Hypertension
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association®
Learn and Live℠

Renin Gene Polymorphisms and Haplotypes, Blood Pressure, and Responses to Renin-Angiotensin System Inhibition

Niamh Moore, Patrick Dicker, John K. O'Brien, Milos Stojanovic, Ronán M. Conroy, Achim Treumann, Eoin T. O'Brien, Desmond Fitzgerald, Denis Shields and Alice V. Stanton

Hypertension 2007;50;340-347; originally published online Jun 11, 2007;
DOI: 10.1161/HYPERTENSIONAHA.106.085563
Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
Copyright © 2007 American Heart Association. All rights reserved. Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/cgi/content/full/50/2/340

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

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

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

Downloaded from hyper.ahajournals.org by on July 23, 2007

Renin Gene Polymorphisms and Haplotypes, Blood Pressure, and Responses to Renin-Angiotensin System Inhibition

Niamh Moore, Patrick Dicker, John K. O'Brien, Milos Stojanovic, Ronán M. Conroy, Achim Treumann, Eoin T. O'Brien, Desmond Fitzgerald, Denis Shields, Alice V. Stanton

Abstract—Renin catalyzes the rate-limiting step of the renin-angiotensin system. A T allele variant at position −5312 within a distal enhancer region has been reported to increase in vitro renin gene transcription. Among 387 White bank employees, ambulatory blood pressures were higher in 133 −5312T allele carriers than in 254 CC homozygotes—mean differences [99% confidence interval] between carriers and homozygotes for daytime and night-time systolic/diastolic pressure were 2.5[0.4,4.6]/1.7[0.2,3.2] and 2.4[0.5,4.4]/1.5[0.1,2.9] respectively. Ambulatory pressure estimates for the only common renin haplotype including the −5312T variant (−5312T, 5090C, 5912A, 9479A, 10194G), were statistically significantly higher than estimates for all other haplotypes. Among 259 White hypertensive participants in a randomized double-blind clinical trial comparing a renin antagonist, aliskiren, with an angiotensin receptor blocker, losartan, plasma renin activity did not differ with renin −5312C/T genotype. Nocturnal blood pressure reductions with losartan 100 mg daily were significantly greater in −5312T allele carriers than in CC homozygotes (mean[standard error]; −12.9[3.7]/−7.9[2.4] versus −7.1[2.5]/−4.2[1.6]) whereas with aliskiren 150 and 300 mg daily, lesser reductions were observed in −5312T allele carriers than in CC homozygotes (−5.4[2.0]/−4.1[1.3] versus −10.1[1.4]/−6.5[1.1]; $P<0.03$ for treatment×genotype interaction for night-time systolic and diastolic pressures). Hence, the −5312 renin C/T enhancer polymorphism does contribute to blood pressure variation in Whites and also appears to predict responses to inhibition of the renin–angiotensin system. These findings suggest that genotyping at this locus may aid in the identification of susceptibility to hypertension and in the selection of optimal therapy for individual hypertensive patients. (***Hypertension*. 2007;50:340-347.)**

Key Words: blood pressure ■ renin ■ renin–angiotensin–aldosterone system ■ genetics ■ pharmacogenetics ■ renin inhibition ■ angiotensin receptor blockade

The renin-angiotensin system (RAS) plays important roles in the regulation of electrolytes, blood pressure (BP), and atherosclerosis.[1,2] Renin catalyzes the first and rate limiting step of the cascade, the conversion of angiotensinogen to angiotensin I. Hypertensive patients with high plasma renin levels are more likely than those with normal or low renin levels to experience myocardial infarctions.[3] Furthermore BP lowering responses to antihypertensive drugs differ depending on the plasma renin status of the patient.[4]

Much progress has been made in elucidating the molecular mechanisms involved in renin gene expression.[5] In mice a 242-base pair element (nucleotides −2866 to −2625) is capable of stimulating promoter activity approximately 50-fold. In man, however, the homologous element, located approximately 11 kilobases upstream of the transcription start site, is much weaker.[6] This between species difference appears to be attributable to a single nucleotide change in the promoter proximal half site of a retinoic-acid response element.[7] There is a further distal enhancer region (nucleotides −5777 to −5312) which has been reported to activate the human renin promoter approximately 60-fold in primary cultures of human chorionic cells.[8,9] Fuchs and colleagues noted 45% greater rates of renin gene transcription in the presence of a −5312T allele rather than a −5312C allele.[9] This finding stimulated our interest in examining this gene polymorphism for in vivo human functionality.

We hypothesized that this polymorphism would, either by itself or in haplotypic combination with other common renin gene single nucleotide polymorphisms (SNP), be associated with an increased susceptibility to hypertension. Having confirmed that carriers of the renin −5312T allele did indeed demonstrate elevated ambulatory BP, we proceeded to exam-

Received December 6, 2006; first decision January 2, 2007; revision accepted May 14, 2007.

From Molecular and Cellular Therapeutics (N.M., P.D., J.K.O., R.M.C., A.T., A.V.S.), RCSI Research Institute, Royal College of Surgeons in Ireland; Blood Pressure Unit (M.S., A.V.S.), Beaumont Hospital; and the Conway Institute of Biomolecular and Biomedical Research (E.T.O., D.F., D.S.), University College Dublin, Dublin, Ireland.

Correspondence to Dr Alice Stanton, Molecular and Cellular Therapeutics, RCSI Research Institute, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland. E-mail astanton@rcsi.ie

© 2007 American Heart Association, Inc.

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/HYPERTENSIONAHA.106.085563**

Downloaded from hyper.ahajournals.org by on July 23, 2007

ine whether this association was likely mediated by altered plasma renin activity (PRA), and whether BP lowering responses with RAS blockade were greater in persons carrying the variant T allele.

