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SYNERGISTIC POLYMORPHISMS OF β_1- AND α_{2C}-ADRENERGIC RECEPTORS AND THE RISK OF CONGESTIVE HEART FAILURE

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ABSTRACT

Background Sustained cardiac adrenergic stimulation has been implicated in the development and progression of heart failure. Release of norepinephrine is controlled by negative feedback from presynaptic α_2-adrenergic receptors, and the targets of the released norepinephrine on myocytes are β_1-adrenergic receptors. In transfected cells, a polymorphic α_{2C}-adrenergic receptor (α_{2C}Del322–325) has decreased function, and a variant of the β_1-adrenergic receptor (β_1Arg389) has increased function. We hypothesized that this combination of receptor variants, which results in increased synaptic norepinephrine release and enhanced receptor function at the myocyte, would predispose persons to heart failure.

Methods Genotyping at these loci was performed in 159 patients with heart failure and 189 controls. Logistic-regression methods were used to determine the potential effect of each genotype and the interaction between them on the risk of heart failure.

Results Among black subjects, the adjusted odds ratio for heart failure among persons who were homozygous for α_{2C}Del322–325 as compared with those with the other α_{2C}-adrenergic receptor genotypes was 5.65 (95 percent confidence interval, 2.67 to 11.95; $P<0.001$). There was no increase in risk with β_1Arg389 alone. However, there was a marked increase in the risk of heart failure among persons who were homozygous for both variants (adjusted odds ratio, 10.11; 95 percent confidence interval, 2.11 to 48.53; $P=0.004$). The patients with heart failure did not differ from the controls in the frequencies of nine short tandem-repeat alleles. Among white subjects, there were too few who were homozygous for both polymorphisms to allow an adequate assessment of risk.

Conclusions The α_{2C}Del322–325 and β_1Arg389 receptors act synergistically to increase the risk of heart failure in blacks. Genotyping at these two loci may be a useful approach for identification of persons at risk for heart failure or its progression, who may be candidates for early preventive measures. (N Engl J Med 2002;347:1135-42.)

IDIOPATHIC dilated cardiomyopathy and ischemic cardiomyopathy are the major causes of congestive heart failure in the United States. The pathogenesis of the former is unknown; the latter is caused by ischemia or infarction from coronary artery disease. Regardless of the initial insult, studies suggest that sustained sympathetic drive, which is a consequence of depressed cardiac output, plays a part in the progression of heart failure.[1-3] However, there is substantial variation among persons in the expression and function of adrenergic receptors, the development and progression of heart failure, and the response to beta-blocker therapy.[4-7]

We undertook a study to determine whether functional polymorphisms of selected adrenergic receptors may be important factors in such variation. The basis of our hypothesis is summarized in Figure 1. Prejunctional α_2-adrenergic receptors (the α_{2A} and α_{2C} subtypes) regulate the release of norepinephrine from cardiac sympathetic nerves.[8,9] A common coding polymorphism of the gene for the α_{2C}-adrenergic receptor — the deletion of four consecutive amino acids (Del322–325) — results in a substantial loss of agonist-mediated receptor function in transfected cells.[10] A loss of normal synaptic autoinhibitory feedback caused by this dysfunction would result in enhanced presynaptic release of norepinephrine.[9,11,12] We therefore hypothesized that persons with this α_{2C}-adrenergic–receptor variant would be at increased risk for heart failure.

Norepinephrine released from cardiac sympathetic nerves activates myocyte β_1-adrenergic receptors,

