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The Glycine Allele of a Glycine/Arginine Polymorphism in the β_2-Adrenergic Receptor Gene Is Associated With Essential Hypertension in a Population of Chinese Origin

Koustubh Ranade, Wayne H-H. Shue, Yi-Jen Hung, Chao A. Hsuing, Fu-Tein Chiang, Robert Pesich, Joan Hebert, MichaelOlivier, Yii-Der I. Chen, Richard Pratt, Richard Olshen, David Curb, David Botstein, NeilRisch, and David R. Cox

Background: Several studies implicate polymorphisms in the human β_2-adrenergic receptor gene (*ADRB2*) in the susceptibility to hypertension. We sought to replicate these results in a population of Chinese origin primarily from Taiwan and the San Francisco Bay area.

Methods: We genotyped >800 hypertensive subjects and individuals with low-normal blood pressure that were derived largely from the same families as the hypertensive patients for three polymorphisms in the *ADRB2* gene: a C/T transition at position 47 (C-47T) in the 5′ leader cistron; another C/T transition that results in a glycine/arginine substitution at codon 16 (Gly16Arg), and a G/C transversion that causes a glutamate/glutamine substitution at codon 27 (Glu27Gln).

Results: The Gly16Arg was significantly associated with hypertension ($P < .03$). Under a dominant model, for hypertension the relative risk for the Gly/Gly and Gly/Arg genotypes versus the Arg/Arg genotype was 1.35 (95% confidence limits [CL] 1.08, 1.70); for low-normal blood pressure the relative risk was 0.79 (95% CL 0.66, 0.94). This polymorphism explained approximately 1% of the variance in systolic and diastolic blood pressures in our study population. There was no evidence of association between the C-47T and Glu27Gln polymorphisms and hypertension in this population.

Conclusions: The Gly16 allele in the β_2-adrenergic receptor gene is a susceptibility allele for essential hypertension in a population of Chinese origin. Am J Hypertens 2001;14:1196–1200 © 2001 American Journal of Hypertension, Ltd.

Key Words: Hypertension, blood pressure variation, β_2-adrenergic receptor, association, polymorphism.

Despite intense effort, our understanding of heritable factors in the etiology of hypertension is rather poor. Twin, adoption, and epidemiologic studies indicate that variation in blood pressure (BP) is genetically determined to some extent.[1] One of the goals of the Stanford, Asia, and Pacific Program for Hypertension and Insulin Resistance (SAPPHIRe) is to identify genes for essential hypertension. It seems likely that variation in many different genes, each making a small contribution, determine an individual's susceptibility to developing hypertension. The relatively small effect of an individual gene, coupled with the complexity of the phenotype make hunting for genes that affect BP variation a challenging task. To maximize our chances of finding such genes, we took several steps. First, in an attempt to reduce genetic heterogeneity, we focused on a relatively homo-

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From the Department of Genetics (KR, R Pesich, JH, MO, DB, NR, DRC), Stanford University School of Medicine, Stanford, California; Veterans General Hospital (WH-HS), Taichung, Taiwan; Tri-Service General Hospital (Y-JH), National Defense Medical Center, Taipei, Taiwan; National Health Research Institute (CAH), Taipei, Taiwan; National Taiwan University Hospital (F-TC), Taipei, Taiwan; Division of Endocrinology (Y-DIC), Stanford University School of Medicine, Stanford, California; Department of Medicine (R Pratt), Brigham & Women's Hospital, Boston, Massachusetts; Health Research and Policy (RO), Stanford University School of Medicine, Stanford, California; and Hawaii Center for Health Research (DC), Honolulu, Hawaii.

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Address correspondence and reprint requests to Koustubh Ranade, Bristol-Myers Squibb, Pharmaceutical Research Institute, PO Box 5400, Princeton, NJ 08543-5400; e-mail: koustubh.ranade@bms.com

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geneous population of Chinese descent recruited from Taiwan and, to a much smaller extent, from the San Francisco Bay area and Hawaii. Second, we sampled subjects from tails of the BP distribution: the affected hypertensive subjects were recruited from the upper 20% of the distribution, whereas the unaffected "low-normotensive" individuals had BP readings in the lower 30% of the distribution.