Methods

Employee Population

The cohort of 815 current and retired White bank employees and their spouses were free of diagnosed hypertension and vasoactive drugs when recruited to the Allied Irish Bank Study.[10] At the baseline (phase I) examination, conducted between 1989 and 1991, age, gender, smoking habit, alcohol consumption, salt intake, family history of cardiovascular events, past medical history, current drug treatments, height, weight, and clinic BP were recorded, and 24-hour ambulatory BP monitoring (ABPM) was performed. Between 1996 and 2001, after a mean interval of 8.4 years, 441 subjects responded to a written invitation to undergo repeated assessments. At this time, only 4 subjects were taking antihypertensive medication, and these discontinued therapy one week before the phase II assessments. At the phase II examination, blood was additionally drawn for biochemical measurements and for extraction of DNA. We excluded 9 participants because of technically unsatisfactory ABPM recordings and 45 participants in whom DNA extraction was unsuccessful. Thus the total number of participants included in the present analysis was 387. All subjects provided written informed consent to each phase of the study. The study protocols of the 2 phases conform to the Declaration of Helsinki and were approved by the Beaumont Hospital Research Ethics Committee.

Hypertensive Population

The subjects of this population were recruits to a randomized, double-blind, active comparator, parallel group, BP lowering trial. Methods and study design have been described in detail elsewhere.[11] Briefly, 345 White patients, aged between 21 and 70 years, with mild-to-moderate hypertension were recruited from 5 hospital outpatient clinics in Ireland. Participants were required to have an off-treatment average daytime systolic ABPM $\geq$140 mm Hg, and were not eligible if they had secondary hypertension, malignant hypertension, diabetes mellitus, coronary artery disease, or cerebrovascular disease. After an antihypertensive drug free period of at least 2 weeks, the patients were randomly assigned to receive 37.5 mg, 75 mg, 150 mg, or 300 mg aliskiren, or 100 mg losartan, daily for 4 weeks. Clinic BP measurement and ABPM were performed at baseline and after 4 weeks of study treatment. Blood chemistry, PRA, and aliskiren levels were measured from venous blood samples drawn from the patients while seated, at baseline and 24 hours after the last dose of randomized study treatment. Two hundred and fifty-nine individuals provided an additional blood sample at baseline for DNA extraction and anonymous genotyping—of these 47 were not randomized to study treatment, 44, 44, 41, and 43 were assigned to 37.5 mg, 75 mg, 150 mg, and 300 mg aliskiren, respectively, and 40 were assigned to 100 mg losartan. All participants provided written informed consent both for the clinical trial and for further genetic analyses concerning cardiovascular disease. The study protocol conforms to the Declaration of Helsinki, and was approved by the Research Ethics Committees of each participating institution.

Blood Pressure and Laboratory Measurements

Ambulatory BP measurements in both study populations were made every half-hour throughout a 24-hour period using validated oscillometric 90202 or 90207 SpaceLabs recorders (SpaceLabs, Wokingham, Berkshire, UK). Mean daytime (mean of all measurements between 0900 and 2100 hours), and night-time (mean of all measurements between 0100 and 0600 hours) systolic and diastolic pressures were calculated for each individual. Sitting clinic BP was measured from the right arm using a mercury sphygmomanometer (employee population) or a regularly calibrated validated automated sphygmomanometer (Omron HEM-705CP) (hypertensive population). Total cholesterol, triglycerides, glucose, and creatinine were measured using standard enzymatic methods on a Roche/Hitachi 912 automated analyser (Roche Diagnostics, Basel Switzerland). Plasma renin activity was measured by trapping of generated angiotensin I by antibodies and by subsequent radioimmunoassay. Aliskiren was measured by direct radioimmunoassay.

Identification of Renin Gene Sequence Variants and Genotyping

A full description is available in the supplemental materials (available online at http://hyper.ahajournals.org). DNA from 10 normotensive subjects and from 10 hypertensive patients was screened for mutations in the 5′-flanking and protein-coding regions of the renin gene by a combination of ion-pairing reversed-phase partially denaturing high-performance liquid chromatography (HPLC) and direct sequencing. In the employee population, all 10 renin gene SNPs that were detected were genotyped. Only 5 renin gene tagging SNPs (tSNPs), identified using the multiple-marker haplotype r^2 criterion, implemented as Criterion 11 of the TagIT software package (http://popgen.biol.ucl.ac.uk/software),[12] were genotyped in the hypertensive population. Genotyping of SNPs was performed by Kbiosciences (Herts, U.K.) using modified TaqMan assays (http://www.kbioscience.co.uk).

Statistical Analyses

Phenotypic data are expressed as mean$\pm$SD, as median [interquartile range], or as numbers (percentages). Both PRA and plasma aliskiren levels followed a lognormal distribution and were log-transformed before statistical analysis. Departure from Hardy–Weinberg equilibrium was tested by Chi-squared tests. Haplotype frequencies were estimated using the EM algorithm for maximum likelihood.[13]

Genotypic analyses involved comparisons of carriers of the less frequent (minor) allele with subjects that were homozygous for the more frequent allele. Multiple linear regression analyses, with age and sex as covariates, were used to test for associations of genotype of the 5 tSNPs with BP levels within the employee population (both phase I and II BP levels), and to test for associations of −5312C/T genotype with baseline PRA within the hypertensive population. In considering the associations of −5312C/T genotype with on-treatment PRA, and with BP lowering responses, drug treatment group, weight, and baseline PRA were additional covariates. Only data from individuals randomized to effective antihypertensive doses of drug (losartan 100 mg, aliskiren 150 mg or 300 mg,) were included in these latter analyses.

