Scientific Contributions

# Essential Hypertension in African Caribbeans Associates With a Variant of the $\beta_2$-Adrenoceptor

Peter Kotanko, Alexander Binder, Jacquie Tasker, Perry DeFreitas, Sejal Kamdar, Adrian J.L. Clark, Falko Skrabal, Mark Caulfield

*Abstract* Populations of West African ancestry dwelling in Western communities exhibit greater prevalence of human essential hypertension and higher rates of end-organ damage. The sympathetic nervous system influences cardiac output, vascular tone, renal sodium reabsorption, and renin release and could be implicated in enhanced vascular responsiveness observed in African hypertensives. Such an effect could arise from genetic variants that alter agonist response of $\alpha$-adrenoceptors, leading to enhanced vasoconstriction, or attenuate $\beta_2$-adrenoceptor–mediated vasodilatation. Indeed, there is evidence of a blunted vasodilator response to the $\beta$-agonist isoprenaline in African Americans. A variant of the $\beta_2$-adrenoceptor gene that encodes glycine rather than arginine at position 16 (Arg16→Gly) has been shown to confer exaggerated agonist-mediated receptor downregulation, which might attenuate vasodilator response. One hundred thirty-six unrelated hypertensives and 81 unrelated normotensives of African Caribbean origin were identified from primary care on the island of St Vincent. Genomic DNA from these subjects was analyzed for the presence of the Gly16 and Arg16 alleles by using an allele-specific polymerase chain reaction method. We report strong support for association of the prodownregulatory glycine 16 variant of the $\beta_2$-adrenoceptor gene with hypertension in African Caribbeans from St Vincent and the Grenadines ($\chi^2$=18.9, $P$=.000014, 1 *df*). This observation, coupled with reports of attenuated vasodilator responses to $\beta$-agonists among people of West African ancestry, may provide a mechanism for enhanced vascular reactivity and identify a candidate gene for hypertension in this ethnic group. (***Hypertension.* 1997;30:773-776.**)

***Key Words*** • $\beta_2$-adrenoceptor gene • hypertension • genetics • African ethnicity

Human essential hypertension is thought to arise from an interaction between susceptibility genes and environmental factors. To date, investigation of the genetic basis of hypertension has largely focused on candidate genes from the renin-angiotensin system. Indeed, there is support for linkage and association of the angiotensinogen gene to hypertension in two different populations of white European origin and one population of African Caribbean ethnicity.[1-3] The sympathetic nervous system represents a second important regulator of blood pressure through alterations in vascular responsiveness, renin release, renal sodium handling, and cardiac output.[4] Accordingly, genetic variation in either the $\alpha$-adrenoceptor, leading to enhanced vasoconstriction, or $\beta_2$-adrenoceptors, leading to attenuated vasodilatation, might be important for increasing total peripheral resistance and hence blood pressure. In addition, evidence from cultured skin fibroblasts indicates that the expression of $\beta_2$-receptors in normotensive white Europeans with sodium-responsive blood pressure is less than half of that observed in salt-resistant subjects.[5,6] This observation provides a potential link between sodium sensitivity and the sympathetic nervous system. The greater prevalence of hypertension and end-organ damage, such as stroke and renal disease, in populations of West African ancestry has prompted speculation that there may be ethnic differences in the genetic basis of high blood pressure.[7] This hypothesis is supported by observations in people of African origin that the blood pressure response to sodium loading or mental and physical stress is enhanced.[8,9] The human $\beta_2$-adrenoceptor is a member of the guanosine protein–linked, seven-transmembrane-domain–receptor superfamily and has been extensively studied in terms of physiological and pharmacological functions.[10] An amino-terminal variant that encodes glycine instead of arginine at position 16 (Arg16→Gly) within the receptor exhibits exaggerated agonist-mediated receptor downregulation and could therefore lead to enhanced vascular reactivity.[11,12] In this study, we tested whether there is an association between the Arg16→Gly variant of the $\beta_2$-adrenoceptor and essential hypertension in African Caribbeans from St Vincent and the Grenadines.

Received January 14, 1997; first decision February 10, 1997; revision accepted April 1, 1997.

From the Department of Internal Medicine Krankenhaus der Barmherzigen Brüder and Teaching Hospital of the Karl Franzens University Graz, Austria (P.K., A.B., F.S.); the Departments of Clinical Pharmacology (S.K., M.C.) and Chemical Endocrinology (J.T., A.J.L.C.), St Bartholomew's Hospital, West Smithfield, UK; and the Ministry of Health of St Vincent and the Grenadines, Kingstown, West Indies (P.D.).

Reprint requests to Dr Peter Kotanko, MD, Department of Internal Medicine Krankenhaus der Barmherzigen Brüder and Teaching Hospital of the Karl Franzens University Graz, Marschallgasse 12, A-8020 Graz, Austria.