Because the β_2 adrenergic receptor is involved in diverse physiologic pathways that are relevant to the control of BP,[2–6] it is an attractive candidate gene for susceptibility to hypertension. Indeed, several studies implicated glycine/arginine or glutamate/glutamine substitutions at codons 16 (Gly16Arg) and 27 (Glu27Gln), respectively, in the susceptibility to hypertension.[7–11] Other studies, however, failed to find an association between these single nucleotide polymorphisms (SNP) and hypertension.[12–15]

In this study, we evaluated the importance of the Gly16Arg and Glu27Gln polymorphisms in the susceptibility to hypertension in our sample comprised of subjects of Chinese origin. We also determined whether a polymorphism in the leader cistron (C-47T), which is in proximity to these two variants and which was shown to regulate the expression of β_2-receptor mRNA,[16] was associated with hypertension status. Unlike most of the previous case-control studies, our study was family-based, where the unaffected low-normotensive individuals were derived largely from the same families as the affected hypertensive patients.

Materials and Methods

Subjects

Details of our study population have previously been published.[17] In the current study, 264 low-normotensive and 595 hypertensive subjects of Chinese origin were genotyped. These 859 subjects were drawn from 370 families. The distribution of family sizes is as follows: 93 singletons, 151, 71, 38, 13, and four families with two, three, four, five, and six sibs, respectively. The majority of families (160) contributed both hypertensive and low-normotensive sibs; 114 families had only hypertensive sibs; three families had only low-normotensive sibs; and the remainder were singletons. Thus there were 160 independent hypertensive–low-normotensive sib-pairs in the data set. Hypertensive and low-normotensive subjects of Chinese origin were recruited in the San Francisco Bay area (N = 114), Taiwan (N = 700) and Hawaii (N = 45). Hypertension is defined as BP in the upper 20% of the BP distribution, which in our population translates into the following values: systolic BP (SBP) $\geq$160 mm Hg or diastolic BP (DBP) $\geq$95 mm Hg or taking two medications for high BP (stage II hypertension). Alternatively the subject could have uncontrolled hypertension, ie, could be taking one medication for high BP and has either systolic BP $\geq$140 or diastolic $\geq$90 mm Hg. "Low normotension" is defined as BP in the bottom 30% of the age and sex-adjusted BP distribution, which in our population translates into the following BP values: for men $<$45 years of age, systolic BP $\leq$115 mm Hg and diastolic BP $\leq$76. For men $>$45 years of age, systolic BP $\leq$122 and diastolic BP $\leq$78 were used. For women $<$45 years of age, low-normal BP was defined as systolic BP $\leq$107 and diastolic BP $\leq$70 mm Hg. For those $>$45 years, the cut-off was systolic BP $\leq$118 and diastolic BP $\leq$75. Individuals with chronic illnesses such as diabetes, cancer, or heart, liver, or kidney disease were excluded from the study. This study was approved by institutional review committees at Stanford, Taiwan, and Hawaii.

Blood Pressure Measurements

Blood pressure was measured using an oscillometric device, the Dinamap model 1846 SX (Critikon Inc, Tampa, Florida). After a 5-min rest period, BP measurements were taken three times with a $\geq$1-min time lapse between two readings, and the average of the second and third readings was used in the analysis. To ensure uniform BP measurement at the different sites, technicians/clinicians at all the sites were trained to measure BP using this protocol, and they were monitored annually during site visits. Furthermore, there was a centralized retraining and recertification of key technicians each year.

Genotyping

Subjects were genotyped using the 5′ allelic discrimination assay or TaqMan as previously described.[17] Sequences of probes and primers used for genotyping are available upon request.

Statistical Analysis

In preliminary analyses, genotype frequencies between hypertensive and low-normotensive groups were compared using a 3 $\times$ 2 contingency table. If there was significant evidence for association, then this analysis was followed-up by likelihood analysis to evaluate simultaneously the relationship between *ADRB2* genotypes and the BP data for the low-normotensive subjects as well as the hypertension status of affected subjects, essentially as described.[17]

As is well known, BP is affected by age and gender. For this reason, for the low-normotensive subjects, we calculated age- and sex-standardized systolic and diastolic BP measurements by subtracting the population mean BP and dividing by the standard deviation for that age and sex group. Normative data were obtained for the Chinese population of Taiwan. Square-root transformations of both systolic and diastolic BPs were used in the standardization. The transformation and standardization procedures make the distribution normal with mean 0 and variance 1. The measured BP of hypertensive individuals are confounded by medication because most (~90%) of the hypertensive individuals included in our study take antihypertensive medication. For this reason, all the hypertensive subjects

