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Determinants of Plasma Renin Activity

Role of a Human Renin Gene Variant as a Genetic Factor

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Abstract: The plasma renin activity (PRA) is affected by a number of environmental factors. However, significant heritability has been shown for the activity. A hypothesis that a candidate regulatory single-nucleotide polymorphism, C-5312T, of human renin gene should have a significant effect on PRA was elucidated and updating of independent determinants of PRA was attempted.

Cross sectional study.

Outpatient study.

We enrolled consecutive 810 subjects who had consulted our hospitals for lifestyle-related diseases.

Genotypes were assayed with genomic DNA for C-5312T. Among the genetic variants, the difference of PRA was evaluated. Monovariate linear regression analysis was performed to test the correlation between PRA and clinical variables. Finally, stepwise multiple regression analysis was performed to evaluate the independent determinants.

On comparing 2 genotype groups, CC/CT and T allele homozygote, the geometric means of PRA were 0.778 and 0.941 ng/ml/h, respectively ($F = 5.992$, $P = 0.015$). Monovariate linear regression analysis revealed that a number of variables have a significant correlation with the activity, including urinary salt excretion. A stepwise multivariate regression analysis revealed that renin C-5312T variant (TT) is one of the independent determinants of PRA.

Thus, for the first time, a human renin gene variant was associated with a significant increase in PRA as a genetic factor and the independent determinants for the activity were updated including genetic factor.

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Abbreviations: BMI = body mass index, eGFR = estimated glomerular filtration rate, PRA = plasma renin activity, rSNP = regulatory SNP, RAS = renin–angiotensin system.

INTRODUCTION

The renin-angiotensin system (RAS) plays a major role in blood pressure regulation and electrolyte metabolism[1]; however, the overactivation of the RAS is thought to play a pivotal role in the pathophysiology of cardiovascular,[2] renal[3] and metabolic conditions.[4] Plasma renin had been associated with cardiovascular morbidity and mortality in hypertensive patients[5,6] and recent studies also confirmed that higher plasma renin was associated with greater cardiovascular mortality in patients referred for coronary angiography[7] and in community-based cohort studies.[8,9] The plasma renin activity (PRA) is affected by a number of factors, especially quantity of sodium intake.[10] However, significant heritability has been shown for the PRA.[11,12] Recent advance in understanding for transcriptional mechanism of the human renin gene revealed that multiple *trans*-factors and *cis*-elements are involved including a candidate functional single-nucleotide polymorphism (SNP), C-5312T, which is thought to be a transcriptional regulatory SNP (rSNP).[13–21] It has been proven that the transcription levels of the 5312T allele are 45% greater than those of the C allele in cultured cell model.[19] A number of renin gene polymorphisms were reported including *Bgl* I and *Hind* III,[22] *Bgl* II, *Rsa* I, and *Taq* I,[23] *Mbo* I,[24] *Hinf* I,[25] *Sty* I,[26] G1051A,[27] and so forth. However, no previous SNP has been validated on such an experimental model. Thus, this is the solely validated variant among several known variants. So, we selected the variant for the study. Thus, in this study, we substantiated the hypothesis that the genetic variant of human renin gene should have a significant association with PRA and attempted updating of independent determinants for the activity.

METHODS

Subjects and Methods

We enrolled to the study consecutive 810 subjects who had consulted our hospitals for mainly lifestyle related diseases

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(hypertension 61.5%, diabetes mellitus 38.8%, dyslipidemia 41.1%, etc.) with no special selection between June 2000 and September 2013. The study was approved by the ethics committee of Fukui University No. 13–1 and 14–2, which means the first approval of Japanese era name ''Heisei'' 13 (A.D. 2001) and the second approval of ''Heisei'' 14 (A.D. 2002), respectively. Written informed consent for participation was obtained from all individuals. Subjects with age less than 20 years, secondary hypertension, acute phase disorders, and severe organ failure were excluded. Diabetic subjects continued to receive their usual care for diabetes. At the blood sampling, all subjects had been under the condition without any antihypertensive or antidyslipidemic agent for at least 1 week. After 15 minutes' rest in the supine position, blood samples were drawn for the measurement in the morning once for evaluation. Standard conditions for blood sampling for PRA, that is early morning sampling, sufficient untreated span, repeated measurement, etc, are recommended.[28–30] We tried as much as possible to make available the recommended ideal conditions. For PRA measurement, samples were collected and processed avoiding cold activation and incubated at 37°C for adequate number of hours (fundamentally 1.5 hour); generated angiotensin I was measured by radioimmunoassay (BML, Japan). The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Estimated glomerular filtration rate (eGFR) was calculated according to the formula: eGFR (mL/min/1.73 m^2) = 194 × $Cr^{-1.094}$ × $Age^{-0.287}$ (×0.739, in the case of female).[31] The major demographic and baseline clinical characteristics are summarized in Table 1.