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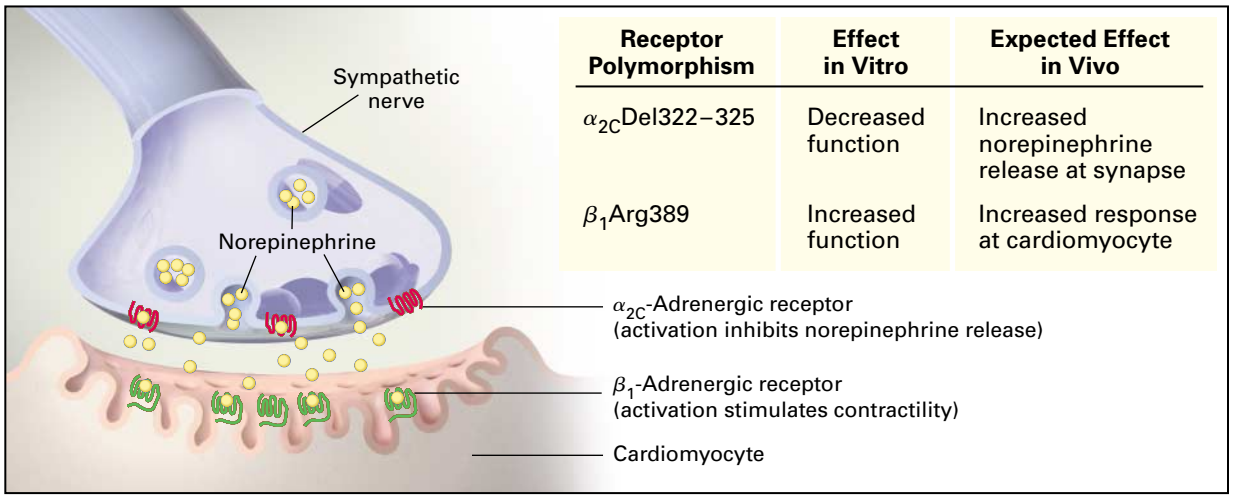

Receptor Polymorphism	Effect in Vitro	Expected Effect in Vivo
α_{2C}Del322–325	Decreased function	Increased norepinephrine release at synapse
β_1Arg389	Increased function	Increased response at cardiomyocyte

Figure 1. Basis of the Hypothesis That the α_{2C}Del322–325 and β_1Arg389 Receptors Act Synergistically as Risk Factors for Heart Failure.
The α_{2C}-adrenergic receptor (along with the α_{2A}-adrenergic receptor) inhibits norepinephrine release at cardiac presynaptic nerve endings through negative feedback. The presence of the dysfunctional α_{2C}Del322–325 receptor would be expected to result in enhanced norepinephrine release. The β_1-adrenergic receptor is the receptor for norepinephrine on the cardiomyocyte, and the presence of the hyperfunctional β_1Arg389 receptor would be expected to increase contractile response at the myocyte. The combination of increased norepinephrine release and increased responsiveness of the receptor was hypothesized to be a risk factor for heart failure.

which couple to the stimulatory G protein G_s, activating adenylyl cyclase and increasing intracellular cyclic AMP (cAMP). Through the subsequent phosphorylation of several intracellular proteins by means of the cAMP-dependent protein kinase A, such activation of β_1-adrenergic receptors culminates in an increase in cardiac inotropy, lusitropy, and chronotropy. Two common polymorphisms of β_1-adrenergic receptors in the human population lead to either a glycine (β_1Gly389) or an arginine (β_1Arg389) at amino acid position 389 within a G_s-coupling domain.[13] In a recombinant cell-based expression system, β_1Arg389 has a much greater ability to couple to adenylyl cyclase than does β_1Gly389.[13] We thus considered the possibility that β_1Arg389 might also predispose persons to heart failure. We hypothesized that persons with both the α_{2C}Del322–325 and the β_1Arg389 variants would have the greatest risk of heart failure, since norepinephrine release and β_1-adrenergic–receptor activity would be simultaneously enhanced. To investigate whether these variants alone or in combination represent risk factors for the development of heart failure, we genotyped these loci in 348 subjects: 159 patients with well-characterized heart failure and 189 controls.