## Methods

### Study Population

The subjects reported here (Table 1) were recruited from semirural primary care clinics on the island of St Vincent[3] after

**TABLE 1. Demographic Characteristics of the 136 Hypertensive and 81 Normotensive African Caribbeans Expressed as the Median (Interquartile Range)**

| Variable | Hypertensives | Normotensives |
|---|---|---|
| Age, y | 63 (52-70) | 53 (40-62) |
| Sex, M/F | 27/109 | 38/43 |
| Body mass index, kg/m$^2$ | 26.7 (22.5-30.9) | 24 (21.2-27.6) |
| Alcohol consumption, units/wk | 0 (0-0) | 0 (0-1) |
| Systolic blood pressure, mm Hg | 170 (160-190) | 130 (110-130) |
| Diastolic blood pressure, mm Hg | 100 (100-110) | 80 (70-85) |

ethical approval from the Ministry of Health of St Vincent and the Grenadines. The subjects were of African ancestry by grandparental and parental ethnicity. During screening, we identified 136 unrelated hypertensives who had a positive family history of hypertension and whose hypertension was defined by diastolic blood pressure greater than 95 mm Hg or by receipt of antihypertensives with documentary evidence of diastolic blood pressure greater than 95 mm Hg. Simultaneously, we identified 81 unrelated normotensives who had a negative family history of hypertension and whose diastolic blood pressure was less than 85 mm Hg. Genomic DNA from these subjects was extracted using phenol-chloroform purification.

## Genetic Analysis

Genotyping of the $\beta_2$-adrenoceptor variant was cross-checked by an individual who was unaware of the subject's phenotype, and all genotypes were repeated twice to confirm assignment. The polymorphism of the $\beta_2$-adrenoceptor encoding the receptor amino acid at position 16 (Gly16 or Arg16) of the $\beta_2$-adrenoceptor was delineated using an allele-specific polymerase chain reaction (PCR) approach.

PCR reactions were carried out in a volume of 25 $\mu$L using 300 ng of genomic DNA; the primer pairs were: 5′-CTTCTTGCTGGCACCCAATA-3′ (sense) and 5′-ACAATCCACACCATCAGAAT-3′ (antisense) or the same antisense primer and 5′-CTTCTTGCTGGCACCCAATG-3′ (sense). Use of these primers resulted in a product with a molecular size of 452 base pairs. One international unit of Dynazyme (Dynal) was used with reaction buffers supplied by the manufacturer and 1.5 mmol/L magnesium chloride. Amplification was over 30 cycles at 95°C for 30 seconds, 68°C for 35 seconds, and 72°C for 35 seconds. Seven microliters of the PCR reactions was then electrophoresed on 1.5% agarose gels and visualized with ethidium bromide staining and ultraviolet illumination. Each DNA sample was analyzed twice, and direct sequencing of the region containing Arg16→Gly variant was undertaken using *Thermus aquaticus* fluorescent cycle sequencing (*Taq FS* sequencing kit, Perkin-Elmer) on an automated (ABI 377) sequencer (Applied Biosystems) to confirm the robustness of the genotypes in 18 individuals.

## Statistical Analysis

The association of the Arg16→Gly polymorphism and essential hypertension was tested by comparison of the distribution of genotypes and alleles with the $\chi^2$ test. The relative risk of hypertension associated with allelic variation at the $\beta_2$-adrenoceptor gene is expressed in terms of an odds ratio with 95% confidence limits, which was computed according to the method of Miettinen with the application of Yates' correction.

# Results

One hundred thirty-six unrelated hypertensives and 81 unrelated normotensives of African Caribbean origin were identified from primary care on the island of St Vincent (demographic data are summarized in Table 1). Genomic DNA from these subjects was analyzed for the presence of the Gly16 and Arg16 alleles by using an allele-specific PCR method. The distribution of $\beta_2$-adrenoceptor alleles did not conform to Hardy-Weinberg equilibrium in this study. However, the careful ascertainment of hypertensive and control subjects from the same population, coupled with our published observations of Hardy-Weinberg equilibrium in the same population with several diallelic markers at other loci, makes selection bias in our cohorts highly unlikely.[3,13] In addition, all genotypes were repeated twice for confirmation, and these results were cross-checked in 18 individuals by direct sequencing, which confirmed our data in all cases and minimized the risk of laboratory error during genotyping.

There was marked disequilibrium in the distribution of genotypes (Gly16/Gly16, Gly16/Arg16, and Arg16/Arg16) between normotensives and hypertensives ($\chi^2$=14.6, $P$=.0007, 2 *df*), with the number of Gly16 homozygotes markedly increased in the hypertensive subjects (Table 2). The frequency of the Gly16 allele in the hypertensive patients was 0.85 and in the normotensive controls 0.66 ($\chi^2$=18.9, $P$=.000014, 1 *df*). The relative risk of hypertension associated with alleles of the $\beta_2$-adrenoceptor gene was increased, with a corrected odds ratio of 2.74 (95% confidence limits, 1.72 to 4.36).

