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 \(\alpha\)-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 460WW polymorphism of \(ADD1\) was significantly worse within the group with the \(I\) genotype of \(ACE\) (Kaplan-Meier, log rank test; \(\chi^2=6.062, P=0.0138\) 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 460WW variant of \(ADD1\) was a highly significant and independent risk factor only for patients with the \(ACE\) \(I_2\) 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. (\textit{Hypertension}. 2003; 42:304-309.)

Key Words: angiotensin-converting enzyme \(\bullet\) blood pressure \(\bullet\) hypertension, genetic \(\bullet\) kidney failure \(\bullet\) polymorphism \(\bullet\) renal disease \(\bullet\) renin-angiotensin system

Immunoglobulin 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,\(^2,3\) 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.\(^4,5\) 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.\(^6,7\) Recently, interactions among multiple genetic variants of complex traits, including blood pressure regulation as well as the progression 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.\(^8-10\) 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 \(\alpha\)-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 \(\alpha\)-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
polymorphism with ACE I/D has been suggested for both blood pressure regulation and renal disease progression\cite{18-20} 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.\cite{21-23}

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 prognosis 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 glomerulosclerosis, 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/dl), serum creatinine level (sCr, mg/dl), and 24-hour creatinine clearance (CrCl, ml/min) were retrospectively investigated. Hypertension was defined by the use of 1 or more antihypertensive medications and/or a blood pressure \( \geq 140 \text{ mm Hg systolic or 90 mm Hg diastolic.} \) 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 agents, angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin II receptor blockers (ARBs) was also recorded for each patient.

DNA Isolation and Genotyping

Genomic DNA was isolated from peripheral blood cells by an automatic DNA isolation system (NA-1000, Kurabo). The I/D polymorphism in intron 16 of the ACE gene was assessed by polymerase chain reaction (PCR) under conditions that have been previously described.\cite{24} Because of preferential amplification of the D allele compared with the I allele, DNA from subjects with the DD genotype was reexamined with an I allele-specific primer (5\'-TTT GAG AGC GGA GTC TG CTC-3\') to avoid mistyping ID heterozygotes as DD homozygotes.\cite{25}

Genotyping of the ADD1 G460W polymorphism was determined by allele-specific oligonucleotide hybridization after PCR amplification. The forward primer and biotin-labeled reverse primer for PCR were 5\'-AGA CAA GAT GGC TGA ACT CTG G-3\' and 5\'-CAC ACC TTA GTC TTC GAC TTT G-3\', respectively. The PCR mixture (25 \( \mu L \)) contained 50 ng DNA, 5 pmol of each oligonucleotide primer, 0.2 mmol/L ddNTPs, 2.5 mmol/L MgSO\(_4\), and 1 U DNA polymerase (KOD plus, Toyobo) in KOD buffer. The amplification protocol consisted of 1 cycle at 94°C for 5 minutes, followed by 40 cycles of denaturation at 93°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 2 minutes. The amplified 88-bp DNA fragment was denatured with NaOH and hybridized with allele-specific capture probes fixed to the bottom of a microtiter plate at 57.5°C for 30 minutes. Specific probes for each single-nucleotide polymorphism were 5\'-GAA GGG CAG CAA TGG CAG AAT GGA AGC A-3\' and 5\'-GAA TGG CAG CAA TGG CAG AAT GGA AGC A-3\'. After being washed, alkaline phosphatase–conjugated streptavidin was added to each well, and the plate was incubated for 15 minutes at 37°C. Then 4-methoxy-4-(3-phosphophenyl)spiro-(1,2-dioxetan 3,2\'-adamantane), a substrate for alkaline phosphatase, was added, and luminescence measured by an automated chemiluminescence assay system (Toyobo).

Statistical Analysis

Hardy-Weinberg equilibrium was tested by the \( \chi^2 \) test with 1 df. \( \chi^2 \) Analysis was also used when comparing allele frequencies and categorical variables between the groups. Continuous variables were expressed as mean±SD or percentage according to clinical features. When the baseline characteristic was continuous (eg, age, blood pressures, duration of observation, urinary protein, sCr, and CrCl), the Kruskal-Wallis test and Mann-Whitney U test were used. The Kaplan-Meier method and the Cox proportional-hazards regression model were used to analyze the time course from renal biopsy to end point. When overall survival was significantly different in the Kaplan-Meier analysis, Greenwood’s estimation was performed at every 12 months of observation to test at which time point the difference between groups became significant. In the Cox regression model, we tested covariates (age, sex, urinary protein, hypertension, steroid therapy, administration of ACEI and/or ARB, and the gene polymorphism) by a stepwise backward method, and several covariates were selected. The effects of these covariates were expressed by a hazard ratio (HR) and 95% confidence interval (CI). 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 460G 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.\cite{26-28} 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 CrCl. Systolic blood pressure and incidence of hypertension at baseline tended to be higher in patients with ADD1 460WW 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-
sive renal disease). There was no difference for each genotype in observation duration, incidence of end point, mean blood pressure, incidence of corticosteroid treatment, and ACEI/ARB administration. As also shown in Table 1, among patients with each ACE genotype, no difference was noted in any clinical characteristic both at the time of diagnosis and during observation.

