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Adrenergic-Pathway Gene Variants Influence Beta-Blocker–Related Outcomes After Acute Coronary Syndrome in a Race-Specific Manner

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Objectives Overcoming racial differences in acute coronary syndrome (ACS) outcomes is a strategic goal for U.S. health care. Genetic polymorphisms in the adrenergic pathway seem to explain some outcome differences by race in other cardiovascular diseases treated with β-adrenergic receptor blockade (BB). Whether these genetic variants are associated with survival among ACS patients treated with BB, and if this differs by race, is unknown.

Background β-adrenergic receptor blockade after ACS is a measure of quality care, but the effectiveness across racial groups is less clear.

Methods A prospective cohort of 2,673 ACS patients (2,072 Caucasian; 601 African-American) discharged on BB from 22 U.S. hospitals were followed for 2 years. Subjects were genotyped for polymorphisms in *ADRB1, ADRB2, ADRA2C,* and *GRK5*. We used proportional hazards regression to model the effect of genotype on mortality, stratified by race and adjusted for baseline factors.

Results The overall 2-year mortality rate was 7.5% for Caucasians and 16.7% for African Americans. The prognosis associated with different genotypes in these BB-treated patients differed by race. In Caucasians, *ADRA2C* 322-325 deletion carriers had significantly lower mortality as compared with homozygous individuals lacking the deletion (hazard ratio: 0.46; confidence interval [CI]: 0.21 to 0.99; $p = 0.047$; race × genotype interaction $p = 0.053$). In African Americans, the *ADRB2* 16R allele was associated with significantly increased mortality (hazard ratio for RG vs. GG: 2.10; CI: 1.14 to 3.86; RR vs. GG: 2.65; CI: 1.38 to 5.08; $p = 0.013$; race × genotype interaction $p = 0.096$).

Conclusions Adrenergic pathway polymorphisms are associated with mortality in ACS patients receiving BB in a race-specific manner. Understanding the mechanism by which different genes impact post-ACS mortality differently in Caucasians and African Americans might illuminate opportunities to improve BB therapy in these groups. (J Am Coll Cardiol 2012; 60:898–907) © 2012 by the American College of Cardiology Foundation

More than 1.6 million patients are hospitalized in North America each year with acute coronary syndrome (ACS) (acute myocardial infarction [MI] or unstable angina [UA]) (1). Evidence-based practice guidelines, developed jointly by the American Heart Association and American College of Cardiology, recommend that β-blocker (BB) therapy be initiated in all patients without a contraindication (2,3), and administration of BB after MI is an accepted performance measure of high-quality health care (4). These recommendations are based on results of older trials with less diverse cohorts, whereas subsequent larger studies in other racial groups (5) as well as meta-analyses (6) have questioned the consistency of BB benefit across the spectrum of MI patients, with the potential for hazard observed in at least 1 study (5). It has been hypothesized that a portion of this variability might be due to genetic variation, which is of particular interest, given the intersection of race with genetics and the disparities in ACS outcomes in the United

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States (7). Identifying genetic predictors of BB-related outcomes in ACS patients could allow more targeted prognostic risk estimates and, hopefully, support more appropriate use of therapy to reduce the public health burden of ACS and heterogeneity of outcomes.

Support for genetic variation contributing to differences in BB response is available in the case of systolic heart failure, where although BBs are a cornerstone of standard therapy due to their overall mortality benefit (8–10), substantial heterogeneity of individual responses—related in part to genetic sequence variants in the adrenergic pathway—have been documented (11–13). For example, functional genetic variants within genes encoding the β-adrenergic receptor 1 (*ADRB1* R389G; rs1801253), the β-adrenergic receptor 2 (*ADRB2* G16R; rs1042713 and *ADRB2* Q27E; rs1042714), the alpha-2c receptor (*ADRA2C* 322-325 deletion; rs2234888), and—more recently—G-protein receptor kinase 5 (*GRK5* L41Q; rs17098707), a critical regulator of β-receptor function, are associated with BB response in heart failure (13–18). These data raise the question as to whether functional adrenergic genetic variants might influence BB-related outcomes after ACS. Consistent with this notion is our previous work, from an exploratory analysis of a small ACS cohort, which suggested an association of *ADRB2* genotypes with survival in patients treated with BB (19). However, this study was inadequately powered to accurately define the importance of each variant within racial subgroups. That most of the candidate variants differ in allele frequency in Caucasians, as compared with African Americans, underscores the importance of defining race-specific associations between genetic variants and outcomes. To address this existing gap in knowledge, we report the results of a large, prospective, longitudinal analysis of the effect of genetic variants on mortality in patients who suffered an ACS and were treated with BB, stratified by race. This analysis represents the first comprehensive study of previously reported functional adrenergic pathway genetic variants in an ACS cohort.