METHODS

Study Subjects

The protocol was approved by the institutional review board of the University of Cincinnati, and subjects provided written informed consent. Both control subjects and patients with heart failure were from the greater Cincinnati area. Patients were recruited from the University of Cincinnati Heart Failure Program between January 2, 1999, and January 2, 2001, through the enrollment of consecutive eligible patients who agreed to participate in the genetic study. Approximately 50 percent of patients in the Heart Failure Program are referred by community cardiologists, approximately 40 percent are referred by physicians within this tertiary-care center, and approximately 10 percent are self-referred. The criteria for enrollment were an age of 20 to 79 years, a left ventricular ejection fraction of less than 35 percent, heart failure of New York Heart Association class II, III, or IV, and a diagnosis of either idiopathic dilated cardiomyopathy or ischemic cardiomyopathy. Patients with nonischemic dilated cardiomyopathy who had antecedent hypertension were characterized as having idiopathic dilated cardiomyopathy. Patients whose heart failure was due to primary valvular disease, myocarditis, or obstructive or hypertrophic cardiomyopathies were not eligible. The control group consisted of persons who were unrelated to one another and were apparently healthy (as assessed by questionnaire); controls were recruited before voluntary blood donation and by newspaper advertisements. Specifically, none of the controls had a history or symptoms of cardiovascular disease or had been taking any medications for long periods. The racial classification of the participants was self-reported.

Genotyping

Genomic DNA was extracted from samples of peripheral blood, and the adrenergic-receptor polymorphisms were detected as previously described.[14] The adrenergic-receptor genotypes are referred to as wild-type α_{2C}-adrenergic receptor (the more common variant without the deletion), α_{2C}Del322–325 (the variant with the deletion of four amino acids), β_1Arg389, and β_1Gly389. To test for population stratification within the two racial groups,[15] the frequencies of alleles at nine highly polymorphic short tandem-repeat loci[16] were determined by multiplexed polymerase chain reactions with detection by multicolor fluorescence (ABI Prism 377 Sequencer, Applied Biosystems).

Statistical Analysis

Allele frequencies were computed by standard gene-counting methods. Chi-square tests of independence within each racial group were used to test for associations between heart failure and genotype or allele. In order to test for interactions between the α_{2C}-adrenergic–receptor and β_1-adrenergic–receptor polymorphisms, we used logistic-regression methods[17] to model the effect of each genotype and their interaction on the risk of heart failure. Likelihood-ratio tests were used to assess the influence of each locus and the interaction between them both before and after adjustment for the potential confounding effects of age and sex. Finally, we used chi-square tests of independence to perform an analysis involving only the patients with heart failure in order to test for associations of single-locus genotype and two-locus genotype with hypertension status and diagnostic group (idiopathic or ischemic). Chi-square tests were used to compare the patients with heart failure with the controls within each racial group in terms of the frequencies of the short tandem-repeat alleles. When appropriate, results are reported as means ±SD. Kaplan–Meier plots and log-rank tests were used to assess whether survival differed significantly among subgroups defined according to genotype.

RESULTS

Characteristics of the Subjects

The characteristics of the patients with heart failure are shown in Table 1. There were 78 black patients with heart failure (mean age, 49±12 years) and 84 black controls (mean age, 53±16 years). There were 81 white patients with heart failure (mean age, 55±11 years) and 105 white controls (mean age, 36±12 years). As we have previously noted,[10,18] there are significant differences between blacks and whites in the allele frequencies of both receptor variants. In the current study, the α_{2C}Del322–325 variant was more than 10 times as common among black controls as among white controls (allele frequency, 0.411 vs. 0.038; $P<0.001$). The β_1Arg389 variant was somewhat less common among black controls than among white controls (frequency, 0.560 vs. 0.762; $P<0.001$). These racial differences in the frequencies of the two polymorphisms, particularly the difference in the frequency of α_{2C}Del322–325, prompted us to perform separate risk analyses for the two racial groups.

TABLE 1. CHARACTERISTICS OF THE PATIENTS WITH HEART FAILURE.*

CHARACTERISTIC	WHITE PATIENTS (N=81)	BLACK PATIENTS (N=78)
Age (yr)	54.6±11.1	48.9±11.5
Male sex (%)	76.5	59.0
NYHA class III or IV (%)	47.5	47.4
Diagnosis (%)		
Idiopathic dilated cardiomyopathy	45.7	83.3
Ischemic cardiomyopathy	54.3	16.7
Age at onset of heart failure (yr)	51.7±10.7	46.3±11.8
Duration of heart failure (yr)	2.62±4.35	2.62±4.77
LVEF at enrollment (%)	24.8±12.6	25.4±11.9
Died after enrollment (%)	25.9	26.9
Received transplant after enrollment (%)	14.8	9.0
Other risk factors or coexisting conditions (%)		
Hypertension	44.8	61.0
Diabetes mellitus	30.9	25.6
History of hypercholesterolemia	44.4	23.1
Obesity	72.5	66.2
Smoking (%)		
History of ≥10 pack-yr	70.8	58.6
Current	17.5	23.4
Medications at entry (%)		
Digoxin	76.5	50.0
Diuretics	91.4	56.4
ACE inhibitor	82.7	92.3
Beta-blocker	60.5	33.3

