

β -2 Adrenergic Receptor Gene Variations, Blood Pressure, and Heart Size in Normal Twins

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Abstract—Genetic variability, which influences cardiovascular phenotypes in normal persons, is likely to be relevant to cardiovascular disease. We studied normal monozygotic and dizygotic twins and found strong genetic influences on blood pressure and heart size. We then relied on the dizygotic twins and their parents to apply molecular genetic techniques. We performed a linkage analysis with markers close to the β -2 adrenergic receptor (AR) gene locus in the dizygotic twins and their parents and found strong evidence for linkage to the quantitative traits of blood pressure and heart size. We then used allele-specific polymerase chain reaction to genotype the subjects further. We performed an association analysis and found that 4 functionally relevant polymorphisms in the β -2 AR gene, namely Arg16/Gly, Gln27/Glu, Thr164/Ile, and a variant in the promoter region ($-47C/T$), were variably associated with blood pressure and heart size differences but were in linkage disequilibrium with each other. A subsequent conditional analysis suggested that the Arg16/Gly polymorphism exerted the predominant effect. These findings underscore the importance of the β -2 AR gene to blood pressure regulation, heart size, and probably to the development of hypertension. We suggest that a combined linkage and association approach will elucidate the genetic variability influencing blood pressure and other cardiovascular phenotypes. (*Hypertension*. 2000;35:555-560.)

Key Words: receptors, adrenergic, beta ■ genetics ■ hypertension, genetic ■ twins ■ blood pressure

The β -2 adrenergic receptor (β -2 AR) has been implicated in the pathogenesis of hypertension, both on the basis of studies suggesting altered β -2-mediated vasodilation¹ and on the basis of molecular genetic association studies.^{2,3} Recently, Kotanko et al⁴ found an association between the Arg16/Gly polymorphism in the β -2 AR gene and hypertension in an African Caribbean population. They showed that the Gly16 allele was more common in hypertensive subjects than in normotensive African Caribbean control subjects. Since the Gly16 allele indicates an increased propensity for downregulation of the receptor,⁵ the authors raised the possibility that an impaired vasodilation in peripheral arteries in response to β -2 AR agonists may play a role in the hypertension of individuals carrying the Gly16 allele. We subsequently examined the firstborn normotensive adult children of couples documented to be normotensive or hypertensive in the Bergen Blood Pressure Study.⁶ Offspring of 2 hypertensive parents had higher blood pressures and a preponderance of the Arg16 allele compared with offspring of 2 normotensive parents. To further examine the genetic variability at the β -2 AR locus and its relevance for the cardiovascular system in northern Europeans, we performed a combined linkage and association study in normotensive twin subjects. We used allele-specific polymerase chain reaction (PCR), which al-

lowed us to examine other polymorphisms in the β -2 AR gene.

Methods

We recruited 166 pairs of twins (monozygotic [MZ] 100 and dizygotic [DZ] 66) by advertisement to participate in studies involving blood pressure regulation and cardiovascular phenotypes.^{7,8} We recruited the parents of the DZ twins and genotyped them as well, to permit an identity by descent (IBD) analysis. The subjects were all Germans, white subjects recruited from various parts of Germany. The protocol was approved by the university committee on the protection of human subjects, and written informed consent was obtained from all participants. Blood was obtained for the determination of zygosity and other molecular genetic studies from all the twins and the parents of the DZ twins. Each participant underwent a medical history and physical examination. Subjects taking antihypertensive medication or being affected by any other chronic medical illness were excluded. Blood pressure was measured after 5 minutes of rest in the sitting position with a standardized mercury sphygmomanometer (World Health Organization criteria) by an experienced physician. Two measurements were obtained 2 minutes apart. The same procedure was performed after the subjects were supine for 5 minutes and after 5 minutes of upright posture. The mean values of the 2 determinations were recorded. Norepinephrine was measured with high-performance liquid chromatography and electrochemical detection. M-mode and 2-dimensional echocardiograms were recorded with patients in the left lateral decubitus position. Interventricular septal thickness and posterior wall thickness were measured in all patients as described earlier.⁸

Received August 27, 1999; first decision September 27, 1999; revision accepted October 8, 1999.

