Genetic Variant of the Renin-Angiotensin System and Diabetes Influences Blood Pressure Response to Angiotensin Receptor Blockers

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OBJECTIVE — Recent studies have proven the favorable effects of angiotensin receptor blockers (ARBs) on cardiovascular and renal disorders. However, determinants of the response to ARBs remain unclear. We substantiated the hypothesis that genetic variants of the renin-angiotensin system (RAS) have significant impacts on the response to ARBs.

RESEARCH DESIGN AND METHODS — Subjects comprised 231 consecutively enrolled hypertensive individuals including 45 type 2 diabetic subjects. Five genetic variants of the RAS, i.e., renin (*REN*) C-5312T, ACE insertion/deletion, angiotensinogen M235T, angiotensin II type 1 receptor A1166C, and angiotensin II type 2 receptor C3123A were assayed by PCR and restriction fragment-length polymorphism. A dose of 40–160 mg/day of valsartan was administered for 3 months as a monotherapy.

RESULTS — Changes in diastolic blood pressure significantly differed between genotypes of *REN* C-5312T: 10.7-mmHg reduction (from 95.9 ± 12.9 to 85.2 ± 11.4) in CC versus 7.0-mmHg reduction (from 94.7 ± 14.0 to 87.7 ± 12.6) in CT/TT ($P = 0.02$ for interactive effects of valsartan and genotype). Responder rates also differed between the genotypes: 72.8% in CC versus 58.0% in CT/TT ($P = 0.03$). Univariate analysis indicated a significant association of response to valsartan with blood pressure, diabetes, plasma aldosterone concentration, and CC homozygotes of *REN* C-5312T. Finally, multiple logistic regression analysis revealed that systolic blood pressure, CC homozygotes of *REN* C-5312T, and diabetes were independent predictors for responders with odds ratios (95% CI) of 2.49 (1.41–4.42), 2.03 (1.10–3.74), and 0.48 (0.24–0.96), respectively.

CONCLUSIONS — This study provides strong support that a genetic variant of *REN* C-5312T and diabetes contribute to the effects of ARBs and are independent predictors for responder. Thus, in treatment of hypertension with ARBs, a new possibility for personalized medicine has been shown.

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The renin-angiotensin system (RAS) plays major roles in blood pressure regulation and electrolyte metabolism (1) and pivotal roles in the pathophysiology of cardiovascular, renal, and metabolic conditions (2,3). Genetic variants of this system have been developed to test their association with cardiovascular and renal conditions. An ACE insertion/deletion polymorphism has been associated with ischemic heart disease (4) and the development of stage 5 chronic kidney disease (5). Angiotensinogen M235T has been associated with the development of hypertension (6). Angiotensin II type 1 (AT1) receptor A1166C has been associated with the development of hypertension and ischemic heart disease (7). Recently, a number of large-scale prospective studies have proven the favorable effects of blockade of the RAS on cardiovascular and renal conditions (8,9). However, the association between genetic variants of the RAS and effects of angiotensin II receptor blockers is still unclear (10) and must be well elucidated. Therefore, we substantiated the hypothesis that some variants of the RAS have influences on the effects of single administration of valsartan and analyzed determinants of responders to angiotensin II receptor blockers including the genetic variants.

RESEARCH DESIGN AND METHODS

— We enrolled 233 consecutive hypertensive patients from our outpatient clinic in the study, and 231 subjects completed the study. They consisted of 101 (43%) men with mean ± SD age, BMI, glucose level, A1C, LDL, and estimated glomerular filtration rate (eGFR) of 64.6 ± 12.6 years, 24.6 ± 3.9 kg/m^2, 107.4 ± 19.8 mg/dl, 5.46 ± 0.87%, 117.1 ± 28.7 mg/dl, and 72.5 ± 18.3 ml/min per 1.73 m^2, respectively. All subjects were Japanese inhabiting Hokuriku, a region of Japan. Subjects aged <20 years old and those with secondary hypertension, target organ disease, severe organ failure, and acute-phase disorders were excluded. All subjects had not taken any antihypertensive or antidyslipidemic agents for at least 1 week before the first sampling for the study, and home blood pressure was measured to exclude subjects with white coat hypertension. In the clinic, with the subject in a sitting position, blood pressure was taken from the left arm at least three times repeatedly using an automated digital device (ES-H51;