# Discussion

In this study, we have demonstrated association of a variant within the $\beta_2$-adrenoceptor gene with essential hypertension in African Caribbeans. The frequency of the prodownregulatory Gly16 allele of the $\beta_2$-adrenoceptor gene is considerably higher in hypertensives than in normotensives. This observation may account for $\beta_2$-adrenoceptor regulation in resistance vessels and might explain the blunted forearm vasodilatation

**TABLE 2. Distribution, Expressed as n (%), of the Gly16 and Arg16 Alleles of the Human $\beta_2$-Adrenoceptor in Hypertensive and Normotensive African Caribbeans Exhibiting Marked Linkage Disequilibrium of the Prodownregulatory Gly16 Variant**

| Population | Gly16/Gly16 | Gly16/Arg16 | Arg16/Arg16 |
|---|---|---|---|
| Hypertensives | 101 (74) | 28 (21) | 7 (5) |
| Normotensives | 42 (52) | 24 (30) | 15 (18) |

$\chi^2$=14.6, $P$=.0007, 2 *df*.

that has been reported in normotensive African Americans in response to infusion of the β-adrenoceptor agonist isoprenaline.[14] Such attenuation of the vasodilatory response in people of West African ancestry could contribute to the increase in total peripheral resistance in response to sympathetic stimulation by mental or physical stress and may elevate blood pressure.[9]

This result is very unlikely to be due to ethnic admixture or a population-stratification artifact, since the distribution of other diallelic polymorphisms and blood-group antigens within St Vincent is very similar to that observed in West Africans and African Americans.[3,15] Furthermore, the hypertensives and normotensives in this study were identified from the same semirural clinics. The normotensive subjects were younger in mean age than the hypertensives but were recruited from decades in which hypertension is manifest. Since hypertension has a variable age of onset, it remains possible that some of the normotensives may subsequently develop hypertension, but this would only serve to dilute any association with the trait. Although the numbers of each sex were not equal in each group, it is unlikely that there is a sex-specific influence on the distribution of this intragenic variant of $\beta_2$-adrenoceptor, since the gene is located on chromosome 5.

Evidence for the regulatory influence of the Arg16→Gly variant of the $\beta_2$-adrenoceptor gene stems from transfection experiments in Chinese hamster (CHW-1102) fibroblasts,[12] which indicate identical affinities of either variant for isoprenaline and adrenaline. In contrast, after prolonged exposure to isoprenaline, the Gly16 allele exhibited enhanced agonist-mediated receptor downregulation.[12] Further support for this finding emerges from quantitative in vivo assessment of pulmonary β-adrenoceptor response to the $\beta_2$-agonist salbutamol, demonstrating that the Gly16 variant associates with enhanced agonist-induced receptor downregulation.[16] Indeed, the Gly16 allele has been recently associated with nocturnal asthma,[17] and therefore, diminished receptor number may be an important genetic factor in that asthmatic phenotype.

Additional support for a role of the $\beta_2$-adrenoceptor gene in hypertension arises from a recent report of association of a restriction fragment length polymorphism (RFLP) at this locus with hypertension in African Americans.[18] Although the Arg16→Gly variant, which may have functional influences, was not studied by that group, it is probable that the RFLP alleles are in linkage disequilibrium with this coding alteration.

The distribution of $\beta_2$-adrenoceptor genotypes in this study deviates from Hardy-Weinberg equilibrium. This law describes the expected distribution of genotypes in a population under the assumptions of random mating, with no change in genotype frequencies from one generation to the next and without mutation, migration, or natural selection. When deviations from this equilibrium arise, the possibility of selection bias in establishing the study or incorrectly assigned genotypes due to laboratory error must be considered. As we have indicated, previous studies in this population have conformed to Hardy-Weinberg criteria, and we have genotyped all subjects twice, which makes these possible explanations less likely.[3,13] Interestingly, the distribution of RFLP genotypes of the $\beta_2$-adrenoceptor reported previously also deviates from Hardy-Weinberg equilibrium in a population of African Americans.[18] Furthermore, in the same report from the same population, the genotypic distribution at the $\alpha_2$-adrenoceptor locus did conform to Hardy-Weinberg criteria. In tandem with our data, this finding may reflect the relationship of genotypes at the $\beta_2$-adrenoceptor locus with hypertension rather than population bias or laboratory error.[18]

This study offered 95% power to demonstrate a change in the distribution of the glycine allele from 0.66 in the normotensives to 0.85 in the hypertensives; $P=.0002$. In spite of the power of this study and the observations reported, it is necessary to remain cautious about these results until they are replicated in other populations of West African ancestry.

In conclusion, essential hypertension in African Caribbeans is associated with an increased frequency of the Gly16 allele of the $\beta_2$-adrenoceptor. This variant may predispose to essential hypertension by conferring enhanced agonist-mediated receptor downregulation and represents an additional candidate for the genetic basis of this complex trait.

## Acknowledgments

This work was supported by the Joint Research Board of St Bartholomew's Hospital, the Mason Medical Foundation, the Fellowship of Postgraduate Medicine, St Bartholomew's Hospital Special Trustees, and the Fonds zur Förderung der wissenschaftlichen Forschung, Austria (SFB 007 "Biomembranes"). We thank the people of St Vincent and the Grenadines.

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