Dysfunctional Corin I555(P568) Allele Is Associated With Impaired Brain Natriuretic Peptide Processing and Adverse Outcomes in Blacks With Systolic Heart Failure

Results From the Genetic Risk Assessment in Heart Failure Substudy

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Background—Corin, a transmembrane serine protease expressed in cardiomyocytes, cleaves pro–atrial natriuretic peptide and pro–brain natriuretic peptide (BNP) into biologically active peptide hormones. The minor corin I555(P568) allele, defined by the T555I and Q568P mutations, is common in persons of African ancestry and associated with increased risk for hypertension and cardiac concentric hypertrophy. The corin gene product containing the T555I and Q568P mutations has significantly reduced natriuretic peptide processing capacity. We hypothesized that the corin I555(P568) allele would be associated with adverse outcomes and impaired BNP processing in blacks with systolic heart failure.

Methods and Results—This is a retrospective study of 354 subjects in the African American Heart Failure Trial Genetic Risk Assessment in Heart Failure substudy. In the corin variant group (n=50) compared with corin nonvariant group (n=300), BNP-32 (amino acids 77 to 108) was lower (190 pg/mL versus 340 pg/mL, P=0.007), but the ratio of unprocessed BNP1 to 108/processed BNP-32 was significantly higher (P=0.05). Stratified analyses were conducted because of evidence of significant interaction between the corin I555(P568) allele and treatment assignment. In the placebo arm, multivariable analysis demonstrated that the corin I555(P568) allele was associated with increased risk for death or heart failure hospitalization (relative risk 3.49; 95% CI, 1.45 to 8.39; P=0.005); however, in the treatment arm (fixed-dose combination isosorbide-dinitrate/hydralazine), the corin I555(P568) allele was not associated with adverse outcomes.

Conclusions—We have identified a pharmacogenomic interaction in blacks with systolic heart failure. The corin I555(P568) allele is associated with an increased risk for death or heart failure hospitalization in patients receiving standard neurohormonal blockade, but the addition of fixed-dose combination isosorbide-dinitrate/hydralazine ameliorates this risk. A plausible mechanism for this pharmacogenomic interaction is the impaired processing of BNP in carriers of the corin I555(P568) allele as compared with noncarriers. (Circ Heart Fail. 2009;2:541-548.)

Key Words: genetics ■ epidemiology ■ heart failure ■ natriuretic peptides ■ pharmacology

Corin is a transmembrane serine protease that is expressed in cardiomyocytes and cleaves pro–atrial natriuretic peptide (ANP) and pro–brain natriuretic peptide (BNP) into biologically active peptide hormones.1,2 A minor allele in corin, defined by 2 coding variants in complete linkage disequilibrium (T555I and Q568P), is common and almost exclusively expressed in persons of African ancestry (allelic prevalence 6.7%). Furthermore, the corin I555(P568) allele is associated with increased systolic blood pressure, increased risk for systemic hypertension, and an enhanced concentric cardiac hypertrophic response to increased blood pressure.3,4

Recently, in vitro experiments have demonstrated that the presence of both the T555I and Q568P amino acid substitutions significantly reduce the natriuretic processing activity of mutant I555(P568) corin.5

Clinical Perspective on p 548

Adequate natriuretic peptide processing is essential for the endogenous natriuretic peptide system to function in maintaining cardiovascular homeostasis. The human gene for BNP encodes a 134-amino acid prepro-BNP precursor, which after removal of a 26-amino acid signal peptide gives rise to a
108-amino acid pro-BNP polypeptide (pro-BNP_{108}). Further processing of pro-BNP_{108} by the type 2 transmembrane serine protease corin results in the physiologically active 32-amino acid carboxyl-terminal BNP molecule (BNP-32), derived from amino acids 77 to 108, and an inactive N-terminal fragment BNP, derived from amino acids 1 to 76. Recently, cell-based studies using recombinant BNP_{108} have shown that unprocessed BNP_{108} is unable to activate the type A natriuretic peptide receptor. Therefore, abnormalities in corin-mediated natriuretic peptide processing would be expected to attenuate the in vivo biological activity of the natriuretic peptide system. Although elevated BNP levels are associated with adverse outcomes in heart failure, the multiple biological actions of ANP and BNP within target tissues are “compensatory” in nature and include vasodilatation of arteries and veins, opposing activation of adverse neurohormonal systems, including the renin-angiotensin-aldosterone and sympathetic systems, enhancement of natriuresis by opposing distal tubule sodium reabsorption and direct inhibition of endothelin release by the renal vascular endothelium.

