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## Race, Common Genetic Variation, and Therapeutic Response Disparities in Heart Failure

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### Abstract

Because of its relatively recent evolution, *Homo sapiens* exhibits relatively little within-species genomic diversity. However, because of genome size, a proportionally small amount of variation creates ample opportunity for both rare mutations that may be disease-causative as well as more common genetic variation that may be important in disease modification or pharmacogenetics. Primarily because of the East African origin of modern humans, individuals of African ancestry (AA) exhibit greater degrees of genetic diversity than more recently established populations, such as those of European ancestry (EA) or Asian ancestry. These population effects extend to differences in the frequency of common gene variants that may be important in heart failure natural history or therapy. For cell-signaling mechanisms important in heart failure, we review and present new data on genetic variation between AA and EA populations. The data indicate that 1) neurohormonal signaling mechanisms frequently (16 of the 19 investigated polymorphisms) exhibit racial differences in the allele frequencies of variants comprising key constituents, 2) some of these differences in allele frequency may differentially affect the natural history of heart failure in AA vs. EA individuals, and 3) in many cases these differences likely play a role in observed racial differences in drug or device response.

### Keywords

Genetic polymorphisms; pharmacogenetics; racial ancestry; heart failure

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## Introduction

Variability in drug response is typical of cardiovascular therapies (1) and may be related to common genetic variation (1–3), operationally defined as a polymorphism with a minor allele frequency of  $\geq 1\%$  (4). Race-associated differences in cardiovascular disease natural history and drug response are well known, and may be associated with common genetic variation in drug targets or pharmacokinetics (2). Such pharmacogenetic variation can potentially explain variability in racially based response to therapies for heart failure, a phenomenon that is becoming more widely appreciated.

We review and provide new information on race-associated differences in heart failure natural history and therapeutic responses that are associated with common genetic variation. The presented information is confined to heart failure with reduced left ventricular ejection fraction (HFrEF, or simply HF), and the race comparisons are between Sub-Saharan African-ancestry (AA) vs. European-ancestry (EA) populations regardless of geographic location. The overall aim is to explore the hypothesis that reported racial differences in HF natural history or therapeutic responses are influenced by differences in genetic variation in relevant cardiac myocyte neurohormonal signaling mechanisms.

## Race and Genetic Variation

An argument for a genetic basis for race-associated differences in disease natural history or therapeutic response is predicated on the idea that different frequencies of genetic variants between racial groups may lead to differentiated outcomes between those groups. Genetic alteration of a disease or therapeutic phenotype within a racially defined population is dependent on a) the frequency of the polymorphism in that population, b) the difference in the allele frequency of the polymorphism between the indicated population and a general, non-rationally selected population, and c) the extent of the biological effect of the polymorphism on the phenotype.

Extrapolating racial identity from common attributes such as skin color can be problematic due to the assumption that physical characteristics are a proper proxy for variation in biologic phenotype-modifying, non-anthropomorphic genes. Nevertheless, extensive clinical experience supports that phenotype heterogeneity in disease risk, prognosis, and response to therapy can be attributed to self-identified race, and it is appropriate to explore to what extent genetic variation contributes to these differences (5,6). Understanding the underlying genetic basis of such heterogeneity will help devise new drugs, devices and diagnostics that improve outcomes.

Genetic diversity in humans is relatively low compared with other species, including other primates (7). This is generally attributed to *Homo sapiens'* relatively recent evolution in East Africa approximately 200,000 years ago, and the subsequent immigration of modern populations from Africa in the past 100,000 years (6). Based on the first detailed single-nucleotide polymorphism (SNP) map of the human genome encompassing 1.42 million variants occurring every 1.9 Kb, humans were estimated to be 99.6% to 99.8% identical at the nucleotide level (6,8). The more recent 1000 Genomes Project, which has the goal of identifying pan-genomic and coding region variations down to respective allele frequencies

of 1% and 0.1%, identified in its recently published pilot phase (9) about 15 million SNPs, 1 every 800 bases, from whole-genome sequencing of 179 individuals in 3 racial categories. The average number of SNPs per individual was about 3 million, and the variation from the reference genome was 0.125% (9). Thus, although the most recent estimate of single-nucleotide variation is about 0.1%, the 3 million SNPs per individual plus other types of genetic variation provide ample potential for genomic diversity within and between populations. Despite the notion that the vast majority of SNPs represent silent (synonymous) variation or an amino acid change (non-synonymous) with no clear biological function effects, substantial effort has been invested in identifying the small fraction of SNPs and other variants that associate with human phenotypes and disease risks.

African-ancestry populations exhibit greater degrees of genetic variation compared with non-African cohorts (10,11). Given that modern European and Asian populations descended from founder groups that diverged from ancestral African populations, it is expected that genetic diversity in non-African groups would be lower since ancestral founder populations would contain only a subset of the total ancestral African variation. However, most of the genetic variation in African populations can also be found in non-African populations. Overall, 10% to 15% of all human genetic variation is explained by differences between Sub-Saharan Africans, Northern Europeans, and East Asians. Stated another way, approximately 85% to 90% of known variation is captured by studying any 1 of the 3 "major" population groups (Africa, Asia, and Europe), and only an additional 10% to 15% can be ascertained by inclusion of the other 2 groups (12). Thus, genetic variation between populations is only slightly more different than variation within a given population (13).

These data have relevance for the evaluation of genetic variation related to health and disease. A priori, for any given variant there is an increased probability of it being represented in an AA vs. a non-AA population. Furthermore, for any variant locus shared between AA and non-AA populations, the observed allele frequencies may differ, sometimes widely, between racial populations.

In the example of the *ADRA2C* 322-325 insertion (Ins)-deletion (Del) polymorphism (rs2234888), various studies have noted a 7- to 10-fold increase in the prevalence of the Del variant in AA populations (14–16). Some of the difference in allele frequency is likely due to the lower frequency of the Del allele in the founder population(s) that immigrated to Northern Europe. Presumably, there may be differential allele frequency across Africa, with lower Del frequencies in East African populations. This question has not been extensively investigated, although one analysis of black South Africans, far removed from the migration point, noted the Del allele to be present in more than 50% of individuals (17). The same logic and arguments apply to other variants that exhibit marked racial differences in frequencies, such as *GRK5* Gln41Leu (rs2230345) (18) and *SCN5A* Ser1103Tyr (rs7626962) (19), both of which have minor alleles of demonstrated functional importance with frequencies that are 10-fold higher in AA vs. EA populations. However, even in these highly minor allele-enriched examples, the major allele has a frequency >0.5. Thus there is a non-trivial percentage of AA individuals that *do not* have the minor allele in the heterozygous or homozygous state. Therefore, one can readily appreciate that skin color

would be a poor method of determining whether a person carries the minor allele for *ADRA2C* Ins322-325Del, *GRK5* Gln41Leu, or *SCN5A* Ser1103Tyr.

In spite of widespread variation in the frequency of various genetic markers, the associated odds ratios for disease risk in different racial groups appears to be less variable (20). However, as will be presented, in HF therapeutics there is good evidence that racially based pharmacogenetic differences exist for both effectiveness and safety.

## Common Genetic Variation in Signaling Mechanisms Important in HF

Numerous reports have documented substantial polymorphic variation in neurohormonal and associated signaling mechanisms important in HF therapeutics. For example, the  $\beta_1$ -adrenergic receptor (*ADRB1*, Figure 1A)—the initiating signal transduction protein in a system that can cause a dilated cardiomyopathy when overexpressed in transgenic mice and is also the primary drug target for a major class of HF therapeutics ( $\beta$ -blockers) (21)—exhibits about 5 times the average SNP percentage detected in the ENCODE regions in the human genome (22) and 2 times that detected in exons in the 1000 Genomes Project (9), with 26 SNPs in the 1431 coding region nucleotides, half of which result in an amino acid change (23). Most genomic common variation has no impact on protein function, so the focus of this study is on variants with well-characterized biologic or pharmacologic effects.

Table 1 is a compilation of allele frequency data from non-synonymous SNPs, insertion-deletions (InDels), or other polymorphisms that have been shown to have functional significance in selected genes encoding signaling pathway proteins of documented or potential importance in HF natural history or therapeutics. The methods for generating the data in Table 1 are given in the Supplement and in the Table 1 footnotes. The basis for combining AA populations from Sub-Saharan Africa and elsewhere is that this alignment forms a distinct genetic group compared with EA populations from the United States, Europe, and elsewhere (44).

As can be seen in Table 1, of the 19 selected polymorphisms in 15 genes in non-HF (NF) populations, 16 variants exhibit evidence of racial differences in minor allele frequency (MAF). Moreover, in every case the difference in allele frequency is maintained in HF populations (i.e., there is good agreement in MAFs between HF and NF populations). The data in Table 1 illustrate that 1) racially based genetic variation is common in genes encoding proteins that are important in neurohormonal signaling systems; and 2) the racial differences in distribution of polymorphic variants are generally not altered in HF.

For *ADRB2* Gln27Glu, *GNB3* C825T, *NOS3* Glu298Asp, and *AGTR1* A1166C, the differences in MAF allele frequencies are very different (i.e., 2.5-fold between races, Table 1); and for *ADRB2* Thr164Ile, *ADRA2C* Ins322-325Del, *ECE1* Thr341Ile, *GRK5* Gln41Leu, and *SCN5A* Ser1103Tyr, the MAF frequencies are extremely different (i.e., >5-fold). Having established that AA and EA racial differences are common in polymorphisms of signaling systems important in HF, does this have any practical importance? We will first consider potential impacts on HF natural history.

## Does Common Genetic Variation Influence HF Natural History in Either AA or EA Populations?

Table 2 lists the Table 1 polymorphisms with a racial difference in MAF, the pharmacologic effects of their minor vs. major alleles, the predicted biologic or pharmacologic effects of the frequency difference in AA vs. EA individuals, and whether the projected relative signaling effect would be cytoprotective or cytopathic in failing human cardiac myocytes based on the assumption that increases in signaling are harmful and decreases are beneficial. In 13 of the 14 polymorphisms listed in Table 2 (exception is *ECE1* Thr341Ile, where functional effects are unknown), the minor alleles have effects that differ from the major allele and could influence HF risk or progression. Race-associated differential genetic effects on HF natural history could in turn affect therapeutic responses.

In contrast to the low frequency (MAF <1%) mutations in contractile protein, cytoskeletal, and other genes that cause inherited dilated cardiomyopathies (45), there is no good evidence that any of the cell-signaling polymorphisms listed in Tables 1 or 2 predispose to the risk of developing HF. The fact that the MAF distributions are quite similar in HF and NF populations indicates that HF populations are not generally enriched in these signaling molecule minor alleles. Although earlier, smaller studies did report enrichment in HF for some of these MAFs (46–48), most subsequent larger studies (49–51), including meta-analyses (52,53), have not confirmed the findings. In general there is a need for well-controlled, prospective studies with large numbers of patients and clinical endpoints to address this issue, and whether any of the variants listed in Table 1 could influence the risk of developing HF is not settled.

In terms of possible effects on disease progression, of the HF studies with enough patients and events to provide adequate statistical power, the adrenergic receptor polymorphism substudy (1040 patients, 193 deaths, and 426 deaths or first HF hospitalizations [HFHs]) (16,25) of the placebo-controlled  $\beta$ -Blocker Evaluation of Survival Trial (BEST) and a large observational study from the Universities of Cincinnati and Pennsylvania (2460 patients and 765 deaths) (18), each with >20% of AA patients, provide the most robust data. Effects of polymorphic variants on natural history of established HF can be assessed in BEST in the 525 placebo-treated patients, and in the Cincinnati/Pennsylvania study by effects in patients untreated with  $\beta$ -blockers, as described in the Supplement.

