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Prevention of Atrial Fibrillation by Bucindolol Is Dependent on the Beta$_1$389 Arg/Gly Adrenergic Receptor Polymorphism

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Objectives This study assessed the impact of bucindolol, a beta-blocker/sympatholytic agent, on the development of atrial fibrillation (AF) in advanced chronic heart failure with reduced left ventricular ejection fraction (HFREF) patients enrolled in the BEST (Beta-Blocker Evaluation of Survival Trial).

Background β-blockers have modest efficacy for AF prevention in HFREF patients. Bucindolol's effects on HF and ventricular arrhythmic endpoints are genetically modulated by β_1- and α_{2c}-adrenergic receptor (AR) polymorphisms that can be used to subdivide HFREF populations into those with bucindolol effectiveness levels that are enhanced, unchanged, or lost.

Methods BEST enrolled 2,708 New York Heart Association (NYHA) class III to IV HFREF patients. A substudy in which 1,040 patients' DNA was genotyped for the β_1-AR position 389 Arg/Gly and the α_{2c}322–325 wild type (Wt)/deletion (Del) polymorphisms, and new-onset AF was assessed from adverse event case report forms or electrocardiograms at baseline and at 3 and 12 months.

Results In the entire cohort, bucindolol reduced the rate of new-onset AF compared to placebo by 41% (hazard ratio [HR]: 0.59 [95% confidence interval (CI): 0.44 to 0.79], p = 0.0004). In the 493 $\beta_1$389 arginine homozygotes (Arg/Arg) in the DNA substudy, bucindolol reduced new-onset AF by 74% (HR: 0.26 [95% CI: 0.12 to 0.57]), with no effect in $\beta_1$389 Gly carriers (HR: 1.01 [95% CI: 0.56 to 1.84], interaction test = 0.008). When $\beta_1$389 Gly carriers were subdivided by α_{2c} Wt homozygotes (n = 413, HR: 0.94 [95% CI: 0.48 to 1.82], p = 0.84) or Del variant carriers (n = 134, HR: 1.33 [95% CI: 0.32 to 5.64], p = 0.70), there was a positive interaction test (p = 0.016) when analyzed with $\beta_1$389 Arg homozygotes.

Conclusions Bucindolol prevented new-onset AF; β_1 and α_{2c} polymorphisms predicted therapeutic response; and the 47% of patients who were $\beta_1$389 Arg homozygotes had an enhanced effect size of 74%. (Beta-Blocker Evaluation in Survival Trial [BEST]; NCT00000560) (J Am Coll Cardiol HF 2013;1:338–44) © 2013 by the American College of Cardiology Foundation. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Atrial fibrillation (AF) is the most common cardiac arrhythmia and is frequently observed in chronic heart failure/reduced ejection fraction (HFREF) populations (1), where the incidence is several-fold higher than in patients without heart failure (2). In the Framingham cohort, new-onset AF was associated with an increase in mortality in patients with heart failure (3). However, in HFREF patients, rhythm control strategies with current antiarrhythmic medications have not been associated with improved outcomes (4). This may be due to multiple adverse effects of current antiarrhythmic agents in HFREF populations (5).

A drug treatment capable of decreasing the incidence of new-onset AF with an improved safety profile would benefit HFREF patients, particularly if such therapy also favorably

affected the underlying pathophysiologic mechanisms that predispose patients to AF. β-blockers are candidates for such a therapy because they both improve heart failure outcomes (6) and have efficacy for AF prevention (7), likely due in part to reverse remodeling in both ventricular (8) and atrial (9,10) chambers. However, currently approved β-blockers exhibit only modest efficacy for reducing new-onset AF in HFREF patients (7).