When testing for associations of haplotype with BP within the employee population, sex, age, and predicted individual haplotypes were included in weighted analysis of covariance (ANCOVA) models with haplotype probability as the weighting factor. Each factor was then assessed for significance using standard F-tests, and least squares estimates of blood pressure were obtained. The associations of haplotype with PRA, and with BP lowering responses, were assessed using similar weighted ANCOVA models.

Analyses were performed using SAS statistical software package versions 9.1 and 9.2 (SAS Institute Inc). All statistical tests were 2-sided. As we tested for associations of the genotypes of 5 renin tSNPs with BP in the employee population, $P<0.01$ was considered statistically significant. For all further analyses which only concerned renin −5312C/T genotype or renin gene haplotype, $P<0.05$ was considered statistically significant.

Results

Populations Characteristics

Fifty-eight percent of the employee population and 63% of the hypertensive population were men. By comparison with the employee population, the hypertensive population was older ($P<0.0001$), more overweight ($P<0.0001$), and had higher fasting cholesterol ($P<0.01$), triglyceride ($P<0.0001$), and creatinine levels ($P<0.0001$; Table 1). There were fewer

Downloaded from hyper.ahajournals.org by on July 23, 2007

TABLE 1. Characteristics of the Employee and Hypertensive Participants

	Employees		Hypertensives	
Variable	Males (n=224)	Females (n=163)	Males (n=164)	Females (n=93)
Age, y	46±9	41±10	50±12	55±9
Body mass index, kg/m^2	27±3	24±4	30±5	30±6
Current smoking, %	71 (32)	45 (28)	27 (16)	17 (18)
Alcohol intake, units/week	15[8–24]	6[2–11]	10[3–20]	2[0–6]
Salt usage at table, %	128 (57)	98 (60)	82 (50)	60 (63)
Total cholesterol, mmol/L	5.7±1.2	5.3±1.2	5.7±1.0	5.9±1.0
Triglycerides, mmol/L	1.4±0.9	1.0±0.6	2.2±1.6	1.7±1.5
Diabetes mellitus	2 (1)	1 (1)	0	0
Creatinine, μmol/L	97±13	79±10	100±13	86±11
Daytime SBP, mm Hg	131±12	120±12	154±10	154±12
DBP, mm Hg	82±8	75±8	95±9	93±11
Night-time SBP, mm Hg	112±12	104±10	132±14	132±15
DBP, mm Hg	67±8	61±7	77±11	76±12
Clinic SBP, mm Hg	130±17	116±19	155±18	156±18
DBP, mm Hg	82±11	74±11	94±11	91±10

Data expressed as mean±SD, median [interquartile range] or as No. (%). Employee participant information pertains to the phase II assessment.

current smokers in the hypertensive population ($P<0.001$), and self reported alcohol intake was lower ($P<0.005$). Both clinic and ambulatory pressures were higher in male employees than female employees ($P<0.0001$), and as expected, untreated BP levels were considerably higher in the hypertensive population ($P<0.0001$).

Renin Gene SNPs and Haplotypes

Screening the renin gene in 20 Irish subjects revealed 2 variants in the 5′ flanking region, one synonymous coding region variant, and 7 intronic variants (Figure 1a). Nine were previously reported variants (http://www.ncbi.nlm.nih.gov/SNP) but the variant in intron 2 (4435 G/A) was novel.

In the employee population, the proportions of the minor allele of these 10 SNPs were as follows: −5434T 0.41, −5312T 0.19, 4280C 0.11, 4435A 0.11, 5090G 0.39, 5912G 0.11, 9088T 0.11, 9479G 0.06, 10194T 0.10, and 10601A 0.22. The r^2 values for linkage disequilibrium between these SNPs ranged from 0.07 to 0.98, allowing the identification of 5 tSNPs (Figure 1b). The distributions of genotypes for these tSNPs were all in Hardy–Weinberg Equilibrium. Combinations of the 5 tSNPs were used to define 6 unique 5-SNP haplotypes in the bank workers (Figure 1c).

In the hypertensive population, the proportions of the minor allele of the 5 tSNPs (−5312T 0.18, 5090G 0.36, 5912G 0.12, 9479G 0.05, 10194T 0.11) were similar to those of the employee population. The 4 downstream tSNPs were in Hardy-Weinberg equilibrium. By contrast, the genotype distribution of the −5312C/T polymorphism in the hypertensive population deviated significantly ($P<0.005$)—there was a relative paucity of TT homozygotes, and a small excess of CT heterozygotes (CC homozygotes, 166 [64%]; CT heterozygotes, 92 [36%]; TT homozygotes, 1 [0.4%]). Estimated renin gene haplotype frequencies were similar in the hypertensive population to those of the employee population (Figure 1c).

Associations of Renin Genotype and Haplotype With BP in the Employee Population

Firstly considering the renin −5312C/T polymorphism alone, unadjusted daytime and nighttime ambulatory pressures were highest among the TT homozygotes and lowest among the CC homozygotes, with CT heterozygotes demonstrating intermediate values (TT homozygotes n=11, CT heterozygotes n=122, and CC homozygotes n=254, mean±SD; daytime, 128±19/82±10, 128±14/80±9, and 126±13/79±9; nighttime, 112±12/67±10, 111±13/65±9 and 108±10/64±9). Mean age and sex-adjusted daytime and nighttime ambulatory pressure differences between carriers of the T allele and CC homozygotes ranged from 2.5 to 1.5 mm Hg ($P<0.01$ for all pressures; Figure 2). The associations with ambulatory pressures were found in younger and older participants and in both males and females (data not shown). Clinic diastolic pressure was also significantly higher in renin −5312 T allele carriers than in CC homozygotes, but the difference in clinic systolic BP was not statistically significant after adjustment for multiple comparisons (Figure 2). Neither demographic characteristics nor cardiovascular risk factors, other than BP, varied with renin −5312C/T genotype within this population (data not shown). Figure 2 also illustrates that none of the other 4 renin gene tSNPs had a statistically significant association with any measure of BP.

