β1-Adrenoreceptor Polymorphisms and Blood Pressure: 49S Variant Increases Plasma Renin But Not Blood Pressure in Hypertensive Patients

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BACKGROUND:
Activation of β1-AR in the kidney releases renin that plays a major role in the maintenance of blood pressure. Genetic variation in β1-AR could therefore alter the physiological and clinical effects of this hormone. We tested this hypothesis in patients from a primary care cohort being screened for primary hyperaldosteronism (n = 467).

METHODS:
Demographic and hemodynamic data were measured and plasma renin was determined by a standard immunoassay. Subjects were genotyped for the 2 common single-nucleotide polymorphisms Arg389Gly (rs1801253) and Ser49Gly (rs1801252), and thus the 4 possible haplotypes in β1-AR gene.

RESULTS:
In patients being screened for hyperaldosteronism, plasma renin was significantly elevated in Ser49 homozygotes (49SS) compared with Gly49 (49G) allele carriers (0.307 ± 0.03 vs. 0.164 ± 0.05; P = 0.01). However, this did not translate into differences in either blood pressure or heart rate. On the other hand, the Arg389Gly polymorphism did not affect either plasma renin or blood pressure in this group. There was also no evidence that the 2 loci were linked in this group of patients.

CONCLUSION:
These data suggest that in this cohort the Ser49 variant of the Ser49Gly β1-AR gene polymorphism associates with higher renin levels. However, these common β1-AR gene polymorphisms do not affect blood pressure in the same cohort.

Keywords: β-adrenergic receptor; blood pressure; haplotypes; hypertension; single-nucleotide polymorphisms.

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Catecholamines modulate a number of aspects of renal physiology, including renal blood flow, glomerular filtration, tubular transport, and renin and erythropoietin release. Renin is released from juxtaglomerular cells in response to the circulating catecholamines that activate β1-adrenoreceptor (β1-AR). Several studies have demonstrated the importance of β1-AR on renin release during dynamic exercise and using selective antagonists. Renin itself has a central role in blood pressure and sodium water homeostasis through angiotensin II and aldosterone as part of the renin–angiotensin–aldosterone system.

The sympathetic nervous system also directly regulates cardiac output and peripheral resistance that in turn determine blood pressure. Hence, sympathetic overdrive from increased function and density of adrenergic receptors contributes to the development and maintenance of elevated blood pressure in hypertensive patients. The role of β1-AR-stimulated plasma renin activity in the development of hypertension is dependent on its action on salt and water homeostasis, leading to increases in plasma volume, vascular tone, and peripheral resistance. This is true for patients with sympathoadrenal overactivity as indicated by elevated levels of renin where specific drugs aimed at reducing the effects of this cascade are widely used to lower blood pressure. Similarly, activation of myocardial β1-AR leads to higher cardiac output as a result of increased inotropy and chronotropy. It is worth noting that β1-AR blocking drugs have been shown to reduce plasma renin activity and hence

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blood pressure, whereas selective β2-AR antagonists have no apparent effect.7,8

Hence, β1-AR gene is crucial to the maintenance and development of blood pressure and variation in the gene coding for this G-protein-coupled receptor could have significant functional effects on blood pressure. Indeed, several such variants have been identified in this gene located on chromosome 10 (OMIM: 190630), but the 2 common single-nucleotide polymorphisms (SNPs) within the coding region of β1-AR that have been most extensively studied are the following: a serine to glycine variation at position 49 (rs1801252, Ser49Gly (49S>G)) and an arginine to glycine variation at position 389 (rs1801253, Arg389Gly (389R>G)).9–12 Hence, we have looked at these 2 polymorphisms in a hypertensive cohort screened to establish the frequency of primary aldosteronism in a primary care setting. The aim was to investigate whether the in vitro functional β1-AR polymorphisms could modulate human renin release and hence susceptibility to hypertension.

METHODS

Study population

All subjects were recruited as part of a screening study from their local General Practices to identify the incidence of primary aldosteronism in mild hypertensives and were all Caucasians. Approval was obtained from the local research ethics committee and written informed consent was obtained from each participant.

Demographic details such as age and current hypertensive treatment (namely diuretic, calcium channel blocker, or angiotensin-converting enzyme (ACE) inhibitors) were noted. Supine blood pressure with heart rate was measured, and a blood sample was taken for plasma renin activity assessment and for genetic analysis. Plasma renin was measured as renin mass by the Nichols Advantage assay in an accredited laboratory at the Addenbrooke’s Hospital. Genomic DNA was extracted using standard method.13 From a total of 844 subjects who originally participated in the study, only 467 subjects who were not taking any β1-AR-selective antagonists were selected and were genotyped for the β1-AR gene polymorphisms.