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TABLE 1. Demographic Data, Phenotypic Values (Mean±SD), Correlations (*r*), Heredity Estimates (*a*²), and Results of Linkage Analysis with the β -2 Locus

Phenotype	MZ Twins	DZ Twins	<i>a</i> ² (<i>r</i> _{MZ} / <i>r</i> _{DZ})	<i>P</i> (Linkage)
n	200	132		
Age, y	29±12	31±12		
Sex, M/F	52/148	85/47		
Height, cm	169±8	170±8		
Weight, kg	65±11	67±12		
BMI, kg/m	22.4±3.5	22.8±3.4		
Systolic BP recumbent, mm Hg	128±17	124±14	0.69 (0.69/0.31)	0.001
Diastolic BP recumbent, mm Hg	71/12	71/11	0.66 (0.66/0.42)	0.001
Systolic BP sitting, mm Hg	125±16	123±13	0.74 (0.74/0.38)	NS
Diastolic BP sitting, mm Hg	73±11	73±10	0.72 (0.72/0.51)	0.001
Systolic BP standing, mm Hg	124±15	122±14	0.67 (0.66/0.48)	0.001
Diastolic BP standing, mm Hg	80±10	79±10	0.64 (0.63/0.40)	NS
Posterior wall thickness, mm	8.7±1.6	8.6±1.6	0.48 (0.48/0.26)	NS
Septum, mm	8.9±1.7	8.8±1.6	0.64 (0.64/0.37)	0.001
Left ventricular mass, mm ³	165±50	176±60	0.68 (0.68/0.27)	0.001
Norepinephrine, pmol/L*	54.7/21.2	58.1/23.1	0.30 (0.60/0.45)	0.001

BMI indicates body mass index; BP, blood pressure.
*pg/mL, 5.458 pmol/L.

For linkage analysis, 3 microsatellite markers spanning 2.3 cM around the β -2 locus on chromosome 5 (D5S413, D5S2090, and D5S2013) were analyzed with the use of the ABI genotyping system. Our techniques for zygosity testing and genotyping of microsatellites have been previously described.^{9,10} We genotyped the functionally relevant polymorphisms of the β -2 AR by means of allele-specific polymerase chain reaction (PCR), as described elsewhere.⁶

Statistical analysis was conducted with the use of the SPSS program. Association analysis was based on quantitative measures. Blood pressure values and all other phenotypes were adjusted for sex and age by multiple linear regression. Cardiac dimensions were also adjusted for blood pressure-related influences. Two different approaches were applied. Phenotypic values were compared between groups defined by their genotype with the use of ANOVA for all 4 polymorphisms independently. To increase the power for the association analysis, mean scores of pairs of MZ twins were included together with 1 randomly selected member of the DZ pairs.¹¹ Because this approach might be prone to false-positive results as the result of population stratification, a second analysis based on sib-pair data was carried out.¹² To test for association, structural equation modeling was used to obtain maximum likelihood estimates for the allelic effect, based on within-family and between-family differences. Significance was tested by computing nested models and comparing the log-likelihood between models. To test for stratification effects, both estimates of allelic effect are constrained to be equal. In the absence of stratification, both estimates were then set to zero. In the presence of stratification effects, setting only the within-family estimate to zero provides an unbiased test for true allelic association.

Haplotypes could not be constructed for all subjects. Furthermore, for polymorphisms in close linkage disequilibrium, not all informative combinations were present in the sample. To estimate the influence of single polymorphisms independent from each other, conditional analyses were carried out within subgroups differing for 1 polymorphism while being equally homozygous for a second polymorphism. This approach controlled for possible influences of the second polymorphism. For the association analysis we accepted a value of $P < 0.05$ as significant.

For linkage analysis, twin pairs were selected and used as ordinary sib-pairs but with the advantage of perfect age matching and reduced environmental variation affecting the phenotype. We assessed linkage

for blood pressure as a continuous trait. Analysis was done by using a structural equation modeling approach,¹³ as implemented in the MX-package developed by Neale.¹⁴ If the locus under study is a quantitative trait locus (QTL), phenotypic similarity of sibs (measured by their covariance) should increase with the number of alleles shared IBD. For each sib-pair, the proportion of alleles IBD for the given locus is calculated on the basis of parental genotypes, with the use of a multipoint approach, as implemented in MAPMAKER/SIBS.¹⁵ To test for a QTL effect, the difference in model fit for models with and those without a QTL effect is calculated as a χ^2 statistic. Testing of multiple correlated phenotypes was used to verify results; thus no adjustment for multiple testing was performed. Because we used a candidate gene approach, we accepted a value of $P < 0.01$ to test for significant linkage in accordance with the criteria defined by Lander and Kruglyak.¹⁶ Parameters of the quantitative genetic models were estimated on the basis of variance/covariance matrixes by structural equation modeling¹⁷ with the use of the MX program.¹⁸ The variability of any given phenotype within a population can be decomposed into genetic influences, environmental influences shared by the twins within a family, and effects of random environment.