Ambulatory and clinic pressures were also found to differ significantly with renin gene haplotype (Table 2). This was in large part attributable to the age and sex adjusted estimates for blood pressure of haplotype 3 being significantly higher than the corresponding pressure esti-

Downloaded from hyper.ahajournals.org by on July 23, 2007

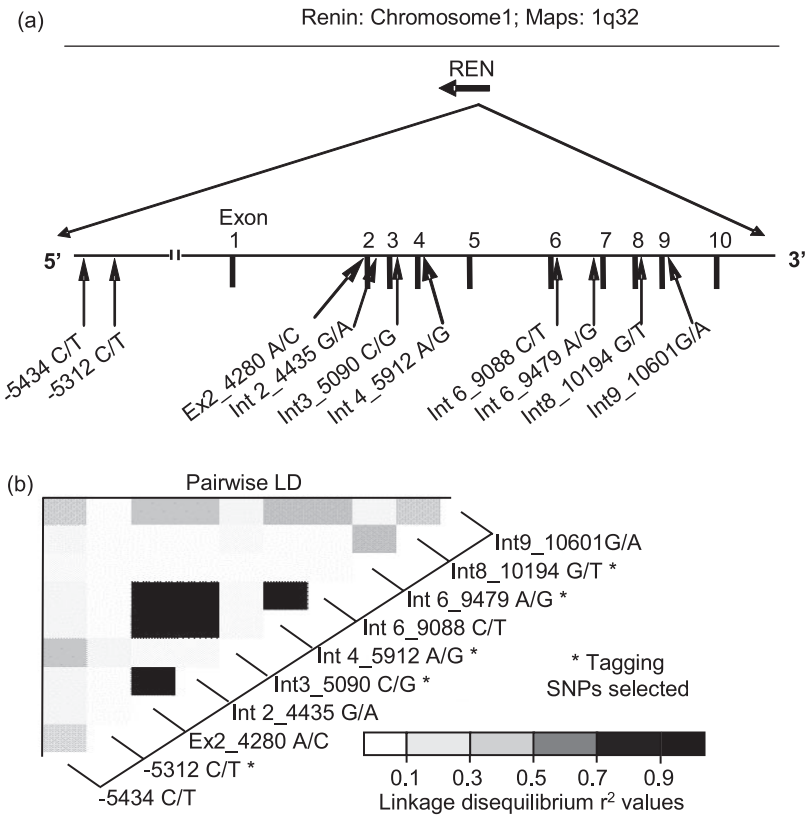

Figure 1. Renin gene polymorphisms and haplotypes. a, A diagrammatic representation of the renin gene (maps: chromosome 1q32). b, The pairwise linkage disequilibrium (LD) plot of the 10 renin SNPs within the employee population. Five tagging SNPs are identified with an asterisk. c, The frequencies of the haplotypes, estimated by applying the EM algorithm for maximum likelihood to the 5 tSNPs, in the employee and hypertensive populations.

(c)

Haplotype	Frequencies	
	Employee Population	Hypertensive Population
1. CGAAG	0.41	0.35
2. CCAAG	0.21	0.23
3. TCAAG	0.18	0.19
4. CCGAG	0.10	0.12
5. CCAAT	0.05	0.05
6. CCAGT	0.05	0.06
Other rare	<0.01	<0.01

mates of the remaining haplotypes. This was in agreement with the results concerning individual renin gene SNP associations, as haplotype 3 (TCAAG) alone carried the −5312T variant.

Associations of Renin −5312C/T Genotype and Renin Gene Haplotype With PRA in the Hypertensive Population

As previously reported, PRA increased in response to losartan, whereas aliskiren suppressed PRA in a dose-dependent manner ($P \leq 0.0001$).[11] We 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]; Figure S1). Baseline PRA and on-treatment PRA also did not differ with renin gene haplotype (data not shown).

Prediction of BP Lowering Responses by Renin −5312C/T Genotype and Renin Gene Haplotype in the Hypertensive Population

Ambulatory BP at baseline was similar across the drug treatment groups and did not differ between REN −5312 CC homozygotes and T-allele carriers (data not shown). With both losartan and aliskiren, the higher the baseline PRA, the greater was the BP lowering response. By contrast the magnitude of BP lowering achieved with the 2 treatments differed according to renin −5312C/T genotype (Figure 3)—BP lowering with losartan, particularly nocturnally, was greater in renin −5312T allele carriers than in CC homozygotes, whereas BP lowering with aliskiren was greatest in CC homozygotes (probability value for treatment×genotype interaction not significant for daytime pressures nor for clinic diastolic BP, $P<0.03$ for nighttime pressures and for clinic systolic pressures). The independent prediction of ambulatory