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Table 1—*Baseline characteristics of subjects and effects of valsartan*

	Baseline	After administration	*P**
Blood pressure (mmHg)			
SBP	162.4 ± 18.2	144.8 ± 18.4	<0.001
DBP	95.1 ± 13.6	86.4 ± 12.2	<0.001
Natrium (mEq/l)	141.4 ± 2.0	141.3 ± 2.3	0.51
Kalium (mEq/l)	4.02 ± 0.35	4.06 ± 0.35	0.12
Chlorine (mEq/l)	104.5 ± 2.4	104.8 ± 2.5	0.04
Creatinine (mg/dl)	0.75 ± 0.28	0.74 ± 0.27	0.07
Urinary natrium (mEq/creatinine)	171.3 (104.9–254.1)	187.3 (123.9–264.6)	0.07
Urinary kalium (mEq/creatinine)	49.8 (36.1–76.4)	54.7 (36.1–76.7)	0.03
Urinary chlorine (mEq/creatinine)	185.5 (116.1–269.1)	193.6 (141.0–288.7)	0.09
Urinary albumin excretion (mg/creatinine)	25.0 (11.4–74.4)	14.9 (8.8–45.0)	<0.001
Plasma renin activity ($ng \cdot ml^{-1} \cdot h^{-1}$)	0.60 (0.21–0.90)	1.10 (0.60–2.30)	<0.001
Angiotensin I (pg/ml)	61.0 (39.5–110.0)	93.0 (55.0–210.0)	<0.001
Angiotensin II (pg/ml)	5.0 (4.0–8.0)	7.0 (4.0–13.0)	<0.001
Plasma aldosterone concentration (pg/ml)	69.6 (50.0–100.0)	58.0 (39.9–76.7)	<0.001
Atrial natriuretic peptide (pg/ml)	25.6 (17.0–39.5)	22.7 (14.7–34.3)	0.001

Data are means ± SD or medians (interquartile ranges). *The differences between two paired continuous variables for before and after administration of valsartan were analyzed by Student's *t* test or a Wilcoxon signed-rank test as appropriate. All *P* values are two-sided.

Terumo) with each recording separated by as much time as practical. If readings varied >5 mmHg, additional readings were taken until the last two were close. Diabetic subjects, 19.5% of the total subjects, continued to receive their usual care for diabetes. A target A1C level of <6.5% was recommended for all subjects. Diabetes was diagnosed according to the criteria of the World Health Organization. Dyslipidemia was diagnosed according to the criteria of the International Diabetes Federation. Estimated glomerular filtration rate (eGFR) was calculated according to the formula for Japanese subjects: eGFR (milliliters per minute per 1.73 m^2) = $194 \times \text{creatinine}^{-1.094} \times \text{age}^{-0.287}$ (× 0.739, for women). BMI was calculated as the weight in kilograms divided by the square of height in meters. Arterial hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg or diastolic blood pressure (DBP) of ≥90 mmHg with the subject in the sitting position on two separate occasions in the morning. Daily 40–80 mg valsartan as a starting dose was administered, and then the dose were augmented to daily 80–160 mg according to the blood pressure in an intention-to-treat manner. At baseline and after 3 months the items shown in Table 1 were examined. After the subjects rested for 15 min in the supine position, blood samples were drawn for the measurements. Plasma samples collected carefully and processed to avoid cold activation were incubated at 37°C for an adequate length of time, and the angiotensin I generated was measured by radioimmunoassay. Responders to valsartan were defined by a strict criterion (subjects who had a decrease in DBP of ≥5 mmHg) and a standard criterion (responders by the strict criteria and subjects who achieved a SBP of <140 mmHg and a DBP of <90 mmHg). The study was approved by the ethics committee of Fukui University (no. 13-1, 14-2), and informed consent for participation was obtained from all individuals.

Genotyping

Genomic DNA was isolated by a commercial kit (QIAamp DNA Blood Mini Kit; Qiagen, Tokyo, Japan) from whole blood. In all DNA amplification, a thermal cycler (PC-700; Astec, Fukuoka, Japan) was used, and PCR were performed fundamentally according to the protocol (rTAQ, Toyobo, Osaka, Japan).