Genetic analysis

Subjects were genotyped using restriction digest of polymerase chain reaction products for the Ser49Gly polymorphism as described previously.14 The Arg389Gly polymorphism was genotyped using the ABI Prism (7700 Sequence Detecting System). Briefly, for each subject 50 ng of genomic DNA was pipetted to 10 μl of 2× Master Mix (including the buffer, dNTPs, ROX reference standard, AmpliTaq Gold, optimized MgCl2, AmpErase UNG), 500 nM of both forward (GCC GGT CTC CGT GGG T) and reverse (GGC TGG GTG ACG CCA AC) primers, 130 nM of each probe (TET-labeled probe CAGAGCAGTCCCTGGAAGGCC for G variant of the allele; FAM-labeled CAGAGCAGTCCCTGGAAGGCC for the C variant of the allele) with MQ H2O to a total volume of 20 μl. During polymerase chain reaction, the probes bind to their chosen allele and the reporter dye cleaved and released into solution by the 3′→5′ exonuclease activity of the Taq polymerase. Reporter dye intensity was then measured in “real time” using the ABI 7700. The thermal cycling conditions consisted of 50 °C for 2 minutes, 95 °C for 10 minutes ×1 followed by 40 cycles each of 95 °C for 15 seconds, and then a final cycle of 62.5 °C for 1 minute. Data were analyzed offline with the sequence detection software (version 1.9).

Data analysis

The haplotype frequencies and evidence of linkage between the 2 loci in β1-AR gene were assessed using SAS software, version 9.0 (SAS Institute, Cary, NC). Other data were analyzed using SPSS software (version 23) and GraphPad Prism (version 5). The distribution of renin measured was significantly skewed; therefore, renin was log transformed and this variable was used in the subsequent analysis. Student’s t-test and one-way analysis of variance were used to compare mean differences in variables for the 3 genotypes and alleles in question. Chi-square tests were performed to calculate genotype and allele frequencies and also to establish the proportion of patients using cardiovascular drugs. General linear models were constructed to test the SNP effect alone, followed by treatment assigned and then SNP by drug interaction effects. Multiple regression analysis was also performed to determine independent associations of the SNPs on renin after adjusting for confounding factors such as age and prescribed cardiovascular drugs. All values are expressed as mean ± SEM, except for age which is presented as mean ± SD. P-value of <0.05 was considered statistically significant.

RESULTS

Population frequency of the β1-AR Ser49Gly and Arg389Gly haplotypes

The genotyping results for the 2 β1-AR polymorphisms are shown in Supplementary Table 1. There was no evidence that the 2 loci were linked. Both polymorphisms were in Hardy–Weinberg equilibrium and estimated haplotype frequencies were not significantly different from those assuming independent segregation. The linkage disequilibrium coefficient (D) was 0.0048 with a D’ of 0.149, and neither P-values reached statistical significance. The SR haplotype was the most frequent β1-AR haplotype (64%) and GG the rarest, with an estimated frequency of just 3%; no subject with a double GG haplotype was actually identified within this cohort. The allele frequencies of β1-AR polymorphisms are 72.8% for Arg389 and 27.2% for Gly389 alleles, and 88.1% for Ser49 and 11.9% for Gly49 alleles.

Hemodynamic and biochemical data by genotypes

There were no significant genotype differences in terms of systolic and diastolic blood pressure, pulse pressure or heart rate.
rate, and log renin for the Arg389Gly polymorphism in this study group (Supplementary Table 2). Hemodynamic data and log plasma renin were also not different between the 2 homozygotes (Arg389Arg, 389RR vs. Gly389Gly, 389GG). In contrast, Ser49Gly polymorphism, although showed no significant genotype differences in the hemodynamic parameters, showed a significant difference for the log renin (Supplementary Table 2, ANOVA $P = 0.002$). Only 8 subjects were identified as carrying the 49GG genotype, so further comparisons were made by comparing the 49SS genotype with the Gly49 carriers (49SG+49GG genotypes). This analysis revealed that plasma renin was significantly higher in 49SS homozygotes compared with the Gly49 allele carriers ($P = 0.01$), but this did not translate into a difference in heart rate or blood pressures. A high proportion of the patients carrying the Arg389Arg and Ser49Ser genotypes were also on cardiovascular drugs (diuretics, ACE inhibitors, and calcium channel blockers; Supplementary Table 2). Because of the study exclusions, none of the patients studied were using any $\beta_1$-AR blocking drugs. Similar to the genotype analysis, there were no statistically significant differences in hemodynamic variables between subjects with different numbers of haplotype copies, except for the plasma renin ($P = 0.01$, data not shown).