Among the potential candidate gene variants listed in Tables 1 and 2, perhaps the most likely to influence the progression of HF is the *ADRB1* Arg389Gly polymorphism (Figure 1A). This is because in human ventricular preparations, compared with its 389Gly counterpart the 389Arg version of the receptor has 3-to-4 times the signal transduction capacity (25), a higher proportion of receptors that are constitutively active as inferred from inverse agonist activity (25), and a much higher affinity for norepinephrine (54) (Table 2). As a result of this marked difference in agonist affinity and the lower affinity of  $\beta_2$  receptors for norepinephrine, the protein product of *ADRB1* 389Arg can be considered the norepinephrine receptor in the human heart (21,54). Increased release and cardiac concentrations of the  $\beta_1$ -selective neurotransmitter norepinephrine produce and lead to the progression of ventricular chamber remodeling and HF (21). Table 3 summarizes the

*ADRB1* Arg389Gly data from placebo-treated patients in BEST (16,25) and MERIT-HF (Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure, 55), as well data from the Cincinnati/Pennsylvania  $\beta$ -blocker untreated patients (18).

In BEST as well as in MERIT-HF (which did not report pharmacogenetic data by race), in placebo-treated patients there was no evidence of an effect of *ADRB1* Arg389Gly genotypes on the outcomes of time to all-cause mortality (ACM) or the combined endpoint of ACM or HFH (Table 3). In contrast, in the Cincinnati/Pennsylvania study, EA (but not AA) patients who were untreated with  $\beta$ -blocking agents had improved transplant-free survival from the time of HF diagnosis if they were *ADRB1* 389Arg homozygotes, compared with 389Gly genotypes (18). One possible difference between the clinical trial/placebo groups data from BEST and MERIT-HF as compared with the Cincinnati/Pennsylvania study is much shorter mean or median follow-up times in BEST (2 years) (25) and MERIT-HF (1 year) (55), compared with 46 months in the observational study (18). It is conceivable that a longer follow-up time in HF is necessary to detect genotype-based differences in outcomes for common genetic variation in neurohormonal cell signaling systems. However, the Cincinnati/Pennsylvania data imply a protective effect of the *ADRB1* 389Arg allele, which is contrary to work in transgenic cardiac overexpressor mice as well as multiple cell biology studies that demonstrate that heightened *ADRB1* signaling is cytopathic to cardiac myocytes (21). Thus, natural history and biologic plausibility data do not consistently support an effect of the *ADRB1* Arg389Gly polymorphism on clinical HF outcomes. However, larger prospective studies with relatively long follow-up periods, of greater duration than is usually the case in therapeutic clinical trials, are needed before reaching firm conclusions. This principle also applies to the other genetic variants listed in Table 1.

The Cincinnati/Pennsylvania study produced a very interesting result in  $\beta$ -blocker-untreated patients regarding the *GRK5* Gln41Leu polymorphism: AA patients (but not EA patients or the entire EA + AA cohort) had improved transplant-free survival (Table 3, Figure 2 L-panels) in 41Leu genotypes compared with 41Gln homozygotes (18). The 41Leu variant of *GRK5* is a gain-of-function polymorphism whose protein product increases  $\beta$ -adrenergic receptor phosphorylation, which produces receptor desensitization and a genetic anti-adrenergic effect (18,56). The approximate 10-fold higher prevalence of *GRK5* 41Leu genotypes in AA patients compared with EA patients may therefore provide a significant amount of "endogenous  $\beta$ -blockade" (18,56), with the effect detectable in patients untreated with  $\beta$ -blocking agents.

Table 3 also gives the BEST placebo-treated patient data for adrenergic receptor polymorphisms listed in Tables 1 and 2. For the entire EA + AA cohort, there were no differences between major allele homozygotes and MAF carriers for the *ADRB1* Ser49Gly, *ADRB2*, or *ADRA2C* Ins322-325Del polymorphisms, for either the ACM or ACM/HFH endpoints. In AA patients the ACM hazard ratio p-value is 0.035 (above the 0.01 critical value, see Supplement) and the interaction test is  $p < 0.10$  for *ADRB2* 27Glu carriers having an increased mortality risk, but this is based on only 15 total events. Although the EA ACM and ACM/HFH hazard ratios of respectively 1.11 and 1.21 are substantially lower than AA values, the race-treatment interaction p-values are not significant for either endpoint. There are no other differences with  $p < 0.05$  in the BEST receptor polymorphism data in Table 3.

In addition to pump dysfunction, the other major clinical component of HF disease progression is the development of serious arrhythmias. The *SCN5A* 1103Tyr variant of the Nav1.5 sodium channel is found in 6% to 9% of AA individuals but is extremely rare in EA subjects (19,44, Table 1), and it has been associated with a 4-fold increase in implantable cardioverter defibrillator (ICD) discharges for sustained ventricular arrhythmias in AA HF patients (43). The *SCN5A* Ser1103Tyr polymorphism clearly has the potential to influence HF progression disproportionately in AA vs. EA populations, and also has implications for device and even drug therapy as discussed below.

Therefore, the cell-signaling polymorphisms denoted in Tables 1 and 2, some of which are capable of major effects on cardiac myocyte function, do not appear to fundamentally affect the risk of developing HF. One likely reason for this is that neurohormonal cell-signaling mechanisms only modulate cardiac myocyte function and are not critical for cell integrity. In addition, when neurohormonal activation occurs in HF, it is typically ontogenetically beyond the reproductive years, obviating any possibility of natural selection eliminating deleterious alleles. In terms of polymorphism effects on HF disease progression, the most compelling evidence is for *GRK5* Gln41Leu producing a beneficial effect and for *SCN5A* Ser1103Tyr producing an adverse effect in AA individuals, due to markedly higher (10-fold) MAF frequencies.

## Genetic Variation and HF Therapy: Interaction of Race, Signaling Polymorphisms, and Therapeutic Responses

There is considerable evidence that several of the polymorphisms in Table 1 exert an influence on drug or device therapeutic response. Table 4 summarizes pharmacogenetic effects on HF clinical, left ventricular remodeling, or biomarker endpoints that have been reported for some of the polymorphisms listed in Table 1, independent of race. Many of these studies were underpowered, but some (16,18,25,54) had adequate prespecified statistical design. In most cases the results are reinforced by biologic plausibility, and in some instances there are large differences in functional activity between the polymorphic variants that have been shown to interact with the therapeutic agent or drug class (15,16,25,54–56).

Table 5 gives some of the evidence for racial effects on specific HF therapeutic responses that may be influenced by the products of genes listed in Table 1. Because HF clinical response-racial data are limited in several circumstances, antihypertensive response is included for some drug classes. Decreased effectiveness in AA HF patient populations has been reported for 3 drug classes: standard  $\beta$ -blockers (18,65); the  $\beta$ -blocker/sympatholytic agent bucindolol (67); and angiotensin-converting enzyme inhibitors (68,69). Blunted antihypertensive responses to  $\beta$ -blockers (66) and angiotensin-converting enzyme inhibitors (70) have also been reported in AA patients. In contrast, the response to fixed-dose hydralazine/isosorbide dinitrate appears to be enhanced in AA HF patients (68), and ICD therapy appears to be more effective in a genetically defined subset of AA HF patients (43).

There is clearly the potential for some of the polymorphisms listed in Tables 1 and 2 to account for the Table 5 racial effects. The potential impact of race-related differences in

MAF on therapeutic response based on estimations of the predicted, possible, or actual observed effects of therapeutic agents or classes is given in the last column of Table 2. The estimates follow the general paradigm that increased cardiac myocyte neurohormonal signaling is harmful, decreased signaling is beneficial, and the predicted therapeutic effect of an inhibitor is directly related to the degree of signaling. Most of the pharmacogenetic interactions are assigned to the *possible* (some clinical evidence in support) or *predicted* (no clinical data in support) categories. Some of the the pharmacogenetic interactions listed in Table 2 that are supported by clinical outcomes data will now be discussed by therapeutic class.

### **β-blockers, including the β-blocker/sympatholytic bucindolol**

***ADRB1* Arg389Gly, Ser49Gly**—The higher-functioning 389Arg allele of *ADRB1* present at lower frequency in AA patients (Tables 1 and 2) was originally hypothesized to confer a greater therapeutic HF response to β-blockers (25) (Table 2). However, thus far for clinical outcomes this seems to be the case only for the β-blocker/sympatholytic agent bucindolol (25,54), an experimental compound in development for HF and prevention of atrial fibrillation. There is no evidence of a differential *ADRB1* Arg389Gly clinical therapeutic effect for standard HF-approved β-blockers (18,55,73), which are devoid of β<sub>1</sub> 389Arg adrenergic receptor inverse agonist effects in isolated preparations of a failing human heart (25) and lack systemic sympatholytic properties in HF patients. The latter property likely contributes to a favorable therapeutic effect of bucindolol on the *ADRB1* 389Arg "norepinephrine receptor" (54). Thus, the *ADRB1* Arg389Gly polymorphism could explain the reduced response to bucindolol in BEST AA patients (66) and the perhaps smaller racial interaction with carvedilol (63) or metoprolol (64). However, the Cincinnati/Pennsylvania study (18), which contained the largest cohort of β-blocker-treated AA HF patients studied to date, found reduced efficacy with carvedilol and metoprolol (18). In addition, in a large (1094 patients, 56% AA, 1368 primary events) well-controlled health care system retrospective study evaluating the effects of all U.S. marketed β-blockers, the reduction in ACM and first re-HFH in AA HF patients, although statistically significant ( $p = 0.024$  compared to the 20% of patients not treated with β-blockers), was 40–50% less than in EA patients (65).

Thus the weight of the evidence favors a blunted effectiveness of β-blockers in AA compared to EA HF patients. Figures 3 and 4 address the question of whether a lower frequency of the favorable response *ADRB1* 389Arg homozygous (Arg389Arg) genotype explains the attenuated response of bucindolol in AA patients (i.e., whether pharmacogenetics alone can explain lower efficacy in AAs) (67). Baseline characteristics for EA or AA patients in the BEST 1040-patient adrenergic receptor polymorphism substudy (16,25,54) are given in the Supplement (Table S1), and the differences are typical of other HF studies (68). For the primary ACM endpoint of BEST, as in the entire cohort (67), there was a statistically significant ( $p=0.023$ ) test for interaction between non-AA and AA patients in the substudy population, with AA patients showing no evidence of a favorable treatment effect. There were relatively few ( $n=36$ ) ACM events in the 207-member AA subgroup, and in order to assess the interaction of race and *ADRB1* Arg389Gly genotype, time to ACM/HFH was evaluated. Figure 3 gives the Kaplan-Meier curves for AA (84 events) and



EA (310 events) patients for ACM/HFH in the substudy parent (all genotypes) population. The AA group has an attenuated hazard ratio (0.87,  $p=0.52$ ) vs. the EA group (0.70,  $p=0.002$ ; interaction  $p=0.39$ ), a pattern similar to the entire cohort (data not shown). In Figure 4 the treatment effects in the *ADRB1* Arg389Arg genotype subgroup are enhanced in both racial groups, but attenuation relative to EA persists in AA patients (hazard ratio 0.76 in AA [C] vs. 0.57 in EA [B]). However, when class-IV HF subjects, who in BEST tended to be volume overloaded/unstable at the time of randomization (74) are excluded, the AA hazard ratio improves further to 0.66 (Figure 4D), approaching the EA hazard ratio in the same patient population (0.58 [95% confidence interval 0.41–0.82], data not shown). Compared with all genotypes (Figure 3C), the 32% of patients who were *ADRB1* Arg389Arg (Figure 4C) have an approximate 2-fold increase in effect size (using the relative effect size [RES] calculation [Supplement]), and then restriction to class-III (Figure 4D) results in an approximate 3-fold increase compared with Figure 3C. These data suggest that differences in  $\beta$ -blocker/sympatholytic response exist between AA and EA patients beyond the 37% lower (32% AA, 51% EA) *ADRB1* Arg389Arg genotype in AA individuals, but that strategies in addition to pharmacogenetics such as more precise clinical phenotyping (3)—in this case excluding volume overloaded, unstable class-IV patients consistent with most HF guidelines could be used to bring the AA therapeutic response in line with that of EA individuals. In the example presented in Figures 3 and 4, the largest increment in effectiveness (2-fold) was achieved by treating patients with a preferred genotype, followed by an additional 1.5-fold achieved by patient selection. The result is an effect size (Supplement) increasing from a negligible 13% to a quite therapeutic 34%, albeit achieved in only 30% of the original AA population.