Genotyping was carried out with genomic DNA isolated from human leukocytes of whole blood drawn with ethylenediaminetetraacetic acid 2Na tubes by a commercial kit (QIAamp DNA Blood Mini Kit, QIAGEN Inc, Japan). Human renin gene C-5312T variant was previously assayed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism with *Dde* I digestion. Primers originally designed by us from the adjacent sequence were as follows: sense oligo, 5'-CGTAGTGCCATTTTTAGGAAC-3' and anti-sense oligo, 5'-AACACCAAAGCAGGCTTAA-3'. The program consisted of 40 cycles of denaturation at 94°C for 40 seconds, annealing at 52°C for 40 seconds, and extension at 72°C for 40 seconds followed by a final extension at 72°C for 5 minutes. PCR products were incubated with *Dde* I overnight at 37°C. The PCR products were loaded on 3.0% agarose gels. Alleles were designated as C or T, indicating digestion by *Dde* I or not. Recently, the variant C-5312T is assayed using the TaqMan method (rs 12750854) with common PCR conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA).

TABLE 1. Characteristics of Subjects*

Characteristics	
Number	810
Female sex, n (%)	424 (52.3 %)
Age, y	62.1 ± 14.3
Height, cm	158.1 ± 9.9
Weight, kg	60.3 ± 13.1
Body mass index†	24.0 ± 4.0
Blood pressure, mmHg	
Systolic	144.8 ± 23.7
Diastolic	85.9 ± 14.8
Mean	105.5 ± 16.5
Blood glucose, mg/dl‡	106.0 (95.0–133.0)
Glycosylated hemoglobin, %	6.46 ± 1.47
Triglyceride, mg/dL‡	98.0 (68.0–142.5)
Cholesterol, mg/dL	
High-density lipoprotein‡	52.0 (44.0–63.0)
Low-density lipoprotein	117.9 ± 32.1
Serum uric acid, mg/dL‡	5.0 (4.0–6.1)
Serum creatinine, mg/dL‡	0.70 (0.59–0.81)
Plasma renin activity, ng/mL/h‡	0.70 (0.30–1.30)
eGFR, mL/min/1.73 m^2	78.5 ± 21.8
Urinary sodium excretion, mEq/creatinine‡	162.6 (102.1–247.9)
Urinary potassium excretion, mEq/creatinine‡	51.2 (36.1–72.7)
Urinary chloride excretion, mEq/creatinine‡	178.5 (114.0–262.6)

eGFR = estimated glomerular filtration rate.
* Plus-minus values are means ± SD.
† The body mass index is the weight in kilograms divided by square of the height in meters.
‡ Values shown are medians (interquartile ranges).