Downloaded from hyper.ahajournals.org by on July 23, 2007

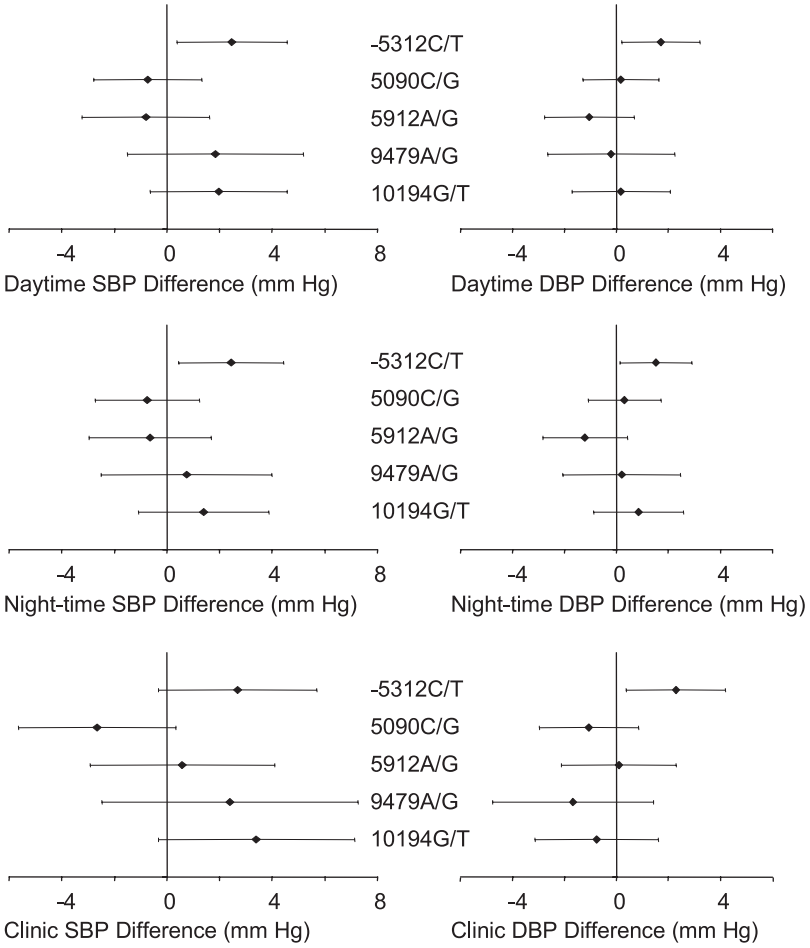

Figure 2. Associations of the 5 renin tSNPs with ambulatory and clinic BP in the employee population. Mean age- and sex-adjusted differences between carriers of the minor allele and subjects homozygous for the major allele, and 99% confidence intervals, are illustrated.

BP lowering responses to losartan and to aliskiren by baseline PRA and by genotype is illustrated in Figure S2.

Age and sex adjusted estimates of BP lowering responses to losartan 100 mg daily for haplotype 3 (TCAAG) were approximately twice the responses to aliskiren 150 mg and 300 mg daily (daytime −13.0/−7.0 versus −7.7/−4.4; nighttime −13.4/−8.6 versus −5.1/−3.8; clinic −15.1/−6.1 versus −5.2/−2.1), whereas estimates of responses to losartan and to aliskiren for all other haplotypes tended to be very similar. However the haplotype 3×treatment interaction was only statistically significant for nighttime systolic pressures (P=0.02).

Interestingly on-treatment plasma aliskiren levels, at all drug doses administered, were lower in renin −5312 T allele carriers compared with CC homozygotes (median ng/mL [interquartile range], 37.5 mg, 2.4[1.1,2.9] versus 3.1[1.6,4.0], 75 mg, 3.9[2.9,6.8] versus 4.4[3.5,5.9], 150 mg, 7.1[5.6,11.3] versus 11.0[6.7,16.0], and 300 mg, 21.0[17.5,39.5] versus 31.5 [17.3, 55.3]). However this trend did not achieve statistical significance (P=0.22).

Discussion

A common SNP in a renin distal enhancer element has recently been reported to influence in vitro gene transcription

TABLE 2. Association Analysis of Renin Haplotypes With Ambulatory and Clinic BP Levels in the Employee Population

Haplotype	Frequency	Day SBP	Day DBP	Night SBP	Night DBP	Clinic SBP	Clinic DBP
1 CGAAG	0.41	$124^{0.11}$	$79^{0.90}$	$106^{0.06}$	$62^{0.89}$	**$119^{0.003}$**	$76^{0.17}$
2 CCAAG	0.21	$124^{0.34}$	$78^{0.29}$	$106^{0.30}$	$62^{0.14}$	$121^{0.56}$	$77^{0.55}$
3 TCAAG	0.18	**$126^{0.03}$**	**$80^{0.002}$**	**$108^{0.01}$**	**$63^{0.009}$**	$122^{0.32}$	**$78^{0.02}$**
4 CCGAG	0.10	$123^{0.09}$	$78^{0.09}$	$106^{0.14}$	**$61^{0.02}$**	$121^{0.62}$	$77^{0.74}$
5 CCAAT	0.05						
6 CCAGT	0.05	$126^{0.09}$	$78^{0.94}$	$108^{0.16}$	$63^{0.17}$	**$123^{0.05}$**	$76^{0.23}$
rare	<0.01						
Haplotype *P* value		**0.02**	**0.03**	**0.02**	**0.01**	**0.02**	0.10

Data are least squares estimates of blood pressure level (age and sex adjusted) for each haplotype. Haplotype *P* value refers to the significance of an association between haplotype and BP. Superscripted *P* values on the BP estimates pertain to contrasts of the BP of that haplotype tested against the BP of all remaining haplotypes. Statistically significant values are emboldened.