Plasma renin was elevated in patients harboring the 49SS and 389RR genotypes as shown in Figure 1a. This finding was significantly more distinct in patients carrying 49SS genotype in the whole group ($P = 0.0109$), and in patients who were not taking diuretics (Figure 1a), as compared with the 498G+49GG genotype group ($P = 0.0117$). Similar nonsignificant trend was also observed in patients who were using ACE inhibitors for this polymorphism and for the Arg389Gly polymorphism. In a separate univariate analysis, we did not find treatment interaction effects for either Ser49Gly or Arg389Gly variants with diuretics or with ACE inhibitors ($P = ns$, data not shown). As expected, we found significantly elevated renin levels in patients treated with hypertensive drugs as compared to those who were not on any medications ($0.312 \pm 0.03$ vs. $0.042 \pm 0.04$ mU/l; $P = 0.001$, data not shown).

As Ser49Ser and Arg389Arg homozygotes had elevated plasma renin, a multiple linear regression analysis was performed including the main confounders for renin (age and cardiovascular drugs). This analysis revealed that the Ser49Gly polymorphism was independently associated and predicted elevated plasma renin in these patients as shown in Figure 1b.

**DISCUSSION**

This work demonstrates for the first time that the $\beta_1$-AR control of renin release is affected by genetic variation of this receptor. The haplotype and allele frequencies for each polymorphism were similar to the published data.$^9$ However, there was no evidence of linkage disequilibrium...

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**Figure 1.** $\beta_1$-AR gene polymorphisms relationship with plasma renin. (a) Plasma renin in subjects taking diuretics and ACEI compared with those not taking these drugs. The average plasma renin was significantly elevated in patients using diuretics and carrying the 49SS genotype compared with those not taking diuretics but carrying the 49SG+49GG genotypes ($P = 0.0117$) for the Ser49Gly polymorphism. Similar nonsignificant trends were found in people using the ACEI for this SNP, and for the Arg389Gly polymorphism in subjects who were on diuretics and ACEI. Bar graphs represent mean ± SEM of plasma renin. (b) Multiple regression analysis results showing the independent association of $\beta_1$-AR gene polymorphisms with log transformed renin. Abbreviations: ACEI, angiotensin converting enzyme inhibitor; SNP, single-nucleotide polymorphism.
between the β1-AR gene polymorphisms in our study cohort, compared to Terra et al., who genotyped 692 women for both variants, observed significant LD for the 2 SNPs, and reported very similar frequencies of 0.65 (SA), 0.26 (SG), 0.09 (GA), and 0 (GG) for the 4 possible haplotypes, including no individual homozygous for both 389G and 49G. Thus, future pharmacogenetic and disease association studies should therefore include analysis by haplotypes in addition to individual SNPs.

Despite the effect of the Ser49Gly polymorphism on renin release, there was no effect of this polymorphism on heart rate or blood pressure that might have been expected, given the significant differences observed in log renin levels. This difference in log renin is similar to the effect of the Ser49Gly polymorphism on heart rate with a 5 heart beat difference reported for the Ser49 variant. This observation may be dependent on the level of circulating catecholamine levels as this polymorphism in the N-terminal of the receptor is more susceptible to agonist-promoted downregulation. Hence, the difference in heart rate and blood pressure could have been easier to define from patients with heart failure where the level and hence degree of receptor downregulation would be greater compared to hypertension. Also, patients with heart failure would be expected to have uncoupled receptors, so the 389 variant may have less impact, giving another reason to study differences in heart rate and blood pressure in these patients. This has been confirmed in a large study of cardiac transplant subjects who underwent exercise and hemodynamic testing as part of the transplant protocol.

The Arg389Gly polymorphism failed to affect plasma renin, heart rate, and blood pressure in our large screening group. It is likely therefore that the difference in renin release is of limited pathological or clinical significance, and other homeostatic mechanisms operate to maintain similar blood pressures and heart rates in 389R vs. 389G homozygotes. Although the data were analyzed with the confounding effect of drug treatment taken into account, this concentrated on the percentages of patients on certain medications rather than the dose administered and its dose effect.

There are many studies that have analyzed the β1-AR phenotype of heart rate and blood pressure. In keeping with these largely negative studies, the Arg389Gly polymorphism had no effect on exercise-promoted renin release, heart rate, or blood pressure. The subjects in this study were young healthy volunteers compared to the patients with hypertension or ischemic heart disease used in other studies. Because of the small numbers of healthy volunteers studied, no haplotypes responses were analyzed for treatment effects, together with the fact that β1-AR function may have been affected by the circulating catecholamine in patients with disease: genotype differences were therefore more apparent in patients as compared to healthy controls. However, this work fails to show any significant difference in heart rate and blood pressure that might have been predicted given the marked in vitro effect of both polymorphisms. This work has practice implications for using the aldosterone–renin ratio in screening for hyperaldosteronism, because the Ser49 genotype would appear to affect the reference range for renin itself. This mirrors the problem in using serum ACE as a diagnostic tool for sarcoidosis where the reference range for ACE is affected by the genotype status for the common InDel polymorphism in the ACE gene.

SUPPLEMENTARY DATA
Supplementary data are available at American Journal of Hypertension online.

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DISCLOSURE
The authors declared no conflict of interest.

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