*Plus–minus values are means ±SD. Hypertension was defined by a blood pressure of 140/90 mm Hg or higher, hypercholesterolemia by a total cholesterol level of 240 mg per deciliter (6.2 mmol per liter) or higher, and obesity by a body-mass index (the weight in kilograms divided by the square of the height in meters) of more than 25. NYHA denotes New York Heart Association, LVEF left ventricular ejection fraction, and ACE angiotensin-converting enzyme.

Association between Polymorphisms and Heart Failure

Given the biologic properties of the polymorphic receptors, the primary hypothesis of the study was that α_{2C}Del322–325, β_1Arg389, and particularly, the combination of the two influence the risk of heart failure. In black persons, among whom both variants are relatively common, single-locus analysis (Table 2) revealed that α_{2C}Del322–325 was more common among patients with heart failure (allele frequency, 0.615) than among controls (allele frequency, 0.411; $P<0.001$). When all three possible genotypes were analyzed together, the association with heart failure remained significant (Table 2). Indeed, 52.6 percent of the black patients with heart failure were homozygous for the α_{2C} polymorphism as compared with only 16.6 percent of the black controls. The unadjusted odds ratio for heart failure among subjects who were homozygous for α_{2C}Del322–325 as compared with subjects who were not was 5.54 (95 percent confidence interval, 2.68 to 11.45; $P<0.001$). There was no evidence of significant confounding by age or sex, and the sex- and age-adjusted odds ratio for heart failure among subjects who were homozygous for α_{2C}Del322–325 was 5.65 (95 percent confidence interval, 2.67 to 11.95; $P<0.001$). There was no evidence of a statistically significant difference between black patients with heart failure and black controls in the frequency of the β_1Arg389 allele (Table 2).

A two-locus analysis indicated a significant interaction between the α_{2C}Del322–325 and β_1Arg389 genotypes in black patients with heart failure. The combination of the two genotypes had a multiplicative association (i.e., more than an additive effect) with the risk of heart failure ($P=0.05$ by the likelihood-ratio test for interaction). Subjects were divided into four subgroups as follows: those who were homozy-

TABLE 2. DISTRIBUTION OF α_{2C}-ADRENERGIC–RECEPTOR VARIANTS AND β_1-ADRENERGIC–RECEPTOR VARIANTS AMONG CONTROLS AND PATIENTS WITH HEART FAILURE.*

ALLELES AND SUBJECTS	ALLELE FREQUENCY	P VALUE	GENOTYPE			P VALUE	ADJUSTED ODDS RATIO FOR HEART FAILURE (95% CI)†
			WT/WT	WT/Del	Del/Del		
			no./total no. (%)				
α_{2C}Del322–325							
Black subjects		<0.001				<0.001	5.65 (2.67–11.95)
Controls	0.411		29/84 (34.5)	41/84 (48.8)	14/84 (16.6)		
Patients with heart failure	0.615		23/78 (29.5)	14/78 (17.9)	41/78 (52.6)		
White subjects		0.01				0.13	3.94 (0.50–31.05)
Controls	0.038		99/105 (94.3)	4/105 (3.8)	2/105 (1.9)		
Patients with heart failure	0.105		70/81 (86.4)	5/81 (6.2)	6/81 (7.4)		
			Gly/Gly	Gly/Arg	Arg/Arg		
			no./total no. (%)				
β_1Arg389							
Black subjects		0.54				0.27	0.90 (0.44–1.84)
Controls	0.560		13/84 (15.5)	48/84 (57.1)	23/84 (27.4)		
Patients with heart failure	0.526		19/78 (24.4)	36/78 (46.2)	23/78 (29.5)		
White subjects		0.64				0.36	0.80 (0.37–1.73)
Controls	0.762		8/105 (7.6)	34/105 (32.4)	63/105 (60.0)		
Patients with heart failure	0.741		4/81 (4.9)	34/81 (42.0)	43/81 (53.1)		