Downloaded from hyper.ahajournals.org by on July 23, 2007

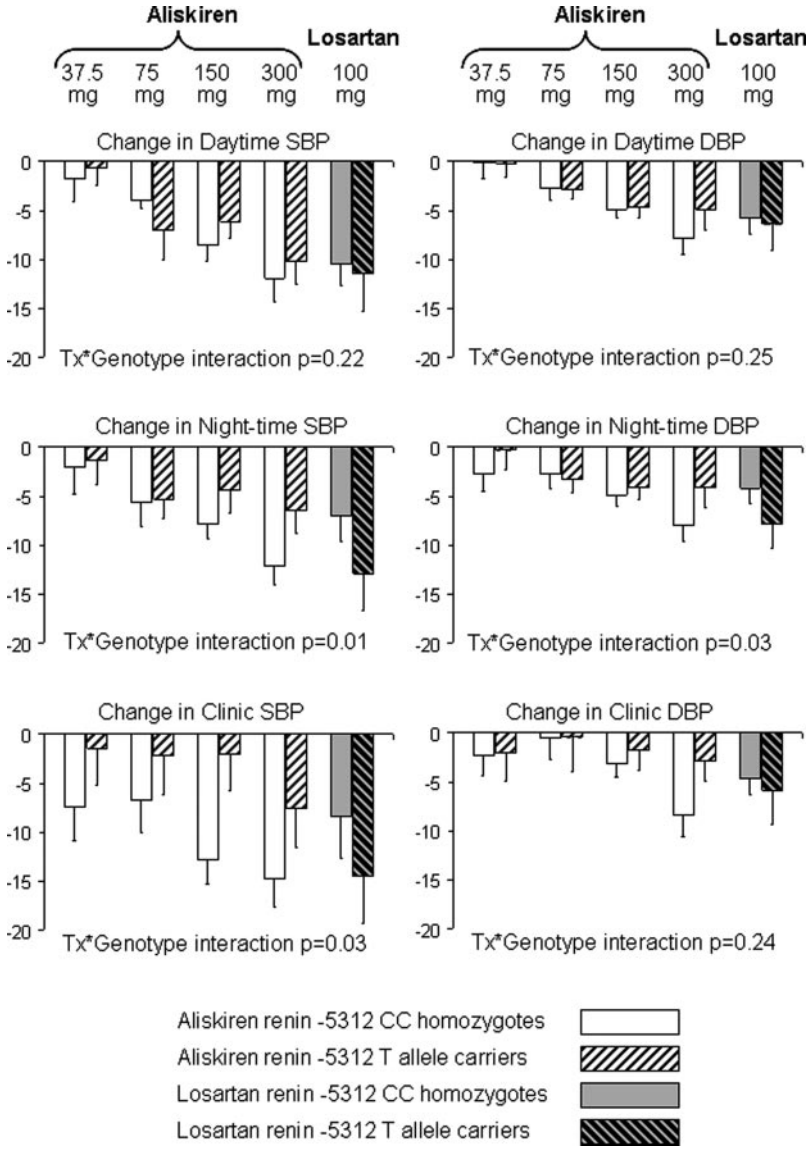

Figure 3. Mean (SE) ambulatory and clinic BP lowering responses to aliskiren 37.5 mg, 75 mg, 150 mg, and 300 mg, and to losartan 100 mg, subgrouped by renin −5312C/T genotype (CC homozygotes vs T allele carriers).

in transfected human choriodecidual cells.[9] In this article, we provide the first evidence that renin −5312C/T has in vivo functional activity. We found carriage of the −5312T allele, a specific marker for a single renin haplotype, to be associated with both elevated ambulatory and elevated clinic BP levels in healthy humans. The associations of renin −5312 C/T genotype with BP were independent of age and sex. The magnitude of the effect associated with carriage of the −5312T allele ranged from 2.7 mm Hg to 1.5 mm Hg. Furthermore we found evidence that the polymorphism may predict BP lowering responses to RAS blockade in hypertensive patients and that this prediction is additional to that of PRA.

Animal model studies have demonstrated that variations in the renin gene can affect blood pressure.[14,15] Renin *Mbo*I, BgII, and *Hind*III restriction fragment length polymorphisms have been found to be associated with essential hypertension in a range of ethnic groups.[16–20] On the other hand, there are as many studies that have reported no association whatsoever between these renin gene restriction fragment length polymorphisms and blood pressure.[21–25] More recently a missense mutation in exon 9 (10501G/A) has been reported to be associated with both hypertension and elevated PRA in a Japanese population,[26] and an association between a renin −4021C/T variant and hypertension was found in African Americans but not in European Americans.[27] The lack of consistent results emerging from studies investigating associations between renin gene polymorphisms and BP is likely contributed to by small sample sizes, genetic heterogeneity between populations, and the studying of different variants by different research groups. Because both populations studied for this article were exclusively White and European, further research across ethnic groups will be needed to determine the generalizability of our findings.

Our genotypic analyses showed carriage of a renin−5312T allele to be associated with 1.5 to 2.7 mm Hg higher ambulatory and clinic, systolic, and diastolic pressures than CC homozygotes. The haplotype analyses supported the individual renin gene SNP analyses. Such apparently modest increases in BP are of importance—a 2-mm Hg rightwards shift

Downloaded from hyper.ahajournals.org by on July 23, 2007

in the population average of clinic systolic BP has been reported to result in a 17% increase in hypertension prevalence, and 14% and 7% increases in stroke and heart attack mortality, respectively.[28,29] We chose ambulatory pressures over clinic pressures as the primary phenotypic end point because of its greater reproducibility and avoidance of the whitecoat effect. Further, ambulatory pressures, particularly nighttime values, have recently been confirmed to more powerfully predict cardiovascular mortality than clinic pressures among hypertensive patients.[30]

We hypothesized that BP lowering with RAS blockade would be greater in renin −5312T allele carriers. This was the case for angiotensin receptor antagonism but not for renin inhibition. Blood pressure lowering with losartan among T allele carriers with a baseline PRA greater than the median value was more than twice that of CC homozygotes with baseline PRA less than the median. Whereas BP responses with aliskiren were also positively correlated with baseline PRA, BP lowering, particularly at night, was greatest among CC homozygotes. If our findings, of independent and disparate predictions of responses to renin inhibition and to angiotensin receptor blockade, by baseline PRA and by renin −5312C/T genotype, are replicated in further larger clinical trials, there may be clinical utility in measuring both PRA and renin genotype, before the prescription of antihypertensive therapy. Also any divergent BP lowering responses with antihypertensive therapy may be paralleled by cardiovascular protection.