Statistical Analysis

The sample size of the study was calculated tentatively with PRA, estimating a standard deviation of about 0.8 ng/mL/h, a difference to be detected between groups of 0.2 ng/mL/h and using a bilateral analysis of variance (ANOVA) with protection against type I error of 5% and 80% of power. Altman's nomogram with δ/σ value for paired *t* test showed that the study required around 750 subjects in total, considering the genetic variant distribution. The allele frequencies for each genotype were tested by contingency table analysis for Hardy–Weinberg equilibrium. The differences between 2 nonpaired continuous variables were analyzed by ANOVA or Wilcoxon signed-rank test as appropriate. When the distribution of measurement values was significantly deviated, the values were transformed so as to be consistent with the normal distribution prior to statistical analysis; logarithmic transformation was performed for blood glucose, triglyceride, serum uric acid, serum creatinine, and PRA. Square root transformation was performed for urinary sodium excretion, etc. Because the distribution of the PRA was deviated (skewness = 3.54, kurtosis = 20.1), we alternatively adopted the log-transformed value, the (log [PRA + 1]) (skewness = 1.10, kurtosis = 1.63), for statistical analysis. Still, the distribution was not normal; however, no further transformation was thought to be adequate. Monovariate linear regression analysis was performed to test the correlation between log-transformed PRA and variables under investigation, which is thought to be indispensable for proceeding to the multiple regression analysis. Finally, stepwise multiple regression analysis was further performed for the log-transformed PRA to evaluate the independent determinant for the activity with the entry threshold of test variables set to be a *P* value of 0.05. At the regression analyses, to female and male sex, 0 and 1 were allocated, respectively and similarly, to CC/CT genotypes and TT genotype subjects, 0 and 1 were allocated, respectively. Data were presented as numbers, percentage, means ± SD, or medians (interquartile ranges), as appropriate. All statistical analyses were conducted using the SPSS Version 17.0 (SPSS Japan Inc., Tokyo, Japan).

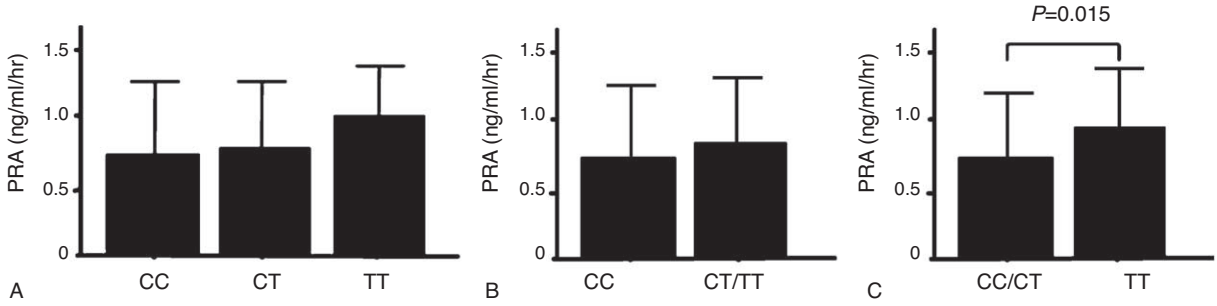

FIGURE 1. Renin gene variant, C-5312T, and plasma renin activity. Closed columns and bars express geometric means and standard errors of plasma renin activity. (A) Comparison among 3 genotype groups (CC, CT and TT). (B) Comparison between 2 genotype groups (C allele homozygote and CT/TT). (C) Comparison between 2 genotype groups (CC/CT and TT allele homozygote). On comparing 3 genotype groups, CC, CT and TT, the geometric means of PRA were 0.776, 0.798 and 0.941 ng/mL/h, respectively ($F = 2.993$, $P = 0.051$). On comparing 2 genotype groups, C allele homozygote and CT/TT, the geometric means of PRA were 0.776 and 0.821 ng/mL/h, respectively ($F = 0.678$, $P = 0.411$). On comparing 2 genotype groups, CC/CT and T allele homozygote, the geometric means of PRA were 0.778 and 0.941 ng/mL/h, respectively ($F = 5.992$, $P = 0.015$). Statistical analysis was performed with analysis of variance for log-transformed value. PRA = plasma renin activity.

RESULTS

Renin Gene Variant, C-5312T and Plasma Renin Activity

The numbers of individuals with each genotype were 255, 406, and 149 for CC, CT, and TT, respectively. The distributions were similar to those expected from Hardy–Weinberg equilibrium. Thus, minor allele frequency is about 43.5%. First, among human renin gene variant, C-5312T, the difference of log-transformed PRA was evaluated with ANOVA (Fig. 1). We performed comparisons between 2 genotype groups as well as between 3 genotype groups to evaluate the additive model and the recessive model. On comparing 3 genotype groups, CC, CT, and TT, the geometric means of PRA were 0.776, 0.798 and 0.941 ng/mL/h, respectively ($F = 2.993$, $P = 0.051$). On comparing 2 genotype groups, C allele homozygote and CT/TT, the geometric means of PRA were 0.776 and 0.821 ng/mL/h, respectively ($F = 0.678$, $P = 0.411$). On comparing 2 genotype groups, CC/CT and T allele homozygote, the geometric means of PRA were 0.778 and 0.941 ng/mL/h, respectively ($F = 5.992$, $P = 0.015$).