The 49Gly minor allele of *ADRB1* (Figure 1A) has an approximate 70% higher MAF in AA vs. EA patients (Table 1), and this would lead to a greater degree of cardiac myocyte *ADRB1* receptor down-regulation in AA HF patients (Table 2) that could attenuate  $\beta$ -blocker treatment effects (Table 2) by reducing *ADRB1* signaling. There are conflicting reports of effects of the *ADRB1* Ser49Gly polymorphism on  $\beta$ -blocker effect. A recent report found that open-label carvedilol-treated, Brazilian, AA patients who were 49Gly carriers had improved hospitalization-free survival compared with 49Ser homozygotes, while non-black patients treated with the same 25 mg bi-daily target dose showed no difference between genotypes (75). However, in a large, 75%-EA HF patient population studied prospectively and treated with carvedilol or metoprolol, no effect of *ADRB1* Ser49Gly was found, including no racial interaction (73). In BEST, there was no effect of the *ADRB1* Ser49Gly polymorphism on bucindolol vs. placebo treatment effects in either AA or EA patients, with respective interaction  $p$ -values of 0.23 and 0.74 for ACM/HFH events. In AA patients, the hazard ratio was numerically higher in 49Gly carriers (1.26 [95% confidence interval 0.60–2.63]) compared with 49Ser homozygotes (0.72 [0.42–1.26]), indicating a trend for AA *ADRB1* 49Gly carriers to do worse on bucindolol, the opposite of the result with carvedilol in the Brazilian study (75). A Swedish study that did not report racial demographics but was presumably conducted in predominately EA patients found a therapeutic advantage to the the 49Gly genotype in patients treated with low doses of open-label metoprolol, which disappeared on higher doses (76). However, the possible enhanced effects of  $\beta$ -blockers in

*ADRB1* 49Gly genotypes (75,76) are not supported by biologic plausibility, which would predict an attenuated effect (Table 2), at least in placebo-controlled studies.

***GRK5* Gln41Leu**—The other good candidate for a racial-genetic interaction to  $\beta$ -blocker therapy is the *GRK5* Gln41Leu polymorphism investigated in the large (n=711 AA HF patients, n=1749 EA HF patients) Cincinnati/Pennsylvania observational study (18). Similar to bucindolol in BEST (67, Figure 3), carvedilol or metoprolol exhibited no evidence of a beneficial treatment effect in the all-genotype AA population (Figure 5). In AA patients with *GRK5* 41Leu genotypes who were not treated with  $\beta$ -blocking agents, transplant-free survival from the time of HF diagnosis was better (age/sex-adjusted hazard ratio 0.325, p=0.01, Figure 2) than in 41Gln homozygotes. However, in the  $\beta$ -blocker-treated cohort, AA patients with 41Leu genotypes did equally well compared with 41Gln homozygotes, while EA patients with 41Leu genotypes (limited by only 10 events) may have done worse than their Gln homozygous counterparts (p=0.019 in an unadjusted model, upper right panel Figure 2). These data were interpreted as the *GRK5* 41Leu polymorphism conferring endogenous  $\beta$ -blockade preferentially in AA patients by virtue of their approximate 10-fold higher prevalence of 41Leu genotypes (18), which then obviated potentially favorable effects of  $\beta$ -blockade.

***ADRB2* Gln27Glu, Gly16Arg, Thr164Ile**—These 3 polymorphisms are depicted in Figure 1B. Two studies have reported that the minor allele of *ADRB2* Gln27Glu predicts a positive reverse remodeling response to the  $\beta_1/\beta_2/\alpha_1$  receptor blocker carvedilol: 27Glu carriers in one (57) and Glu homozygotes (58) in the other. The *ADRB2* 27Glu variant is resistant to agonist-induced receptor down-regulation and actually up-regulates in response to agonist exposure (77), which may make the human  $\beta_2$  adrenergic receptor and its subsequent blockade more important in 27Glu genotypes. Although *ADRB2* genotype data relative to race and  $\beta$ -blocker response have not been reported, the allele frequency data in Tables 1 and 2 would predict that AA patients may be at a possible disadvantage to treatment with nonselective  $\beta$ -blockers (Table 2) due to their lower *ADRB2* 27Glu allele frequency.

There is no evidence that *ADRB2* Gly16Arg, which exhibits only a slight difference (increase by about 20%, Table 2) in MAF in AA vs. EA HF patients, affects  $\beta$ -blocker response (Table 2). The *ADRB2* 164Ile allele is rare and was detected in only one AA patient in BEST (Table 1); hence, there are no data on race-associated pharmacogenetic effects (Table 2). Also, the *ADRB2* facilitates norepinephrine release, but in BEST there were no effects of *ADRB2* polymorphisms on baseline norepinephrine or 3-month change, for either race or treatment group (data not shown).

***ADRA2C* Ins322-325Del**—In HF patients, the *ADRA2C* Ins322-325Del polymorphism affects the cardiac sympatholytic response to bucindolol (16), which is a secondary pharmacodynamic property of this nonselective  $\beta$ -blocker (78). The *ADRA2C* receptor is located prejunctionally on cardiac adrenergic neurons, where it provides tonic inhibition to norepinephrine release (79), and a 4-amino acid deletion in the third intracytoplasmic loop at positions 322-325 completely abolishes functionality (15). In patients with advanced HF and elevated adrenergic activity, the *ADRA2C* Ins322-325Del polymorphism does not

appreciably affect circulating norepinephrine levels, but influences bucindolol's sympatholytic effects (16). The norepinephrine-lowering effects of bucindolol, and potentially other sympatholytic agents, are markedly enhanced in HF patients with 322-325Del genotypes (Figure 6) (16).

Similar to *GRK5* 41Leu genotypes, in AA vs. EA subjects the 322-325Del or minor allele genotypes are present at a 10-fold higher frequency (14–16, Tables 1 and 2). In advanced HF, marked sympatholysis can be associated with increased cardiovascular adverse events, due to adrenergic support being reduced to levels below what are required to support the failing heart (16,78). Because of the markedly higher *ADRA2C* 322-325Del frequency, AA patients treated with bucindolol are potentially more prone to this type of adverse event, manifested as increased mortality from sudden death or precipitation of HFH (78).

Figure 6 shows the large effect on bucindolol-associated systemic norepinephrine lowering conferred by *ADRA2C* 322-325 genotypes, and that the effect is present in both EA and AA patients. Systemic plasma norepinephrine is a biomarker for cardiac norepinephrine, which directly participates in the pathophysiology of adverse remodeling as well as in the support of cardiac function (21). As can be seen in Figure 6, the sympatholytic effect of bucindolol is similar in AA and EA patients, but the clinical impact would be much greater in the former population because of the ~10-fold higher frequency of *ADRA2C* 322-325Del genotypes. The exaggerated sympatholysis in *ADRA2C* 322-325Del genotypes in advanced HF patients is associated with a loss in favorable effects of bucindolol on mortality (16) (Figure 6), which is especially pronounced in the presence of *ADRB1* 389Gly genotypes and is not evident in patients who are *ADRB1* Arg389Arg (54). Since AA populations have a much higher frequency of *ADRA2C* 322-325Del as well as *ADRB1* 389Gly genotypes, this may unfavorably impact the response of bucindolol, a situation that can be avoided by confining treatment to patients with an *ADRB1* Arg389Arg genotype (54). The data presented in Figure 6 and their clinical implications underscore how gene variant differences in allele frequency may impact drug response (2), and how a second gene variant's pharmacologic effects can impact outcomes associated with a functionally important polymorphism (54).

Standard HF  $\beta$ -blockers devoid of sympatholytic activity such as carvedilol or metoprolol do not have potential for adverse interactions with *ADRA2C* 322-325Del genotypes, which was borne out in a large clinical trial (73).

***ACE Del/intron16/Ins, EDN1 Lys198Asn, ECE1 Thr341Ile***—A significant effect on  $\beta$ -blocker therapy was shown for the *ACE* Del/intron16/Ins polymorphism (47), which is discussed in the renin-angiotensin-aldosterone system (RAAS) inhibitor section below. In a 309-patient, nonischemic cardiomyopathy subgroup of the BEST DNA bank population, 9 polymorphisms in 6 genes within the endothelin system were measured and tested against the outcome of ACM and HFH (42). A haplotype including an exon 5, non-synonymous SNP that is correlated with elevated endothelin-1 levels, *EDN1* Lys198Asn was highly related to outcome in the bucindolol group, with the minor allele associated with an adverse treatment effect (42). The finding appeared to be independent of any *ADRB1* effects, but the data were generated in only 30% of the total number of DNA substudy patients. In addition,

since the *EDN1* Lys198Asn allele frequencies don't differ by race (Table 1), differential effects in AA vs. EA patients would not be expected. However, another endothelin system polymorphism, *ECE1* Thr341Ile does exhibit substantial racial differences, with an approximate 10-fold higher MAF in AA patients (Tables 1 and 2). Both the *ACE* Del/Ins association with standard  $\beta$ -blocker responses (47) and the *EDN1* Lys198Asn or *ECE1* Thr341Ile pharmacogenetic interactions with bucindolol are likely examples of indirect (from the primary target[s]) pharmacogenetic interactions, based on inhibitory effects of  $\beta$ -blockers on the renin-angiotensin system and cross-regulation of the endothelin and  $\beta$ -adrenergic systems.

### Hydralazine/isosorbide dinitrate and *NOS3* Glu298Asp, *CYP11B2* T-344C, or *GNB3* C825T

In a 354-patient DNA bank substudy of the African-American Heart Failure Trial (A-HeFT, 80), McNamara and associates have published data indicating that polymorphisms in *NOS3* (endothelial nitric oxide synthase) (30), *CYP11B2* (aldosterone synthase) (32), and *GNB3* (guanine nucleotide  $\beta$ 3 subunit) (28) influence the response to fixed-dose hydralazine/isosorbide dinitrate (trade name BiDil) in AA patients. The more favorable response genotypes are the major allele homozygotes in *NOS3* and *CYP11B2* and the minor allele homozygote in *GNB3* that is the major allele in AA populations (Tables 1 and 2). In each case, the favorable response genotype is present at a higher frequency in AA populations as compared with EA populations, and there is evidence that BiDil is more effective in AA compared with EA patients (68). Although the A-HeFT DNA/pharmacogenetic substudy was relatively small, these data are highly intriguing and suggest that at least for one type/class of HF therapy, AA patients are at a pharmacogenetic advantage (81). However, because there are no comparable EA data, these findings are classified as *possible* in Table 2.

***NOS3* Glu298Asp**—The biologic/pharmacologic plausibility for the pharmacogenetic interactions of BiDil are not as obvious as for  $\beta$ -blockers, as the modulating polymorphisms are more remote from (28,30) or even unrelated to (32) the drug target. However, the exon 7 *NOS3* Glu298Asp polymorphism results in a loss of function (82) in an enzyme that may partially mediate (83) or at least modulates NO donor pharmacologic action, and the more favorable effect in the higher-function *NOS3* Glu298Glu genotype that is enriched in AAs (Tables 1 and 2) provides biologic plausibility for the pharmacogenetic interaction observed in the A-HeFT DNA substudy (30). The observed pharmacogenetic effect was an improvement in the primary endpoint composite score (mortality, HFH, and change in quality of life at 6 months) by BiDil vs. placebo in patients with a Glu298Glu genotype but not in 298Asp carriers (30).