In patients with HFREF, the Arg389Gly polymorphism in the β_1-adrenergic receptor (-AR) (*ADRB1*) gene affects the therapeutic response to bucindolol, a nonselective β-AR blocker with sympatholytic properties (11). Compared to the 389 glycine (Gly) minor allele, the 389 arginine (Arg) major allele gene protein product has a 3- to 4-fold higher signal transduction capacity (11), higher affinity for agonists including norepinephrine (NE) (12), and a larger proportion of constitutively active ARs (11). In a genetic substudy of the BEST (Beta-Blocker Evaluation of Survival Trial), bucindolol exhibited $\beta_1$389 Arg/Gly genotype-dependent differential effects on mortality, heart failure hospitalizations, and ventricular arrhythmias (11–13). In addition, in HFREF patients who were $\beta_1$389 Gly carriers (having at least one copy of the dominant negative 389 Gly allele), an insertion/deletion polymorphism at amino acid position 322–325 of the α_{2c}-AR, alleles commonly referred to as either wild type (Wt) or deletion (Del), affects bucindolol's response for both heart failure (12,14) and ventricular arrhythmia (13) endpoints by regulating bucindolol's sympatholytic effects (14–16).

We hypothesized that $\beta_1$389 Arg/Gly and α_{2c}322–325 Wt/Del AR polymorphisms may modulate bucindolol's effects on new-onset AF in HFREF patients, as they do for heart failure (12) and serious ventricular arrhythmia endpoints (13).

Methods

Study population. The BEST was a randomized trial of bucindolol versus placebo in HFREF patients with NYHA class III to IV heart failure and left ventricular ejection fractions (LVEF) ≤0.35 (15). The current study analyzed patients who were not in AF at study entry, including 2,176 patients in sinus rhythm (SR) plus 216 patients with other rhythms to yield a study population of 2392 from the entire 2,708 patient cohort, and 925 patients from the 1,040 DNA substudy (846 SR and 79 other rhythms). In the 925 AF-free DNA bank substudy patients, the development of new-onset AF was investigated in $\beta_1$389 Arg/Gly and α_{2c}322–325 Wt/Del genetic subgroups as previously described for heart failure (12) and ventricular arrhythmic (13) endpoints. The BEST protocol, patient population, and main outcomes have been previously described (15). The DNA bank and the AR polymorphism substudy protocols and patient populations have also been previously described (11–14). This study used the DNA substudy of BEST, a prospectively planned investigation (n = 1,040) with a separate consent form and ethical committee review designed to test the effects of AR polymorphisms on clinical outcomes. All patients signed written consent forms for both the parent BEST protocol and the DNA substudy. Although DNA analysis was performed after the trial ended, clinical data remained blinded from the investigators until the coded genetic data results were submitted to the data coordinating center and analyzed by trial statisticians.

Abbreviations and Acronyms

AF = atrial fibrillation
AR = adrenergic receptor
Arg/Arg = arginine homozygote
Del = deletion
HFREF = heart failure with reduced left ventricular ejection fraction
HR = hazard ratio
NE = plasma norepinephrine
SR = sinus rhythm
Wt = wild type

The current substudy is a post hoc analysis investigating the incidence of new-onset AF. Cases of AF were prospectively identified from adverse event case report forms that included electrocardiograms (ECGs) and were reviewed and certified by cardiologist investigators at each site. In patients for whom no adverse event had been recorded, new-onset AF event was also obtained from study ECGs performed at baseline and at 3 and 12 months. In order to assess the total number of new-onset AF events, the separate adverse event and study ECG datasets were combined. Time of onset of the AF event was taken as the day of detection, with the duration of AF-free follow-up determined by comparison to the randomization date.

Genotyping and norepinephrine measurements. Genotyping for $\beta_1$389Arg/Gly and α_{2c}322–325 Wt/Del polymorphisms was performed with archived DNA (11–14), and plasma norepinephrine (NE) was measured from systemic venous samples as previously described (16).

Statistical analysis. The primary analysis was the measure of time to first event of AF for patients free of AF at study entry. A log rank statistic was used to generate treatment comparison p values, and a Cox proportional hazards model was used to estimate hazard ratios (HRs) and confidence intervals (CIs) between bucindolol and placebo groups. Per the study regulatory statistical analysis plan, all analyses were adjusted for the covariates of presence/absence of coronary artery disease, LVEF ≤20% to >20%, black and non-black race, and gender, which are the 4 strata used in the treatment randomized assignment. Follow-up was by intention-to-treat, with censoring for cardiac transplantation, death, nonfatal lost to follow-up, or study end on July 26, 1999. For baseline characteristics, continuous variables were compared using Student *t* test and presented as the mean ± SD. Categorical variables were compared using the chi-square test. As previously reported (14), 66% of patients entered the DNA substudy after randomization and had DNA collection after being enrolled in the parent treatment protocol. In these "late entry" patients, postrandomization AF events that occurred prior to DNA collection were counted in the statistical analysis.

