other hand, negative results on the significance of both the ADD1 G460W polymorphism alone and its interaction with ACE I/D on blood pressure and renal disease have been reported in multiple ethnic groups.

Taken together, genetic polymorphisms of ACE I/D and ADD1 G460W have been suggested, but not confirmed, as candidate markers for the progression of renal disease, and their possible synergistic interaction also remains to be fully investigated in IgAN. Previous studies on this matter included various kidney diseases. Therefore, the aim of this study was to evaluate the role of the ADD1 G460W gene polymorphism, as well as its possible synergistic interaction with the ACE I/D gene polymorphism, on the progression of renal function in Japanese patients with histologically proven IgAN.

Methods

Subjects and Clinical Data
The ethics committee of our institution (Niigata University Graduate School of Medical and Dental Sciences) approved the protocol for the genetic study. Japanese patients were eligible for inclusion in the analysis when (1) they had been diagnosed as having IgAN by kidney biopsy at our institute between 1976 and 2001; (2) they had no evidence of systemic diseases, such as hepatic glomerulonephrosis, Schönlein-Henoch purpura, or rheumatoid arthritis; (3) they had been followed up for at least 12 months at our institute; and (4) written, informed consent for the genetic study was obtained. Patients who received immunosuppression therapy other than corticosteroids were excluded from the analysis. In total, 276 patients were analyzed. In all cases, the diagnosis of IgAN was based on immunofluorescence microscopy of a kidney biopsy specimen, which showed dominant or codominant deposition of IgA in the glomerular mesangium.

The clinical characteristics of the patients at the time of diagnosis, including gender, age, office blood pressure, urinary protein excretion (g/d), serum creatinine level (sCr, mg/dL), and 24-hour creatinine clearance (CCr, mL/min) were retrospectively investigated. Hypertension was defined by the use of 1 or more antihypertensive medications and/or a blood pressure of 140 mm Hg systolic or 90 mm Hg diastolic. The primary end point (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 93.0±67.3 months. Administrations of glucocorticoids, antihypertensive therapy, including gender, age, office blood pressure, urinary protein excretion, sCr, and CCr, were 0.629 and 0.371, respectively. During the observation period of 93.0±67.3 months, 21–23 the expected genotype frequencies of heterozygotes of ADD1 were 0.475 and 0.525, and those of ACE I/D were 0.49. 25 The allele frequencies of DD were 0.56, GW (n=150), and WW (n=70); and those of ACE were II (n=106), ID (n=135), and DD (n=35). The allele frequencies of ADD1 G460G and W were 0.475 and 0.525, and those of ACE I and D were 0.629 and 0.371, respectively. These findings are compatible with previous reports of a general Japanese population. Statistical analysis was performed with Statview 5.0J software (SAS Institute, Inc) on a personal computer (Apple Macintosh G4). A value of P<0.05 was considered significant.

Results

Demographic Data at the Time of Diagnosis and During Observation
Two hundred seventy-six patients with histologically proven IgAN were all genotyped for ADD1 G460W and ACE I/D polymorphisms. The genotype frequencies of ADD1 were GG (n=56), GW (n=150), and WW (n=70); and those of ACE were II (n=106), ID (n=135), and DD (n=35). The allele frequencies of ADD1 G460G and W were 0.475 and 0.525, and those of ACE I and D were 0.629 and 0.371, respectively. These findings are compatible with previous reports of a general Japanese population. The expected genotype frequencies of heterozygotes of ADD1 and ACE, according to Hardy-Weinberg equilibrium, were no different from those observed in this study.

The clinical characteristics of the patients at the time of diagnosis and during the observation period are listed in Table 1. No difference was noted among patients with each genotype of ADD1 G460W with respect to gender, age, blood pressure, urinary protein excretion, sCr, and CCr. Systolic blood pressure and incidence of hypertension at baseline tended to be higher in patients with ADD1 G460WW than in those with other genotypes, but the difference was not significant. During the observation period of 93.0±67.3 months, 31.2% (86 patients) reached the end point (progres-
Table 1: Clinical Data of the Subjects at the Time of Diagnosis and During Observation