Methods

Subjects. Between March 2001 and December 2008, 8,037 patients with ACS (7,517 acute MI, 520 UA) from 31 U.S. hospitals were prospectively screened and enrolled into 3 consecutive observational cohort studies: 1) the INFORM (INvestigation oF Outcomes from acute coronary syndRoMes) study (n = 1,199) from March 2001 to October 2002; 2) the PREMIER (Prospective Registry Evaluating outcomes after Myocardial Infarction: Events and Recovery) study (n = 2,498) from January 2003 to June 2004; and 3) the TRIUMPH (Translational Research Investigating Underlying disparities in acute Myocardial infarction Patients' Health status) study (n = 4,340) from April 2005 to December 2008, as previously described (19–22). The MI patients were identified by an elevated troponin blood test and either diagnostic electrocardiogram changes or ischemic symptoms. The UA patients had a normal troponin but presented with electrocardiogram changes (left bundle branch block 4%, ST-segment elevations 9%, ST-segment depression 12%, T-wave inversions 22%) and cardiac symptoms, defined as new-onset angina (<2 months) of at least Canadian Cardiovascular Society Classification class III, prolonged (>20 min) rest angina, recent (<2 months) worsening of angina, or angina that occurred within 2 weeks of a previous MI (23). To further increase the specificity of the UA diagnosis, those patients with a diagnostic study that excluded obstructive coronary disease (e.g., coronary angiography, nuclear or echocardiographic stress testing) (n = 125) or confirmed an alternative explanation for their presentation (e.g., esophagogastroduodenoscopy) were excluded. Three physicians adjudicated the charts of those patients with diagnostic uncertainty (n = 45) and attained consensus on the final diagnosis.

Abbreviations and Acronyms

ACS = acute coronary syndrome(s)
BB = β-adrenergic receptor blockade
CI = confidence interval
HR = hazard ratio
I/D = insertion/deletion
MI = myocardial infarction
UA = unstable angina

Within these combined cohorts, a subset of 3,373 patients were approached for and consented to genetic testing and were genotyped for adrenergic pathway polymorphisms. The genetics cohort was representative of the entire cohort (24). Of these, 2,946 (87%) were discharged alive on BB and were included in the present analyses. We further excluded 94 patients who were either transferred to other hospitals or who left against medical advice because their use of BB at discharge could not be definitively ascertained. Finally, given the large frequency differences for several genotypes of interest across race, we restricted the analyses to self-identified Caucasian and African-American patients, yielding a final sample size of 2,673 patients (2,469 MI; 204 UA; 2,072 Caucasians; 601 African Americans) from 22 centers.

Each patient was prospectively interviewed during hospital stay to ascertain their sociodemographic (including self-identified race), economic, and health status characteristics. Detailed chart abstractions were performed to obtain medical history of patients, laboratory results, disease severity, and the processes of inpatient care. All 3 studies received institutional review board approval at all participating sites, and written informed consent was obtained from each participant.

Mortality assessment. The Social Security Administration Death Master File was queried to determine vital status of patients as of December 30, 2009, and was available for all patients in this study.

Genotyping. Deoxyribonucleic acid (DNA) was isolated and purified from whole blood with the Qiagen QIAamp DNA purification kit (Quiagen, Germantown, Maryland). The DNA segments containing the region of interest were amplified with the polymerase chain reaction (PCR). The

PCR primers were designed with Primer3 online software (25), and pyrosequencing primers were designed with the Pyrosequencing SNP Primer Design software (version 1.01, QIAGEN, Hilden, Germany). Before use, PCR primer sequences were screened across the human genome with the National Center for Biotechnology Information Basic Local Alignment Search Tool program to ensure their specificity for the gene of interest. The PCR was carried out with Amplitaq Gold PCR master mix (ABI, Foster City, California), 1 pmol of each primer (IDT, Coralville, Iowa), and 1 ng of DNA. The PCR primers and conditions are listed in Online Table S1. Pyrosequencing was performed with the PSQ_HS 96A system with MA v2.0 software (Qiagen, Kungsgatan, Germany), as previously described (26). Data were automatically transferred from the PSQ_HS 96A to a Microsoft Access (Richmond, Washington) database for permanent storage and merging with the clinical datasets through SAS (version 9.1, SAS, Cary, North Carolina).