There was no difference in PRA at baseline between renin −5312T allele carriers and CC homozygotes in hypertensive patients. Plasma renin activity of T allele carriers was neither more resistant to suppression by aliskiren nor more elevated with losartan therapy than that of CC homozygotes. We found the associations of genotype and baseline PRA, with nocturnal systolic and diastolic and clinic systolic BP lowering responses to renin inhibition and to angiotensin receptor blockade, to be independent of each other. These 3 findings suggest that the renin −5312C/T polymorphism does not influence the highly regulated secretion of active renin from renal juxtaglomerular cells into the systemic circulation. It is noteworthy that mice with 2 renin genes do demonstrate higher BP levels, but have lower levels of both plasma and renal renin, than mice with only one renin gene.[31] A plausible explanation is that functionality of the renin −5312C/T polymorphism is mediated by altered local tissue or even intracellular renin levels.[32,33] Possibly in keeping with this suggestion is our observation of a nonsignificant trend for aliskiren levels to be lower in T allele carriers—we speculate that high affinity binding of aliskiren by elevated tissue renin in −5312T allele carriers could have contributed to the lesser BP lowering achieved in these individuals after 4 weeks of aliskiren. Alternatively or additionally, a failure by aliskiren to inhibit the catalytic activity or the intracellular signaling that occurs when renin or prorenin binds to the recently discovered (pro)renin receptor, could have contributed to the lesser BP lowering seen in renin −5312T allele carriers with aliskiren.[34,35]

Some potential discrepancies and limitations of this article should be addressed. In the hypertensive population, baseline BP was not elevated in renin −5312T allele carriers by comparison with CC homozygotes. A likely explanation for this lack of difference was the requirement for the daytime systolic BP of the clinical trial participants to be at least 140 mm Hg. There was no difference in renin −5312 C/T allele frequencies between the employee and hypertensive populations. However there was a statistically significant paucity of TT homozygotes within the latter population—with a sample size of 259 and a T allele frequency of 18%, 6 to 10 TT homozygotes were anticipated rather than the single homozygous individual that we observed. A possible explanation is that TT homozygotes were more likely to be excluded from the clinical trial, because of severity of hypertension or because of a previous atherosclerotic event. In the employee cohort, there was considerable power to detect small between-genotype differences in blood pressure, as ABPM was performed on 2 occasions in a large group of subjects. By contrast, the BP lowering clinical trial was relatively underpowered—sample size was originally chosen so that differences between drug treatments and doses could be detected, rather than to identify between-genotype differences. Hence the reported differences in responses to treatment, according to genotype and haplotype, although large in magnitude and consistent in direction, were not uniformly statistically significant. The hypertensive participants were not taking a standardized sodium diet, and previous antihypertensive therapy was only withdrawn 2 weeks before estimation of PRA. These factors probably increased PRA variability. However, even with the magnitude of PRA variability observed, with this sample size, and with a renin −5312T allele carrier prevalence of 36%, our study had 80% power to detect a 30% difference in baseline PRA.

Perspectives

This study demonstrates that a common genetic variant of renin contributes to BP variation in a Caucasian population. Carriers of the renin −5312T allele had higher systolic and diastolic BP. The variant does not appear to influence circulating plasma renin levels, but its effects may be mediated by local tissue or intracellular renin–angiotensin–aldosterone systems. Our data also suggest that this renin gene variant predicts blood pressure lowering responses to angiotensin receptor blockade and to renin inhibition, independently of PRA. If these findings are confirmed by further epidemiological studies and clinical trials, genotyping for the renin −5312C/T polymorphism may have value in the early identification of those at risk of hypertension and cardiovascular events. Further, renin −5312C/T genotyping in combination with measurement of PRA may improve the selection of optimal antihypertensive therapy in individual patients.

Sources of Funding

The Higher Education Authority (Ireland), the Charitable Infirmary Charitable Trust (Ireland), and the Royal College of Surgeons in Ireland Research Committee supported these studies. The clinical trial was sponsored by Speedel Pharma AG, Basel, Switzerland.

Disclosures

N.M. and A.V.S. hold a patent concerning renin genotype, blood pressure lowering, and prognosis. Other potential conflicts are as

Downloaded from hyper.ahajournals.org by on July 23, 2007

follows: A.V.S., E.T.O., and D.F. have received honoraria and/or research support from Speedel Pharma and from Novartis. The remaining authors report no conflicts.