***GNB3* C825T**—This *GNB3* polymorphism affects a gene product in vascular smooth muscle contraction signal transduction pathways (84). A synonymous C→T transition in exon 10 of *GNB3* in some unknown way leads to the formation of a splice variant that has a 41-amino acid deletion, and is a gain-of-function change (84). Thus the *GNB3* 825T favorable response polymorphism for BiDil is for a gene variant that would lead to greater vasoconstriction, resulting in biologic plausibility for the observed favorable effects (28). In

the A-HeFT DNA substudy, BiDil vs. placebo improved the composite score and HF hospitalization-free survival in 825T homozygotes, but not in C825 carriers (28).

**CYP11B2 T-344C**—Although there is no known mechanism of action for BiDil modulating aldosterone synthase, the –344T homozygous promoter polymorphism in *CYP11B2* is related to increased aldosterone urinary excretion (85), and it was associated with deterioration in the composite score in the placebo arm of the A-HeFT DNA substudy (32). This is potentially an example of a pharmacogenetic effect being manifested through effects on increasing event rate in placebo-treated patients, making drug effectiveness easier to detect (3). In the A-HeFT DNA substudy, BiDil vs. placebo improved the composite score in –344T homozygotes but not in –344C carriers (32).

### ACEIs, ARBs, or MRAs and polymorphisms in the RAAS

The RAAS is of undeniable importance in the natural history of myocardial remodeling and HF, and inhibitors of angiotensin II formation (ACEIs), angiotensin AT-1 receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs) all produce substantial favorable effects on HF natural history. With the exception of *AGT* Thr174Met (40,86), all the RAAS polymorphisms listed in Table 1 have been associated with effects on cognate gene expression or hormone levels (36,40,85–89), and *AGT* Thr174Met has been associated with hypertension (40). The *AGTR1* A1166T and the 2 *AGT* polymorphisms exhibit racial differences in allele frequencies, while *ACE* Del/intron16/Ins and *NR3C2* Ile180Val do not. However, in racially unselected HF populations, relatively few of these gene variants have been shown to be associated with differential responses to RAAS inhibitors.

In 2 medium-sized observational studies conducted at the University of Pittsburgh, McNamara et al reported that either  $\beta$ -blockers (n=328) (47) or higher doses of ACEIs (n=479) (59) were required to reduce an elevated risk of mortality or cardiac transplantation in HF patients who had an *ACE* Del homozygous genotype. These findings are consistent with the *ACE* Del allele being associated with higher circulating (36) angiotensin-II levels and higher cardiac *ACE* gene expression (87), and with  $\beta$ -blockers inhibiting renin release (90). There weren't enough (about 10%) AA subjects in the Pittsburgh studies (47,59) to assess a racial difference in response, and the *ACE* Del/Ins polymorphism does not exhibit a racial difference in allele frequencies (Table 1). However, a racial difference in ACEI response could result from different allele frequencies in *AGT* Met235Thr, *AGT* Thr174Met, *AGTR1* A1166C, or *CYP11B2* T-344C polymorphisms (Tables 1 and 2).

The Veterans Affairs Vasodilator-Heart Failure (V-HeFT) II study provided evidence of a lesser response to enalapril in AA vs. EA HF patients (68), but the larger Valsartan Heart Failure Trial (Val-HeFT) trial did not observe differences between captopril responses in AA vs. EA patients (71). The definitive information on race and ACEI response in HF comes from a 1196 patient matched-cohort design report from the Studies of Left Ventricular Dysfunction (SOLVD) trial, where compared to EA 800 AA patients had a reduction in enalapril efficacy for prevention of HFHs but not mortality (69). RAAS polymorphisms were not measured in any of these multicenter, placebo-controlled HF trials, so a possible relationship between race-associated RAAS genetic variation and blunted

response can only be inferred. However, assuming that the effects of ACEIs are attenuated in HF just as they are in blood pressure responses in hypertension (70), the polymorphism that best predicts such an effect is *AGTR1* A1166C, whose 70% lower 1166C MAF in AAs would predict lower AGTR1 receptor density (88) and RAAS drug response (60) (listed as *possible* in Table 2).

Val-HeFT also reported no effect of race on the response to the ARB valsartan (71), but support for a possible pharmacogenetic interaction between ARBs and the *AGTR1* A1166C polymorphism does come from a small Canadian study in EA HF patients (60). This study demonstrated that 1166C genotypes had a more favorable N-terminal pro-brain natriuretic peptide response to candesartan compared with A1166 homozygotes (Table 4) (60). Therefore, Table 2 lists a racial interaction of ARBs via *AGTR1* A1166C as *possible*. Surprisingly, there are no reports of racial effects of MRAs in HF, but the potential for such heterogeneity exists via the approximate 2-fold difference in MAF of the promoter region *CYP11B2* T-344C polymorphism (Table 1).

### Device therapies and signaling polymorphisms

Improvement in left ventricular chamber function by synchronizing contraction with biventricular pacing is expectedly accompanied by decreases in neurohormonal biomarkers (91,92), and so polymorphic variation in signaling mechanisms could affect cardiac resynchronization therapy (CRT) responses. There are 2 polymorphisms, *ADRB2* Gln27Glu (61) and *NR3C2* Ile180Val (62), whose minor allele genotypes have been reported to be associated with a CRT reverse remodeling response (Table 4). As seen in Table 1, there is no obvious racial difference in MAF for *NR3C2* Ile180Val, but there is a modest 2-fold lower frequency of the higher response *ADRB2* 27Glu allele in AA patients that could affect CRT response (Tables 1 and 2). However, thus far only CRT-treated EA patients have been genetically investigated for either polymorphism (61,62). CRT responses by racial subgroup have been reported from the Multicenter Automatic Defibrillator Implantation Trial–Cardiac Resynchronization Therapy (MADIT-CRT) (93) study in the absence of any genetic information, where no differences in effectiveness were observed.

The other device that has proved to be invaluable in HF therapy is the ICD. AA compared with EA HF patients may have a disproportionately higher rate of ventricular arrhythmias and sudden cardiac death (94), which is likely due in part to genetic factors. The nearly exclusive presence in AAs of the Ser1103Tyr variant of *SCN5A* (Tables 1 and 2)—the gene that encodes the  $\alpha$  subunit of the human Nav1.5 sodium channel and plays a central role in cardiac conduction and repolarization as well as in regulation of  $[Ca^{2+}]_i$  through downstream effects on  $Na^+$ - $Ca^{2+}$  exchange (which is also under  $\beta$ -adrenergic regulation)—is especially intriguing. It has been proposed that the metabolic abnormalities and cellular derangements accompanying HF may serve as proarrhythmic substrates for 1103Tyr minor allele carriers, and the variant has been shown to increase the “late” or sustained  $Na^+$  current under certain conditions (95). The *SCN5A* 1103Tyr variant is associated with a 4-fold increase in sustained ventricular arrhythmias in HF patients with ICDs (43), essentially making ICDs more clinically effective (more lives saved from sudden cardiac death) in AA patients with an *SCN5A* 1103Tyr genotype (43). Moreover, these observations suggest that a

pharmacogenetic approach to sudden cardiac death prevention in HF may be indicated, with agents such as ranolazine (96) that diminish the sustained Na<sup>+</sup> current potentially having a role. In HF there is precedent for major antiarrhythmic pharmacogenetic effects via the *ADRB1* Arg389Gly polymorphism, for prevention of ventricular tachycardia/fibrillation (97) and atrial fibrillation (98).

### Non-Cardiac disease common genetic variation

This review does not address common genetic variation important in non-cardiac pathophysiologic settings, but based on population genetics it is likely that at least some of the polymorphism differences between AA and EA extend to affecting non-cardiac disease mechanisms or their treatment. It would be expected that some signaling systems that were shown to have AA vs. EA differences in nonfailing/presumably healthy populations, such as those involving *ADRB1* and *ADRB2*, would exhibit similar genetic variation in non-cardiac diseases in which they may be important, for example in CNS disorders.

### Conclusions

Due to population genetics, AA vs. EA HF patients exhibit substantial differences in frequencies of functionally important alleles that code for important constituents of neurohormonal cell-signaling pathways. For at least two polymorphisms this may differentially affect the progression of HF in AA vs. EA patients. Importantly, the effectiveness or safety of several drugs and a device may be influenced by AA vs. EA self-identified race, and it is likely that genetic variation in signaling pathways that are important in HF contribute to this therapeutic heterogeneity. Compared to EA, AA race can be associated with allele frequency-based genetic profiles that 1) can lead to therapeutic response attenuation (standard  $\beta$ -blockers and the experimental  $\beta$ -blocker/sympatholytic bucindolol); 2) possibly decrease therapeutic response (ACEIs and ARBs); 3) enhance effectiveness (fixed-dose hydralazine/isosorbide dinitrate); or 4) increase event rate, making the therapy more clinically effective (ICDs). In order to better understand the basis for heterogeneous therapeutic responses, race, pharmacogenetics and their interaction need to be considered in the development of drugs and devices for the treatment of HF. As demonstrated, identifying genetic subgroups with an enhanced response is a key to delivering optimal therapies for racial cohorts, regardless of the net effect of the therapeutic agent in the parent population and frequency of the preferred genotype.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations and Acronyms

<b>AA</b>	African ancestry
<b>ACEI</b>	angiotensin-converting enzyme inhibitor
<b>ACM</b>	all-cause mortality
<b>A-HeFT</b>	African-American Heart Failure Trial
<b>ARB</b>	angiotensin-receptor blocker
<b>BEST</b>	$\beta$ -Blocker Evaluation of Survival Trial
<b>CCDS</b>	Consensus coding sequence
<b>CRT</b>	cardiac resynchronization therapy
<b>Del</b>	deletion
<b>EA</b>	European ancestry
<b>ENCODE</b>	Encyclopedia of DNA Elements (Project)
<b>HF</b>	heart failure
<b>HFH</b>	heart failure hospitalization
<b>HF<sub>r</sub>EF</b>	heart failure with reduced left ventricular ejection fraction
<b>ICD</b>	implantable cardioverter defibrillator
<b>InDel</b>	insertion-deletion
<b>Ins</b>	insertion
<b>MADIT-CRT</b>	Multicenter Automatic Defibrillator Implantation Trial–Cardiac Resynchronization Therapy
<b>MAF</b>	minor allele frequency
<b>MERIT-HF</b>	Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure
<b>MRA</b>	mineralocorticoid receptor antagonists
<b>NF</b>	non-heart failure
<b>RAAS</b>	renin-angiotensin-aldosterone system
<b>RES</b>	relative effect size
<b>SNP</b>	single-nucleotide polymorphism
<b>SOLVD</b>	Studies of Left Ventricular Dysfunction
<b>Val-HeFT</b>	Valsartan Heart Failure Trial
<b>V-HeFT</b>	Veterans Affairs Vasodilator-Heart Failure



