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Association of Regulatory Genetic Variants for Protein Kinase C α with Mortality and Drug Efficacy in Patients with Heart Failure

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Structured Abstract

Purpose: Protein kinase C alpha (gene:*PRKCA*) is a key regulator of cardiac contractility. Two genetic variants have recently been discovered to regulate *PRKCA* expression in failing human heart tissue (rs9909004 [T→C] and rs9303504 [C→G]). The association of those variants with clinical outcomes in patients with heart failure (HF), and their interaction with HF drug efficacy, is unknown.

Methods: Patients with HF in a prospective registry starting in 2007 were genotyped by whole genome array (n=951). The primary outcome was all-cause mortality. Cox proportional hazards models adjusted for established clinical risk factors and genomic ancestry tested the independent

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Disclosures

None.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution (Henry Ford Health System institutional review board approval number 4562) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

association of rs9909004 or rs9303504 and the variant interactions with cornerstone HF pharmacotherapies (beta-blockers or angiotensin-converting enzyme inhibitors/angiotensin receptor blockers) in additive genetic models.

Results: The minor allele of rs9909004, but not rs9303504, was independently associated with decreased risk for all-cause mortality: adjusted HR = 0.81 (95% CI = 0.67 – 0.98) p = 0.032. The variants did not significantly interact with mortality benefit associated with cornerstone HF pharmacotherapies (p > 0.1 for all).

Conclusions: A recently discovered cardiac-specific regulatory variant for *PRKCA* (rs9909004) was independently associated with decreased risk for all-cause mortality in patients with HF. The variant did not interact with mortality benefit associated with cornerstone HF pharmacotherapies.

Keywords

heart failure; protein kinase C α (*PRKCA*); regulatory variants; mortality

Introduction

The protein kinase C (PKC) family plays an integral role in the pathophysiology of heart failure [1]. This family of nine serine/threonine kinases transmits neurohormonal and mechanical signals in the cardiac myocyte [2]. The alpha member of the PKC family (PRKCA) is a critical mediator of cardiomyocyte calcium handling, and thereby regulates cardiac contractility and the development of heart failure [3-5]. Increased expression of *PRKCA*, which encodes for PRKCA, decreased cardiac contractility in transgenic mice [6]. Alternatively, knockout of *PRKCA* in mice increased cardiac contractility and protected against dilated cardiomyopathy and heart failure [6]. In humans, *PRKCA* expression is increased by 70% in failing human hearts [3]. Pharmacologic inhibition of PRKCA enhances cardiac contractility, and affords cardioprotection, in animal models of heart failure [7-9]. The sum of this experimental evidence suggests that *decreased PRKCA* activity/expression may be protective in heart failure.

Over 200 genetic variants are significantly associated with *PRKCA* expression in human left ventricle [10]. Until recently, the true causative variant(s) among the over 200 associated was/were unknown. Two independent studies recently discovered two single nucleotide polymorphisms (SNPs) as the likely causative variants: rs9909004 (T→C) [11] and rs9303504 (C→G) [12]. These variants are common (global minor allele frequencies = 42% and 40%, respectively) and in strong linkage disequilibrium (r^2 = 55% - 98%, depending on ancestry) [13]. Both SNPs are intronic and located in cardiac specific enhancer regions [14]. In both studies, the minor allele at either locus was significantly associated with decreased cardiac expression of *PRKCA*. Given the prior experimental evidence suggesting that *decreased* cardiac expression of *PRKCA* would be associated with improved heart failure outcomes, the minor alleles of these two SNPs could be protective in patients with heart failure. However, the association of rs9909004 and rs9303504 with clinical outcomes in patients with heart failure is unknown. Therefore, the primary objective of this study was to assess the independent association of *PRKCA* rs9909004 and rs9303504 with all-cause

mortality in patients with heart failure. Based on the prior experimental data, our hypothesis was that the minor alleles of rs9909004 and rs9303504 would be protective.