Results

Demographic data, blood pressure values, heredity estimates of blood pressure, and echocardiographic variables in 200

TABLE 2. Position, Consequence, and Genotype Frequencies for Polymorphisms

Position (Nucleotide base)	Polymorphism/Frequency	Amino Acid Change/Position
-47	C/T (38%/62%)	(regulatory region)
+46	A/G (47%/53%)	Arg/Gly/16
+79	C/G (61%/49%)	Gln/Glu/27
+491	G/A (99%/1%)	Thr/Ile/164

TABLE 3. Frequencies for Genotype Combinations

Genotype	Arg16/Gly Arg/Arg	Arg/Gly	Gly/Gly	<i>P</i>
-47CT				
C/C	0 (0%)	3 (0.9%)	38 (17.8%)	0.01
C/T	3 (1.4%)	56 (26.3%)	24 (11.3%)	
T/T	55 (25.8%)	26 (12.2%)	9 (4.2%)	
Gln27/Gln				
	Glu/Glu	Glu/Gln	Gln/Gln	
-47CT				
C/C	0 (0%)	1 (0.5%)	39 (18.3%)	0.01
C/T	1 (0.5%)	78 (36.6%)	4 (1.9%)	
T/T	87 (40.8%)	3 (1.4%)	0 (0%)	
Thr164/Ile				
	Thr/Thr	Thr/Ile	Ile/Ile	
-47CT				
C/C	40 (18.8%)	0 (0%)	0 (0%)	NS
C/T	79 (37.1%)	3 (1.4%)	1 (0.5%)	
T/T	88 (41.3%)	2 (0.9%)	0 (0%)	
Gln27/Gln				
	Glu/Glu	Glu/Gln	Gln/Gln	
Arg16/Gly				
Arg/Arg	55 (25.8%)	3 (1.4%)	0 (0%)	0.01
Arg/Gly	25 (11.7%)	58 (27.2%)	1 (0.5%)	
Gly/Gly	8 (3.8%)	21 (9.9%)	42 (19.7%)	
Thr164/Ile				
	Thr/Thr	Thr/Ile	Ile/Ile	
Arg16/Gly				
Arg/Arg	58 (27.2%)	0 (0%)	0 (0%)	NS
Arg/Gly	83 (39.0%)	1 (0.5%)	0 (0%)	
Gly/Gly	66 (31.0%)	4 (1.9%)	1 (0.5%)	
Thr164/Ile				
	Thr/Thr	Thr/Ile	Ile/Ile	
Gln27/Gln				
Glu/Glu	86 (40.4%)	2 (0.9%)	0 (0%)	NS
Glu/Gln	79 (37.1%)	2 (0.9%)	1 (0.5%)	
Gln/Gln	42 (19.7%)	1 (0.5%)	0 (0%)	

MZ and 132 DZ twins are given in Table 1. There were no significant differences between MZ and DZ twins for any of the variables examined. Systolic and diastolic blood pressures were heritable. The echocardiographic parameters also demonstrated strong evidence of heritability. The multipoint linkage analysis gave significant ($P < 0.001$) results for systolic blood pressure recumbent and standing, diastolic blood pressure sitting and recumbent, and left ventricular mass index, as shown in Table 1. These data establish the β -2 AR gene locus as a QTL for blood pressure and heart size in normal humans.

We next performed allele-specific PCR for 4 polymorphisms resulting in an amino acid exchange. The gene frequencies are given in Table 2. The test for Hardy-Weinberg equilibrium gave evidence for an excess of homozygous individuals for polymorphisms in the 5' leader cistron and the first extracellular loop of the gene (-47C/T, Arg16/Gly, Gln27/Glu), whereas the polymorphism in the fourth transmembrane domain (Thr164/Ile) showed no deviation from expected frequencies. We performed an analysis to assess the interdependency of the polymorphisms, as shown in Table 3. The strongest linkage was found between polymorphisms in close spatial relation to one another, namely -47C/T, Arg16/Gly, and Gln27/Glu. Thr164/Ile was independent of -47C/T, Gln27/Glu, and Arg16/Gly.