Table 1. *ADRB2* SNP genotype frequencies

SNP	Low-Normotensive *N* (%)	Hypertensive *N* (%)
C-47T		
C/C	5 (1.9)	8 (1.4)
C/T	33 (12.6)	73 (12.6)
T/T	223 (85.4)	499 (86.0)
Gly16Arg		
Gly/Gly	55 (20.8)	114 (19.2)
Gly/Arg	110 (41.7)	312 (52.4)
Arg/Arg	99 (37.5)	169 (28.4)
Glu27Gln		
Glu/Glu	5 (1.9)	7 (1.2)
Glu/Gln	37 (14.1)	92 (15.6)
Gln/Gln	220 (84.0)	492 (83.2)

SNP = single nucleotide polymorphisms.

were assumed to have a BP value above the threshold used in the recruitment (see earlier here). Because the frequency of the Gly allele of the Gly16Arg SNP is not significantly different for subjects recruited in the San Francisco Bay area (Gly allele frequency = 0.47), Hawaii (0.42), and Taiwan (0.44), we could pool all the samples and analyze them together.

Results

We typed a sample of 859 subjects of Chinese origin for three SNP in the *ADRB2* gene, as follows: a C/T transition at position 47 (C-47T) in the 5′ leader cistron; another C/T transition that results in a glycine/arginine substitution at codon 16 (Gly16Arg); and a G/C transversion that causes a glutamate/glutamine substitution at codon 27 (Glu27Gln). The frequencies of each genotype class for each SNP are given in Table 1. The difference in genotype frequencies between the low-normotensive and hypertensive groups for the C-47T and Glu27Gln SNP was not significant ($\chi^2_{2df} < 5.9$). In contrast, for the Gly16Arg SNP there was a significant difference in genotype frequencies between the low-normotensive and hypertensive groups ($\chi^2_{2df} = 9.4$, $P \sim .01$). The frequency of the Gly/Arg heterozygotes was higher in the hypertensive (52.4%) than the low-normotensive (41.7%) class; there was a concomitant decrease in the frequency of the Arg/Arg homozygotes in the hypertensive subjects (28.4%) compared to the unaffected, low-normotensive individuals (37.5%). These results suggest that the Gly allele is dominant.

Because the above preliminary analysis ignored correlations in sib genotypes (which tend to inflate significance), we analyzed data using a likelihood model that took into account familial correlations in genotypes. In addition, in this likelihood model we evaluated simultaneously BP of low-normotensive individuals and the hypertensive status of affected individuals: that is, we asked whether the difference in genotype frequencies between the low-normotensive and hypertensive groups together with the difference in BP in the low-normotensive group was significant. We used measured BP of the low-normotensive subjects (Table 2) and assumed that the BP of hypertensive subjects was greater than a certain threshold (see Materials and Methods). We could not use the measured BP of hypertensive patients because most (~90%) were taking antihypertensive medication. This analysis was confined to the Gly16Arg data because this was the only SNP that showed significant association with hypertension in the preliminary analysis.

Consistent with the data in Table 2, there was significant evidence for a dominant model ($P < .03$) for both systolic and diastolic BP (Table 3). There was suggestive evidence for an additive model but no evidence to suggest that the Gly allele is recessive. We also calculated the absolute and relative risks for hypertension and low BP associated with the Gly/Gly and Gly/Arg genotypes vesus the Arg/Arg genotype. For hypertension (upper 20% of the BP distribution), the risks were 22.1% and 16.4% respectively, or a relative risk of 1.35 (95% confidence limits [CL] 1.08, 1.70). For low BP (lower 30% of the BP

Table 2. Gly16Arg genotype frequencies and blood pressures (BPs)

Genotype	Hypertensive *N* (%)	Low-Normotensive *N* (%)	Low-Normotensive BP* (SD)	Low-Normotensive BP† (SD)
Gly/Gly	114 (19.2)	55 (20.8)	105 (7.8) 67 (6.4)	−1.20 (0.412) −1.23 (0.464)
Gly/Arg	312 (52.4)	110 (41.7)	106 (7.6) 65 (6.7)	−1.13 (0.372) −1.38 (0.527)
Arg/Arg	169 (28.4)	99 (37.5)	104 (8.8) 64 (6.1)	−1.22 (0.454) −1.42 (0.484)
Total595	264			

Number of individuals (*N*) in each genotype class is given.
Data for genotype frequencies are taken from Table 1.
* BP in millimeters of mercury.
† Age- and sex-standardized BP. In each case, the first line is systolic BP and the second line diastolic. BP of hypertensive subjects is not given because it is confounded by antihypertensive medication.