The renin (*REN*) C-5312T was assayed by PCR-restriction fragment-length polymorphism (RFLP) with *Dde*I digestion. Primers as originally designed by us from the adjacent sequence (11) were the following: sense oligo 5′-CGTAGTGCCATTTTTAGGAAC-3′ and antisense oligo 5′-AACACCAAAGCAGGCTTAA-3′. The program consisted of 40 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s, and extension at 72°C for 40 s followed by a final extension at 72°C for 5 min. PCR products were incubated with *Dde*I overnight at 37°C. The PCR products were loaded on 3.0% agarose gels. Alleles were designated C or T, indicating digestion by *Dde*I or not.

The *ACE* gene polymorphism was assayed by DNA amplification followed by confirmation with insertion-specific primers. Primers were the same as those in a previous report with minor modifications (12). To confirm the absence of insertion allele, samples from the DD genotype were subjected to an independent DNA amplification with an insertion allele–specific primer. Primers were the same as those in a previous report with minor modifications (13). Alleles were designated I or D, indicating the presence or absence of the insertion sequence.

AGT M235T was assayed by mismatch primer PCR-RFLP with *Sfa*NI digestion. Primers were the same as those in a previous report with minor modifications (14). Alleles were designated M or T, indicating digestion by *Sfa*NI or not.

AT1 A1166C was assayed by PCR-RFLP with *Dde*I digestion. Primers were the same as those in a previous report with minor modifications (7). Alleles were designated C or A, indicating digestion by *Dde*I or not.

AT2 C3123A was assayed by PCR-RFLP with *Alu*I digestion. Primers were the same as those in a previous report with minor modifications (15). Alleles were designated A or C, indicating digestion by *Alu*I or not. Because the *AT2* gene is harbored on the X chromosome, alleles were A or C in men and AA or AC or CC in women, respectively.

Statistical analysis

The sample size of the study was calculated, assuming an SD for DBP of 10 mmHg and a difference to be detected between groups of at least 5 mmHg, and used a bilateral paired Student's *t* test with protection against type I error of 5 and 80% of power. It was calculated that the study required 200 subjects in total. Statistical analyses were performed with SPSS (version 11.0J; SPSS Japan, Tokyo, Japan). Data are presented as numbers, percentages, means ± SD, or medians (interquartile ranges) as appropriate. The differences between two paired continuous variables for before and after administration of valsartan were analyzed by Student's *t* test or Wilcoxon's signed-rank test as appropriate. The difference for the interactive effect of valsartan and genotype in subjects on blood pressure was

Table 2—*Genetic variants of the RAS and reduction in blood pressure

Genetic variants allele (no.)	SBP (mmHg)			DBP (mmHg)		
	Baseline	Valsartan	*P**	Baseline	Valsartan	*P**
REN C-5312T			0.07			0.02
CC (81)	162.5 ± 19.7	141.9 ± 17.5		95.9 ± 12.9	85.2 ± 11.4	
CT/TT (150)	162.4 ± 17.5	146.4 ± 18.8		94.7 ± 14.0	87.7 ± 12.6	
ACE insertion/deletion			0.45			0.54
II (96)	162.9 ± 19.6	146.4 ± 17.6		94.8 ± 14.8	85.9 ± 13.7	
ID/DD (135)	162.1 ± 17.3	143.7 ± 19.0		95.4 ± 12.8	87.4 ± 11.0	
AGT M235T			0.87			0.25
TT (160)	161.7 ± 17.9	144.3 ± 18.5		95.4 ± 13.5	86.5 ± 11.9	
MM/MT (71)	163.9 ± 19.1	146.1 ± 18.2		94.6 ± 14.1	87.6 ± 12.9	
AT1 A1166C (AA)			0.50			0.52
AA (195)	162.3 ± 18.6	144.8 ± 18.9		94.7 ± 13.8	86.1 ± 12.5	
AC/CC (36)	162.9 ± 16.4	145.2 ± 16.3		97.8 ± 12.9	90.5 ± 9.7	
AT2 C3123A			0.37			0.14
C/CC(105)	162.3 ± 17.8	143.5 ± 17.3		95.7 ± 13.6	86.1 ± 12.0	
A/AA/AC(126)	162.6 ± 18.7	146.0 ± 19.3		94.7 ± 13.7	87.4 ± 12.3	