Tables 4 and 5 show the results of the association analysis. Probability values for both ANOVA and maximum likelihood estimation are given, although the results with the 2 approaches were relatively similar. Arg16/Gly and Gln27/Glu showed strong associations to systolic blood pressure and norepinephrine levels as well as with septum and posterior wall thickness. Thr164/Ile was associated with recumbent systolic blood pressure (ANOVA) and all cardiac dimensions. The associations with cardiac dimensions for the polymorphisms remained significant, even when corrected for systolic blood pressure by multiple linear regression analysis (data not shown). Homozygous Ile164/Ile was observed in only 1 person. The biallelic polymorphism in the regulatory region was weakly associated with systolic blood pressure, norepinephrine levels, and septum thickness.

Table 6 shows the results of a conditional analysis for the Arg16/Gly and Gln27/Glu polymorphisms. In subjects homozygous for Gly/Gly, the Gln27/Glu polymorphism showed no effect on any of the phenotypes. In contrast, when we examined subjects with the Gln27/Glu polymorphism who were homozygous Gln/Gln, Arg16/Arg subjects differed from Gly16/Gly subjects for all 3 blood pressure values and for cardiac dimensions. These results favor the interpretation that the Arg16/Gly polymorphism is more likely to be responsible for the effects on the phenotypes.

Discussion

The important findings in this study were that the β -2 AR gene locus is linked to the quantitative traits systolic blood pressure, diastolic blood pressure, and cardiac size, indicating that this gene locus is a QTL for blood pressure and heart size in normotensive individuals. Furthermore, the 4 polymorphisms in the β -2 AR gene that we examined, namely Arg16/Gly, Gln27/Glu, Thr164/Ile, and a variant in the promoter region (-47C/T), were all variably associated with systolic blood pressure and heart size differences in these normal subjects, whereas 3 of the 4 were associated with differences in norepinephrine levels. We report on 4 biallelic polymorphisms in the β -2 AR gene, which result in amino acid substitutions.¹⁸ A fifth, Val34/Met, was not encountered in our subjects. We believe that our combined linkage-association approach is unique and strongly supports an important role for the β -2 AR gene in blood pressure regulation. The significant associations we observed were for systolic, not diastolic, blood pressure. This finding should not

TABLE 4. Three Biallelic Polymorphisms in the β -2 AR Gene Resulting in Amino Acid Change

Phenotype	Arg16/Arg/Arg	Gly Arg/Gly	Gly/Gly	<i>P</i> , ANOVA	<i>P</i> , ML
n, subjects	58	84	71		
Systolic BP recumbent	131±15	125±16	123±14	<0.05	<0.05
Systolic BP sitting	128±14	124±14	122±14	<0.05	<0.05
Systolic BP standing	127±14	123±14	120±14	<0.05	<0.05
Norepinephrine	58.4±23.8	61.9±23.5	50.7±17.0	<0.05	<0.05
Septum thickness	9.3±1.6	8.9±1.6	8.6±1.4	<0.05	<0.05
Posterior wall thickness	8.9±1.4	8.8±1.5	8.4±1.3	0.08	<0.05
Left ventricular mass	174±32	173±45	167±42	NS	<0.05
	Gln27/Glu/Glu	Glu Glu/Gln	Gln/Gln	<i>P</i> , ANOVA	<i>P</i> , ML
n, subjects	88	82	43		
Systolic BP recumbent	130±14	124±16	123±17	<0.05	<0.05
Systolic BP sitting	127±13	122±15	122±16	0.053	NS
Systolic BP standing	126±13	121±15	120±14	<0.05	<0.05
Norepinephrine	61.2±24.0	56.2±21.6	50.4±16.1	<0.05	<0.05
Septum thickness	9.3±1.6	8.6±1.5	8.7±1.4	<0.05	<0.05
Posterior wall thickness	8.9±1.5	8.6±1.4	8.4±1.3	NS	<0.05
Left ventricular mass	176±40	166±42	172±38	NS	NS
	Thr164/Thr/Thr	Ile Thr/Ile	Ile/Ile*	<i>P</i> , <i>t</i> test	<i>P</i> , ML
n, subjects	208	5	(1)		
Systolic BP recumbent	126±16	122±4		<0.05	NS
Systolic BP sitting	124±14	124±5		NS	NS
Systolic BP standing	123±14	124±8		NS	NS
Norepinephrine	57.2±22.2	58.3±18.3		NS	NS
Septum thickness	8.9±1.6	10.3±1.9		<0.05	<0.05
Posterior wall thickness	8.6±1.4	9.7±2.0		<0.05	<0.05
Left ventricular mass	170±39	220±60		<0.05	<0.05

BP, blood pressure.