Correlation Between Log-transformed PRA and Variables Under Investigation

As shown in Table 2, monovariate linear regression analysis between log-transformed PRA and variables under investigation revealed that a number of variables have a significant correlation with PRA. Age, blood pressure, urinary sodium excretion, urinary potassium excretion, and urinary chloride excretion showed significant negative correlation. On the contrary, sex, height, weight, blood glucose, glycosylated hemoglobin, triglyceride, low-density lipoprotein, serum uric acid, and eGFR showed significant positive correlation.

Stepwise Multiple Regression Analysis for Log-transformed PRA and Variables Under Investigation

Finally, a stepwise multivariate regression analysis was performed in a forward stepwise selection manner (Table 3). Considering collinearity, sex, age, height, weight, mean blood pressure, blood glucose, triglyceride, serum uric acid, eGFR, urinary sodium excretion, and renin C-5312T variant (TT) were set into this model. Fundamentally, we selected the variables that showed P value less than 0.05. At the same time, blood glucose and glycosylated hemoglobin, systolic diastolic and mean blood pressure, and urinary minerals are presumed to have

TABLE 2. Correlation Between Log-transformed PRA and Variables Under Investigation

Variables	*r*	*P* Value
Sex*	0.167	<0.001
Age, y	−0.225	<0.001
Height, cm	0.168	<0.001
Weight, kg	0.081	0.022
Body mass index†	−0.020	0.566
Blood pressure, mmHg		
Systolic	−0.238	<0.001
Diastolic	−0.151	<0.001
Mean	−0.205	<0.001
Blood glucose, mg/dL‡	0.100	0.005
Glycosylated hemoglobin, %	0.139	<0.001
Triglyceride, mg/dL‡	0.099	0.005
Cholesterol, mg/dL		
High-density lipoprotein‡	−0.67	0.074
Low-density lipoprotein	0.125	0.001
Serum uric acid, mg/dL‡	0.084	0.019
Serum creatinine, mg/dL‡	0.068	0.053
eGFR, mL/min/1.73 m^2	0.083	0.018
Urinary sodium excretion, mEq/creatinine§	−0.257	<0.001
Urinary potassium excretion, mEq/creatinine‡	−0.133	<0.001
Urinary chloride excretion, mEq/creatinine§	−0.263	<0.001

''*r*'' at the top of the central column represents the Pearson's correlation coefficient. eGFR = estimated glomerular filtration rate, PRA = plasma renin activity.

* To female and male sex, 0 and 1 were allocated, respectively.

† The body mass index is the weight in kilograms divided by square of the height in meters.

‡ Logarithmic transformation was performed.

§ Square root transformation was performed.

TABLE 3. Stepwise Multiple Regression Analysis for Log-transformed PRA and Variables Under Investigation

	Unstandardized Coefficient		Standardized Coefficient		
Included Variables	**B**	**Standard Deviation Error**	β	***T* Value**	***P***
(constants)	0.420	0.114		3.690	<0.001
Urinary sodium excretion*	−0.009	0.001	−0.224	−6.300	<0.001
Age	−0.003	0.000	−0.236	−6.156	<0.001
Mean blood pressure	−0.002	0.000	−0.178	−4.916	<0.001
Triglyceride†	0.082	0.025	0.115	3.200	0.001
Sex‡	0.045	0.014	0.131	3.341	0.001
Blood glucose†	0.109	0.047	0.083	2.323	0.020
C-5312T variant (TT)§	0.033	0.015	0.073	2.139	0.033
Weight	−0.001	0.001	−0.093	−2.094	0.037
Excluded variables		**Coefficient**		***T* Value**	***P***
Height		−0.051		−0.809	0.419
eGFR		0.030		0.755	0.450
Serum uric acid†		0.025		0.630	0.529

eGFR = estimated glomerular filtration rate.
* Square root transformation was performed.
† Logarithmic transformation was performed.
‡ To female and male sex, 0 and 1 were allocated, respectively.
§ To CC/CT genotypes and TT genotype, 0 and 1 were allocated, respectively.