<table>
<thead>
<tr>
<th>Genotype of ADD1G460W</th>
<th>Genotype of ACEI/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients</td>
<td>GG</td>
</tr>
<tr>
<td>Gender, male, %</td>
<td>46.7</td>
</tr>
<tr>
<td>Age, y</td>
<td>37.0±13.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>127.9±18.4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>77.3±13.5</td>
</tr>
<tr>
<td>UP, g/d</td>
<td>1.3±1.2</td>
</tr>
<tr>
<td>sCr, mg/dL</td>
<td>1.0±0.6</td>
</tr>
<tr>
<td>Ccr, mL/min per 1.73 m²</td>
<td>88.9±33.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>35.9</td>
</tr>
</tbody>
</table>

During observation

| Observation, mo | 93.0±67.3 | 90.2±65.1 | 94.0±70.6 | 92.8±62.3 | 0.9442 | 91.5±59.5 | 96.7±72.8 | 83.1±68.8 | 0.5013 |
| Reached end point, % | 31.2 | 26.8 | 32.7 | 31.4 | 0.7141 | 34.0 | 28.9 | 31.4 | 0.6998 |
| Mean SBP, mm Hg | 128.6±17.0 | 128.2±19.5 | 129.2±16.3 | 127.9±16.3 | 0.6650 | 131.0±16.5 | 127.6±17.6 | 125.6±15.4 | 0.1528 |
| Mean DBP, mm Hg | 77.5±11.8 | 78.3±13.5 | 77.9±11.1 | 76.3±12.0 | 0.7389 | 79.8±11.6 | 75.8±12.0 | 77.0±11.2 | 0.4285 |
| Corticosteroids, % | 28.3 | 30.3 | 28.0 | 27.1 | 0.7885 | 26.4 | 28.1 | 34.3 | 0.6684 |
| ACE/ARB, % | 40.9 | 35.7 | 43.3 | 40.0 | 0.6001 | 37.7 | 40.0 | 54.3 | 0.2146 |

UP indicates urinary protein excretion. All other abbreviations are the same as in text.

ADDAI 460WW was significantly worse than in those with other genotypes (Figure 1B; Kaplan-Meier, log rank test, χ²=6.062, P=0.0138). The difference in survival rate was statistically significant after 7 years of observation. The survival rates of patients with ADD1 WW and GW/GG at 84 months were 53.0±11.0% and 79.3±5.0%, respectively (P=0.0307 by Greenwood's estimation). In contrast, in patients with ACE ID or DD, patients with 460 WW tended to have a better survival curve, but the difference was not significant (Figure 1C; χ²=2.238, P=0.1386).

The prognostic significance of the ADD1 460WW genotype on the advance to the progressive renal disease end point was further evaluated after adjusting for other clinical risk factors by the multivariate Cox proportional-hazards regression model (Figure 2). In all patients, significant risk factors were identified, including urinary protein >1.0 g/d, hypertension, and no administration of ACEI/ARB, whereas the 460WW variant of ADD1 had no prognostic influence on renal survival (Figure 2A). These 3 clinical risk factors were significant in both groups with ACE II and those with ID or DD genotypes, with the exception that the significance of no administration of ACEI/ARB was much higher in patients with the II genotype. ADD1 460WW was found to be an independent risk factor only for the group with the ACE II genotype (Figure 2B; HR, 3.65; 95% CI, 1.63 to 8.20; P=0.0016). In contrast, in the group with the ID or DD variant of ACE, the hazard ratio of the 460WW variant of ADD1 was much lower and not significant (Figure 2C; HR, 0.65; 95% CI, 0.29 to 1.45; P=0.2902).

Discussion

This study shows the significance of the ADD1 G460W polymorphism on the progression of renal dysfunction in Japanese patients with IgAN, which was specific in patients...
carrying the II genotype of ACE. In patients with other ACE genotypes, the ADD1 polymorphism had no influence on renal survival. There is a possibility that the association between the ADD1 polymorphism and renal disease progression observed in this study was secondary to its effect on blood pressure regulation, because for patients with the ACE II genotype, the frequency of hypertension in the ADD1 460WW genotype was higher than that in other genotypes. However, the prognostic effect of ADD1 460WW was significant even after adjusting for other clinical risk factors, including urinary protein excretion, hypertension, and no administration of ACEI/ARB. In this study ACE II carriers presented with numerically but not significantly higher blood pressures at the time of diagnosis and the lowest frequency of ACEI/ARB administration. Although this study is based on a retrospective observation and we cannot clearly define the reason for these indistinct deviations, the results of the Cox regression model, which takes these variables into account, show an independent prognostic effect of the genetic polymorphism ADD1 WW only in patients with the ACE II genotype.