*P values for the comparisons of allele frequencies between controls and patients with heart failure were determined by two-by-two chi-square tests; P values for the comparisons of genotype distributions between controls and patients with heart failure were determined by two-by-three chi-square tests. CI denotes confidence interval, WT wild-type α_{2C}-adrenergic receptor, Del α_{2C}Del322–325, Gly β_1Gly389, and Arg β_1Arg389.

†Odds ratios are for heart failure among subjects who were homozygous for the given polymorphism as compared with those who were not; odds ratios are adjusted for sex and age.

gous for both α_{2C}Del322–325 and β_1Arg389; those who were homozygous for α_{2C}Del322–325 only; those who were homozygous for β_1Arg389 only; and those who were not homozygous for either variant (the reference group). Among black subjects, homozygosity for α_{2C}Del322–325 and β_1Arg389 was associated with a substantially increased risk of heart failure relative to the reference group (unadjusted odds ratio, 12.67; 95 percent confidence interval, 2.70 to 59.42; P=0.001) (Table 3). When age and sex were controlled for in the model, the odds ratio was reduced slightly but remained significant (adjusted odds ratio, 10.11; 95 percent confidence interval, 2.11 to 48.53; P=0.004). To assess whether these findings could be explained by differences in the frequency of this two-locus genotype according to diagnostic group (idiopathic dilated cardiomyopathy vs. ischemic cardiomyopathy) or according to the hypertension status of patients with heart failure, we performed an analysis involving only the patients with heart failure. Among the black patients, there were no differences in the frequency of the dual-polymorphism genotype between the two subgroups defined according to diagnosis (chi-square = 1.38, P = 0.71) or between patients with hypertension and those without hypertension (chi-square = 0.34, P = 0.95).

Among white subjects, the frequency of the α_{2C}Del322–325 allele was higher among patients with heart failure than among controls (0.105 vs. 0.038, P=0.01) (Table 2). Analysis in which all three possible genotypes were considered did not show a statistically significant association with heart failure, and the unadjusted odds ratio for heart failure among white subjects who were homozygous for α_{2C}Del322–325 relative to those who were not was 4.12 (95 percent confidence interval, 0.89 to 20.98; P=0.08). The sex- and age-adjusted odds ratio was 3.94 (95 percent confidence interval, 0.50 to 30.05; P=0.13). Although these odds ratios are not statistically significant, they are similar in magnitude and of the same direction as the estimate for blacks of 5.65. The lack of statistical significance in the analysis of white subjects may be due to the small number of white subjects who were homozygous for α_{2C}Del322–325 (two controls and six patients with heart failure). As was the case with black subjects, the frequency of the β_1Arg389 variant did not differ significantly between white patients with heart failure and white controls. There was no associ-

ation between the genotype consisting of α_{2C}Del322–325 and β_1Arg389 and the risk of heart failure in white subjects.

Testing for Genetic Stratification of the Populations

The possibility that there was unequal genetic admixture in the control and patient populations, which could have resulted in a spurious association in black subjects,[15] was explored by means of genotyping at nine highly polymorphic short tandem-repeat loci (Fig. 2 and Table 4). Since all subjects were from the same geographic area and associations were sought within racial groups, the likelihood of such stratification of the populations was considered a priori to be