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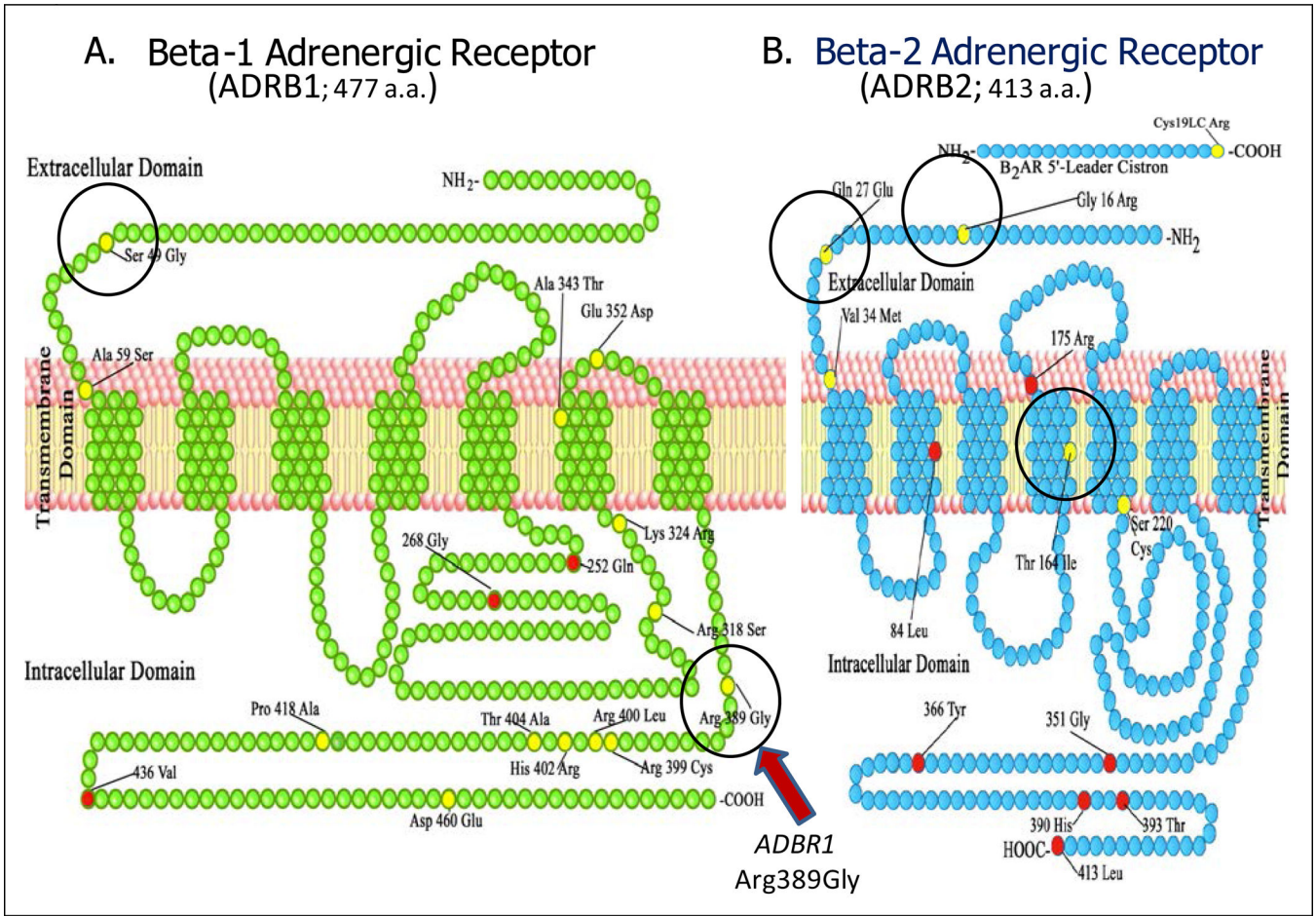
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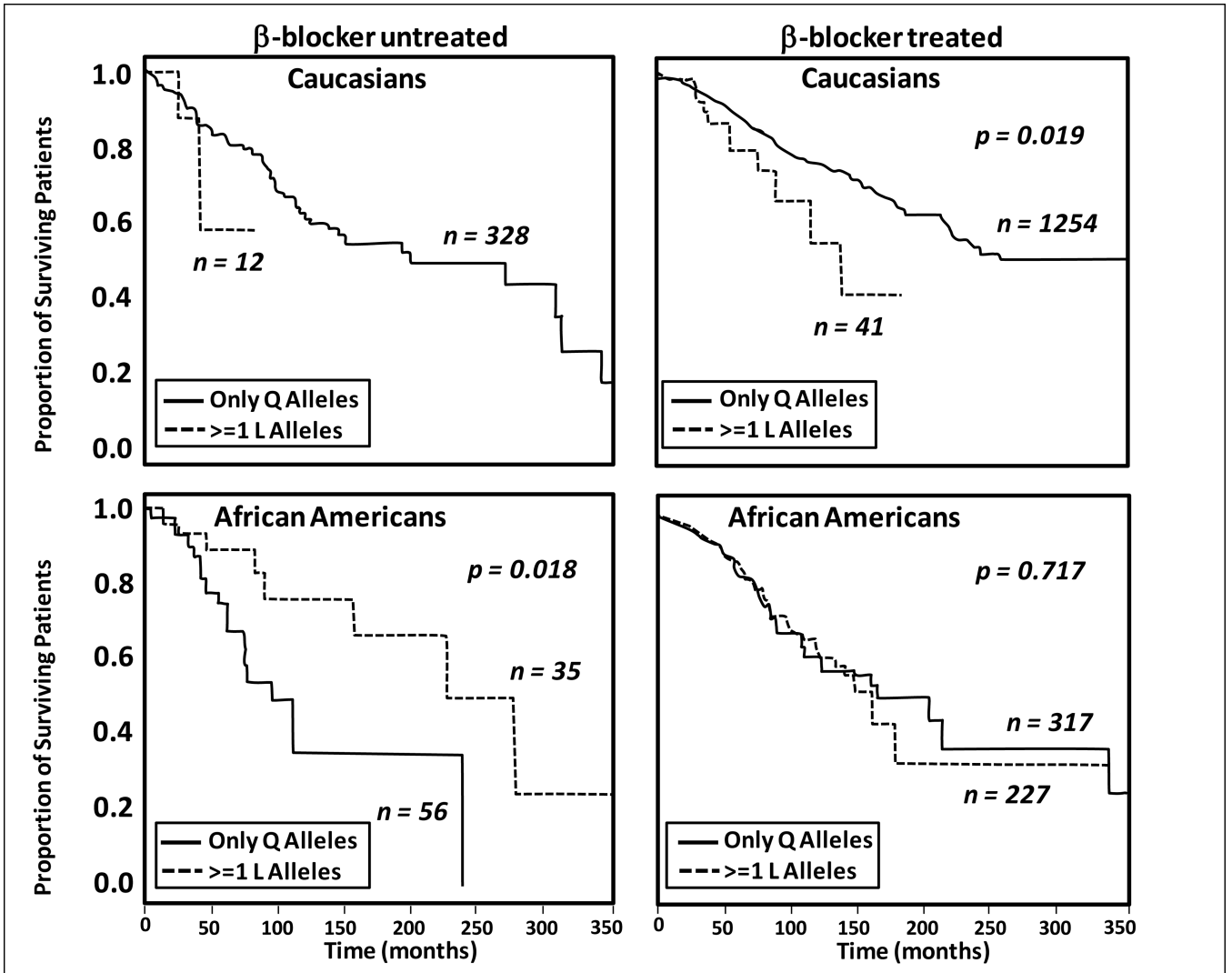
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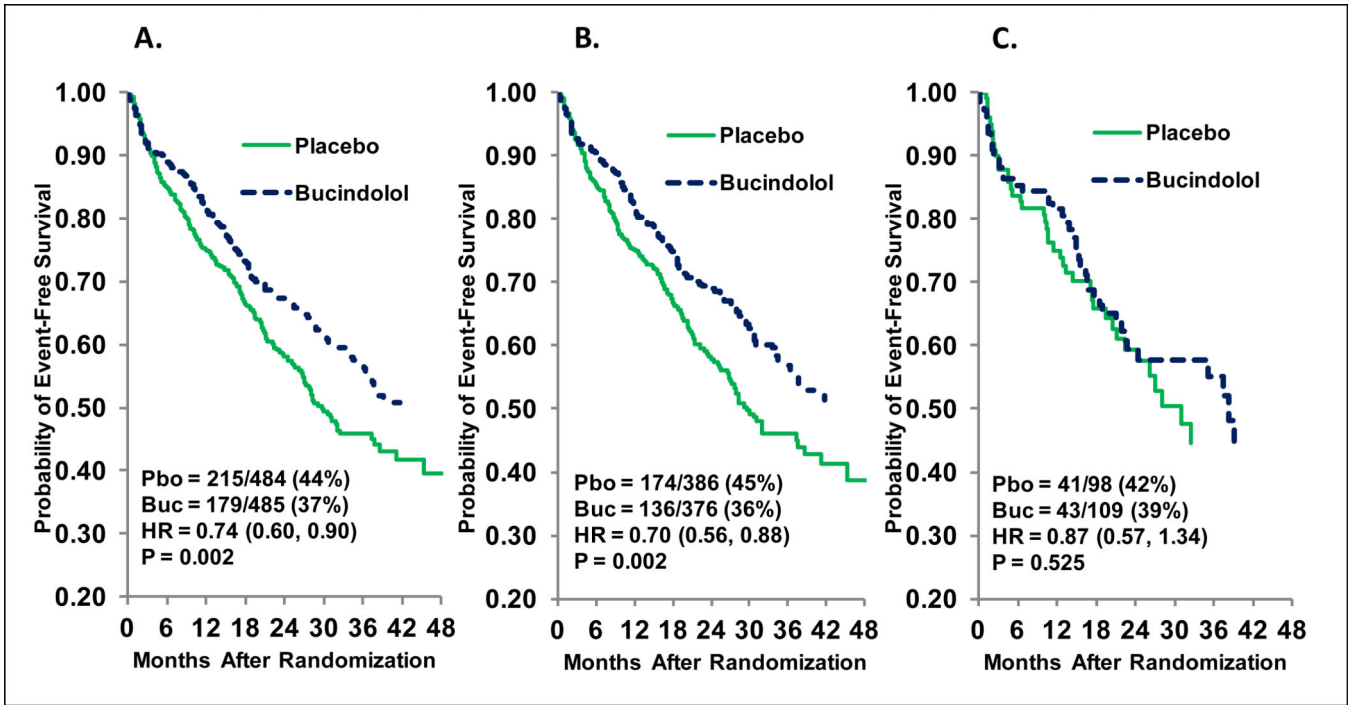


**Figure 1.** Consensus coding sequence (CCDS, <http://www.ensembl.org/info/genome/genebuild/ccds.html>) for *ADRB1* (A) and *ADRB2* (B). Amino acid (a.a.) changes resulting from nonsynonymous SNPs are in yellow, while unchanged amino acids at the site of synonymous SNPs are in red. Polymorphisms discussed in the text are circled, and the *ADRB1* Arg389gly locus is highlighted by an arrow. Reproduced and modified from reference (23), with the permission of Le Jacq Communications, Inc and *Congestive Heart Failure*.