The association analysis was performed both with ANOVA and a maximum likelihood (ML) approach.

*This subject was deleted from the analysis.

be surprising, because earlier studies indicate that systolic and diastolic blood pressures may be influenced by different genes.⁷ Furthermore, systolic blood pressure may be more a function of aortic elasticity and pulse-wave velocity, whereas diastolic blood pressure may be more a function of peripheral vascular resistance.¹⁹

The significant deviation from Hardy-Weinberg equilibrium detected in our sample prompted us to test allele frequencies from other studies for the Arg16/Gly polymorphisms. In the Bergen Blood Pressure study⁶ we found the same excess of homozygous individuals as in this twin study, but because of sample size this deviation did not reach statistical significance. Another sample of healthy subjects⁵ showed a deviation from expected genotype frequencies pointing in the same direction. Interestingly, the hypertensive persons described by Kotanko et al⁴ also deviated significantly from Hardy-Weinberg equilibrium. The confirmation of excess homozygosity in independent samples makes us confident that this finding is neither a genotyping error nor an effect specific for twin subjects. At this point, we can only speculate that there may be some disadvantage in having 2

different variants of the β -2 AR as the result of heterozygosity.

The Gly16 variant has been shown to represent a β -2 AR, which is more likely to be downregulated in response to β -2 agonists.⁵ The Gly16 variant was more common in hypertensive compared with normotensive African Caribbeans than the Arg16 variant and was also found to indicate a lesser propensity to vasodilatation in response to salbutamol infusion in normotensive white subjects from Austria.²⁰ Lang et al²¹ observed that forearm blood flow responses to isoproterenol were markedly attenuated in normotensive black subjects compared with white subjects, indicating a blunting of vasodilatation mediated by the β -2 AR. In that study, the β -2 AR alleles were not examined. Possibly, the Gly16 variant is associated with salt sensitivity; however, that hypothesis has not been prospectively tested.

Arg16 wild-type subjects had recumbent, sitting, and standing systolic blood pressures 7 to 8 mm Hg higher than homozygous Gly16 subjects, whereas heterozygous subjects were intermediate. Gly16 was also associated with a lesser cardiac septum thickness and lower norepinephrine levels.

TABLE 5. Biallelic Polymorphism in the Regulatory Region of the β -2 AR Gene

Phenotype	-47C/T			P, ANOVA	P, ML
	WW	WP	PP		
n, subjects	40	83	90		
Systolic BP recumbent	124 \pm 17	124 \pm 16	129 \pm 14	<0.05	NS
Systolic BP sitting	123 \pm 16	122 \pm 15	126 \pm 13	0.08	NS
Systolic BP standing	120 \pm 14	122 \pm 15	126 \pm 13	0.06	NS
Norepinephrine	50.9 \pm 17.0	55.9 \pm 21.4	61.2 \pm 23.8	<0.05	NS
Septum thickness	8.7 \pm 1.4	8.7 \pm 1.5	9.2 \pm 1.6	<0.05	<0.05
Posterior wall thickness	8.3 \pm 1.3	8.6 \pm 1.4	8.9 \pm 1.5	NS	<0.05
Left ventricular mass	169 \pm 37	168 \pm 43	175 \pm 39	NS	NS

BP, blood pressure; ML, maximum likelihood.

We have no explanation for the discrepancy between our observations and those reported earlier for African Caribbeans. Ethnic differences may be responsible and suggest that the hypertensive mechanisms may be quite different in black Africans and white subjects. Our conditional analysis gave support to the notion that the Arg16/Gly polymorphism is responsible for the effects on blood pressure and heart size rather than the Gln27/Glu polymorphism. Thus differences in Gln27/Glu distributions between the 2 populations would not serve to explain the discrepancy. Nevertheless, genotyping the African Caribbean population for other β -2 AR polymorphisms would be of interest.