TABLE 3. GENOTYPE AND GENE–GENE INTERACTIONS OF α_2- AND β_1-ADRENERGIC–RECEPTOR VARIANTS IN RELATION TO HEART FAILURE.*

RACIAL GROUP	α_{2C}-ADRENERGIC RECEPTOR	β_1-ADRENERGIC RECEPTOR	CONTROLS	PATIENTS WITH HEART FAILURE	ADJUSTED ODDS RATIO FOR HEART FAILURE (95% CI)	P VALUE
			no. of subjects			
Black subjects			84	78		
	≥1 Wild-type	≥1 Gly389	49	29	1.00	
	≥1 Wild-type	Arg389/Arg389	21	8	0.55 (0.21–1.44)	0.23
	Del322–325/Del322–325	≥1 Gly389	12	26	3.87 (1.65–9.05)	0.002
	Del322–325/Del322–325	Arg389/Arg389	2	15	10.11 (2.11–48.53)	0.004
White subjects			105	81		
	≥1 Wild-type	≥1 Gly389	42	35	1.00	
	≥1 Wild-type	Arg389/Arg389	61	40	0.85 (0.39–1.85)	0.68
	Del322–325/Del322–325	≥1 Gly389	0	3	Undefined	—
	Del322–325/Del322–325	Arg389/Arg389	2	3	2.14 (0.13–36.85)	0.60

*Subjects with at least one wild-type α_{2C}-adrenergic receptor allele and at least one β_1Gly389 allele served as the reference group. Odds ratios are adjusted for sex and age. In the analysis of white subjects, because there were no subjects in one of the cells, the odds ratios for the other two genotypes represent single (two-by-two) comparisons with the reference group.

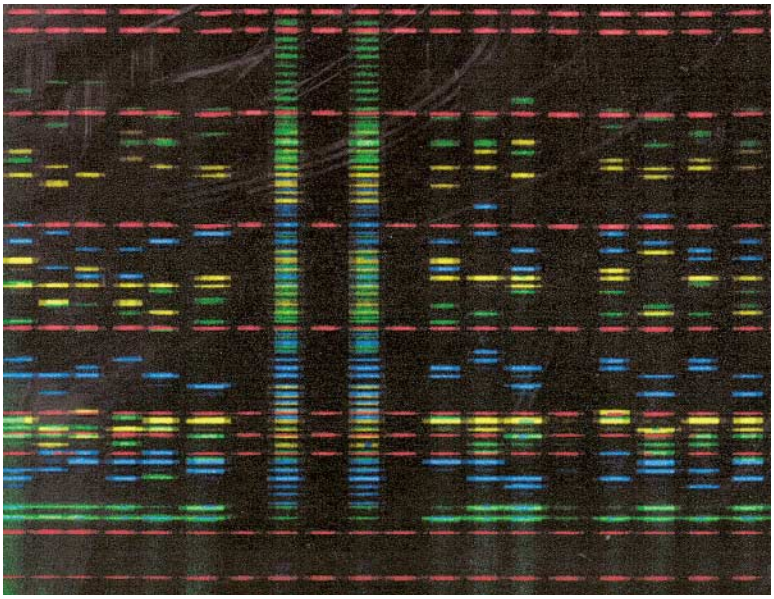

Figure 2. Multiple PCR Detection of Short Tandem-Repeat Alleles.

The middle two lanes are ladders that represent all possible alleles from nine short tandem-repeat loci. Each multicolored lane represents fluorescence output from a single patient, which is scored by a computer algorithm. The red signals are molecular-size markers. The results shown are representative of output from a single gel.

TABLE 4. FREQUENCIES OF SHORT TANDEM-REPEAT ALLELES IN BLACK CONTROLS AND BLACK PATIENTS WITH HEART FAILURE.*

LOCUS AND ALLELE	CONTROLS	PATIENTS WITH HEART FAILURE	P VALUE
D3S1358			0.53
15	0.250	0.227	
16	0.381	0.340	
17	0.169	0.240	
Other	0.050	0.067	
D21S11			0.47
27	0.094	0.067	
28	0.206	0.240	
29	0.175	0.240	
30	0.125	0.153	
31	0.100	0.067	
31.2	0.050	0.040	
32.2	0.119	0.067	
Other	0.131	0.127	
D13S317			0.48
11	0.272	0.304	
12	0.418	0.466	
13	0.146	0.108	
Other	0.165	0.122	
vWA			0.73
14	0.099	0.093	
15	0.191	0.193	
16	0.276	0.233	
17	0.237	0.220	
18	0.105	0.100	
19	0.033	0.067	
Other	0.059	0.093	
FGA			0.45
19	0.068	0.074	
20	0.041	0.101	
21	0.103	0.122	
22	0.171	0.169	
23	0.178	0.169	
24	0.171	0.149	
25	0.096	0.108	
27	0.062	0.020	
Other	0.110	0.088	
D5S818			0.40
8	0.063	0.033	
10	0.050	0.040	
11	0.263	0.227	
12	0.294	0.407	
13	0.275	0.240	
Other	0.056	0.053	
D18S51			0.29
12	0.056	0.081	
13	0.056	0.068	
14	0.056	0.054	
15	0.160	0.182	
16	0.215	0.182	
17	0.090	0.182	
18	0.118	0.095	
19	0.132	0.088	
Other	0.118	0.068	
D7S820			0.78
8	0.230	0.264	
9	0.079	0.074	
10	0.316	0.311	
11	0.243	0.257	
12	0.099	0.054	
Other	0.033	0.041	
D8S1179			0.31
12	0.094	0.107	
13	0.163	0.253	
14	0.394	0.387	
15	0.231	0.153	
16	0.050	0.047	
Other	0.069	0.050	