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Results

Clinical characteristics of patient cohorts. Baseline characteristics for the entire 2,392 BEST AF-free cohort at entry are given in Table 1, and they do not differ from previously reported characteristics of the patients in SR at study entry (17). The average follow-up of the 2,392 non-AF patients was 2.0 years, with a maximum of 4.1 years. Table 1 also gives the baseline characteristics of the 925 non-AF patients in the DNA substudy (average follow-up 2.1 years) and in selected genotype groups. The 69 patient ($\beta_1$389 Arg/Arg + α_{2c}322–325 Del carrier) group contained too few events (n = 6) for analysis, and the $\beta_1$389 Arg/Arg group was therefore not subdivided by α_{2c}322–325 Wt/Del polymorphism. In the DNA substudy, there were 441 patients who were $\beta_1$389 Arg homozygotes ($\beta_1$389 Arg/Arg) and 484 Gly carriers ($\beta_1$389 Gly/Gly or Arg/Gly). Within the $\beta_1$389 Gly carrier patient group, 358 were α_{2c} Wt homozygotes and 126 were α_{2c}322–325 Del carriers. There were no clinically relevant differences between baseline characteristics in the DNA substudy and the entire cohort non-AF patients. As previously reported for all baseline rhythms (12,13), there were significant differences in race and hypertension history between $\beta_1$389 Arg/Arg and Gly carriers groups, as well as between the 2 $\beta_1$389 Gly carrier/α_{2c}322–325 groups that were related to the $\beta_1$389 Gly and α_{2c}322–325, deletion alleles being more prevalent in blacks (11–14).

Outcomes in the BEST cohort and DNA substudy. There were 190 new-onset AF events in the entire 2,392 patient cohort, for an overall event rate of 7.9%. In the 925 DNA substudy patients, there were 80 new-onset AF events (rate, 8.6%). In the entire BEST cohort, there was a lower incidence of new-onset AF in the bucindolol group than in the placebo group (n = 75 [6.2%] vs. n = 115 [9.7%] HR: 0.59 [95% CI: 0.44 to 0.79]), corresponding to a 41% risk reduction (Table 2). There was a similar decrease in the incidence of new-onset AF in the DNA substudy in the bucindolol group compared to the placebo group (n = 31 [6.7%] vs. n = 49 [10.7%]; HR: 0.57 [95% CI: 0.36 to 0.90]) (Table 2). Data presented in Table 2 indicate that 85% of events were detected from adverse event forms as opposed to routine ECGs only; thus, most of the events were symptomatic. Time to first event curves for the entire cohort and DNA substudy are given in Figure 1.

Table 3 gives the reduction in new-onset AF analyzed by event duration. AF events were classified as short duration paroxysmal (<24 h), longer duration paroxysmal (between 24 h and 7 days), or persistent (longer than 7 days). Greater than two-thirds (67.9%) of the events were persistent AF, with 23.2% of events longer paroxysmal and only 8.9% of events being short paroxysmal. By HR, bucindolol treatment effects were similar for the 3 AF durations, with HR of 0.51 (p = 0.183), 0.57 (p = 0.066), and 0.62 (p = 0.007) for shorter paroxysmal, longer paroxysmal, and persistent AF, respectively (Table 3). However, event rates were low in the paroxysmal groups, and the persistent AF group was the only one that attained statistical significance.