References

1. Oparil S, Haber E. The renin-angiotensin system. *N Engl J Med.* 1974; 291:381–401/446–457.
2. Dzau VJ. Tissue Angiotensin and pathobiology of vascular disease, a unifying hypothesis. *Hypertension*. 2001;37:1047–1052.
3. Brunner HR, Baer L, Newton MA, Goodwin FT, Krakoff LR, Bard RH, Buhler FR. Essential hypertension: renin and aldosterone, heart attack and stroke. *N Engl J Med*. 1972;286:441–449.
4. Laragh JH. Vasoconstriction-volume analysis for understanding and treating hypertension: the use of renin and aldosterone profiles. *Am J Med*. 1973;55:261–274.
5. Petrovic N, Black TA, Fabian JR, Kane C, Jones CA, Loudon JA, Abonia JP, Sigmund CD, Gross KW. Role of proximal promoter elements in regulation of renin gene transcription. *J Biol Chem*. 1996;271: 22499–22505.
6. Pan L, Gross KW. Transcriptional regulation of renin: an update. *Hypertension*. 2005;45:3–8.
7. Itani HA, Liu X, Pratt JH, Sigmund CD. Functional characterization of polymorphisms in the kidney enhancer of the human renin gene. *Endocrinology*. 2007;148:1424–1430.
8. Germain S, Bonnet F, Philippe J, Fuchs S, Corvol P, Pinet F. A novel distal enhancer confers chorionic expression on the human renin gene. *J Biol Chem*. 1998;273:25292–25300.
9. Fuchs S, Philippe J, Germain S, Mathieu F, Jeunemaitre X, Corvol P, Pinet F. Functionality of two new polymorphisms in the human renin gene enhancer region. *J Hypertens*. 2002;20:2391–2398.
10. O'Brien E, Murphy J, Tyndall A, Atkins N, Mee F, McCarthy G, Staessen J, Cox J, O'Malley K. Twenty-four-hour ambulatory blood pressure in men and women aged 17 to 80 years: the Allied Irish Bank Study. *J Hypertens*. 1991;9:355–360.
11. Stanton A, Jensen C, Nussberger J, O'Brien E. Blood pressure lowering in essential hypertension with an oral renin inhibitor, aliskiren. *Hypertension*. 2003;42:1137–1143.
12. Weale ME, Depondt C, Macdonald SJ, Smith A, Lai PS, Shorvon SD, Wood NW, Goldstein DB. Selection and evaluation of tagging SNPs in the neuronal-sodium-channel gene SCN1A: implications for linkage-disequilibrium gene mapping. *Am J Hum Genet*. 2003;73:551–565.
13. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evolution*. 1995; 12:921–927.
14. Rapp JP, Wang SM, Dene H. A genetic polymorphism in the renin gene of Dahl rats cosegregates with blood pressure. *Science*. 1989;243: 542–544.
15. Kurtz TW, Simonet L, Kabra PM, Wolfe S, Chan L, Hjelle BL. Cosegregation of the renin alleles of the spontaneously hypertensive rat with an increase in blood pressure. *J Clin Invest*. 1990;85:1328–1332.
16. Frossard PM, Lestringant GG, Elshahat YI, John A, Obineche EN. An MboI two-allele polymorphism may implicate the human renin gene in primary hypertension. *Hypertension Res*. 1998;21:221–225.
17. Frossard PM, Lestringant GG, Malloy MJ, Kane JP. Human renin gene BglI dimorphism associated with hypertension in two independent populations. *Clin Genet*. 1999;56:428–433.
18. Ahmad U, Saleheen D, Bokhari A, Frossard PM. Strong association of a renin intronic dimorphism with essential hypertension. *Hypertension Res*. 2005;28:339–344.
19. Barley J, Carter ND, Cruickshank JK, Jeffery S, Smith A, Charlett A, Webb DJ. Renin and atrial natriuretic peptide restriction fragment length polymorphisms: association with ethnicity and blood pressure. *J Hypertens*. 1991;9:993–996.
20. Chiang FT, Hsu KL, Tseng CD, Lo HM, Chern TH, Tseng YZ. Association of the renin gene polymorphism with essential hypertension in a Chinese population. *Clin Genet*. 1997;51:370–374.
21. Naftilan AJ, Williams R, Burt D, Paul M, Pratt RE, Hobart P, Chirgwin J, Dzau VJ. A lack of genetic linkage of renin gene restriction fragment length polymorphisms with human hypertension. *Hypertension*. 1989;14: 614–618.
22. Soubrier F, Jeunemaitre X, Rigat B, Houot AM, Cambien F, Corvol P. Similar frequencies of renin gene restriction fragment length polymorphisms in hypertensive and normotensive subjects. *Hypertension*. 1990;16:712–717.
23. Fu Y, Katsuya T, Asai T, Fukuda M, Inamoto N, Iwashima Y, Sugimoto K, Rakugi H, Higaki J, Ogihara T. Lack of correlation between MboI restriction fragment length polymorphism of the renin gene and essential hypertension in Japanese. *Hypertension Res*. 2001;24:295–298.
24. Berge KE, Berg K. No effect of a BglI polymorphism at the renin (REN) locus on blood pressure level or variability. *Clin Genet*. 1994;46: 436–438.
25. Jeunemaitre X, Rigat B, Charru A, Houot AM, Soubrier F, Corvol P. Sib pair linkage analysis of renin gene haplotypes in human essential hypertension. *Hum Genet*. 1992;88:301–306.
26. Hasimu B, Nakayama T, Mizutani Y, Izumi Y, Asai S, Soma M, Kokubun S, Ozawa Y. Haplotype analysis of the renin gene and essential hypertension. *Hypertension*. 2003;41:308–312.
27. Zhu X, Chang YPC, Yan D, Weder A, Cooper R, Luke A, Kan D, Chakravarti A. Associations between hypertension and genes in the renin-angiotensin system. *Hypertension*. 2003;41:1027–1034.
28. Cook NR. Implications of small reductions in diastolic blood pressure for primary prevention. *Arch Intern Med*. 1995;155:701–709.
29. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903–1913.
30. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, Den Hond E, McCormack P, Staessen JA, O'Brien E. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin Outcome Study. *Hypertension*. 2005;46:156–161.
31. Lum C, Shesely EG, Potter DL, Beierwaltes WH. Cardiovascular and renal phenotype in mice with one or two renin genes. *Hypertension*. 2004;43:79–86.
32. Bader M, Peters J, Baltatu O, Müller DN, Luft FC. Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med*. 2001;79:76–102.
33. Re RN. Intracellular renin and the nature of intracrine enzymes. *Hypertension*. 2003;42:117–122.
34. Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest*. 2002;109:1417–1427.
35. Saris JJ, 't Hoen PAC, Garrelds IM, Dekkers DHW, den Dunnen JT, Lamers JMJ, Danser AHJ. Prorenin induces intracellular signaling in cardiomyocytes independently of angiotensin II. *Hypertension*. 2006;48: 564–571.

Downloaded from hyper.ahajournals.org by on July 23, 2007