If rs9909004 and rs9303504 impact heart failure clinical outcomes, it needs to be considered whether those variants are acting by modulating the efficacy of cornerstone heart failure medications. Based on their potential to affect *PRKCA* expression, rs9909004 and rs9303504 may alter the efficacy of medications targeting elements of PRKCA signaling pathways. The mechanisms of action of three cornerstone heart failure medication classes [15], angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor type 1 (AT_1) blockers (ARB), and beta-adrenergic receptor blockers, all involve PRKCA. ARB and ACEI either directly or indirectly target AT_1, which has been shown to regulate contractility in cardiomyocytes via PRKCA signaling [16-18]. Moreover, PRKCA is known to regulate two proteins (phospholamban and protein phosphatase inhibitor-1) involved in the beta-adrenergic signaling cascade and the associated contractile response [6, 19]. Increased cardiomyocyte PRKCA activity can therefore increase therapeutic response to ACEI, ARB, and beta-blockers. Therefore our second hypothesis is that the minor alleles of rs9909004 and rs9303504, which reduce *PRKCA* expression, will be associated with decreased response to these cornerstone heart failure therapies. Accordingly, the secondary objectives of this study were to assess the potential for *PRKCA* rs9909004 or rs9303504 to modify benefit from ACEI/ARB or beta-blockers in heart failure patients (i.e., ACEI/ARB or beta-blocker associated reductions in all-cause mortality).

Methods

Patients

Patients for this study came from a prospective, heart failure registry designed to discover novel ways to predict prognosis and the response to heart failure therapies. The registry is based at Henry Ford Health System in Detroit, MI, USA, and it started in October 2007 and completed enrollment in March 2015. Patients were included in the heart failure registry if they were 18 years of age or older, had health insurance coverage, and met the definition for heart failure as defined by the Framingham Heart Study [20]. Patients included in the analysis had at least one documented left ventricular ejection fraction <50%. Patients were excluded if they required dialysis or supplemental oxygen. Detailed clinical information (e.g., demographics, physical examination results, past medical history, laboratory values, functional status, and medication use) and blood samples were collected at the time of study enrollment. Patient deaths through July 28, 2016 were identified using the Social Security Administration Death Master File, the Michigan State Division of Vital Records, and administrative data from Henry Ford Health System. This study was approved by the institutional review board of the Henry Ford Health System, and all patients gave written informed consent prior to participating.

Calculation of Drug Exposure

Exposure of the patients to beta-blockers and ACEI or ARB was calculated using dose standardization and pharmacy claims data as previously described [21]. Briefly, doses of specific drugs were standardized into dose equivalents by the target dose used in clinical

trials of heart failure with reduced ejection fraction (HFrEF) or, if the drug was not tested in a HFrEF clinical trial (e.g., atenolol), by the maximum daily dose. For example, 25 mg of carvedilol per day (i.e., 12.5 mg twice daily) was considered a 0.5 beta-blocker standardized dose equivalent. Drug exposure was then calculated by multiplying the standardized dose equivalent by the quantity of medication dispensed from a pharmacy in a 6-month time block, divided by the total number of days in the 6-month time block. Drug exposure was calculated for each patient for each day of observation, and thus this method accounts for both dose and adherence over a rolling period of time (6 months). For example, if a patient was prescribed 12.5 mg of carvedilol twice daily, and had picked up their prescription from the pharmacy so that there was continuous availability over the previous 6 months, then their calculated average beta-blocker exposure over this time period would be 0.5. We have previously demonstrated that this approach for calculating drug exposure is superior to a single time point, dichotomous classification of drug exposure (e.g., discharge medication status) [22].

Genotyping

Blood samples were collected at enrollment into the heart failure registry and were immediately processed and stored at −70°C. All patients donated blood and were genotyped using the Axiom™ Biobank array (ThermoFisher Scientific). The array includes ~600K polymorphisms: 1) ~300K genome-wide association study (GWAS) markers with minor allele frequencies >1%; 2) ~250K low frequency (<1%) coding variants selected from global exome sequencing projects; and an 3) additional ~50K variants to improve African ancestry coverage (Yoruba in Ibadan, Nigeria [YRI] booster). The two *PRKCA* SNPs rs9909004 and rs9303504 were not directly genotyped by the array, and imputed genotypes for the SNPs were used. Genome-wide imputation using the 1000 Genomes Project (1KGP) haplotype reference panel was performed using the Michigan Imputation Server with Minimac3 [23]. The accepted imputation score was >0.3. The accuracy of the genotypes for rs9909004 and rs9303504 was checked by performing the chi-square test for Hardy Weinberg equilibrium within each self-reported race group and by comparing the minor allele frequencies in each self-reported race group in this registry to minor allele frequencies reported in the 1KGP.