Outcomes by genotype group. Table 4 gives HR data by genotype group. In the 441 $\beta_1$389 Arg/Arg patients, bucindolol was associated with a marked decrease in the incidence of new-onset AF (HR: 0.26 [95% CI: 0.12 to 0.57], p = 0.0003). In contrast, bucindolol had no impact on the incidence of new-onset AF in the 484 $\beta_1$389 Gly carriers (HR: 1.01 [95% CI: 0.56 to 1.84], p = 0.97). In the time to first event curves shown in Figure 2, the 74% risk reduction by bucindolol in $\beta_1$389 Arg/Arg patients was associated with an early divergence of curves. There was no reduction in new-onset AF in the $\beta_1$389 Gly carriers who

Table 1 Baseline Patient Characteristics for Patients Who Were Not in AF at the Time of Study Entry, in the Entire Cohort, DNA Substudy and Within Genotype Groups

Characteristic	Entire Cohort (n = 2,392)	DNA Substudy (n = 925)	$\beta_1$389 Arg/Arg (n = 441)	$\beta_1$389 Gly Carrier (n = 484) (p vs. Arg/Arg)	β_1 Gly carrier + α_{2c} Wt/Wt (n = 358)	β_1 Gly carrier + α_{2c} Del (n = 126) (p vs. Wt/Wt)
Age (yrs)	59.6 ± 12.4	59.7 ± 12.2	59.7 ± 11.9	59.8 ± 12.4	60.2 ± 12.3	58.5 ± 12.8
Male (%)	1,829 (76%)	718 (78%)	345 (78%)	373 (77%)	281 (78%)	92 (73%)
Black (%)	586 (25%)	198 (21%)	63 (14%)	135 (28%)*	47 (13%)	88 (70%)*
Resting HR (beats/min)	82.5 ± 13.4	81.7 ± 13.4	81.5 ± 13.7	81.9 ± 13.2	81.6 ± 13.4	82.8 ± 12.7
HTN (%)	1,424 (60%)	523 (57%)	238 (54%)	225 (59%)	191 (53%)	94 (75%)*
Diabetes (%)	876 (37%)	333 (36%)	165 (38%)	168 (35%)	120 (34%)	48 (38%)
Ischemic cause (%)	1411 (59%)	550 (59%)	259 (59%)	291 (60%)	230 (64%)	61 (48%)†
LVEF (%)	22.9 ± 7.3	23.5 ± 7.1	23.3 ± 7.1	23.7 ± 7.1	23.9 ± 7.1	23.0 ± 7.1
HF duration (months)	48.2 ± 47.9	45.1 ± 47.4	47.8 ± 51.3	42.7 ± 43.4	41.5 ± 42.5	46.3 ± 45.7
NYHA class III (%)	2,200 (92%)	857 (93%)	418 (95%)	439 (91%)‡	325 (91%)	114 (90%)
Digoxin (%)	2,197 (92%)	827 (89%)	398 (90%)	429 (89%)	319 (89%)	110 (87%)

Values are mean ± SD or n (%). p values for comparisons of genotype subgroups consist of chi-square test results for categorical variables and the Wilcoxon test results for continuous variables. *p < 0.001; †p < 0.01; ‡p < 0.05.

AF = atrial fibrillation; Arg = arginine; Del = deletion; Gly = glycine; HF = heart failure; HR = heart rate; HTN = history of hypertension; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; Wt = wild type.

Table 2 Prevention of New-Onset Atrial Fibrillation by Bucindolol in BEST

Treatment Group	Patients Free of AF at Baseline Assessed by ECG	Patients With New-Onset AF Reported as AE During the Trial	Total No. of Patients With New-Onset AF During the Trial
Entire cohort			
Placebo (%)	1,190 (88.3%)	100 (8.4%)	115 (9.7%)
Bucindolol (%)	1,202 (89.2%)	61 (5.1%)	75 (6.2%)
Time to first event of new-onset AF		0.55 (0.44 to 0.76), p = 0.0002	0.59 (0.44 to 0.79), p = 0.0004
DNA substudy			
Placebo (%)	460 (88.0%)	45 (9.8%)	49 (10.7%)
Bucindolol (%)	465 (90.6%)	25 (5.4%)	31 (6.7%)
Time to first event of new-onset AF		0.50 (0.31 to 0.82), p = 0.005	0.57 (0.36 to 0.90), p = 0.014

Values are n (%) or hazard ratio (95% confidence interval), p value.
AE = adverse event; AF = atrial fibrillation; BEST = Beta-Blocker Evaluation of Survival Trial.

received bucindolol compared to placebo. These results yielded a significant statistical interaction (p = 0.008) between treatment and $\beta_1$389 Arg/Gly genotypes.