Data are means ± SD. Statistical analysis of the difference was performed by two-way repeated-measures ANOVA. *A *P* value was calculated for the statistics showing the interactive effect of valsartan administration and category in patients on DBP.

analyzed by two-way repeated-measures ANOVA. Dichotomous and polychotomous characteristics were compared with use χ^2 analysis for tests for Hardy-Weinberg equilibrium and responder and sex distributions in *REN* C-5312T genotypes. The differences between continuous characteristics of genotype groups were assessed by one-way ANOVA or a Mann-Whitney *U* test was used as appropriate. Crude odds ratios (ORs) and 95% CI for responders to valsartan were calculated using logistic regression for each dichotomous characteristic. Then, multivariate logistic regression was performed to adjust for potential confounding factors for responders to valsartan. All *P* values are two-sided.

RESULTS

Effects of valsartan on clinical characteristics

A total of 231 subjects received monotherapy with valsartan for 3 months and completed the study. Two patients dropped out because of palpitation or malaise. However, including these two patients, no serious adverse effects occurred in the study term. Changes in clinical and biochemical characteristics with valsartan administration are summarized in Table 1. Significant reductions in systolic and DBP were achieved. Blood pressure changes induced by valsartan treatment showed unimodal distribution (SBP [mean ± SD] −17.6 ± 18.5 mmHg, kurtosis 1.582, and skewness −0.496; DBP −8.4 ± 11.4 mmHg, kurtosis 1.221, and skewness 0.236). Urinary albumin excretion was significantly reduced from a median of 25.0 to 14.9 mg/creatinine, an ~40% reduction. Significant elevations of plasma renin activity, plasma angiotensin I concentration, and plasma angiotensin II concentration were observed, whereas significant reductions in the plasma aldosterone concentration and plasma atrial natriuretic peptide concentration were observed. The responder rate is calculated to be 71.0% (164 of 231 cases), according to the standard criteria for this study and 63.2% (146 of 231 subjects) according to the strict criteria for this study.

Genetic variants of the RAS and reduction in blood pressure with valsartan

Changes in blood pressure with valsartan monotherapy in each genetic variation of the RAS are summarized in Table 2. The distributions of each genotype were similar to those expected from Hardy-Weinberg equilibrium. Among the genetic variants of RAS elucidated, the change in DBP was significantly different according to the genetic variant of renin C-5312T: CC ($n = 81$) from 95.9 ± 12.9 to 85.2 ± 11.4 mmHg versus CT/TT ($n = 150$) from 94.7 ± 14.0 to 87.7 ± 12.6 mmHg ($P = 0.02$ for the interactive effect of valsartan and genotype on DBP). Thus, it was revealed that the reductions in DBP in C allele homozygotes were significantly larger than those in T allele carriers with a difference of about 3.6 mmHg (CC −10.7 vs. CT/TT −7.1). The responder rate by the strict criteria for C allele homozygotes was significantly larger than that for other genotypes (CC 59 of 81 subjects [72.8%] vs. CT/TT 87 of 150 subjects [58.0%]; $\chi^2 = 4.98$, $P = 0.03$).

Genetic variants of *REN* C-5312T and characteristics at baseline and changes from baseline

The baseline demographic, clinical, and biochemical characteristics of the subjects were almost balanced among genotypes of *REN* C-5312T except for differences in the serum chlorine level and in urinary albumin excretion, including the doses of valsartan and the duration of administration. Unexpectedly, no difference was observed in levels of plasma renin activity, angiotensin I, angiotensin II, or plasma aldosterone. The drug doses of all genotypes also balanced (data not shown).