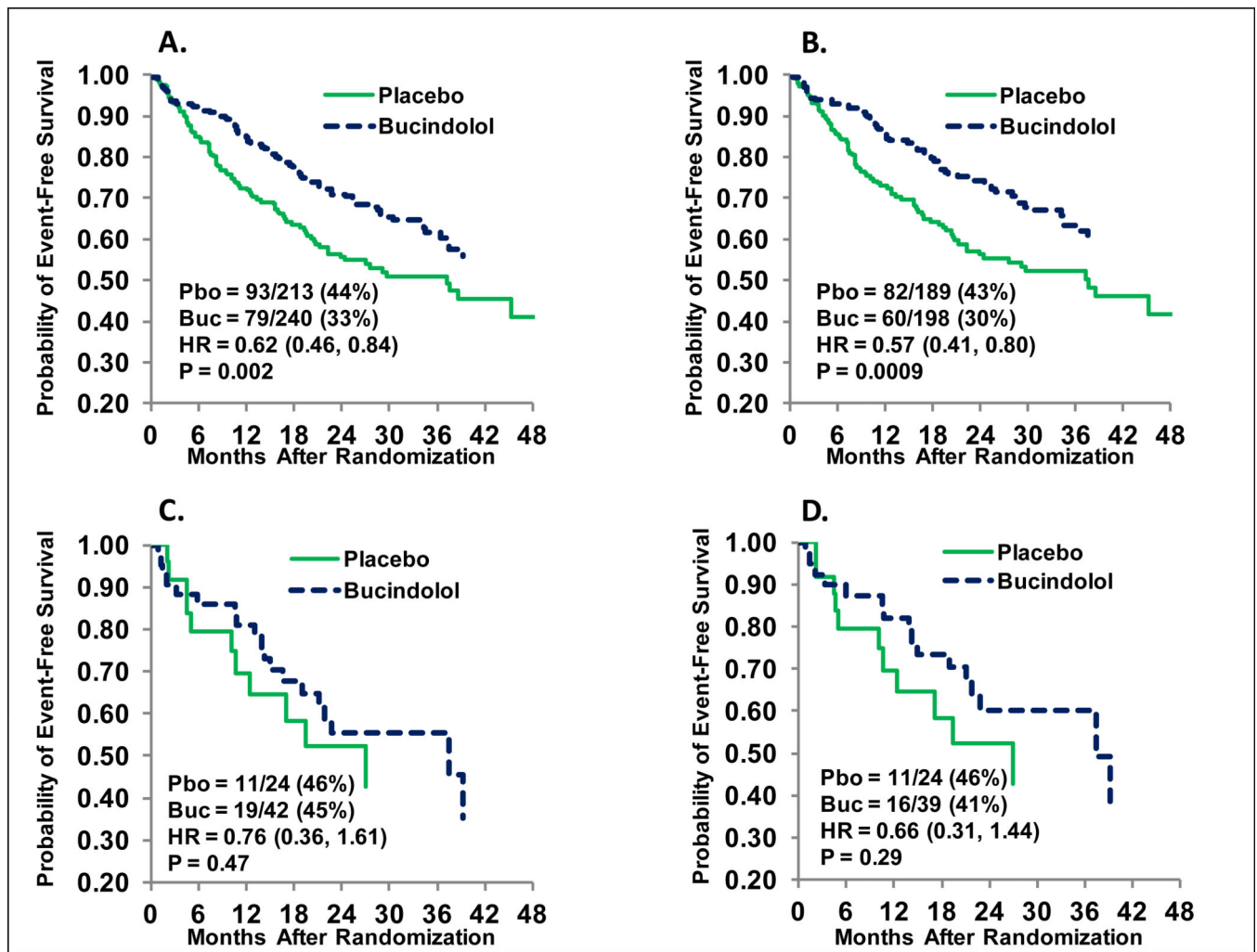


**Figure 2.** Kaplan-Meier curves for time to all-cause mortality or cardiac transplantation from the time of heart failure diagnosis of patients with *GRK5* Gln(Q)41Leu(L) genotypes (18). P-values are from an unadjusted model log-rank statistic. Reproduced with permission of Elsevier and the *Journal of the American College of Cardiology*.



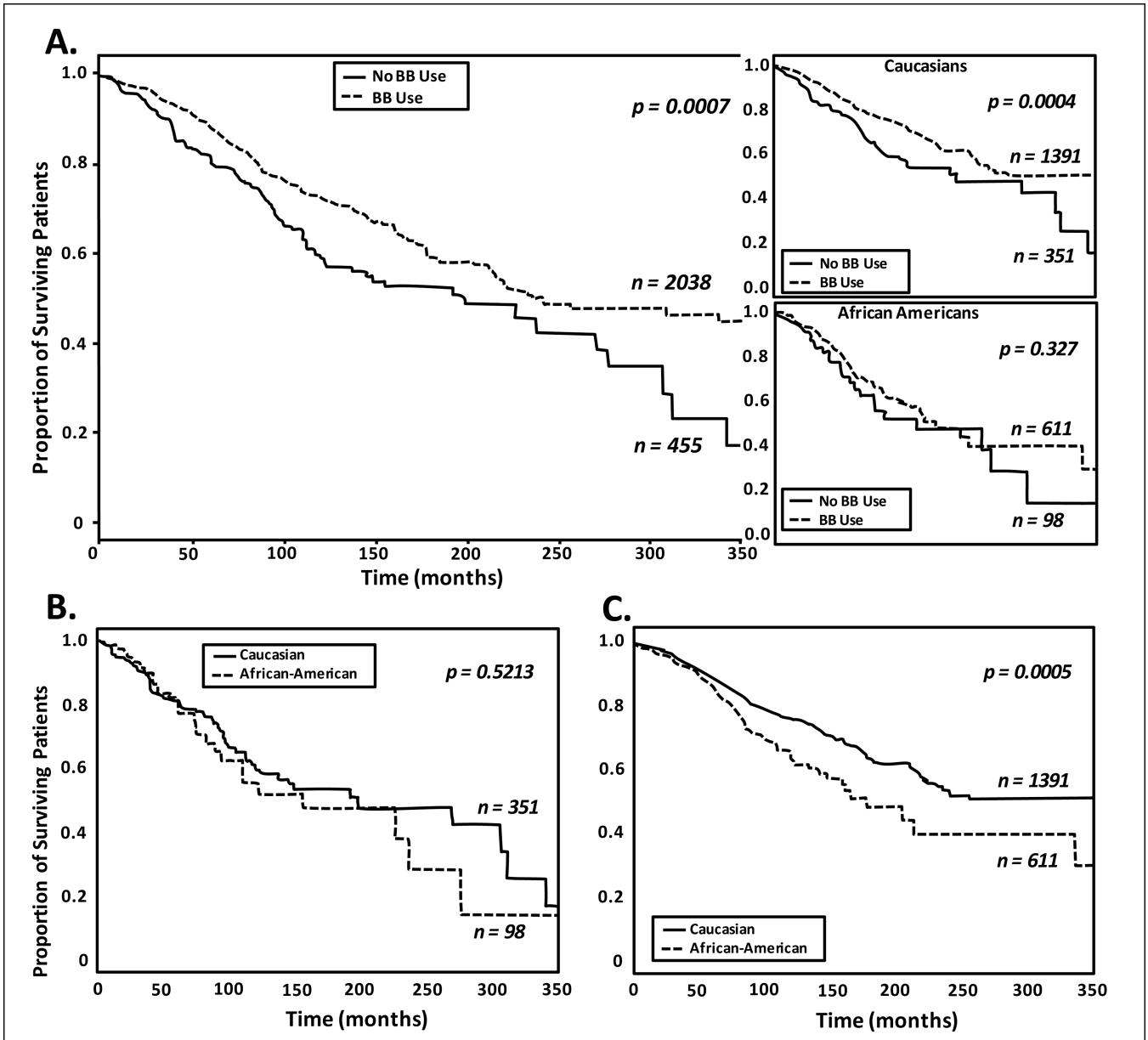


**Figure 3.** Time to all-cause mortality or first heart failure hospitalization in the BEST “Adrenergic Receptor Polymorphism Substudy.” A. European-ancestry (EA) + African-ancestry (AA) patients; B. EA patients; C. AA patients. Pbo = placebo events/patients; buc = bucindolol events/patients; HR = hazard ratio. Cox modeling was unadjusted.

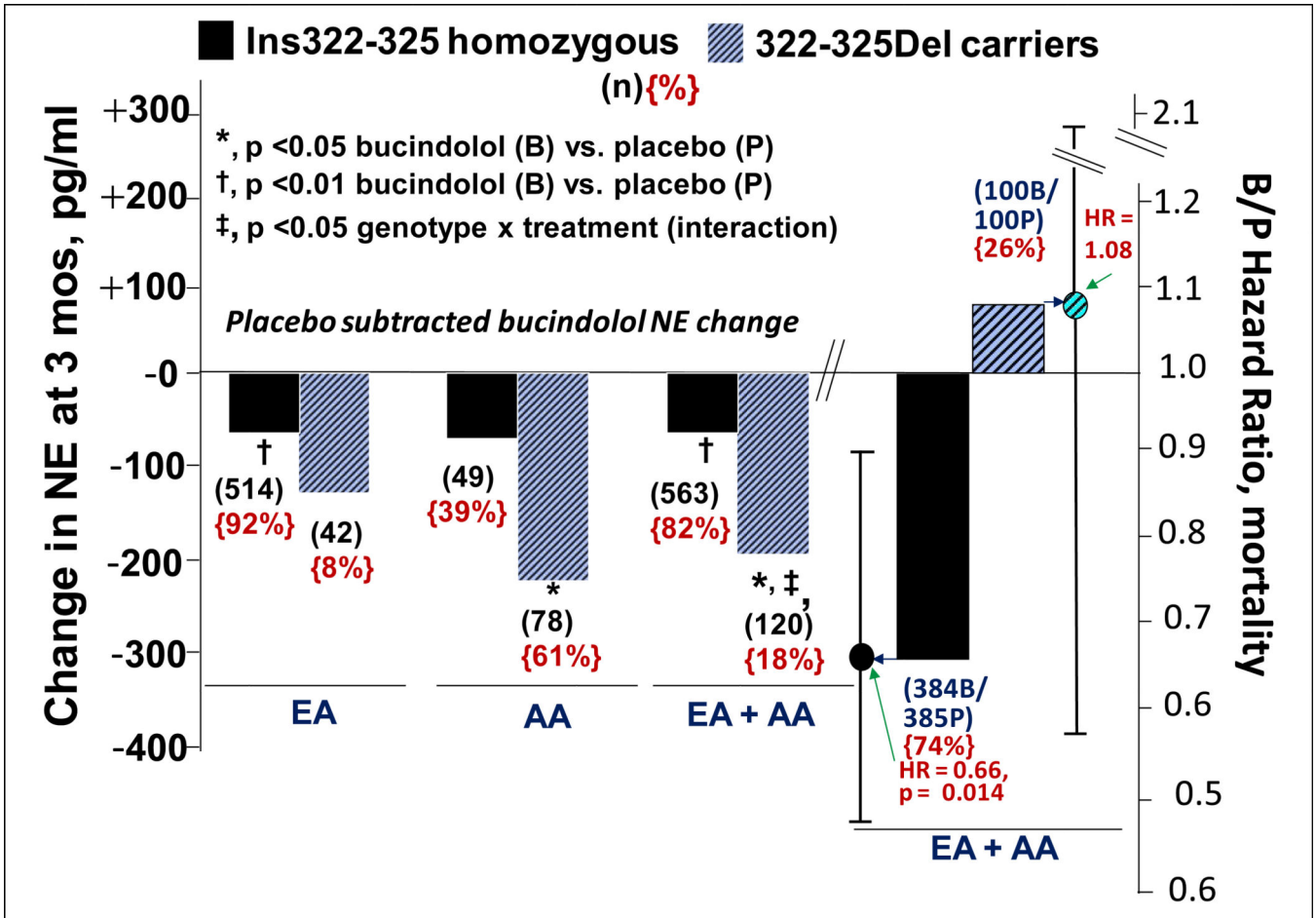


**Figure 4.**

Time to all-cause mortality or first heart failure hospitalization in the BEST “Adrenergic Receptor Polymorphism Substudy,” *ADRB1* Arg389Arg cohort. A. European-ancestry (EA) + African-ancestry (AA) New York Heart Association Class III and IV patients; B. EA patients Class III and IV; C. AA patients Class III and IV; D. AA patients Class III only. Pbo = placebo events/patients; Buc = bucindolol events/patients; HR = hazard ratio. Cox modeling was unadjusted.