Statistical Analysis:

Continuous baseline variables were summarized by the mean ± standard deviation and categorical baseline variables were summarized by counts and percentages. The primary outcome was time-to all-cause mortality. Each SNP was shown to have an additive effect on *PRKCA* cardiac expression [12, 11], and thus they were coded in the additive genetic model for the number of minor alleles (i.e., rs9909004 genotypes TT, TC, and CC were coded as 0, 1, and 2; rs9303504 genotypes CC, CG, and GG were coded as 0, 1, and 2). Each SNP was tested individual in time-dependent Cox proportional hazards regression models adjusted for known clinical risk factors for mortality in patients with heart failure: the Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC) risk score [24], self-reported race, and N-terminal pro b-type natriuretic peptide (NT pro-BNP) level. To overcome the influence of population stratification on genetic effects [25], principal components (PC) analysis was performed by PLINK on the SNP array data after linkage disequilibrium pruning [26]. The top three most significant PCs were included in the association models as covariates. The

secondary objective, evaluating a potential interaction of each of the *PRKCA* SNPs with beta-blocker or ACEI/ARB exposure, was tested by incorporating a multiplicative interaction term between the *PRKCA* SNPs and the drug exposure variable in the Cox proportional hazards models for all-cause mortality (i.e., number of minor alleles of each SNP*beta-blocker exposure and number of minor alleles of each SNP*ACEI/ARB exposure in separate models). Linkage disequilibrium between rs9909004 and rs9303504 was calculated among African American and white study participants using PLINK [26]. All statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC) unless noted otherwise above; $p < 0.05$ was considered statistically significant for main effects and $p < 0.1$ was considered statistically significant for interaction effects. Given a level of statistical significance of $p < 0.05$ and our sample size, we estimated 80% power to detect a hazard ratio ≤ 0.83 for each SNP.

Results

This analysis consisted of 951 heart failure patients with a mean duration of follow-up of 1104 days (standard deviation [SD] ±701 days) and a total of 239 deaths (25%). Baseline characteristics of the registry overall and stratified by rs9909904 and rs9303504 genotypes are presented in Tables 1 and 2, respectively. The genotyping results and accuracy check stratified by patients' self-reported race are displayed in Table 3. The minor allele frequencies in the registry were similar to publicly reported frequencies in 1KGP [13], and the genotypes did not significantly deviate from Hardy Weinberg equilibrium for either SNP. The two SNPs were in strong linkage disequilibrium in both the self-reported African American ($r^2 = 0.86$; D' = 0.95) and white ($r^2 = 0.96$; D' = 0.99) patients.

In the survival analyses (adjusted for MAGGIC risk score, self-reported race, NT pro-BNP level, and the first three principal components of genomic ancestry), rs9909004 was significantly associated with all-cause mortality in the additive genetic model: rs9909904 adjusted HR = 0.81 (95% CI = 0.67 – 0.98) p = 0.032. rs9303504 was not significantly associated: adjusted HR = 0.83 (95% CI = 0.69 – 1.01) p = 0.059. Kaplan-Meier curves for each individual SNP are displayed in Figures 1 and 2, and the log rank p-values for rs9909004 and rs9303504 were p = 0.019 and p = 0.038, respectively.

The secondary analysis of interactions between the *PRKCA* SNPs and beta-blocker or ACEI/ARB associated benefit for all-cause mortality did not yield statistically significant findings. For an interaction with beta-blockers, the adjusted p-values for rs9909004 and rs9303504 were p = 0.500 (β = 0.23) and p = 0.445 (β = 0.26), respectively. For an interaction with ACEI/ARB, the adjusted p-values for rs9909004 and rs9303504 were p = 0.157 (β = 0.37) and p = 0.301 (β = 0.27), respectively. The associations of beta-blocker exposure and ACEI/ARB exposure with a reduction in all-cause mortality were independent of the total number of minor alleles of the *PRKCA* SNPs (adjusted HR comparing zero to target beta-blocker exposure in the model including the *PRKCA* SNPs = 0.47 [0.29 – 0.74] p = 0.001; adjusted HR comparing zero to target ACEI/ARB exposure in the model including the *PRKCA* SNPs = 0.63 [0.44 – 0.90] p = 0.011). These results suggested that the *PRKCA* SNPs did not modify the effectiveness of beta-blockers or ACEI/ARB in patients with heart failure.