For both heart failure endpoints (12) and serious ventricular arrhythmias (13), when HFREF patients are $\beta_1$389 Gly carriers, the type of associated α_{2c}322–325 Wt/Del polymorphism can alter bucindolol treatment effects. Data in Table 4 suggest this is also the case for prevention of AF, where Del carriers have a HR >1.0. Moreover, the 3-genotype group construct that included $\beta_1$389 Arg/Arg patients had an interaction p value of 0.016, supporting the validity of subdividing the $\beta_1$389 Gly carrier group by α_{2c} polymorphism.

Plasma norepinephrine and new-onset AF. In order to assess the relationship of adrenergic drive and outcomes, systemic venous plasma NE levels were measured at baseline and at months 3 and 12. Of the entire 2,392 patient cohort, 1,868 had baseline NE measured. Compared to patients who remained free of AF, patients who developed AF had higher baseline NE levels in the bucindolol group (581 ± 304 pg/ml vs. 514 ± 344 pg/ml, respectively, p = 0.009) and in the combined treatment groups (530 ± 231 pg/ml vs. 498 ± 326 pg/ml, respectively, p = 0.015). Bucindolol produced a significant reduction in NE levels at 3 months in patients who developed AF (by 129 ± 49 pg/ml, p = 0.0009 vs. placebo change) and in patients who remained free of AF (by 74 ± 12 pg/ml, p <0.0001 vs. placebo change), with no differences between the 2 groups (p = 0.23). Placebo-treated patients exhibited increases in NE in both the new-onset AF subgroup (by 88 ± 46 pg/ml) and in patients who remained free of AF (by 21 ± 11 pg/ml, p = 0.29 vs. new-onset AF). Table 4 gives NE changes at 3 months within the

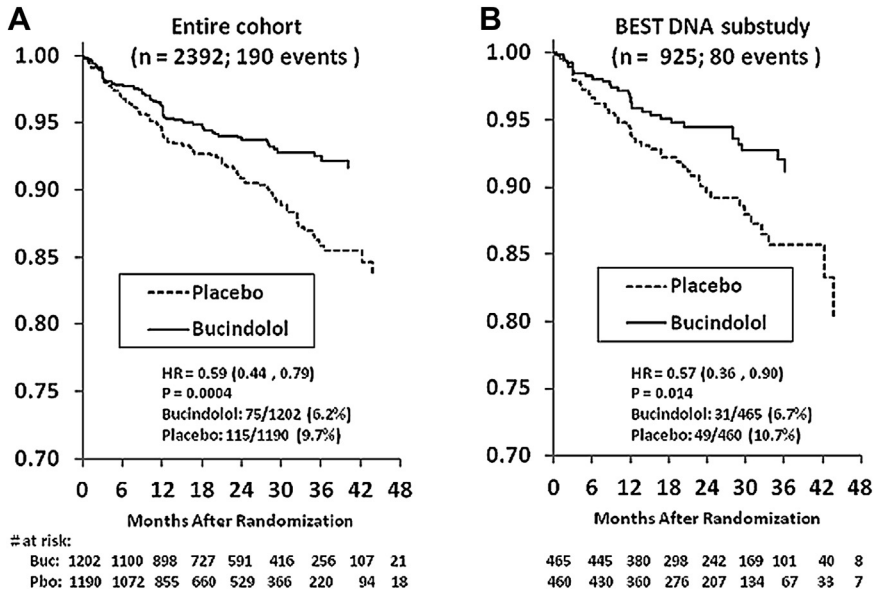

Figure 1 Time to New-Onset AF in Bucindolol and Placebo Arms of BEST

Time to event curves for new-onset atrial fibrillation (AF) in the BEST entire cohort **(A)** and the DNA substudy **(B)**. **Dashed line** = placebo; **solid line** = bucindolol. HR = hazard ratio.