Logistic regression analysis for responders to valsartan

Univariate analysis was performed among dichotomous variables of the subjects to determine the predictors for responders to valsartan (Table 3). The analysis showed that high SBP and DBP, diabetes, high plasma aldosterone concentration, and C allele homozygote of *REN* C-5312T were univariate predictors for responders to valsartan. Consequently, a multivariate logistic regression was performed with the four variables shown in Table 4. Considering that the criteria for the respond-

Table 3—*Univariate analysis of predictors for responder to valsartan*

Variables (criteria, %)	OR (95% CI)	*P*
Age (≥65 years, 51.2%)	1.27 (0.74–2.16)	0.39
Sex (male, 43.7%)	0.94 (0.55–1.61)	0.82
BMI (≥25 kg/m^2, 38.3%)*	1.22 (0.70–2.13)	0.48
SBP (≥160 mmHg, 48.1%)	2.47 (1.42–4.31)	0.001
DBP (≥95 mmHg, 48.1%)	4.47 (2.48–8.05)	<0.001
Diabetes (19.5%)	0.48 (0.25–0.93)	0.03
Dyslipidemia (27.4%)	1.15 (0.61–2.16)	0.67
Urinary albumin excretion (≥30 mg/g creatinine, 44.4%)	0.76 (0.44–1.31)	0.32
eGFR (<60 ml/min per 1.73m^2, 20.8%)	0.85 (0.42–1.71)	0.64
Urinary natrium (≥170 mEq/creatinine, 50.2%)	1.28 (0.74–2.22)	0.37
Plasma renin activity (≥0.5 ng · ml^{-1} · h^{-1}, 56.3%)	1.08 (0.63–1.85)	0.77
ACE (>15.0 IU/l, 38.3%)	1.22 (0.70–2.13)	0.48
Angiotensin I (≥50.0 pg/ml, 59.9%)	1.07 (0.59–1.93)	0.83
Angiotensin II (≥5.0 pg/ml, 48.7%)	1.63 (0.91–2.93)	0.10
Plasma aldosterone concentration (≥75.0 pg/ml, 44.6%)	1.83 (1.06–3.18)	0.03
Atrial natriuretic peptide (≥20.0 pg/ml, 69.5%)	0.77 (0.41–1.46)	0.42
REN C-5312T (CC, 35.1%)	1.94 (1.08–3.49)	0.03
ACE deletion/insertion (II, 41.6%)	0.95 (0.55–1.63)	0.85
AGT M235T (TT, 69.3%)	1.08 (0.61–1.92)	0.80
AT1 A1166C (AA, 84.4%)	0.84 (0.39–1.77)	0.64
AT2 C3123A (C/CC, 45.5%)	1.53 (0.89–2.64)	0.12

Crude ORs for responder to valsartan and 95% CIs were calculated using logistic regression for each dichotomous characteristic. Responders to valsartan were defined as subjects who had a decrease in DBP of ≥5 mmHg. *BMI is the weight in kilograms divided by square of the height in meters.

ers included DBP itself and that a strong collinearity between SBP and DBP is assumed, DBP was excluded from the final model of the analysis. Only high SBP, C allele homozygote of *REN* C-5312T, and diabetes were confirmed to be independent predictors for responders to valsartan. In particular, there was a twofold increase in the odds to be a responder for subjects with the C allele homozygote of *REN* C-5312T (OR 2.03 [95% CI 1.10–3.74], $P = 0.02$).

CONCLUSIONS — The results of the present study revealed for the first time that a genetic variant of the human renin gene, C-5312T, independently affects the blood pressure reduction with angiotensin II receptor blockers in an adequate-sized sample. As results with pharmacogenomic approaches in the area of hypertension show, only a small fraction of the genes probably contribute to the antihypertensive responses. The genetic loci for α-adducin to diuretics and for β_1-adrenoceptor to β-blocker have emerged as strong candidates as one polymorphism for a specific class of antihypertensive agents (16). The genetic variants of the RAS are plausible candidates for the pharmacogenomic predictors for ACE inhibitors and angiotensin II receptor blockers and have been evaluated (17–19). However, no consistent conclusions were obtained regarding this issue, and the problems contributing to the discordance have been noted (10). Our study was designed with the intention of avoiding biases, especially those from sample size for adequate power and adjustments for confounding factors. To our knowledge, only one study has analyzed the relationship between genetic variants of the RAS and angiotensin II receptor blockers in an adequate-sized sample (206 subjects) (18). These authors found no significant association between the presence of any genetic variant (ACE insertion/deletion, angiotensinogen A-6G, AT1 A1166C, and AT1 C573T) and a reduction in blood pressure with concordance to our results. However, no study regarding the relationship of the renin gene to the effects of angiotensin II receptor blocker in an adequate-sized sample other than our present study has been reported thus far.