*Alleles at a given short tandem-repeat locus with frequencies of less than 0.05 in both groups were combined and denoted as "other." P values were derived from chi-square tests comparing the allele frequency between controls and patients with heart failure.

low. There were no significant differences between black controls and black patients with heart failure in the frequencies of these markers (Table 4 and Fig. 2), indicating that genetic stratification does not account for our finding of an association.

Testing for Survivor Effect

Finally, the unlikely possibility that the results in black subjects were biased by early death attributable to genotype (i.e., a "survivor effect") was considered in four analyses. Contingency tables were used to assess the relations between genotype and the age at onset of heart failure, genotype and distribution of left ventricular ejection fraction, and genotype distribution at enrollment. Survival analysis was used to test for an association between genotype and survival after enrollment. Subjects with the various α_{2C}-adrenergic–receptor genotypes did not differ in terms of age at the time of enrollment in the study (data not shown). However, the odds ratio for the onset of heart failure before 40 years of age among carriers of α_{2C}Del322–325 as compared with subjects who were homozygous for the wild-type α_{2C}-adrenergic receptor was 4.07 (95 percent confidence interval, 1.25 to 13.30; P=0.02). Further analysis used the median left ventricular ejection fraction among all black patients with heart failure (22.0 percent) to define two groups with different predicted mortality rates.[19] The odds ratio for a left ventricular ejection fraction of 22.0 percent or lower among patients who were homozygous for α_{2C}Del322–325 as compared with those who were homozygous for the wild-type α_{2C}-adrenergic receptor was 3.63 (95 percent confidence interval, 1.17 to 11.22; P=0.03). Furthermore, Kaplan–Meier survival analysis indicated that the survival distribution after enrollment did not vary according to the α_{2C}-adrenergic–receptor genotype (P=0.23). These results do not suggest the influence of a survivor effect and support the conclusion that it is the α_{2C}Del322–325 allele, as opposed to the wild-type α_{2C}-adrenergic re-

ceptor, that is associated with the heart-failure phenotype.

DISCUSSION

In this study, we identified genetic variants of the β_1-adrenergic receptor and the α_{2C}-adrenergic receptor that jointly represent a major risk factor for the development of heart failure. In black subjects, among whom the α_{2C}Del322–325 and β_1Arg389 polymorphisms are relatively common, the α_{2C}Del322–325 genotype alone represented some degree of risk (odds ratio for heart failure, 5.65), whereas the β_1Arg389 genotype alone was not associated with heart failure. However, when the two polymorphisms occurred together in the homozygous state, the risk was substantial and significant, with an adjusted odds ratio of 10.11. Given the low prevalence of the α_{2C}Del322–325 polymorphism among the white subjects,[10] we did not expect to find a significant association in this racial group after further stratification according to β_1-adrenergic–receptor genotype. Nevertheless, among the white subjects, the frequency of the α_{2C}Del322–325 allele was indeed greater among patients with heart failure than among controls. On the basis of this observation and the molecular properties of the α_{2C}Del322–325 and β_1Arg389 receptors that have been delineated in transfected cells, we suggest that the findings for this two-locus genotype in black subjects are most likely applicable to the white population as well, but the extent of the risk in this racial group remains less well defined.