Discussion

Two previous studies recently discovered two SNPs (rs9909004 and rs9303503) regulating expression of *PRKCA* in heart tissue [12, 11], but the association of those SNPs with clinical outcomes in patients with heart failure was unknown. Our results from a prospective registry of patients with heart failure showed that rs9909004 was significantly associated with the risk of all-cause mortality, and the association was independent of established clinical risk factors. The association of rs9909004 is in the direction expected from previous experimental evidence: the minor allele, which decreases cardiac *PRKCA* expression [12, 11], was the protective allele. Mechanistically, rs9909004 is in enhancer regions in 12 different tissue types, DNAse hypersensitivity sites in 14 different tissues, and it also changes the regulatory motif for transcription factor Pou3f1 [14]. These regulatory effects are supported by data from the Genotype-Tissue Expression (GTEx) project, in which the minor allele of rs9909004 is significantly associated with decreased expression of *PRKCA* in left ventricular and atrial tissues [10]. Based on the additive effects of each SNP on cardiac expression of *PRKCA* [12, 11], we *a priori* defined the additive genetic model in our statistical analysis. However, upon inspection of the Kaplan-Meier survival curves, the association of rs9909004 with all-cause mortality appears to more closely follow a dominant genetic model. Thus, future replication studies with clinical outcomes should consider using the dominant genetic model.

Our results are consistent with several previous human *ex vivo* and animal *in vivo* studies suggesting that increased cardiac expression of *PRKCA* could cause adverse effects related to heart failure [1]. Pharmacologic inhibition of PRKCA enhances cardiac contractility and cardioprotection in animal models [6-9], further supporting our findings of a protective association for the minor alleles. A mixed PRKCA/PRKCB inhibitor is currently in phase I/II clinical trials for patients with heart failure (clinicaltrials.gov identifier: NCT02769611), and our study supports inhibiting PRKCA as a potential mechanism for treating heart failure. These data may also indicate a potential opportunity for precision therapy with PRKCA pharmacologic inhibition. Specifically, heart failure patients without the protective minor allele of *PRKCA* rs9909004 may derive greater benefit from PRKCA inhibition.

Despite our results being consistent with previous experimental studies, our results are not consistent with a clinical association study by Hu *et al.* [12]. Hu *et al.* found the reverse association with similar clinical outcomes: the minor alleles of these *PRKCA* SNPs were associated with adverse left ventricular remodeling, impaired contractile function, and dilated cardiomyopathy. It remains unclear why the findings by Hu *et al.* are inconsistent with the human *ex vivo* studies, animal *in vivo* studies, and our study. Hu *et al.* suggested that there could be species-specific differences in *PRKCA* effects, and the timing of *PRKCA* effects is important (i.e., acute phase of heart failure in animal models versus chronic increased *PRKCA* exposure in affected humans). The difference between our results and the results by Hu *et al.* could be influenced by differences in the patient populations and the clinical outcomes studied. For example, all patients in our study had documented heart failure, while Hu *et al.* excluded patients with heart failure. It is well known that heart failure induces dramatic changes in cardiac gene expression [27], and the expression of PRKCA in normal hearts is known to be much lower when compared to expression in heart failure [3].

The effects of the *PRKCA* SNPs may also differ depending on the clinical outcome investigated. Specifically, Hu *et al.* investigated left ventricular remodeling, contractile function, and new onset dilated cardiomyopathy, whereas we investigated all-cause mortality (in patients with established heart failure). Our results regarding mortality are consistent with an aggregate analysis of clinical trials of a PRKCA inhibitor in patients with diabetes [28]. There were fewer deaths in the groups treated with the PRKCA inhibitor than the groups treated with placebo [28].