The Glu27/Gln variant was the second most common polymorphism we encountered. This variant has been associated with an increase in IgE concentrations in the serum of asthmatic families.²² In contrast to Arg16/Gly, the Glu27/Gln variant is apparently resistant to downregulation, whereas the combination mutant downregulates to the same extent as the Gly16 variant. The most functionally altered β -2 AR is found in persons with a Thr-to-Ile switch at amino acid 164. This receptor exhibits a small decrease in binding affinity for agonists but a substantial decrease in basal and epinephrine-stimulated adenylyl cyclase activities caused by defective coupling of the receptor to the stimulatory G protein G_s and impaired agonist-promoted sequestration.^{23,24} In transgenic mice expressing either the wild-type Thr164 or the Ile164

mutation in the heart, the Ile164 mice displayed depressed contractile function compared with controls.²⁵ Recently, Liggett et al²⁶ were able to show that patients with heart failure who were heterozygous for the Ile164 mutation had a strikingly worse survival compared with patients with the wild-type Thr at this position. Liggett et al²⁶ had no homozygous Ile164 subjects in their study. We encountered 1 such subject, who was clinically well. Ile164 may only become a relevant risk factor when persons develop congestive heart failure but may not necessarily be associated with conditions engendering heart failure.

We first observed the -47C/T polymorphism of the 5' leader cistron in the β -2 AR gene, which results in either Arg or Cys being encoded at the terminal amino acid of the receptor peptide, in our subjects from the Bergen Blood Pressure Study.²⁷ McGraw et al²⁸ recently reported that the Cys variant results in increased β -2 AR expression. Thus the -47C/T polymorphism may represent the genetic basis of variable physiological sympathetic responses or variations in cardiovascular phenotypes.

We cannot state for certain which polymorphism we examined is responsible for the association with blood pressure, cardiac dimensions, and norepinephrine concentrations, nor can we speculate whether or not the functional polymorphisms exert independent effects. Additional interactions between these polymorphisms may be elucidated by larger

TABLE 6. Conditional Analysis for Arg16/Gly and Gln27/Glu

Phenotype	Within Gly16/Gly			Within Gln27/Gln		
	Glu27/Glu	Gln27/Gln	P	Arg16/Arg	Gly16/Gly	P
n (subjects)	8	42		55	8	
Systolic BP recumbent	126 \pm 7	124 \pm 18	NS	132 \pm 17	126 \pm 7	<0.05
Systolic BP sitting	120 \pm 7	122 \pm 17	NS	128 \pm 15	120 \pm 7	<0.05
Systolic BP standing	122 \pm 8	120 \pm 16	NS	128 \pm 15	122 \pm 8	<0.05
Norepinephrine	58.6 \pm 15.6	48.6 \pm 15.9	NS	57.2 \pm 32.1	58.6 \pm 15.6	NS
Septum thickness	8.8 \pm 1.9	8.7 \pm 1.4	NS	9.3 \pm 1.7	8.8 \pm 1.9	<0.05
Posterior wall thickness	8.6 \pm 1.9	8.4 \pm 1.3	NS	8.9 \pm 1.4	8.6 \pm 1.9	<0.05
Left ventricular mass	173 \pm 65	162 \pm 48	NS	177 \pm 53	173 \pm 65	<0.05

BP, blood pressure.

The analysis compares persons homozygous for Gly16/Gly in terms of Gln27/Gln as well as persons homozygous for Gln27/Gln in terms of Arg16/Gly.

studies with complex haplotype analyses. Nevertheless, our study shows that at least 2 functionally relevant, β -2 AR polymorphisms are associated with blood pressure, cardiac dimensions, and norepinephrine levels in normal healthy dizygotic twin subjects. Furthermore, the β -2 AR gene locus is a QTL for these variables in normal humans. Finally, our study suggests that the Gly16 rather than the Arg16 variant is associated with lower blood pressure and a lesser risk to develop hypertension in white subjects. Longitudinal outcome studies incorporating haplotype analyses will be elucidative in this regard.

Acknowledgments

Contributions of Andreas Busjahn and Guo-Hua Li were equal. This study was supported by a grant-in-aid from the Bundesministerium für Bildung und Forschung, Bonn, FRG.

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