We explored these two genes as candidate risk factors for heart failure because of the results of a number of basic studies as well as studies in animals and humans. α_2-Adrenergic receptors expressed on presynaptic cardiac sympathetic nerves in humans inhibit the release of the neurotransmitter norepinephrine.[8] Studies of mice in which the genes for α_{2A}-adrenergic receptors and α_{2C}-adrenergic receptors have been inactivated indicate that the α_{2C}-adrenergic receptor inhibits norepinephrine release under basal conditions (i.e., with low stimulation frequencies).[9] Severe cardiomyopathy develops in such mice.[9] These studies thus suggest that factors that depress α_{2C}-adrenergic–receptor function, leading to sustained norepinephrine release, might represent factors predisposing persons to the development of heart failure. The human α_{2C}-adrenergic–receptor polymorphism Del322–325 results in the deletion of four amino acids within a G-protein–coupling domain, which greatly decreases the function of these receptors (by approximately 85 percent) in transfected cells.[10]

The β_1-adrenergic receptor is the predominant β-adrenergic receptor expressed on the cardiomyocyte and is responsive to circulating epinephrine and to local norepinephrine derived from cardiac sympathetic nerves.[1,2] In rodents, sustained activation of β_1-adrenergic receptors from infusions of β-agonists results in hypertrophy,[20] and transgenic cardiac overexpression of β_1-adrenergic receptors causes progressive cardiomyopathy and heart failure.[21,22] We therefore considered the β_1Arg389 receptor as a possible risk factor for heart failure, since it results in an increase of approximately 200 percent in agonist-stimulated activity in transfected cells as compared with the β_1Gly389 receptor.[13]

Our results revealed a substantial risk of heart failure in black subjects who were homozygous for both polymorphisms. The interaction between the two variants is most likely attributable to the fact that the receptors represent two critical signal-transduction pathways in series: local norepinephrine production and norepinephrine-induced activation of a target receptor. The synergistic, rather than simply additive, nature of the interaction may derive from the fact that activation of receptors that couple to G proteins involves marked signal amplification.[23,24] As more polymorphisms of genes within multistep pathways are detected and characterized, other examples of such interactions may become apparent.

Our study raises a number of provocative questions regarding the potential clinical usefulness of determining these genotypes in patients with heart failure or left ventricular hypertrophy and in asymptomatic persons without evidence of cardiac pathophysiology. Given the magnitude of the findings, and the signaling properties of these variant receptors in vitro, the presence of this specific two-locus genotype may indicate the need for targeted pharmacologic therapy with α_2-adrenergic–receptor agonists, β-adrenergic–receptor antagonists, or both. The current study was not designed to ascertain these potential effects of genotype on the response to treatment. Clinical studies with α_2-adrenergic–receptor agonists such as clonidine have not shown a consistent improvement in indexes of cardiac output.[25-29] The observed heterogeneity in agonist response may have been caused by the presence in some subjects of the α_{2C}-adrenergic–receptor polymorphism. Although β-adrenergic–receptor antagonists have been shown to be efficacious in the treatment of heart failure, there is nevertheless a broad range of responsiveness, and even in those who ultimately benefit, a prolonged and complex period of titration (two to four months) is required.[7]

Patients with the combined α_{2C}Del322–325 and β_1Arg389 genotype may represent a subgroup of patients with a differential response to treatment, and thus genotyping at these loci could be used to tailor pharmacologic therapy to those with the greatest likelihood of having a favorable outcome. Indeed, it could be argued that such persons might benefit from treatment at early stages of the syndrome, even if they have

relatively preserved left ventricular ejection fraction and minimal symptoms, since they may be at the greatest risk for progression. A similar approach might also be indicated in persons with asymptomatic left ventricular hypertrophy, with the objective of halting the transition to clinical heart failure. Finally, one must consider whether persons without left ventricular hypertrophy or heart failure who are homozygous for both polymorphisms and are therefore at risk for heart failure might benefit from prophylaxis. Clinical studies will be required in order to establish whether specific pharmacologic therapy can modify the risk of heart failure, the transition of hypertrophy to heart failure, or the decompensation of stable heart failure in those who are homozygous for α_{2C}Del322–325 and β_1Arg389.

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