Table 3. Likelihood analysis of blood pressures and genotype frequencies

Blood Pressure*	Model†	Maximum Likelihood Estimates: Allele Frequency‡	Maximum Likelihood Estimates: Displacement§	χ^2‖	*P* Value¶
Systolic					
	Dominant	0.407 (0.022)	0.223 (0.082)	7.2	<.03
	Additive	0.413 (0.026)	0.219 (0.136)	2.5	>.05
	Recessive	0.460 (0.021)	−0.082 (0.092)	0.8	>.05
	Null	0.448 (0.016)	0	0.0	
Diastolic					
	Dominant	0.415 (0.02)	0.201 (0.076)	6.8	<.03
	Additive	0.415 (0.023)	0.224 (0.119)	3.5	>.05
	Recessive	0.45 (0.021)	0.021 (0.088)	0.06	>.05
	Null	0.448 (0.016)	0	0.0	

* Square-root transformations of systolic and diastolic blood pressures were used in the likelihood analyses. Hypertensives were assumed to have a BP greater than a certain threshold (see Materials and Methods).

† The null hypothesis is that there is no difference in the mean blood pressures between the two homozygotes.

‡ Frequency and standard error (in parentheses) of the Gly allele.

§ Difference and standard error (in parentheses) in mean values between the Gly/Gly and Arg/Arg homozygotes. A positive value means that the mean blood pressure of Gly/Gly homozygotes is greater than that of the Arg/Arg homozygotes.

‖ The χ^2 value is calculated as twice the difference between the log-likelihood for the indicated model and that for the null model.

¶ The *P* Value is for a χ^2 with 2 df.

distribution), the risks were 27.4% and 34.8%, respectively, or a relative risk of 0.79 (95% CL 0.66, 0.94). Similarly, we calculated from the model the expected genotype frequencies (Gly/Gly and Gly/Arg versus Arg/Arg) among hypertensive and low-normotensive subjects. For hypertensive subjects, the frequencies were 72.0% and 28.0%, respectively, versus 59.6% and 40.4%, respectively, for low-normotensive individuals. These numbers are comparable to those observed (Table 1).

The likelihood analysis also permitted us to estimate the contribution of this particular SNP to the total genetic variance of BP. Approximately 1.1% and 0.9% of the variance in systolic and diastolic BP, respectively, was explained by the Gly16Arg polymorphism.

Discussion

In light of studies by Green et al,[18] the association between the Gly16Arg SNP and hypertension appears to be biologically meaningful. Their in vitro studies revealed that β_2-AR bearing glycine at position 16 undergo enhanced downregulation in response to prolonged exposure to agonists. Thus, it is plausible that over time the vasodilatory response to catecholamines might become blunted in heterozygotes and Gly/Gly homozygotes, thereby resulting in elevated BP.[9]

We have replicated in our population the association between the Gly allele and essential hypertension found in populations of White and African-Caribbean origin.[7,9] Given that our subjects are of Chinese origin from Taiwan and the San Francisco Bay area, this consistency in results is reassuring. On the other hand, although our results implicate the same polymorphism in the *ADRB2* gene, they should not be construed as a replication of findings reported by two other groups, because these studies reported an association between the Arg16 allele and a history of hypertension[8] or elevated BP,[10] whereas statistical significance in our study depends on the Gly allele acting in a dominant and predisposing fashion. The discrepancy between our results and those reported in these two studies may be due to differences in ethnicities or study designs. Further studies based on different populations will help to resolve this issue.

Finally, it should be stressed that although the β_2-adrenergic receptor explains only a small proportion of the genetic variance in BP in our population, several such genes could make a substantial contribution. Our strategy is to use genes (such as the β_2-adrenergic receptor) that make individually significant contributions to the phenotype in such combinatorial analyses.