Statistical analyses. Baseline and follow-up characteristics were compared by genotype. Categorical data are reported as frequencies, and differences between groups were compared with chi-square or Fisher exact tests, as appropriate. Continuous data are reported as mean ± SD, and differences between groups were tested with 1-way analysis of variance. Hardy-Weinberg equilibrium was assessed with chi-square test or Monte Carlo permutation with 10,000 iterations, as appropriate. Kaplan-Meier estimates and Cox proportional hazards models were used to describe the effect of genotype on patient survival. A dominant model was used for *ADRA2C* 322-325 deletion and *GRK5* L41Q genotypes due to the low minor allele frequencies of these variants. For all other genotypes, we used an additive model. Follow-up began at the time of discharge from the index hospital stay. Stratified proportional hazards models were used, adjusting for study and site. To estimate the independent contribution of genotype, after adjusting for potential confounders and other clinical predictors, the proportional hazards models included covariates that were thought to be clinically important (age, sex, type of ACS, hypertension, diabetes, heart failure, chronic obstructive pulmonary disease, coronary angiography, and coronary revascularization) as well as those that differed significantly by genotype (Online Tables S1 to S4). To mitigate the possibility of "over fitting," we also tested models adjusted for age and sex only, for the variants that had significant associations (minimally adjusted model) (Online Table S6); these were consistent with the results of the primary analysis.

Analyses were performed separately in Caucasians and African Americans to minimize the risk of false-positive findings due to population stratification. We further assessed the issue of population stratification with a subgroup of 492 TRIUMPH subjects (382 Caucasian; 110 African-American) who were genotyped as part of a separate project with a custom array containing 3,351 single nucleotide polymorphisms and 100 ancestry informative markers (27,28). With these single nucleotide polymorphisms, we estimated 10 principal components with Eigenstrat and repeated our analyses in this subgroup to assess whether population stratification impacted our findings. Including principal components in the model did not meaningfully alter the findings, providing support for our categorization of race. For primary effects, p values <0.05 were considered statistically significant (29). Analyses were performed with SAS (version 9.2, SAS) and R (version 2.11.1) (30).

Results

Baseline characteristics of the cohort, stratified by race, are shown in Table 1. Types of BBs prescribed at discharge are shown in Table 2. Of the cohort, 91.6% was discharged on either metoprolol (82%; mean dose 73 ± 58 mg/day) or carvedilol (9.6%; mean dose 18 ± 14 mg/day); dose distribution is shown in Table 3. The 2-year mortality rate was 7.5% for Caucasians (n = 152) and 16.7% for African Americans (n = 96). We genotyped the study subjects for functionally significant polymorphisms within the adrenergic pathway that have been previously linked to outcomes after BB use, specifically variants within the genes encoding β-adrenergic receptors 1 (*ADRB1*) and 2 (*ADRB2*), alpha-2c adrenergic receptor (*ADRA2C*), and G-protein receptor kinase 5 (*GRK5*). For all variants, genotype call rates were >96%. Variants, genotype frequencies in Caucasians and African Americans, and the test of Hardy-Weinberg equilibrium are shown in Table 4. Consistent with prior reports, *GRK5* L41 and *ADRA2C* deletion allele frequencies were much higher among African Americans as compared with Caucasians (15). The frequencies of the other genotypes examined were similar to those previously reported (31).

Among the 2,072 Caucasian subjects, 2-year mortality differences across genotypes were assessed with Kaplan-Meier estimates (Figs. 1 to 4A) and proportional hazards regression modeling (Fig. 5) with adjustment for a priori selected baseline characteristics and clinical characteristics that differed significantly between genotype groups (Online Tables S1 to S5). The *ADRA2C* 322-325 insertion/deletion (I/D) genotype was significantly associated with 2-year mortality among Caucasians in the adjusted analyses (hazard ratio [HR]: 0.46; confidence interval [CI]: 0.21 to 0.99; p = 0.047) with *ADRA2C* ID and *ADRA2C* DD (i.e., deletion carriers) subjects having greater survival compared with *ADRA2C* II homozygous individuals. By contrast, none of the other genetic variants tested were significantly associated with 2-year mortality among Caucasians in either adjusted or unadjusted analyses (all p > 0.3).

In similarly constructed models, the association of each polymorphism with mortality was tested in a total of 601 African Americans (Figs. 1 to 4B and 5). Although neither the *ADRB1* nor *ADRA2C* variants were associated with mortality (p > 0.5), *ADRB2* variants were. The *ADRB2* G16R variant was significantly associated with mortality among BB-treated African Americans in both unadjusted