It is also important to note that the PRKCA association with mortality was not mediated via heart failure medication effectiveness (i.e., it was not a pharmacogenetic effect). Despite the biologic plausibility [16-18], the studied *PRKCA* SNPs did not interact with beta-blocker or ACEI/ARB exposure in terms of survival benefit. This suggests that the mechanism through which these *PRKCA* SNPs may affect mortality in patients with heart failure is independent of the effects of these treatments. It could be that PRKCA is acting in the drug pathways, but that the effect is simply proportionate across genotypes. PRKCA plays a myriad of roles within the cardiac myocyte, including the transmission of neurohormonal, mechanical, and electrical signals [29]. So alternatively, the mechanism could be completely outside the beta-blocker and ACEI/ARB pathways, such as via calcium cycling or phosphorylation of ion channels.

Our study has several limitations. The registry enrolled patients from a single site, and thus our results may not be generalizable to broader patient populations. While our cohort is quite diverse in terms of race and stage of heart failure, our findings need to be replicated in an independent heart failure patient population. To our knowledge, a similar dataset consisting of heart failure patients with highly granular clinical and genomic data is not available. Our registry is underpowered to perform a genome-wide association study, or GWAS, which is why we relied on previous experimental data to select these candidate genetic variants. It is possible that rs9303504 is also associated with all-cause mortality in patients with heart failure, but our study was underpowered to detect it. Given the strong linkage disequilibrium between the two *PRKCA* SNPs, our study was also underpowered to distinguish the independent associations of the two SNPs. We used imputed genotypes for both SNPs; however, these were common alleles and the imputation used a well-described reference sample with good confidence. Finally, since this study was an observational registry, the interactions of the *PRKCA* SNPs with beta-blocker, ACEI, or ARB efficacy are limited by confounding by indication. We tried to mitigate this using extensive covariate adjustment, and our cohort includes a significant proportion of untreated patients, enhancing power. However, due to this fact we could not truly test causality, which would require a randomized controlled trial.

Conclusions

The minor allele of *PRKCA* rs9909004, but not rs9303504, was independently associated with decreased risk for all-cause mortality in patients with heart failure. The SNPs did not significantly interact with benefit from beta-blockers or ACEI/ARB; suggesting a mechanism that is independent of adrenergic or angiotensin signaling. Further studies are

needed to replicate this association in additional heart failure patient populations. These results support PRKCA as a drug target in patients with heart failure.

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Abbreviations

ACEI	angiotensin converting enzyme inhibitor
ARB	angiotensin receptor blocker
AT_1	angiotensin II receptor type 1
CI	confidence interval
eQTLs	expression quantitative trait loci
GWAS	genome-wide association study
HFrEF	heart failure with reduced ejection fraction
HR	hazard ratio
MAGGIC	Meta-Analysis Global Group in Chronic Heart Failure risk score
NT pro-BNP	N-terminal pro b-type natriuretic peptide
PKC	protein kinase C family
PRKCA	alpha member of the protein kinase C family
PRKCA:	gene for the alpha member of the protein kinase C family
SNP	single nucleotide polymorphism

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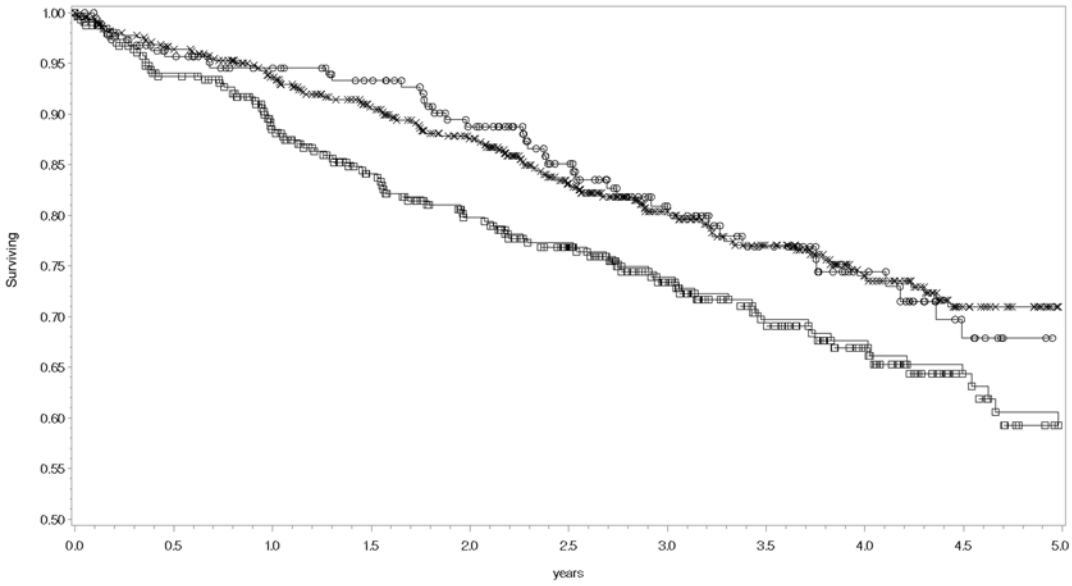

Figure 1.