Table 4—*Multivariate logistic regression analysis for responder to valsartan*

	β	SE	Wald's χ^2	OR (95% CI)	*P*
SBP (≥160 mmHg)	0.91	0.29	9.79	2.49 (1.41–4.42)	0.002
REN C-5312T (CC)	0.71	0.31	5.10	2.03 (1.10–3.74)	0.02
Diabetes	−0.74	0.35	4.36	0.48 (0.24–0.96)	0.04
Plasma aldosterone concentration (≥75.0 pg/ml)	0.54	0.29	3.42	1.72 (0.97–3.07)	0.06

Multivariate logistic regression was performed to adjust for potential confounding factors for responders to valsartan. Responders to valsartan were defined as subjects who had a decrease in DBP of ≥5 mmHg or more. All factors in this table were included in the final model.

Recent advances in understanding of the human renin gene revealed that multiple *trans*-factors and *cis*-elements are involved in transcriptional regulation (20–22). Germain et al. (23) showed that a 225-bp region located about 6 kb upstream from the transcription starting point of the human renin gene gave ~57-fold higher transcription rates and is a distal enhancer with human choriodecidual cells. *REN* C-5312T was discovered as a functional new polymorphism in the distal enhancer region of the human renin gene (11). The levels of transcription were 45% greater with the 5312T variant than with the 5312C variant. Thus, the variant was validated as a functional single nucleotide polymorphism. The results of the present study demonstrated that C allele homozygotes showed greater blood pressure reduction with angiotensin II receptor blockers than T allele carriers and, thus, C allele homozygosity is a predictor for a responder. One possible explanation is that C allele homozygotes might have lower levels of renin and a sufficient drug effect compared with T allele carriers. However, no difference in plasma renin activity was observed between subjects with the two genotypes. An alternative explanation is that the transcriptional difference demonstrated in the in vivo model may not be reflected in the plasma level, but the local concentration is different in the tissue RAS. Recently, expression of renin in larger arteries outside the kidney was shown with human renin gene promoter/LacZ constructs for a trans-gene model (24). Furthermore, a functional receptor for (pro)renin, which mediates intracellular signaling by activating mitogen-activated protein kinases and extracellular signal–regulated kinases 1 and 2 and acts as a cofactor by increasing the efficiency of angiotensinogen cleavage,

was cloned recently (25). These recent discoveries raise the possibility that tissue renin expression exerts a significant effect on the tissue RAS and relates to the pharmacogenetic effects of the C-5312T variant in our study. On the other hand, one plausible supposition is that blood pressure reduction is larger in the 5312T variant than in C allele homozygotes. However, the results were different. We supposed that 160 mg valsartan daily, the approved maximal dose in Japan, might not be sufficient for T allele carriers. We also demonstrated an upregulation of renal tissue RAS with ACE expression (3). This would be one explanation for the fact that diabetes is an independent predictor for nonresponders at a twofold OR.

Several limitations of this study should be noted. We have calculated the sample size considering a type I error of 5 and 80% power; however, the sample number is still relatively small. In particular, the power calculation was not sufficient for AT1 because of a small number for minor alleles in 36 of 231 subjects. Although population admixture is thought to contribute to concordant results among studies, our study comprises only a Japanese population. We evaluated five independent genetic variants. Accordingly, a correction for multiple testing might be needed. On this point, the *P* value threshold of 0.05 for significance might be relatively large. Another limitation is that only one genetic variant for each gene was assayed.

In summary, this study provides strong support that a genetic variant of the renin gene, C-5312T, and diabetes contribute to the effects of angiotensin II receptor blockers. In other words, the variant is able to be an independent predictor for a responder to an angiotensin II receptor blocker. Thus, a new possibility for personalized medicine by genetic variants of the RAS has been shown in treatment of hypertension with angiotensin II receptor blockers. Further studies are needed, including studies on other single nucleotide polymorphisms in each gene to be able to generalize the results to other races and evaluate prorenin concentrations.

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