Table 1 Patient Characteristics by Race

	White/Caucasian (n = 2,072)	Black/African-American (n = 601)	p Value
Age, yrs	59.8 ± 12.3	56.9 ± 11.9	<0.001
Sex			<0.001
Male	1,476 (71.2%)	324 (53.9%)	
Female	596 (28.8%)	277 (46.1%)	
BMI, kg/m^2	29.4 ± 6.0	30.0 ± 7.3	0.043
Smoking status			<0.001
Current (<30 days)	782 (37.9%)	273 (45.9%)	
Former (≥30 days)	702 (34.0%)	148 (24.9%)	
Never (or <100 total)	580 (28.1%)	174 (29.2%)	
Dyslipidemia	1,072 (51.7%)	262 (43.6%)	<0.001
Hypertension	1,255 (60.6%)	463 (77.0%)	<0.001
Diabetes	518 (25.0%)	246 (40.9%)	<0.001
Prior PCI	457 (22.1%)	94 (15.6%)	<0.001
Prior CABG	275 (13.3%)	52 (8.7%)	0.002
Prior MI	414 (20.0%)	147 (24.5%)	0.018
Peripheral arterial disease	109 (5.3%)	29 (4.8%)	0.671
Prior CVA	67 (3.2%)	44 (7.3%)	<0.001
Prior TIA	47 (2.3%)	9 (1.5%)	0.245
Chronic heart failure	109 (5.3%)	110 (18.3%)	<0.001
Chronic renal failure	74 (3.6%)	106 (17.6%)	<0.001
Chronic lung disease	154 (7.4%)	59 (9.8%)	0.057
LV systolic function			<0.001
Normal	1,178 (57.7%)	367 (61.8%)	
Mild	458 (22.4%)	89 (15.0%)	
Moderate	288 (14.1%)	51 (8.6%)	
Severe	117 (5.7%)	87 (14.6%)	
Diseased vessels			<0.001
0	101 (5.1%)	82 (17.5%)	
1	818 (41.6%)	192 (41.0%)	
2	562 (28.6%)	96 (20.5%)	
3	485 (24.7%)	98 (20.9%)	
ACS type			<0.001
STEMI	1,006 (48.6%)	166 (27.6%)	
NSTEMI	912 (44.0%)	385 (64.1%)	
UA	154 (7.4%)	50 (8.3%)	
BB on arrival	638 (30.8%)	224 (37.3%)	0.003
GRACE risk score	97.9 ± 29.1	100.7 ± 30.1	0.043
Revascularization type			<0.001
None	377 (18.2%)	308 (51.2%)	
PCI	1,513 (73.0%)	254 (42.3%)	
CABG	182 (8.8%)	39 (6.5%)	

Values are mean ± SD or n (%).

ACS = acute coronary syndrome(s); BB = β-adrenergic receptor blockade; BMI = body mass index; CABG = coronary artery bypass graft surgery; CVA = cardiovascular accident; GRACE = Global Registry of Acute Coronary Events; LV = left ventricular; NSTEMI = non–ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; STEMI = ST-segment elevation myocardial infarction; TIA = transient ischemic attack; UA = unstable angina.

and adjusted analyses. Compared with African-American *ADRB2* G16 allele homozygous individuals treated with BB, *ADRB2* R16 allele carriers had greater mortality in both unadjusted (RG vs. GG HR: 1.74; CI: 0.96 to 3.16; RR vs. GG HR: 2.25; CI: 1.21 to 4.21; p = 0.039) (Fig. 3B) and fully adjusted analyses (RG vs. GG HR: 2.10; CI: 1.14 to 3.86; RR vs. GG HR: 2.65; CI: 1.38 to 5.08; p = 0.013) (Fig. 5). Notably, patients homozygous for the R allele had the greatest risk, and heterozygous patients had an intermediate risk, consistent with a gene–dose effect.

Because our pilot data suggested that both of the *ADRB2* variants (*ADRB2* G16R and *ADRB2* Q27E)—which are in linkage disequilibrium—might contribute to BB-related outcomes, we retested the mortality association of the compound genotype that most strongly correlated with risk in this earlier study. Among African Americans treated with BB, the results were similar to our pilot data, whereas this was not the case in Caucasians (Fig. 4). In adjusted analyses among African Americans, the compound genotype was associated with a 6-fold risk of mortality in the high-risk

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Table 2 BB on Discharge

Metoprolol	2,193 (82.0%)
Carvedilol	256 (9.6%)
Atenolol	191 (7.1%)
Labetalol	19 (0.7%)
Sotalol	8 (0.3%)
Nadolol	3 (0.1%)
Propranolol	3 (0.1%)
Pindolol	2 (0.1%)
Betaxolol	1 (0.0%)
Bisoprolol	1 (0.0%)
BB not recorded	14 (0.5%)
Total	2,673 (100%)

Values are n (%).
BB = β-adrenergic receptor blockade.

group and approximately a 4-fold risk in the intermediate group (Fig. 5) (p = 0.048).

In African Americans, the *GRK5* Q41L variant had a borderline-significant association with mortality in adjusted analyses. Although not achieving statistical significance, the *GRK5* L41 African-American patients treated with BB trended toward lower mortality as compared with homozygous *GRK5* Q41 patients (adjusted HR: 0.68; CI: 0.45 to 1.04; p = 0.07). We also tested for but found no significant gene-gene interaction between *ADRB2* and *GRK5* and outcomes in African-American ACS patients (interaction p value between *ADRB2* G16R and *GRK5* Q41L = 0.86).