To investigate a possible interaction between the ADD1 and ACE polymorphisms, analyses were performed by subdividing the patients according to ACE genotype. Because the ACE I/D polymorphism has been reported to be associated with some clinical phenotypes, such as hypertension and cardiovascular diseases, with an additive effect of the D allele and because the frequency of the ACE D allele in our population was too low to separately analyze patients who were DD homozygotes, the patients were divided into 2 groups: patients who were II homozygotes and those who were heterozygous or homozygous for the D allele. Table 2 shows comparisons of clinical data between patients with each combined genotype. In patients with the ACE II genotype, hypertension at the time of diagnosis was more frequently observed ($\chi^2=4.350$, $P=0.0370$), and observation duration was shorter ($P=0.0412$) in the WW genotype of ADD1 than in other genotypes. However, these differences were not observed in patients with ACE ID or DD genotypes ($\chi^2=0.015$, $P=0.9024$).

**ADD1 Polymorphism as a Risk Factor for Progression of Renal Dysfunction**

Figure 1A shows the renal survival rate of patients with each ADD1 genotype for all patients studied. There was no difference between them. However, when the analysis was performed for a subgroup of patients with the ACE II genotype, the prognosis of renal function in patients with ADD1 460WW was significantly worse than in those with other genotypes (Figure 1B; Kaplan-Meier, log rank test, $\chi^2=6.062$, $P=0.0138$). 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; $\chi^2=2.238$, $P=0.1386$).

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 the ADD1 polymorphism was not 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.30

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 al19 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 homoyzous 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>Patients With ACE II Genotype</th>
<th>Patients With ACE ID or DD Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GW or GG (n=79)</td>
</tr>
<tr>
<td>At time of diagnosis</td>
<td></td>
</tr>
<tr>
<td>Gender, male, %</td>
<td>48.1</td>
</tr>
<tr>
<td>Age, y</td>
<td>37.2±12.8</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>128.3±18.7</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>78.3±13.9</td>
</tr>
<tr>
<td>UP, g/d</td>
<td>1.5±1.6</td>
</tr>
<tr>
<td>sCr, mg/dL</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>Ccr, mL/min per 1.73 m²</td>
<td>87.8±38.6</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>32.9</td>
</tr>
<tr>
<td>During observation</td>
<td></td>
</tr>
<tr>
<td>Observation, mo</td>
<td>99.0±62.1</td>
</tr>
<tr>
<td>Reached end point, %</td>
<td>36.7</td>
</tr>
<tr>
<td>Mean SBP, mm Hg</td>
<td>130.1±17.0</td>
</tr>
<tr>
<td>Mean DBP, mm Hg</td>
<td>79.6±12.1</td>
</tr>
<tr>
<td>Corticosteroids, %</td>
<td>22.9</td>
</tr>
<tr>
<td>ACEI/ARB, %</td>
<td>34.2</td>
</tr>
</tbody>
</table>

UP indicates urinary protein excretion. All other abbreviations are the same as in text.
presence of the 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 ACE I/D and 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. In fact, the frequencies of ACE I and 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, respectively). Thus, the population in this study might be more suitable to investigate a possible interaction between these 2 loci. Conversely, there is a possibility that our negative data in patients with the ACE D allele might be a consequence of its low frequency in the population studied. In addition, the numbers of patients with the combined genotypes shown in Table 2 might be insufficient to draw a conclusion. Therefore, further investigation with a large-scale population of other ethnic origins is needed. This study could not provide data on plasma renin and angiotensin II levels in patients with each genotype. However, RAS is mostly a paracrine and autocrine system, and the plasma level of its constituents poorly reflects its activity, particularly in local kidney tissues.

A limitation of the present study is that we could not make any adjustment for other possible factors that might have had an influence on the prognosis of renal function, such as smoking, dietary factors, insulin sensitivity, and other medications, including 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, fish oil administration, antiplatelet agents, and diuretics. However, the major clinical risk factors established by previous studies were investigated as confounding factors for disease progression. In addition, the reduced sample size over time shown in Figure 1 might...
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 significant importance in Japanese 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 Research Grants for Research on Specific Diseases from the Ministry of Health, Labor and Welfare. Health and Labor Science Research Grants for Research on Specific Diseases from the Ministry of Health, Labor and Welfare are necessary for confirming the present results.

References

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 and Fumitake Gejyo

Hypertension. 2003;42:304-309; originally published online July 28, 2003; doi: 10.1161/01.HYP.0000085193.25617.78

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/42/3/304

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/