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References

1. Lifton RP: Molecular genetics of human blood pressure variation. Science 1996;272:676–680.
2. Insel PA: Adrenergic receptors: evolving concepts and clinical applications. N Engl J Med 1996;334:580–585.
3. Modan M, Haalkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z: Hyperinsulinemia: A link between hypertension, obesity and glucose intolerance. J Clin Invest 1985;75:809–817.

4. Haffner SM, Ferrannini E, Hazuda HP, Stern MP: Clustering of cardiovascular risk factors in confirmed prehypertensive individuals: The San Antonio Heart Study. Am J Epidemiol 1992;41:715–722.
5. Meehan P, Buchanan TA, Shargill N, Hsueh WA: Effect of chronic insulin administration on blood pressure. Hypertension 1994;23: 1012–1017.
6. Chruscinski AJ, Rohrer DK, Schauble E, Desai KH, Bernstein D, Kobilka BK: Targeted disruption of the beta2 adrenergic receptor gene. J Biol Chem 1999;274:16694–16700.
7. Kotanko P, Binder A, Tasker J, DeFreitas P, Kamdar S, Clark AJL, Skrabal F, Caulfield M: Essential hypertension in African Caribbeans associates with a variant of the β_2-adrenoreceptor. Hypertension 1997;30:773–776.
8. Timmerman B, Mo R, Luft FC, Gerdts E, Busjahn A, Omvik P, Guo-Hua L, Schuster H, Wienker T, Hoehe M, Margret R, Lund-Johansen P: β_2-adrenoreceptor genetic variation is associated with genetic predisposition to essential hypertension: The Bergen Blood Pressure Study. Kidney International 1998;53:1455–1460.
9. Gratze G, Fortin J, Labugger R, Binder A, Kotanko P, Timmermann B, Luft FC, Hoehe M, Skrabal F: β2-Adrenergic receptor variants affect resting blood pressure and agonist-induced vasodilation in young adult Caucasians. Hypertension 1999;33:1425–1430.
10. Busjahn A, Guo-Hua L, Faulhaber H-D, Rosenthal M, Becker A, Jeschke E, Schuster H, Timmermann B, Hoehe M, Luft FC: β-2 adrenergic receptor gene variations, blood pressure and heart size in normal twins. Hypertension 2000;35:555–560.
11. Bray MS, Krushkal J, Li L, Ferrell R, Kardia S, Sing CF, Turner ST, Boerwinkle E: Positional genomic analysis identifies the β2-adrenergic receptor gene as a susceptibility locus for human hypertension. Circulation 2000;101:2877–2882.
12. Herrmann V, Buscher R, Go MM, Ring KM, Hofer JK, Kailasam MT, O'Connor DT, Parmer RJ, Insel PA: Beta2-adrenergic receptor polymorphisms at codon 16, cardiovascular phenotypes and essential hypertension in whites and African Americans. Am J Hypertens 2000;13:1021–1026.
13. Xie HG, Stein CM, Kim RB, Gainer JV, Sofowora G, Dishy V, Brown NJ, Goree RE, Haines JL, Wood AJ: Human beta2-adrenergic receptor polymorphisms: no association with essential hypertension in black or white Americans. Clin Pharmacol Ther 2000; 67:670–675.
14. Jia H, Sharma P, Hopper R, Dickerson C, Lloyd DD, Brown MJ: Beta2-adrenoceptor gene polymorphisms and blood pressure variations in East Anglian Caucasians. J Hypertens 2000;18:687–693.
15. Candy G, Samani N, Norton G, Woodiwiss A, Radevski I, Wheatley A, Cockcroft J, Hall IP: Association analysis of beta2 adrenoceptor polymorphisms with hypertension in a black African population. J Hypertens 2000;18:167–172.
16. McGraw DW, Forbes SL, Kramer LA, Liggett SB: Polymorphisms of the 5′ leader cistron of the human beta2-adrenergic receptor regulate receptor expression. J Clin Invest 1998;102:1927–1932.
17. Ranade K, Hsuing AC, Wu KD, Chang MC, Chen YT, Hebert J, Chen YI, Olshen R, Pratt R, Curb D, Dzau V, Botstein D, Cox D, Risch N: Lack of evidence for an association between alpha-adducin and blood pressure regulation in Asian populations. Am J Hypertens 2000;13:704–709.
18. Green S, Turki J, Innis M, Liggett SB: Amino terminal polymorphisms of the human β2-adrenergic receptor impart distinct agonist-promoted regulatory properties. Biochemistry 1994;33:9414–9419.