Because each of the 2 sequence variants that showed a significant survival association did so in only 1 racial group, we performed formal interaction tests to further assess the significance of the racial distinction. The race × genotype interaction approached significance for both *ADRA2C* I/D (p = 0.053) and *ADRB2* G16R (p = 0.096).

Discussion

In accordance with consensus guidelines and quality performance measures, it is recommended that all patients without contraindications be discharged on BB therapy after MI (4,32–34). However, the consistent efficacy of BB in all groups has been questioned, and this variability might be related in part to genetic heterogeneity (5,6). Our data suggest that variability in post-ACS outcomes among BB-treated patients is associated with genetic variation in the adrenergic pathway. Importantly, we identified different prognostically important genes in African Americans, as compared with Caucasians. Specifically, the *ADRA2C* I/D genotype was significantly associated with 2-year mortality in post-ACS Caucasians treated with BB, whereas the *ADRB2* G16R and *GRK5* Q41L genotypes were associated with 2-year mortality in post-ACS African Americans treated with BB. These findings suggest that race-specific adrenergic-pathway variants might be useful in defining prognosis after BB therapy for Caucasians and African-American ACS patients.

It is important to note that the race specificity of our findings is not due to differences in allele frequency. For example, although the *ADRA2C* D allele is much more common in African Americans, there was no risk relationship between this gene and mortality in these patients, whereas it was associated with risk in Caucasians (race × genotype interaction p = 0.053). This contrasts with our pre-study hypothesis that this allele likely would have similar effects regardless of race. Our data suggest something more complex: that the allele effects might be specific to the Caucasian genetic environment. This is a novel observation requiring replication. If verified in other populations, then mechanistic studies are needed to understand whether ancestry-specific linkage or other genetic modifiers might explain the observed differences in allelic associations across race.

Beyond the prognostic difference between races, the association of the *ADRA2C* I/D variant with 2-year mortality in Caucasian patients discharged on BB is the first report of this finding in ACS patients. *ADRA2C* D creates a hypo-functioning form of this presynaptic inhibitory receptor, which has been previously associated with increased catecholamine release in vitro (35) and in vivo (36). Consistent with this, the *ADRA2C* D allele has also been previously associated with increased risk of heart failure (35). Our findings could be consistent with this line of evidence, considering that patients with the *ADRA2C* D allele might have a higher adrenergic state and thus display enhanced benefit from BB (37), potentially resulting in the lower mortality we observed when comparing genotype groups treated with BB. Conversely, our findings contrast with recent observations in heart failure patients where the BB bucindolol provided survival benefit in *ADRA2C* II

Table 3 Dose Distribution of Metoprolol and Carvedilol on Discharge

Dose (mg/day)	%
Carvedilol	
3.125	6.25
6.25	23.75
12.5	32.08
25	25.42
50	12.08
62.5	0.42
Metoprolol	
12.5	2.49
25	22.06
37.5	0.23
50	39.52
75	1.93
100	21.97
112.5	0.05
125	0.05
150	3.36
200	6.86
300	0.83
400	0.64

Table 4 Genotype Frequencies and HW p Values

SNP	Genotype	% Frequency Caucasian	HW p Value	% Frequency African Americans	HW p Value
ADRA2C INDEL	II	90.46	0.2033	38.63	0.0334
	ID	8.99		42.51	
	DD	0.55		18.85	
ADRB1 R389G	RR	52.85	0.1747	34.77	0.2237
	RG	38.63		50.43	
	GG	8.52		14.80	
ADRB2 G16R	GG	37.01	0.5009	23.84	0.6096
	GR	48.24		48.89	
	RR	14.75		27.27	
ADRB2 Q27E	QQ	34.39	0.3663	67.98	0.3525
	QE	49.33		28.25	
	EE	16.28		3.77	
GRK5 Q41L	QQ	96.50	0.3427	54.99	0.2403
	QL	3.35		37.06	
	LL	0.15		7.95	

HW = Hardy-Weinberg equilibrium; SNP = single nucleotide polymorphism.

homozygous individuals but not in deletion carriers, possibly due to enhanced sympatholysis in *ADRA2C* D carriers (18). This inconsistency might be attributable to differences between the pathophysiologic role of adrenergic signaling in ACS patients as compared with heart failure. For example, it has been proposed that sympatholysis might be particularly detrimental in heart failure patients (38). Alternatively, the inconsistency could be due to pharmacological differences in the specific agents used (no bucindolol in the present study [Table 2] vs. 100% bucindolol use in BEST [Beta-Blocker Evaluation of Survival Trial]), especially given that bucindolol is unique among BB agents for causing sympatholysis. Our findings warrant further investigation of the role of genetic variation in *ADRA2C* and the possible variability by type of BB agent, in Caucasian ACS patients.