**Figure 5.** Effect of race on transplant-free survival in the presence or absence of P-blocking agents in the Cincinnati/Pennsylvania study (18). A. Combined European-ancestry (EA) and African-ancestry (AA) heart failure cohort (18), stratified by +/- P-blocker (carvedilol 65%, metoprolol 25%, other 10%) use; (top inset, EA ["Caucasians"]; bottom inset, AA ("African-Americans")). Solid line = P-blocker-untreated; dashed line = P-blocker-treated. B. Transplant-free survival of P-blocker-untreated heart failure subjects, stratified by race. C. Transplant-free survival of P-blocker-treated heart failure subjects, stratified by race. Solid line = EA; dashed line = AA. P-values are from an unadjusted model log-rank statistic. Reproduced with permission of Elsevier and the *Journal of the American College of Cardiology*. BB =  $\beta$ -blocker.



**Figure 6.** Bucindolol-associated norepinephrine (NE)-lowering and effects on all-cause mortality by *ADRA2C* Ins322-325Del genotype. L-y axis and panel, systemic venous NE changes (pg ± SEM) at 3 months, placebo subtracted in European-ancestry (EA) or African-ancestry (AA) patients or the combination in BEST. R-y axis and panel, bucindolol:placebo hazard ratio for all-cause mortality in EA + AA patients. There were no differences in baseline NE, which ranged from 436 pg/mL to 557 pg/mL in the various groups. The treatment × genotype interaction p-value for all-cause mortality is 0.29.

**Table 1**

Racial distribution of selected signaling pathway alleles in patients with and without HF

Gene, amino acid or nucleotide position, SNP reference (rs) number, cohort, clinical trial	*MAF, AA <i>a,b</i> populations		*MAF, EA <i>c,d</i> populations		Subject Ns		<sup>†</sup> p-value AA vs EA
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	AA	EA	
<sup>24-26</sup> <i>ADRB1</i> Arg389Gly (rs1801253), (NF)	<i>a</i> , <i>b</i>	0.43 Gly	<i>c</i>	0.28 Gly	269	399	<0.0001
<i>ADRB1</i> Arg389Gly (HF, BEST)	<i>a</i>	0.43 Gly	<i>c</i>	0.28 Gly	207	762	<0.0001
<sup>14,24</sup> <i>ADRB1</i> Ser49Gly (rs1801252), (NF)	<i>a</i> , <i>b</i>	0.22 Gly	<i>c</i>	0.12 Gly	119	147	0.002
<i>ADRB1</i> Ser49Gly (HF, BEST)	<i>a</i>	0.24 Gly	<i>c</i>	0.14 Gly	205	756	<0.0001
<sup>24</sup> <i>ADRB2</i> Gly16Arg (rs1042713), (dbSNP)	<i>a</i> , <i>b</i>	0.49 Arg	<i>c</i>	0.35 Arg	210	160	0.0001
<i>ADRB2</i> Gly16Arg (HF, BEST)	<i>a</i>	0.47 Arg	<i>c</i>	0.39 Arg	207	762	0.003
<sup>24</sup> <i>ADRB2</i> Gln27Glu (rs1042714), (dbSNP)	<i>a</i> , <i>b</i>	0.16 Glu	<i>c</i>	0.45 Glu	107	107	<0.0001
<i>ADRB2</i> Gln27Glu (HF, BEST)	<i>a</i>	0.19 Glu	<i>c</i>	0.39 Glu	207	762	<0.0001
<sup>24</sup> <i>ADRB2</i> Thr164Ile (rs1800888), (dbSNP)	<i>a</i> , <i>b</i>	0.00 Ile	<i>c</i>	0.01 Ile	166	272	0.09
<i>ADRB2</i> Thr164Ile (HF, BEST)	<i>a</i>	0.002 Ile	<i>c</i>	0.014 Ile	207	762	0.053
<sup>14,15</sup> <i>ADRA2C</i> Ins322-325Del (rs61767072), (NF)	<i>a</i>	0.40 Del	<i>c</i>	0.036 Del	132	179	<0.0001
<sup>16</sup> <i>ADRA2C</i> Ins322-325Del (HF, BEST)	<i>a</i>	0.43 Del	<i>c</i>	0.04 Del	207	762	<0.0001
<sup>24,27</sup> <i>GNB3</i> C825T (rs5443), (NF)	<i>b</i> , <i>e</i>	0.86 T	<i>d</i>	0.30 T	159	1914	<0.0001
<sup>28</sup> <i>GNB3</i> C825T (HF, A-HeFT)	<i>a</i>	0.72 T	<i>c</i>	0.34 T	350	424	<0.0001
<sup>24-29</sup> <i>NOS3</i> Glu298Asp (rs1799983), (NF)	<i>ab</i>	0.11 Asp	<i>c</i>	0.35 Asp	277	295	<0.0001
<sup>30</sup> <i>NOS3</i> Glu298Asp (HF, A-HeFT)	<i>a</i>	0.11 Asp	<i>c</i>	0.37 Asp	352	424	<0.0001
<sup>24,31</sup> <i>CYP11B2</i> T-344C (rs1799998), (dbSNP), (NF)	<i>ab</i>	0.20 C	<i>c</i>	0.45 C	624	592	<0.0001
<sup>32</sup> <i>CYP11B2</i> T-344C (HF, A-HeFT)	<i>a</i>	0.22 C	<i>c</i>	0.43 C	354	424	<0.0001
<sup>24</sup> <i>NR3C2</i> Ile180Val (rs5522), (dbSNP)	<i>a</i> , <i>b</i>	0.083 Val	<i>c</i>	0.103 Val	241	195	0.38
<sup>33</sup> <i>NR3C2</i> Ile180Val (HF)	-	-	<i>c</i>	0.12Val	-	156	-
<sup>34-36</sup> <i>ACE</i> Del/intron16/Ins (rs1799752), (NF)	<i>a</i>	0.41 Ins	<i>d</i>	0.43 Ins	467	196	0.48
<sup>37,38</sup> <i>ACE</i> Del/intron16/Ins (HF)	<i>a</i>	0.37 Ins	<i>c</i>	0.36 Ins	145	324	0.97

Gene, amino acid or nucleotide position, SNP reference (rs) number, cohort, clinical trial	*MAF, AA <sup>a,b</sup> populations	*MAF, EA <sup>c,d</sup> populations	Subject Ns		<sup>†</sup> p-value AA vs EA
			AA	EA	
<i>AUKI</i> A1166C (3 UTR), (rs5186), (dbSNP), (NF)	a <sub>0.051</sub> C	c <sub>0.26</sub> C	242	242	<0.0001
<i>AUKI</i> A1166C (3 UTR), (HF)	a <sub>0.10</sub> C	c <sub>0.32</sub> C	145	384	<0.0001
<sup>24,35</sup> <i>AGT</i> Thr174Met (rs4762), (NF)	a <sub>0.05</sub> Met	c <sub>0.096</sub> Met	316	469	0.001
<sup>41</sup> <i>AGT</i> Thr174Met (Htn)	a <sub>0.05</sub> Met	–	187	–	–
<sup>38</sup> <i>AGT</i> Thr174Met (HF)	–	i <sub>0.18</sub> Met	–	58	NA
<sup>24,35</sup> <sup>40</sup> <i>AGT</i> Met235Thr (rs699), (NF)	a <sub>0.86</sub> Thr	c <sub>0.38</sub> Thr	299	450	<0.0001
<sup>41</sup> <i>AGT</i> Met235Thr (Htn)	a <sub>0.83</sub> Thr	c <sub>0.41</sub> Thr	187	611	<0.0001
<sup>38</sup> <i>AGT</i> Met235Thr (HF)	–	i <sub>0.48</sub> Thr	–	58	–
<sup>24</sup> <i>EDV1</i> Lys198Asn (rs5370), (dbSNP), (NF)	a <sub>0.18</sub> Asn	c <sub>0.22</sub> Asn	266	220	0.120
<sup>42</sup> <i>EDV1</i> Lys198Asn (HF, BEST)	a <sub>0.23</sub> Asn	c <sub>0.23</sub> Asn	69	212	0.99
<sup>24</sup> <i>ECE1</i> Thr341Ile (rs1076669), (dbSNP), (NF)	a <sub>0.00</sub> Ile	c <sub>0.076</sub> Ile	157	216	<0.0001
<sup>42</sup> <i>ECE1</i> Thr341Ile (HF, BEST)	a <sub>0.007</sub> Ile	c <sub>0.09</sub> Ile	69	212	0.0009
<sup>24</sup> <i>GRK5</i> Gln41Leu (rs2230345), (dbSNP), (NF)	a <sub>0.31</sub> Leu	c <sub>0.025</sub> Leu	141	141	<0.0001
<sup>18</sup> <i>GRK5</i> Gln41Leu (rs2230345), (HF)	a <sub>0.23</sub> Leu	c <sub>0.017</sub> Leu	711	1749	<0.0001
<sup>19,24</sup> <i>SCN5A</i> Ser1103Tyr (rs7626962), (dbSNP), (NF)	a <sub>0.065</sub> Tyr	c <sub>0.001</sub> Tyr	527	493	<0.0001
<sup>43</sup> <i>SCN5A</i> Ser1103Tyr (rs7626962), (dbSNP), (HF)	a <sub>0.09</sub> Tyr	–	112	–	–

\* Minor allele frequency.

<sup>†</sup> Chi-square 2-sided test (Fisher exact test substituted for low cell counts) performed on the number of alleles.

AA = African ancestry; A-HeFT, African-American Heart Failure Trial; BEST =  $\beta$ -Blocker Evaluation of Survival Trial; dbSNP = Database of Single-Nucleotide Polymorphisms; EA = European ancestry; HF = heart failure with reduced left ventricular ejection fraction; Htn, hypertension; NF = nonfailing; UTR = untranslated region; SNP = single-nucleotide polymorphism.

<sup>a</sup> African-American;

<sup>b</sup> Sub-Saharan African;

<sup>c</sup> European-American (Caucasian);

<sup>d</sup> European (Caucasian);

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- <sup>e</sup> South African or Zimbabwean;
- <sup>f</sup> Brazilian-African;
- <sup>g</sup> Brazilian-European (Caucasian);
- <sup>h</sup> United Kingdom-European;
- <sup>i</sup> French-Canadian European.