Kaplan-Meier curves for all-cause mortality stratified by rs9909004 genotypes. Censored patients with the T/T genotype are symbolized as squares,, C/T genotype as the letter X, and C/C genotype as circles. The logrank p-value = 0.0189.

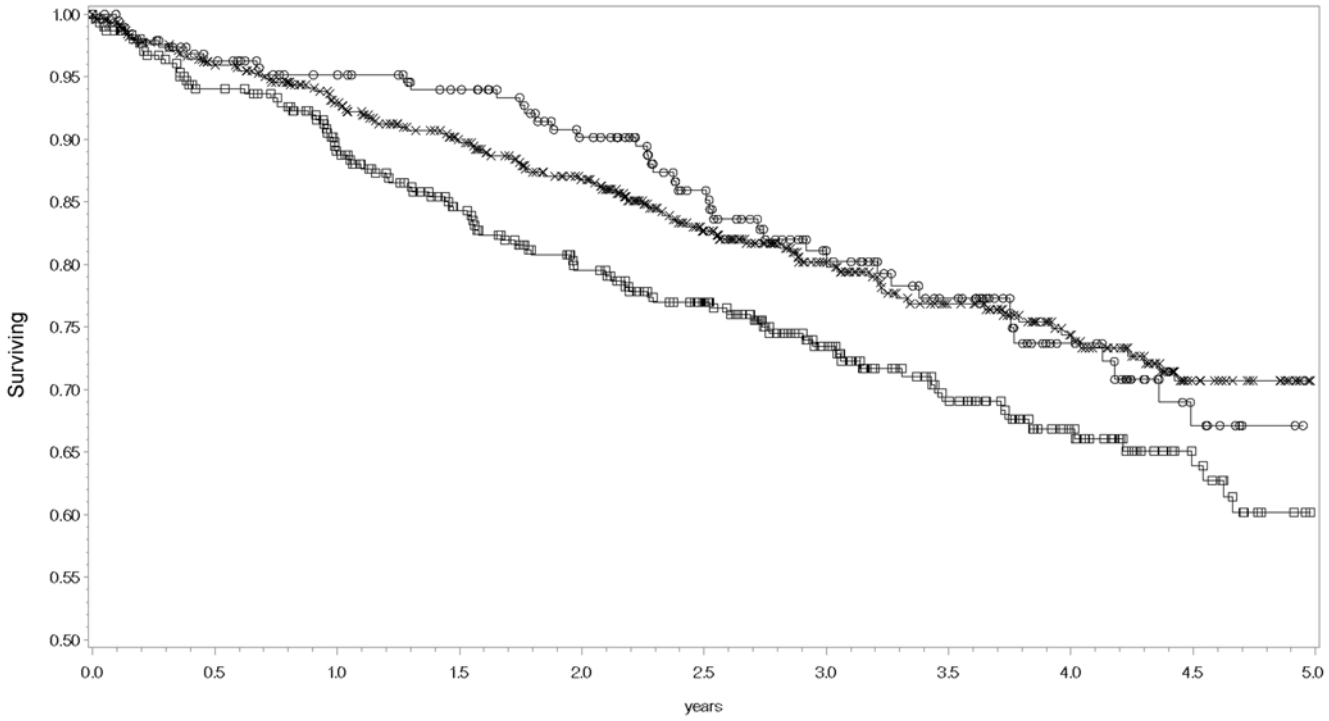

Figure 2.

Kaplan-Meier curves for all-cause mortality stratified by rs9303504 genotypes. Censored patients with the C/C genotype are symbolized as squares, C/G as the letter X, and G/G genotype as circles. The logrank p-value = 0.0375.

Table 1.