Beta-adrenergic receptor signaling is down-regulated by G-protein receptor kinases (GRKs) (39). The GRK 5 phosphorylates, uncouples, and internalizes β1-adrenergic receptors, and the *GRK5* L41 variant has greater activity than the *GRK5* Q41 variant for all of these functions (15). We previously described a pharmacogenomic effect of GRK5 in subjects with ACS (15). The *GRK5* Q41L polymorphism was previously found to be a determinant of β-blocker responsiveness in heart failure among African Americans, with a protective β-blocker–mimetic effect seen in *GRK5* L41 allele carriers (13). Similarly, we found a strong trend toward improved outcomes in post-ACS African-American patients carrying this allele. Our findings are also consistent with our previous observations in heart failure patients in terms of the magnitude of effect: roughly one-half the mortality risk as compared with the *GRK5*

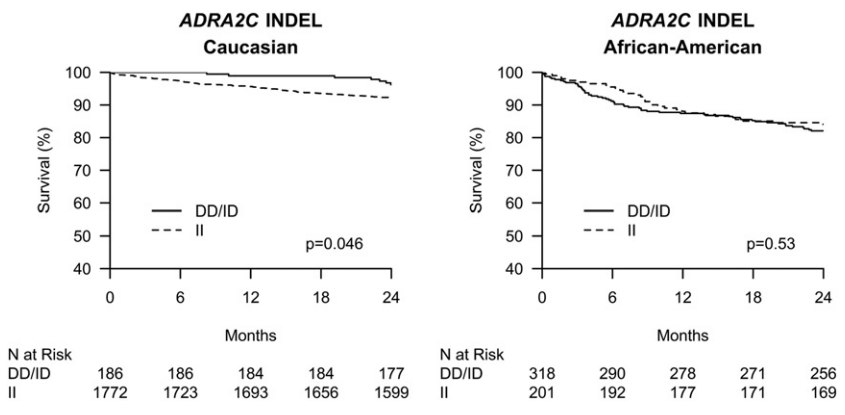

Figure 1 Kaplan-Meier Curves of *ADRA2C* Insertion/Deletion Effect on Post-ACS 2-Year Survival

(A) Caucasian β-blocker–treated acute coronary syndrome (ACS) subjects stratified by *ADRA2C* deletion carrier status. **(B)** African-American β-blocker–treated ACS subjects stratified by *ADRA2C* deletion carrier status.

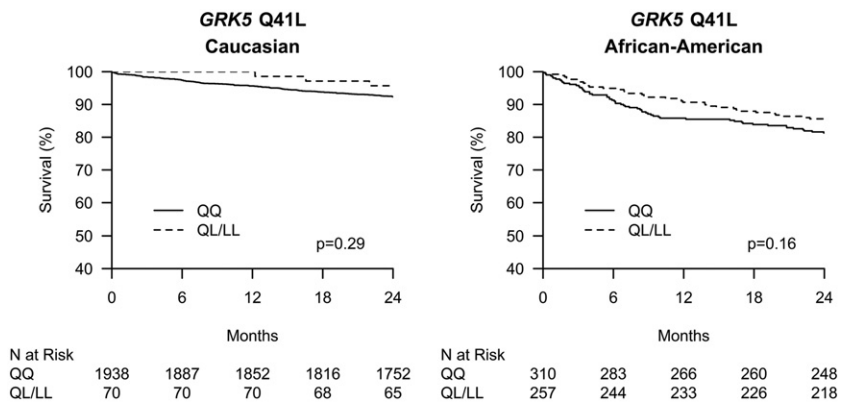

Figure 2 **Kaplan-Meier Curves of *GRK5* Gln/Leu 41 Effect on Post-ACS 2-Year Survival**

(A) Caucasian β-blocker–treated acute coronary syndrome (ACS) subjects stratified by *GRK5* 41Leu carrier status. **(B)** African-American β-blocker–treated ACS subjects stratified by *GRK5* 41Leu carrier status.

Q41 allele homozygous patients. The borderline statistical significance, with a similar effect size, likely reflects suboptimal power for this particular analysis as well as our inability to contrast BB-treated patients with those not treated with BB (due to the near universal compliance with BB as a performance measure of quality in ACS).