To investigate the risk factors for progression of renal dysfunction, this study used a time-to-event approach rather than an analysis of mean slope of renal function, because a substantial proportion of our patients had stable or slowly declining renal function. In fact, only one third of the patients progressed to the end point during the follow-up duration in this study. It has been suggested that an analysis of mean slope of renal function is favored when a negligible proportion of patients have stable or slowly declining renal function.29

The present study could not detect any independent effect of the ACE I/D polymorphism solely in terms of clinical manifestations and renal prognosis in patients with IgAN. This negative result for the role of the genetic polymorphism of ACE I/D alone on renal prognosis is compatible with a previous report in a large-cohort, multicenter trial in Japanese patients with IgAN.12

Interestingly and unexpectedly, our data suggest that the ACE genotype has an influence on sensitivity to the ADD1 polymorphism and that the effect of the ADD1 polymorphism might be enhanced in cases with lower activity of the RAS, because there is general agreement that the D allele of ACE is associated with increased plasma and tissue levels of ACE. We do not have data to explain the precise molecular mechanism of the observed interaction between the ADD1 and ACE polymorphisms. However, it is not surprising that patients with low RAS activity are more susceptible to the effect of the ADD1 polymorphism than are those with high RAS activity, because it is well known that RAS has a direct influence on salt sensitivity.30 Patients with the ACE D allele might be resistant to the effect of the ADD1 polymorphism because of their high salt sensitivity owing to high RAS activity.

Adversely, there is a possibility that patients with low α-adducin activity, which is known to be associated with the ADD1 460G allele, would be more susceptible to the influence of the ACE I/D polymorphism. Although our study could not confirm this notion, Nicod et al29 have recently reported, in an unselected renal population, a more rapid progression with the ACE DD genotype compared with ACE ID and II genotypes in patients homozygous for the ADD1 460G allele. In their study, although the ADD1 460WW genotype was observed in only 10 of 260 patients in their population, they also found that the average time from diagnosis to the onset of ESRD tended to be shorter in the

### Table 2. Clinical Data at the Time of Diagnosis and During Observation: Comparisons According to Each Combined Genotype

<table>
<thead>
<tr>
<th></th>
<th>Patients With ACE II Genotype</th>
<th></th>
<th>Patients With ACE ID or DD Genotype</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GW or GG (n=79)</td>
<td>WW (n=27)</td>
<td>P</td>
<td>GW or GG (n=127)</td>
</tr>
<tr>
<td>At time of diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender, male, %</td>
<td>48.1</td>
<td>59.3</td>
<td>0.3167</td>
<td>44.9</td>
</tr>
<tr>
<td>Age, y</td>
<td>37.2±12.8</td>
<td>40.1±15.8</td>
<td>0.3512</td>
<td>36.2±13.6</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128.3±18.7</td>
<td>133.8±17.4</td>
<td>0.2188</td>
<td>126.6±18.6</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>78.3±13.9</td>
<td>79.1±12.3</td>
<td>0.6233</td>
<td>76.3±13.2</td>
</tr>
<tr>
<td>UP, g/d</td>
<td>1.5±1.6</td>
<td>1.6±1.3</td>
<td>0.2353</td>
<td>1.3±1.2</td>
</tr>
<tr>
<td>sCr, mg/dL</td>
<td>1.0±0.5</td>
<td>1.2±1.0</td>
<td>0.2474</td>
<td>1.0±0.7</td>
</tr>
<tr>
<td>CCr, mL/min per 1.73 m²</td>
<td>87.8±38.6</td>
<td>75.1±27.7</td>
<td>0.2288</td>
<td>92.0±31.4</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>32.9</td>
<td>55.6</td>
<td>0.0370</td>
<td>33.9</td>
</tr>
<tr>
<td>During observation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation, mo</td>
<td>99.0±62.1</td>
<td>69.1±45.2</td>
<td>0.0412</td>
<td>107.6±67.6</td>
</tr>
<tr>
<td>Reached end point, %</td>
<td>36.7</td>
<td>48.1</td>
<td>0.2941</td>
<td>27.6</td>
</tr>
<tr>
<td>Mean SBP, mm Hg</td>
<td>130.1±17.0</td>
<td>133.7±14.9</td>
<td>0.3433</td>
<td>128.1±17.4</td>
</tr>
<tr>
<td>Mean DBP, mm Hg</td>
<td>79.6±12.1</td>
<td>80.3±10.3</td>
<td>0.6934</td>
<td>76.9±11.6</td>
</tr>
<tr>
<td>Corticosteroids, %</td>
<td>22.9</td>
<td>26.9</td>
<td>0.6783</td>
<td>23.1</td>
</tr>
<tr>
<td>ACEI/ARB, %</td>
<td>34.2</td>
<td>48.1</td>
<td>0.1960</td>
<td>45.7</td>
</tr>
</tbody>
</table>