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Table 3 Duration of New-Onset Atrial Fibrillation Events in BEST

Patient Group	Total Placebo	Total Bucindolol	Hazard Ratio (95% CI)	Logrank p Value
Entire cohort (n = 2392), all AF	115/1,190 (9.7%)	75/1,202 (6.2%)	0.59 (0.44 to 0.79)	0.0004
<24 h (n = 17 of 190 [8.9%])	11/1,190 (0.9%)	6/1,202 (0.5%)	0.51 (0.19 to 1.39)	0.183
>24 h to ≤7 days (n = 44 of 190 [23.2%])	27/1,190 (2.3%)	17/1,202 (1.4%)	0.57 (0.31 to 1.05)	0.066
>7 days (n = 129 of 190 [67.9%])	77/1,190 (6.5%)	52/1,202 (4.3%)	0.62 (0.43 to 0.88)	0.007

Values are n/N (%).
AF = atrial fibrillation; BEST = Beta-Blocker Evaluation of Survival Trial; CI = confidence interval.

pharmacogenetic subgroups, where it can be observed that there are similar degrees of NE lowering in the bucindolol $\beta_1$389 Arg/Arg and Gly carrier genetic groups (respectively, 71 and 78 pg/ml and both p <0.010 vs. placebo change). Within the Gly carrier group, the α_{2c}322–325 Del carrier subgroup has a large degree of bucindolol-associated NE reduction (by 164 pg/ml) as previously reported for the full 1,040, all rhythms DNA substudy population (12), which is due to the exclusive presence of the α_{2c}322–325 Del carrier genotype (14).

Discussion

Treatment effects of bucindolol on new-onset AF in the BEST entire cohort and the DNA substudy. The DNA substudy and the entire cohort parent populations were very similar in baseline characteristics, length of follow-up (2.0 vs. 2.1 years), overall event rates (respectively, 7.9% and 8.6%), and placebo event rates (respectively, 9.7% and 10.7%). Thus, there was no evidence that late entry of most in the DNA substudy relative to their randomization dates had any impact on the study population from the standpoint of development of new-onset AF.

For new-onset AF, bucindolol demonstrated respective risk reductions of 41% (p = 0.0004) and 43% (p = 0.014) in the entire and DNA substudy cohorts of BEST. In placebo controlled HFREF trials, the effect of β-blockade on AF episodes by event duration has not been previously reported, and we evaluated effects on both paroxysmal and persistent AF. In the entire cohort the majority (68%) of AF episodes were >7 days duration or persistent, exhibiting a 38% reduction (p = 0.007) by bucindolol. Shorter or paroxysmal episodes of AF were not significantly reduced, although they had lower HRs than in the persistent group. Thus, there was observational evidence of a bucindolol treatment effect regardless of AF duration, and statistical significance of a favorable effect in persistent AF.

Pharmacogenetic treatment effects. Reduction in new-onset AF was driven by a large bucindolol treatment effect in patients with a $\beta_1$389 Arg/Arg genotype who had a 74% reduction (p = 0.0003) when treated with bucindolol compared to those treated with placebo. There was no reduction in event rate (HR: 1.01) in bucindolol patients who were $\beta_1$389 Gly carriers, and the treatment × genotype group interaction p value was 0.008. Subdividing the $\beta_1$389 Gly carrier genotype by α_{2c}322–325 Wt/Del genotype appeared to further differentiate bucindolol response as it does for heart failure (12) and serious ventricular arrhythmia (13) endpoints, with a significant (p = 0.016) test for interaction when $\beta_1$389 Arg/Arg patients were included in the 3-group analysis. Although differences in race and/or history of hypertension could have affected the analysis between genotypes, the ($\beta_1$389 Gly carrier + α_{2c}322–325 Wt/Wt) group had prevalence rates for black patients and cases of hypertension that were similar to those of the $\beta_1$389 Arg/Arg group but markedly different HRs (0.94, p = 0.84 and 0.26, p = 0.0003, respectively). This indicates that the differentially enhanced treatment effect of bucindolol on AF prevention is mediated through $\beta_1$389 Arg vs. Gly ARs and is not directly related to race or history of hypertension.