In this study, the *ADRB2* G16R genotype was significantly associated with mortality in African-American ACS patients, such that patients homozygous for the R allele had greatest risk and heterozygous patients had an intermediate risk, consistent with a gene–dose effect and our previous findings (19). There was an even greater stratification in risk when both functional G16R and Q27E *ADRB2* variants were accounted for, showing approximately a 4-fold and 6-fold risk in the intermediate- and high-risk groups, respectively. However, unlike our pilot data, in which the association was found in a much smaller cohort that included both African-American and Caucasian patients, our current analysis suggests that the association is specific to African Americans. Due to this distinction, the *ADRB2* association is not a strict validation and requires confirmation in additional, adequately sized, cohort(s) of African-American ACS patients. Nonetheless, these data extend previous reports and are the first description, to our knowledge, of an African-American–specific effect of *ADRB2* genotype in ACS patients. Conversely, the lack of association in Caucasians is strong, suggesting that additional inquiry into this association is unlikely to reveal a strong effect of *ADRB2* genotype among Caucasian ACS patients treated with BB.

Study limitations. Our study should be interpreted in the context of several potential limitations. First, due to the high level of adherence to guidelines recommendations, only a small number of subjects were not treated with BB.

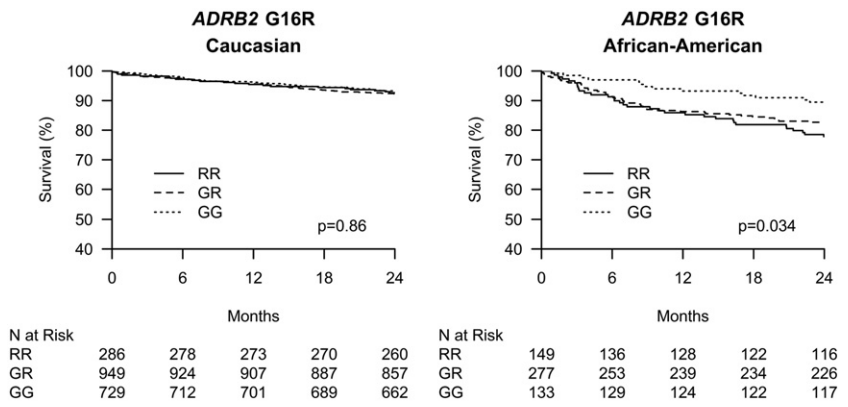

Figure 3 **Kaplan-Meier Curves of *ADRB2* G16R Effect on Post-ACS 2-Year Survival**

(A) Caucasian β-blocker–treated acute coronary syndrome (ACS) subjects stratified by *ADRB2* G16R status. **(B)** African-American β-blocker–treated ACS subjects stratified by *ADRB2* G16R status.

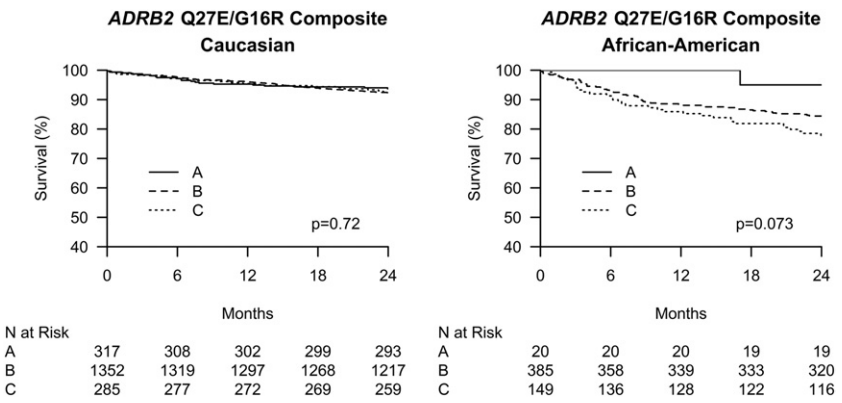

Figure 4 **Kaplan-Meier Curves of *ADRB2* Composite Genotype Effect on Post-ACS 2-Year Survival**

(A) Caucasian β-blocker–treated acute coronary syndrome (ACS) subjects stratified by *ADRB2* composite genotype status. **(B)** African-American β-blocker–treated ACS subjects stratified by *ADRB2* composite genotype status. *ADRB2* composite genotype as follows: **(A)** subjects homozygous for both *ADRB2* G16 allele and *ADRB2* E27 allele; **(C)** subjects homozygous for both *ADRB2* R16 allele and *ADRB2* Q27 allele; **(B)** subjects heterozygous for *ADRB2* G16R and/or *ADRB2* Q27E.