strong collinearity; we selected blood glucose, mean blood pressure, and urinary sodium excretion from each group. Urinary sodium excretion, age, mean blood pressure, triglyceride, sex, blood glucose, renin C-5312T variant (TT), and weight were adopted as independent determinants in this order. On the contrary, height, serum uric acid, and eGFR were excluded from this model. The adjusted R^2 was 1.76, and the standardized partial regression coefficient (β) of the variant was 0.073. We analyzed the same model without C-5312T and, as a result, the same factors were adopted and the adjusted R^2 was 1.72.

DISCUSSION

The results of the present study revealed that a genetic variant of the human renin gene, C-5312T, independently affects the PRA. A previous study investigated this issue as a supplemental evaluation in a very limited number of subjects and hence could not detect the significant difference. The Dublin group found no difference between renin-5312 CC homozygotes and T allele carriers in either baseline PRA (logarithmic mean [95% confidence interval], 11% [−15,42]) or on-treatment PRA (3% [−24, 38]) reported among 387 white bank employees with mild-to-moderate hypertension. It is possible that the difference between their study and ours could be attributed to ethnic difference, subjects' recruitment (their bank employees and our consecutive outpatients), or hypertensive status. However, most plausible explanation might be the statistical power of subjects' number.[32,33] Our data could confirm its importance. Recent studies of the human renin gene revealed that multiple *trans*-factors and *cis*-elements are involved in the transcriptional regulation.[14–21] It was showed that a 225-bp region located about 6 kb upstream from the transcription starting point of the human renin gene gave about 57-fold higher transcription and is a distal enhancer with a human cell model.[18] Our results showed a recessive model for the PRA. The explanation for this recessive model seems to be unclear from the cell model transcription data. *REN* C-5312T of human renin gene was discovered as functional new polymorphism in the distal enhancer region of the gene.[19] The levels of transcription were 45% greater with the 5312T allele than with the C allele. Recently, it has been reported that C-5312T can change binding pattern of Sp1 to renin enhancer that is very likely to affect renin gene expression.[34] In other words, the variant was validated as a functional rSNP. So, the possible mechanism for the results is as follows: subjects with the 5312T allele are thought to have stronger transcriptional power constitutionally and higher PRA from genetic transcriptional level. Thus, this variant is a plausible candidate for a genetic determinant for the PRA. As a result, T allele homozygote has a significantly higher PRA compared with other genotypes.

For a long time, factors affecting PRA have been evaluated and conventional determinants including sodium intake, age, and blood pressure, have been confirmed.[10] The present study confirmed again that these factors have a strong impact on the PRA. With a stepwise statistic method, we could rank the affecting factors. The strongest factor was urinary sodium excretion, implying that sodium intake is the most important factor, which negatively associates with PRA. Then, age, blood pressure, triglyceride, male sex, blood glucose, the gene variant, and weight are important factors in this order. And the gene variant, C-5312T, is confirmed to be an independent factor among the conventional factors.

Several limitations of this study should be noted. The deviated PRA value (skewness = 3.54, kurtosis = 20.1) was log-transformed and the deviation was improved (skewness = 1.10, kurtosis = 1.63), but the distribution was still not normal. We have calculated the sample size in consideration of type I error of 5% and 80% of power; however, the sample number is still relatively small. Although population admixture is thought to contribute to concordant results among studies, our study comprised only Japanese population. Next limitation was

that only one genetic variant was assayed. A cross-sectional study design is another limitation of the present study.

This study emphasizes that a human renin gene variant should have a significant effect on plasma renin activity as a genetic factor; independent determinants for the activity were updated including conventional environmental factors with genetic factor. It has been shown that the variant influences blood pressure response to angiotensin receptor blocker, C allele homozygotes being better responders.[35,36] Thus, a new possibility for personalized medicine by genetic variants of the renin gene type has been shown.

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