Baseline characteristics of the heart failure genetic registry overall and stratified by rs9909004 genotypes

		rs9909004 genotype			
Baseline characteristics	**All patients (n = 951)**	**TT (n = 309; 32%)**	**CT (n = 452; 48%)**	**CC (n = 190; 20%)**	***p-value**
Female	341 (35.9%)	104 (33.7%)	165 (36.5%)	72 (37.9%)	0.584
Self-reported African-American race	481 (50.6%)	171 (55.3%)	231 (51.1%)	79 (41.6%)	**0.011**
Age (years)	68.3 ± 11.7	67.5 ± 11.5	68.2 ± 11.5	69.7 ± 12.2	0.119
Left ventricular ejection fraction (%)	34.7 ± 10.9	34.7 ± 10.9	34.3 ± 11.0	35.7 ± 10.8	0.368
Ischemic etiology	416 (43.7%)	123 (39.8%)	199 (44.0%)	94 (49.5%)	0.106
Hypertension	855 (89.9%)	283 (91.6%)	402 (88.9%)	170 (89.5%)	0.480
Chronic obstructive pulmonary disease	220 (23.1%)	74 (24.0%)	95 (21.0%)	51 (26.8%)	0.256
Chronic kidney disease	222 (23.3%)	81 (26.2%)	106 (23.5%)	35 (18.4%)	0.136
Atrial fibrillation	267 (28.1%)	94 (30.4%)	120 (26.6%)	53 (27.9%)	0.505
Stroke/transient ischemic attack	122 (12.8%)	36 (11.7%)	65 (14.4%)	21 (11.1%)	0.388
Diabetes	399 (42.0%)	119 (38.5%)	198 (43.8%)	82 (43.2%)	0.324
Body mass index (kg/m^2)	31.0 ± 7.3	31.4 ± 7.5	30.8 ± 7.3	30.9 ± 7.3	0.457
Systolic blood pressure (mmHg)	129 ± 23	129 ± 24	129 ± 23	129 ± 21	0.988
Heart rate (beats per minute)	71.2 ± 12.9	72.4 ± 13.7	70.8 ± 12.3	70.2 ± 12.8	0.131
NT pro-BNP (pmol/L)	368 ± 384	368 ± 389	393 ± 404	308 ± 316	**0.038**
Serum creatinine (mg/dL)	1.29 ± 0.93	1.26 ± 0.76	1.36 ± 1.15	1.15 ± 0.46	0.050
MAGGIC risk score (w/o beta-blocker)	18.2 ± 7.3	17.9 ± 7.1	18.2 ± 7.4	18.5 ± 7.1	0.645
1st principal component	0.0007 ± 0.035	0.0017 ± 0.037	−0.0004 ± 0.037	0.0014 ± 0.025	0.687
2nd principal component	0.0038 ± 0.042	0.0053 ± 0.057	0.0030 ± 0.031	0.0034 ± 0.032	0.737
3rd principal component	−0.0002 ± 0.032	0.0015 ± 0.040	0.0010 ± 0.026	−0.0057 ± 0.029	**0.029**
Beta-blocker exposure	0.27 ± 0.29	0.26 ± 0.29	0.28 ± 0.30	0.27 ± 0.30	0.453
Any beta-blocker exposure	623 (65.5%)	206 (66.7%)	291 (64.4%)	126 (66.3%)	0.782
Any ACEI/ARB exposure	580 (61.0%)	194 (62.8%)	272 (60.2%)	114 (60.0%)	0.733
Length of follow-up (days)	1104 ± 701	1052 ± 712	1145 ± 714	1092 ± 646	0.194
Deaths	239 (25.1%)	93 (30.1%)	99 (21.9%)	47 (24.7%)	**0.037**

ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MAGGIC: Meta-Analysis Global Group in Chronic Heart Failure risk score; NT pro-BNP: N-terminal pro b-type natriuretic peptide

* p-value is for difference among the three genotypes. Bolded p-values indicate $p < 0.05$.

Table 2.