**Table 2**

Impact of AA vs. EA MAF differences on cardiac myocyte cell signaling\* and biologic effect on cardiac myocytes, adrenergic nerve terminals, vascular smooth muscle or endothelium

Gene polymorphism	Effect of minor vs major allele	AA vs. EA MAF	AA vs. EA cardiac myocyte relative effect (cytoprotective or cytopathic/harmful)*	Pharmacogenetic effect in AA vs. EA* ( <i>Actual Possible or Predicted</i> )
<i>ADRB1</i> Arg389Gly	↓NE <sup>†</sup> affinity ↓ signal transduction ↓ constitutive activity	~50% ↑ in Gly	Protective (↓ <i>ADRB1</i> signaling)	↓ response to β-blockers ( <i>Actual</i> for bucindolol, <i>Predicted</i> for standard β-blockers but not supported by empirical data)
<i>ADRB1</i> Ser49Gly	↑ internalization, downregulation	~70% ↑ in Gly	Protective (↓ <i>ADRB1</i> signaling)	↓ response to β-blockers ( <i>Predicted</i> but not supported by empirical data)
<i>ADRB2</i> Gly16Arg	↑ internalization, downregulation	~20% ↑ in Arg	Protective	↓ response to nonselective β-blockers ( <i>Predicted</i> , not supported by empirical data)
<i>ADRB2</i> Gln27Glu	↓ internalization, downregulation	~ 50% ↓ in Glu	Protective (↓ <i>ADRB2</i> signaling)	↓ response to carvedilol, CRT ( <i>Possible</i> , no data in AA)
<i>ADRB2</i> Thr164Ile	↓ signal transduction ↓ NE, EPI <sup>‡</sup> affinity ↑ vasoconstriction	Ile ↓, rare in AA	Harmful (restoration of cardiac <i>ADRB2</i> signaling); Protective (↓ afterload)	↑ response to nonselective β-blockers ( <i>Predicted</i> , no reported data)
<i>GRK5</i> Gln41Leu	↑ <i>ADRB1,2</i> phosphorylation, uncoupling	~10-fold ↑ in Leu	Protective (↑ <i>ADRB1,2</i> phosphorylation and desensitization)	↓ response to carvedilol, metoprolol ( <i>Actual</i> )
<i>ADRA2C</i> Ins322-325Del	↑ sympatholysis ↑ adrenergic drive	~10-fold ↑ in Del	Harmful (↑ adrenergic drive)	↓ response to bucindolol, ↑ CV adverse events to bucindolol ( <i>Actual</i> )
<i>GNB3</i> C825T	↑ vasoconstriction	~2.5-fold ↑ in T	Harmful (↑ afterload)	↑ response to BiDil ( <i>Possible</i> based on AA data, no data in EA)
<i>NOS3</i> Glu298Asp	↓ <i>NOS3</i> activity	~70% ↓ in Asp	Protective <sup>§</sup> (↑ <i>NOS3</i> activity)	↑ response to BiDil ( <i>Possible</i> based on AA data, no data in EA)
<i>CYP11B2</i> T-344C	↑ aldosterone synthesis	~50% ↓ in C	Harmful (↑ aldosterone)	↑ response to BiDil ( <i>Possible</i> based on AA data, no data in EA) ↑ response to RAAS inhibition ( <i>Predicted</i> , no data in AA or EA)
<i>AGTR1</i> A1166C	↑ <i>AGTR1</i> density	~70% ↓ in C	Protective (↓ <i>AGTR1</i> density)	↓ response to ARBs ( <i>Possible</i> based on data in EAs, no AA data); ↓ response to ACEIs ( <i>Possible</i> , based on non-genotyped empirical data in AAs)
<i>AGT</i> Met235Thr	↑ <i>AGT</i> levels	~2-fold ↑ in Thr	Harmful (↑ <i>AGT</i> )	↑ response to ACEIs, ARBs ( <i>Predicted</i> , for ACEIs not supported by non-genotyped empirical data in AAs; no support for ARBs)
<i>ECE1</i> Thr341Ile	?	~10-fold ↓ in Ile	?	↑ Response to bucindolol ( <i>Possible</i> , based on EA data)
<i>SCN5A</i> Ser1103Tyr	↑ late I <sub>Na</sub> ; Ca <sup>2+</sup> overload	>10-fold ↑ in Tyr	Harmful; pro-arrhythmic	↑ indication for prevention of sudden cardiac death by ICDs ( <i>Actual</i> )

\* Based on biologic or pharmacologic effects between genotypes, and allele frequency differences between AA (African-ancestry) and EA (European-ancestry) patients.



<sup>†</sup>Norepinephrine (NE).

<sup>‡</sup>Epinephrine (EPI).

<sup>§</sup>Minor allele frequency (MAF) difference may be an adaptive response to *NOS3* uncoupling in AA; *Actual* means by-genotype differences in response in AA and EA, which would imply a differential response in AA vs. EA based on the differences in MAF. *Possible* means 1) by-genotype response differences for drug class in AA, EA, or EA + AA populations, or 2) decreased response in AA predicted by MAF signaling effects. *Predicted* means no clinical data, based entirely on cell-signaling effects; ACEI = angiotensin converting enzyme inhibitors; ARB = angiotensin-receptor blockers; CRT = cardiac resynchronization therapy; ICD = implantable cardioverter defibrillator; RAAS = renin-angiotensin-aldosterone system; CV = cardiovascular

Table 3

Impact of adrenergic signaling genetic variation on outcomes in placebo-treated HF patients in the BEST and MERIT-HF clinical trials or  $\beta$ -blocker-untreated patients in the Cincinnati/Pennsylvania observational study. HRs are expressed as minor allele carriers vs. major allele homozygotes

Gene polymorphism	HR [no. events] (95% CI), ACM/Tx <sup>†</sup>			HR [no. events] (95% CI), ACM* or ACM/HFH <sup>‡</sup>			
	EA	AA	EA + AA	EA	AA	EA + AA	
	Interaction p=0.51						
ADRB1 Arg389Gly, BEST*	1.25 [84] (0.81,1.93) p=0.32	0.85 [15] (0.27,2.63) p=0.77	1.14 [97] (0.76,1.69) p=0.79	1.03 [174] (0.76,1.39) p=0.84	0.77 [41] (0.38,1.54) p=0.45	1.02 [215] (0.79,1.32) p=0.88	
	Interaction p=0.42						
	NA	NA	NA	NA	NA	1.00 <sup>§</sup> [NA] (0.61,1.64) p=0.99	
ADRB1 Arg389Gly, † Cincinnati/Pennsylvania18	1.98 [NA] (1.07,3.65) p=0.03	unadjusted p=0.55 <sup>¶</sup>	1.76 [NA] (1.09,2.85) p=0.02	NA	NA	NA	
	ADRB1 Ser49Gly, BEST*	0.64 [84] (0.37,1.12) p=0.11	0.94 [15] (0.32,2.76) p=0.91	0.68 [99] (0.42,1.11) p=0.12	0.78 [174] (0.54,1.13) p=0.19	0.61 [41] (0.31,1.22) p=0.16	0.74 [215] (0.54,1.03) p=0.07
		Interaction p=0.49					
1.05 [84] (0.68,1.63) p=0.82		0.62 [15] (0.21,1.82) p=0.38	0.95 [99] (0.63,1.42) p=0.80	1.06 [174] (0.78,1.43) p=0.72	0.85 [41] (0.42,1.69) p=0.63	1.02 [215] (0.77,1.35) p=0.89	
ADRB2 Gly16Arg, BEST*	Interaction p=0.38						
	1.11 [84] (0.71,1.74) p=0.65	2.91 [15] (1.03,8.19) p=0.035	1.37 [99] (0.91,2.07) p=0.13	1.21 [174] (0.88,1.67) p=0.23	1.72 [41] (0.92,3.21) p=0.085	1.27 [215] (0.97,1.68) p=0.085	
	Interaction p=0.086						
ADRB2 Gln27Glu, BEST*	1.34 [84] (0.42,4.24) p=0.62	n=1 164Ile	1.29 [99] (0.41,4.09) p=0.66	1.20 [174] (0.53,2.70) p=0.67	n=1 164Ile	1.06 [215] (0.47,2.40) p=0.88	
	Interaction p=NA						
	0.96 [84] (0.44,2.07) p=0.91	1.13 [15] (0.36,3.60) p=0.83	0.86 [99] (0.51,1.43) p=0.55	0.79 [174] (0.45,1.39) p=0.41	0.98 [41] (0.51,1.89) p=0.95	0.91 [215] (0.65,1.76) p=0.58	
ADRA2C Ins322-325Del, BEST*	Interaction p=0.74						
	Interaction p=0.62						

Gene polymorphism	HR [no. events] (95% CI), ACM* or ACM/Tx <sup>†</sup>			HR [no. events] (95% CI), ACM/HFH <sup>‡</sup>		
	EA	AA	EA + AA	EA	AA	EA + AA
<i>GRR5</i> Gln41Leu, Cincinnati/Pennsylvania <sup>18</sup> (adjusted for age, sex)	NA [3] (Too few events)	0.32 [NA] (0.13,0.80) p=0.01	0.76 [NA] (0.40,1.32) p=0.39	NA	NA	NA

\* All-cause mortality (ACM).

<sup>†</sup> ACM or cardiac transplantation.

<sup>‡</sup> ACM or heart failure hospitalization (HFH).

§ All races.

<sup>¶</sup> Events too few for Cox modeling.

CI = confidence interval; HR = hazard ratio; NA = not available; Tx = cardiac transplantation.

**Table 4**

Summary of signaling molecule polymorphism effects by drug or device class, irrespective of races

Drug or class	Polymorphism (effects on response in HF)
Standard $\beta$ -blockers	<i>GRK5</i> Gln41Leu ( $\downarrow$ by Leu carriers); <sup>18</sup> <i>ADRB2</i> Gln27Glu (carvedilol $\uparrow$ in Glu genotypes) <sup>57,58</sup> <i>ACE</i> Del/intron16/Ins ( $\uparrow$ in Del homozygotes) <sup>47</sup>
Bucindolol ( $\beta$ -blocker/sympatholytic)	<i>ADRB1</i> Arg389Gly ( $\uparrow$ in Arg homozygotes, <sup>25</sup> $\downarrow$ in {389Gly + <i>ADRA2C</i> 322-325Del} genotypes; <sup>54</sup> <i>EDN1</i> Lys198Asn (gene dose-related $\downarrow$ in Asn genotypes); <sup>42</sup> <i>ECE1</i> Thr341Ile ( $\downarrow$ in Ile genotypes)
Angiotensin-converting enzyme inhibitors	<i>ACE</i> Del/intron16/Ins ( $\uparrow$ in Del homozygotes) <sup>59</sup>
Angiotensin AT-1 receptor blockers	$\uparrow$ biomarker (NT-proBNP) response in <i>AGTR1</i> 1166C genotypes <sup>60</sup>
Mineralocorticoid receptor blockers	No effects reported
Hydralazine/isosorbide dinitrate	<i>NOS3</i> Glu298Asp ( $\uparrow$ in Glu homozygotes); <sup>30</sup> <i>CYP11B2</i> T-344C ( $\uparrow$ in -344T homozygotes); <sup>32</sup> <i>GNB3</i> 825T ( $\uparrow$ in TT homozygotes) <sup>28</sup>
Cardiac resynchronization therapy	<i>ADRB2</i> Gln27Glu ( $\uparrow$ in Glu homozygotes); <sup>61</sup> <i>NR3C2</i> Ile180Val ( $\uparrow$ in Ile homozygotes) <sup>62</sup>
ICD appropriate discharge for VT/VF	<i>SCN5A</i> Ser1103Tyr ( $\uparrow$ events in Tyr carriers) <sup>43</sup>

HF = heart failure; ICD = implantable cardioverter defibrillator; NT-proBNP = N-terminal pro-brain natriuretic peptide; VF = ventricular fibrillation; VT = ventricular tachycardia.

**Table 5**

Racial effects (AA vs. EA) on HF or hypertension response by drug or device class

Drug or class	Racial effect in HF (AA or EA)	Racial effect in hypertension (AA or EA)
β-blockers	Cresci et al, <sup>18</sup> ↓ in AA (carvedilol, metoprolol) Yancy et al, <sup>63</sup> ↔ in AA (carvedilol) Goldstein et al, <sup>64</sup> ↔ ↓ in AA (metoprolol) Lanfear et al, <sup>65</sup> ↓ in AA (all standard P-blockers)	Cubeddu et al, <sup>66</sup> ↓ in AA (propranolol)
Bucindolol (β-blocker/sympatholytic)	BEST, <sup>67</sup> ↓ in AA	No data
Angiotensin-converting enzyme inhibitors	Carson et al, <sup>68</sup> ↓ in AA Exner et al, <sup>69</sup> ↓ in AA	Weir et al, <sup>70</sup> ↓ in AA
Angiotensin AT-1 receptor blockers	Prisant et al, ↔ in AA	Ofili et al, <sup>72</sup> ↔ in AA
Mineralocorticoid receptor blockers	No data	No data
Hydralazine/isosorbide dinitrate	Carson et al, <sup>68</sup> ↑ in AA	No data
Cardiac resynchronization therapy	No data	No data
ICD	↑appropriate discharge in a subset of AA, by virtue of markedly higher SCN5A 1103Tyr frequency <sup>43</sup>	No data

AA = African ancestry; EA = European ancestry; HF = heart failure; BEST = β-Blocker Evaluation of Survival Trial.