There appears to be a class affect of β-blockers for reduction of new-onset AF in HFREF patients. A meta-analysis by Nasr et al. (7) of new-onset AF in HFREF trials demonstrated an average 27% reduction of new-onset AF for five different β-blockers and evidence for a treatment effect

Table 4 Prevention of New-Onset AF by Bucindolol in BEST by Genotype, Total Number of Events, and Norepinephrine Change at 3 Months in Patients in Genetic Groups

Measure	($\beta_1$389Arg/Arg + any α_{2c})* (P = 206, B = 235)	($\beta_1$389Gly Carrier + any α_{2c}) (P = 254, B = 230)	(β_1 Gly carrier + α_{2c} Wt/Wt)* (P = 183, B = 175)	(β_1 Gly carrier + α_{2c} Del)* (P = 71, B = 55)
HR (95% CI), no. of events, p value	0.26 (0.12 to 0.57) 36 events, p = 0.0003	1.01 (0.56 to 1.84) 44 events, p = 0.97	0.94 (0.48 to 1.82) 36 events, p = 0.84	1.33 (0.32 to 5.64) 8 events, p = 0.70
NE change at 3 months (pg/ml)	P = 14 ± 20 B = 71 ± 22 p = 0.0013	P = 31 ± 22 B = 78 ± 24 p = 0.0019	P = 38 ± 25 B = 54 ± 22 p = 0.0039	P = 7 ± 45 B = 164 ± 79 p = 0.23

Data show prevention of new-onset atrial fibrillation (AF) by bucindolol in BEST by genotype (hazard ratio [R], 95% confidence interval [CI]), total number of events, and p value. Norepinephrine (NE) change at 3 months in patients in genetic groups. *Member of 3-group construct tested for interaction.
B = bucindolol; Del = deletion; P = placebo; Wt = wild type; other abbreviations as in Table 1.

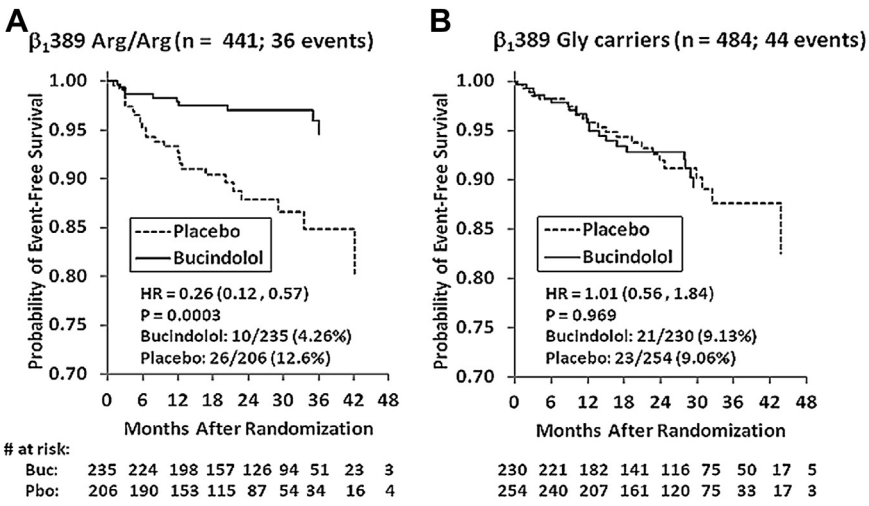

Figure 2 Time to New Onset by $\beta_1$389 Arg/Gly Genotype

Time to event curves are shown for new-onset AF in the BEST DNA substudy by $\beta_1$389 Arg/Gly genotype. There is a significant interaction between genotype and treatment. The benefit of bucindolol is seen exclusively in the $\beta_1$389 Arg/Arg genotype **(A)**, with a risk reduction of 74% compared to placebo ($p = 0.008$ for interaction vs. Gly carrier group). **(B)** There was no impact of bucindolol in the β1 Gly carriers compared to placebo. **Dashed line** = placebo; **solid line** = bucindolol. Abbreviations as in Figure 1.

for all β-blockers except nebivolol. This relatively modest reduction in new-onset AF across all β-blocker HFREF trials is in contrast to the marked 74% reduction in new-onset events in the $\beta_1$389 Arg/Arg group observed in this analysis.