UP indicates urinary protein excretion. All other abbreviations are the same as in text.
presence of the \textit{ADD1} 460WW than with the GW and GG genotypes. These partly differing results between our study and those of Nicod et al are likely to be a consequence of the differences in genotype distribution of \textit{ACE I/D} and \textit{ADD1 G460W} polymorphisms between Japanese and whites. It is well known that a polymorphism with a higher allele frequency has more statistical power in an association study.\textsuperscript{31} In fact, the frequencies of \textit{ACE I} and \textit{ADD1 460W} alleles in our population were 0.629 and 0.525, respectively, and both were much higher than in Nicod’s study (0.525 and 0.185, respec-

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Renal survival rate in IgAN patients with 460WW and other genotypes of \textit{ADD1} polymorphism. In all patients studied (A), there was no difference between patients with 460WW (dotted line; \(n=70\)) and those with 460GW or GG genotypes (solid line; \(n=206\); Kaplan-Meier, log rank test, \(\chi^2=0.655, P=0.9958\)). Within patients carrying the \textit{ACE II} genotype (B, \(n=106\)), however, survival of patients with the WW genotype (dotted line; \(n=27\)) was significantly worse than in those with other \textit{ADD1} genotypes (solid line; \(n=79\); Kaplan-Meier, log rank test, \(\chi^2=6.062, P=0.0138\)). By contrast, in patients with \textit{ACE ID} or DD (C, \(n=170\)), patients with 460 WW (dotted line; \(n=43\)) tended to have a better but not significantly better survival curve than those with other \textit{ADD1} genotypes (solid line; \(n=127\); \(\chi^2=2.238, P=0.1386\)). Numbers of available observations for each genotype are indicated. The primary endpoint (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.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Cox proportional-hazards regression model to test significance of clinical covariates and genotypes of the \textit{ADD1 G460W} polymorphism as predictors of renal survival. The end point 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. Clinical covariates were selected by a stepwise backward method, and analyses were performed in all patients (A, \(n=276\)), in patients with the \textit{ACE II} genotype (B, \(n=106\)), and in patients with the \textit{ACE ID} or DD genotypes (C, \(n=170\)). HRs and 95% CIs are indicated. UP indicates urinary protein excretion.\textsuperscript{31}}
indicate that an even longer observation period and a larger sample size are necessary for confirming the present results. However, overall renal survival was significantly associated with the ADD1 polymorphism in patients with the ACE II genotype, and it was confirmed by the multivariate Cox proportional-hazards regression model.

Perspectives

Although a prospective, randomized, control study with a fixed medication protocol and sufficiently long-term observation is necessary to confirm the present results, this study suggests an interaction between genetic polymorphisms of ACE I/D and ADD1, not only on blood pressure regulation but also on the progression of renal dysfunction in Japanese patients with IgAN. Despite the fact that the effect of each single polymorphism might not be sufficiently significant, this study supports the notion that interindividual variation in susceptibility to the effect of a single-gene polymorphism of ADD1 G460W can be modified by the ACE I/D polymorphism. Genotyping both polymorphisms might have provisional importance in patients with IgAN.

Acknowledgments

This work was supported in part by Health and Labor Science Research Grants for Research on Specific Diseases from the Ministry of Health, Labor and Welfare to F. Gejyo and by a Grant-in-Aid for Scientific Research (No. 11671032) from the Ministry of Education, Culture, Sports, Science and Technology of Japan to I. Narita.

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Hypertension. published online July 28, 2003;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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