Given the recognized importance of the natriuretic peptide system for the maintenance of homeostasis in systolic heart failure and the almost exclusive presence of the 1555(P568) allele in blacks, we undertook this analysis as a substudy within the African American Heart Failure trial (A-HeFT) to test the hypothesis that in patients with systolic heart failure the corin I555(P568) allele would be associated with an increased risk of death or hospitalization for heart failure. Furthermore, we sought to determine whether there was an increase in the degree of impaired processing of BNP in carriers of the corin I555(P568) allele relative to noncarriers. To estimate the degree of impaired processing of BNP, we measured unprocessed pro-BNP_{108} using a novel immunoassay specific for BNP_{108} (Bio-Rad, Hercules, Calif) in 695 of 1050 subjects in the A-HeFT (239 of the 350 subjects who consented for genetic studies in GRAHF). Using a novel immunoassay, we measured baseline plasma levels of pro-BNP_{108} (Bio-Rad) in 695 of 1050 subjects in the A-HeFT (239 of the 350 subjects who consented for genetic studies in GRAHF) who consented for measurement at baseline and at 6-month follow-up. This immunoassay has been demonstrated to have no cross-reactivity with either recombinant BNP-32 or N-terminal fragment-BNP and is therefore highly specific for pro-BNP_{108}. BNP was also measured in these patients using the Biosite Triage immunoassay. A recent Expert Consensus Panel analyzed the cross-reactivity patterns of available commercial BNP immunoassays. The Biosite Triage immunoassay has minimal cross-reactivity (19%; 95% CI, 18% to 20%) with glycosylated recombinant BNP_{108} expressed in mammalian cells. We reasoned that the ratio of BNP_{108} to BNP-32 (Biosite) would serve as a reasonable assessment of BNP processing efficiency.

**Methods**

**Study Design**

The A-HeFT study design, patient characteristics, end point definitions, and complete methodology have been published previously in detail. In brief, A-HeFT was a randomized, placebo-controlled, double-blind trial with self-identified black patients recruited at 169 centers in the United States. The study protocol was reviewed and approved by appropriate institutional review boards. All patients gave written informed consent. Independent committees adjudicated all primary and secondary end points, reviewed data for safety, and oversaw the 2 prespecified interim analyses done to assess adequacy of sample size only.

**Inclusion Criteria**

Patients 18 years and older, self-identified as black, and with New York Heart Association class III or IV heart failure for at least 3 months, were eligible for screening. Patients were required to be undergoing standard background heart failure therapy, as determined by their physician, which included angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, β-blockers for at least 3 months before randomization, digoxin, spironolactone, and diuretics. Evidence of left ventricular (LV) dysfunction within the 6 months preceding randomization was required and consisted of either a resting LV ejection fraction <35% or a resting LV ejection fraction <45% with an LV internal diastolic diameter >2.9 cm/m² of body surface area or >6.5 cm by echocardiography.

**End Points**

The primary efficacy end point for the A-HeFT trial was a novel composite score that weighted all-cause mortality, first hospitalization for heart failure throughout the 18-month follow-up period, and change in quality-of-life at 6 months. For this analysis, we used the composite end point of death from any cause or first hospitalization for heart failure.

**Genotyping**

Subjects were enrolled in the Genetic Risk Assessment in Heart Failure (GRAHF) substudy at the 6-month visit, and DNA was isolated from peripheral blood by leukocyte centrifugation and cell lysis (PureGene, Gentra Systems, Minn). The corin Q568P polymorphism (in complete linkage disequilibrium with the T555I locus) was genotyped in 350 subjects participating in A-HeFT using the TaqMan SNP Genotyping Assay and the Applied Biosystems 7000 (Applied Biosystems, Foster City, Calif).

**Measurement of Pro-BNP and BNP**

Using a novel immunoassay, we measured baseline plasma levels of unprocessed BNP_{108} (Bio-Rad) in 695 of 1050 subjects in the A-HeFT (239 of the 350 subjects who consented for genetic studies in GRAHF) who consented for measurement at baseline and at 6-month follow-up. This immunoassay has been demonstrated to have no cross-reactivity with either recombinant BNP-32 and N-terminal fragment-BNP and is therefore highly specific for pro-BNP_{108}. BNP was also measured in these patients using the Biosite Triage immunoassay. A recent Expert Consensus Panel analyzed the cross-reactivity patterns of available commercial BNP immunoassays. The Biosite Triage immunoassay has minimal cross-reactivity (19%; 95% CI, 18% to 20%) with glycosylated recombinant BNP_{108} expressed in mammalian cells. We reasoned that the ratio of BNP_{108} to BNP-32 (Biosite) would serve as a reasonable assessment of BNP processing efficiency.

**Statistical Analysis**

Subjects were followed to an end point of death or heart failure hospitalization. LV function was assessed by thoracic echocardiography at baseline and 6 months. We compared continuous variables by linear ANOVA when appropriate. Pro-BNP (BNP_{108}) and BNP values were log transformed to produce a normal distribution of values permitting standard parametric testing (Student’s t test) of means. In the case of the ratio of pro-BNP to BNP defined as BNP_{108}/BNP-32, this variable did not satisfy assumption of normality; therefore, we statistically tested for differences in the pro-BNP/BNP ratio between the corin variant (I555/P568) and nonvariant groups using the nonparametric Van der Waerden normal quantile test.