Role of adrenergic drive in the development of new-onset AF and the pharmacotherapeutic effects of bucindolol. Patients who developed AF had higher baseline NE levels than patients who remained free of AF, similar to data for AF development in an animal model of heart failure (18). Bucindolol's well-known sympatholytic effects (14–16) were observed in patients who developed AF and in those who did not and to the same extent in patients with $\beta_1$389 Arg/Arg and $\beta_1$389 Gly carrier genotypes. Thus, NE reduction by bucindolol may play a role in its AF prevention effects, but a difference in degree of sympatholysis does not explain the highly selective therapeutic effects of bucindolol in patients with the $\beta_1$389 Arg/Arg genotype. In this genotype patients express only the $\beta_1$389 Arg receptor, which is the "NE receptor" in the heart (12). A reduction in NE will therefore have a selectively greater therapeutic effect in this genotype, and patients are also protected from the adverse effects of marked sympatholysis (12).

In the ($\beta_1$389 Gly carrier + α_{2c}322–325 Del carrier) group, relatively low prevalence (13.6% of the total) combination genotype that exhibited a statistically insignificant 33% numerical increase in new-onset AF, there was a large reduction in NE due to the α_{2c}322–325 Del carrier polymorphism (12,14). The adverse affects of sympatholysis (12,14,16) may have canceled any therapeutic effect of bucindolol in $\beta_1$389 Gly carriers and led to a nonsignificant increase in AF in patients with a [$\beta_1$389 Gly carrier + α_{2c}322–325 Del carrier] genotype.

Mechanisms of atrial fibrillation prevention by bucindolol as modulated by the $\beta_1$389 Arg/Arg genotype. There are multiple lines of evidence linking high levels of β_1-adrenergic signaling, as predicted for $\beta_1$389 Arg/Arg homozygotes, to the development of AF. Higher adrenergic activity has been shown to increase the inducibility of AF in humans and dogs in a dose-dependent manner (19,20), and in a model of ischemic cardiomyopathy, dogs that developed AF had higher NE levels (18). Furthermore, in isolated human right atrial preparations, isoproterenol infusion has been shown to increase the frequency of atrial early and delayed afterdepolarizations, phenomena that have been implicated in initiating AF (21). Bucindolol is especially effective in inhibiting signaling through $\beta_1$389 Arg ARs, through the novel mechanisms of facilitating inactivation of constitutively active receptors (the property of inverse agonism) (11) and NE lowering (12), as well as through high-affinity competitive antagonism (6).

Study limitations. The primary limitation of the current substudy is the post hoc nature of the analysis. AF was not a prespecified efficacy endpoint, and the data were not adjudicated but rather collected from investigator-reviewed adverse event case report forms and serial ECGs, similar to the approach used by van Veldhuisen et al. (22). Thus, some AF events were likely missed, and in the case of the 15% of events that were detected by ECG, only the onset of AF could have been much earlier than the recorded date. On the other hand, using adverse event forms and ECGs to capture new-onset AF events represented a blinded, nonbiased way to assess arrhythmia occurrence with 85% of the events being symptomatic. Based on the use of adverse event case report forms and ECGs, it is likely that most AF events of more than

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several hours duration were detected, with the onset contemporaneous to detection in a substantial majority of cases.

Another limitation of the current analysis is the relatively small number of new-onset AF events. Although the entire cohort contained 190 events, the largest number reported in any HFREF β-blocker trial (7), the DNA substudy had only 80 events, and after pharmacogenetic subgrouping the number of events in each group was further reduced by ~50%. These limitations will be addressed in a planned study of AF prevention in $\beta_1$389 Arg/Arg genotype HFREF patients who are randomized to bucindolol versus. metoprolol, a β-blocker that does not exhibit pharmacogenetic modulation of clinical therapeutic responses (23).

Conclusions

Bucindolol was associated with a significant, quantitatively large decrease in new-onset AF in the entire BEST cohort that was observed exclusively in the $\beta_1$389 Arg/Arg genotype.

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