Baseline characteristics of the heart failure genetic registry overall and stratified by rs9303504 genotype

		rs9303504 genotype			
Baseline characteristics	**All patients (n = 951)**	**CC (n = 307; 32%)**	**CG (n = 451; 48%)**	**GG (n = 193; 20%)**	***p-value**
Female	341 (35.9%)	107 (34.9%)	161 (35.7%)	73 (37.8%)	0.793
Self-reported African-American race	481 (50.6%)	169 (55.1%)	227 (50.3%)	85 (44.0%)	0.056
Age (years)	68.3 ± 11.7	67.7 ± 11.6	68.4 ± 11.4	69.0 ± 12.4	0.445
Left ventricular ejection fraction (%)	34.7 ± 10.9	34.9 ± 10.9	34.4 ± 11.0	35.2 ± 10.8	0.691
Ischemic etiology	416 (43.7%)	121 (39.4%)	202 (44.8%)	93 (48.2%)	0.130
Hypertension	855 (89.9%)	282 (91.9%)	400 (88.7%)	173 (89.6%)	0.362
Chronic obstructive pulmonary disease	220 (23.1%)	73 (23.8%)	95 (21.1%)	52 (26.9%)	0.255
Chronic kidney disease	222 (23.3%)	79 (25.7%)	107 (23.7%)	36 (18.7%)	0.184
Atrial fibrillation	267 (28.1%)	94 (306%)	119 (26.4%)	54 (28.0%)	0.444
Stroke/transient ischemic attack	122 (12.8%)	36 (11.7%)	65 (14.4%)	21 (10.9%)	0.368
Diabetes	399 (42.0%)	120 (39.1%)	199 (44.1%)	80 (41.5%)	0.381
Body mass index (kg/m^2)	31.0 ± 7.3	31.4 ± 7.5	30.7 ± 7.3	30.9 ± 7.3	0.430
Systolic blood pressure (mmHg)	129 ± 23	130 ± 24	129 ± 23	129 ± 20	0.885
Heart rate (beats per minute)	71.2 ± 12.9	72.1 ± 13.8	70.8 ± 12.3	70.6 ± 12.6	0.312
NT pro-BNP (pmol/L)	368 ± 384	370 ± 387	389 ± 406	315 ± 319	0.082
Serum creatinine (mg/dL)	1.29 ± 0.93	1.23 ± 0.59	1.38 ± 1.22	1.15 ± 0.47	**0.014**
MAGGIC risk score (w/o beta-blocker)	18.2 ± 7.3	17.8 ± 7.2	18.3 ± 7.4	18.3 ± 7.0	0.661
1st principal component	0.0007 ± 0.035	0.0019 ± 0.038	-0.0002 ± 0.037	0.0003 ± 0.026	0.726
2nd principal component	0.0038 ± 0.042	0.0055 ± 0.058	0.0028 ± 0.031	0.0034 ± 0.032	0.672
3rd principal component	−0.0002 ± 0.032	0.0018 ± 0.040	0.0008 ± 0.026	−0.0057 ± 0.028	**0.023**
Beta-blocker exposure	0.27 ± 0.29	0.26 ± 0.29	0.28 ± 0.29	0.28 ± 0.31	0.663
Any beta-blocker exposure	623 (65.5%)	204 (66.5%)	288 (63.9%)	131 (67.9%)	0.565
Any ACEI/ARB exposure	580 (61.0%)	191 (62.2%)	271 (60.1%)	118 (61.1%)	0.840
Length of follow-up (days)	1104 ± 701	1056 ± 714	1138 ± 721	1103 ± 627	0.286
Deaths	239 (25.1%)	91 (29.6%)	100 (22.2%)	48 (24.9%)	0.066

ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MAGGIC: Meta-Analysis Global Group in Chronic Heart Failure risk score; NT pro-BNP: N-terminal pro b-type natriuretic peptide

* p-value is for difference among the three genotypes. Bolded p-values indicate $p < 0.05$.

Table 3.

Genotyping results and accuracy check stratified by patients' self-reported race

	Self-reported race	
	African-American (n = 575) 51%	White (n = 547) 49%
Minor allele frequencies		
rs9909004 (C)		
registry	0.40	0.47
1000 Genomes	0.35 (African)	0.47 (European)
rs9303504 (G)		
registry	0.41	0.47
1000 Genomes	0.37 (African)	0.47 (European)
Hardy Weinberg Equilibrium p-value		
rs9909004	0.839	0.265
rs9309504	0.422	0.516
Linkage disequilibrium		
r^2	0.86	0.96
D'	0.95	0.99