This precluded a formal analysis to assess the interaction of genotype with BB treatment. Thus, we cannot specifically comment on BB efficacy, and have focused solely on the genetic association with outcomes in BB-treated patients. In light of these data, however, it might be worthwhile to consider a randomized trial of BB in the genetic subgroups that show poor outcomes with BB therapy in order to exclude the possibility of harm from BB treatment. Alternatively, new study approaches such as using quantified drug exposure metrics (as opposed to simply present vs. absent) or identification of a non-U.S. cohort of ACS patients where BB treatment is less common might show greater variability in

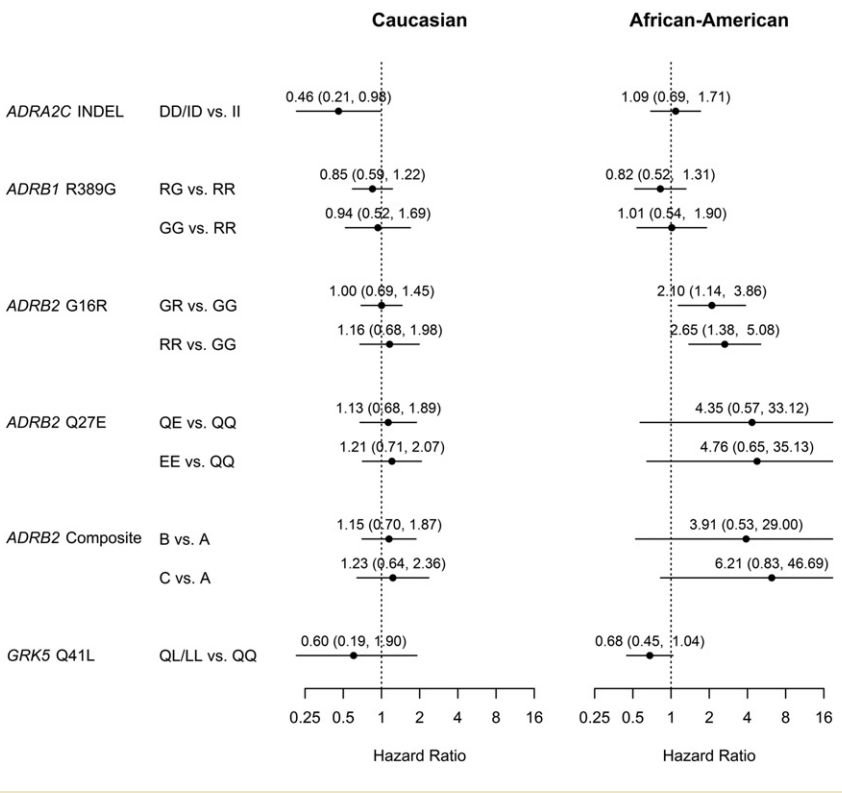

Figure 5 **Model-Adjusted Hazard Ratios for 2-Year All-Cause Mortality**

ADRB2 composite genotype as in Figure 4.

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BB use (i.e., more subjects off BB) and allow drug × genotype interaction analyses. Despite this inherent limitation, our findings are important, because they identify genetic subgroups with worse outcomes despite BB treatment and in whom more aggressive interventions to improve mortality might be warranted. An additional limitation is that we had insufficient sample size to differentiate between BB agents, and thus agent-specific effects are not obtainable from this study. A third limitation is that the current study includes 735 subjects previously described in our preliminary analysis testing the hypothesis that *ADRB1* and *ADRB2* polymorphisms would be associated with mortality in BB-treated patients (19). However, in the current study, we have increased the total cohort by more than 4-fold (including a quadrupling of the African-American population), enabling us to examine race-specific associations between genetic variation and mortality. We have also included additional published variants in our analysis (*GRK5* and *ADRA2C*), enabling a more complete analysis of genes within the adrenergic pathway, and have performed analyses that provide evidence that population stratification is not confounding our findings. Finally, we were not able to account for changes in medications over time. However, classifying patients by discharge medication status is a well-recognized and often-used approach, because most patients remain on their discharge regimen after hospital stay (40).

Conclusions

In summary, our findings represent the first comprehensive analysis of established functional genetic variants within adrenergic pathway genes in an ACS cohort and show that several of these variants are associated with mortality among BB-treated patients and that these associations vary by race. Among Caucasian patients that have suffered an ACS and are treated with BB, *ADRA2C* II homozygous individuals had increased mortality as compared with *ADRA2C* ID and *ADRA2C* DD subjects. In African-American ACS patients treated with BB, *GRK5* L41 allele carriers trended toward having lower mortality compared with *GRK5* Q41 homozygous patients, whereas *ADRB2* 16R allele carriers had significantly increased mortality in a gene–dose response manner. Further study is needed to replicate our findings in African-American patients; randomized trials of post-ACS BB treatment in race-specific, genotype-defined subgroups might be warranted to insure the benefits of BB therapy in these high-risk individuals.

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Key Words: β-blocker therapy ■ pharmacogenetics ■ racial disparities.

APPENDIX

For supplementary tables, please see the online version of this article.