Comparison of event-free survival (death or first hospitalization for heart failure) by genotype class was analyzed by Kaplan-Meier survival analysis and log-rank methods. Multivariable survival analysis were conducted using Cox proportional hazards modeling, and randomization assignment was adjusted for using the intention-to-treat principle. In these models, the risk for persons in the corin variant group, defined as GRAHF participants heterozygous for the corin I555(P568) allele, was compared with the nonvariant group. In GRAHF, all carriers of the corin I555(P568) allele were heterozygous, a finding that is consistent with our results examining the genotype prevalence in two, large, and independent population-based cohorts of self-identified blacks. The multivariable Cox propor-
Table 1. Baseline Characteristics According to Presence or Absence of Corin I555(P568) Allele

<table>
<thead>
<tr>
<th></th>
<th>Corin\textsuperscript{+/+}</th>
<th>Corin\textsuperscript{+/−}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>300 (86)</td>
<td>50 (14)</td>
<td></td>
</tr>
<tr>
<td>Age, mean, SD</td>
<td>57.9</td>
<td>53.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Men, %</td>
<td>62</td>
<td>48</td>
<td>0.12</td>
</tr>
<tr>
<td>EF, %</td>
<td>34.8</td>
<td>33.5</td>
<td>0.35</td>
</tr>
<tr>
<td>LVDD</td>
<td>6.37</td>
<td>6.65</td>
<td>0.19</td>
</tr>
<tr>
<td>SBP</td>
<td>127.2</td>
<td>125.2</td>
<td>0.44</td>
</tr>
<tr>
<td>DBP</td>
<td>76.6</td>
<td>77.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Heart rate</td>
<td>73.1</td>
<td>74.8</td>
<td>0.34</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.23</td>
<td>1.20</td>
<td>0.70</td>
</tr>
<tr>
<td>QOL score</td>
<td>49.9</td>
<td>53.4</td>
<td>0.38</td>
</tr>
<tr>
<td>Causative factor, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>26.7</td>
<td>16.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>37.3</td>
<td>30.0</td>
<td>0.42</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>24.0</td>
<td>32.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Valve-related</td>
<td>2.7</td>
<td>0.0</td>
<td>0.61</td>
</tr>
<tr>
<td>Diabetes</td>
<td>44.0</td>
<td>42.7</td>
<td>0.88</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blocker</td>
<td>83.0</td>
<td>92.0</td>
<td>0.14</td>
</tr>
<tr>
<td>ACEI</td>
<td>77.3</td>
<td>70.0</td>
<td>0.31</td>
</tr>
<tr>
<td>ARB</td>
<td>21.3</td>
<td>24.0</td>
<td>0.71</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>36.3</td>
<td>34.0</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Corin\textsuperscript{+/+} indicates wild type; Corin\textsuperscript{+/−}, heterozygous for corin I555(P568) allele; EF, ejection fraction; LVDD, left ventricular internal dimension-diastole; SBP, systolic blood pressure; DBP, diastolic blood pressure; QOL, quality-of-life; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker.

Results

Baseline Characteristics
Approximately 14% of the GRAHF participants were heterozygous for the corin I555(P568) allele consistent with previous findings from population-based samples.\(^3,4\) We did not identify any subjects homozygous for the corin I555(P568) allele. However, this is not unexpected, given the small sample size in A-HeFT and the fact that in our previous population-based studies, the homozygous genotype frequency for the corin I555(P568) allele was extremely low (<0.2%).

The absolute and log-transformed baseline plasma measures of both BNP and pro-BNP were lower in the corin variant compared with nonvariant groups (Table 4). When we calculated the Pearson correlation coefficient, the corin allele was significantly associated with lower BNP values in univariate analysis (P=0.007) and multivariable analysis that adjusted for age, gender, body mass index, ischemic etiology, and use of β-blockers. Likewise, when calculating the Spearman correlation coefficient, the corin I555(P568) allele was associated with adverse outcomes in multivariable analysis regardless of the inclusion or exclusion of BNP levels.

Association of Corin I555(P568) Allele With Outcomes

When we examined the possibility of interaction between the corin I555(P568) allele and treatment assignment with the composite end point (P=0.07) and the end point of first hospitalization for heart failure (P=0.03), there was a suggestion for interaction meriting further exploration. Therefore, we stratified our analyses by treatment assignment. As demonstrated in Figure 1, Kaplan-Meier survival curves for the composite end point (death or first heart failure hospitalization) were not significantly different between the corin variant and nonvariant groups in the overall analysis. However, within the group randomized to placebo, survival free from the composite end point was significantly worse in carriers of the corin I555(P568) allele as compared with noncarriers (P=0.029). There was no significant difference in survival between carriers and noncarriers of the I555(P568) allele in the group randomized to treatment with fixed-dose combination isosorbide-dinitrate/hydralazine (FDC I/H).

In multivariable analysis (Table 3), we conducted 2 models, differing only in the exclusion or inclusion of log-transformed BNP. The other covariates that we included in our multivariable models included sex, ejection fraction, systolic blood pressure, age, ischemic etiology, diabetes mellitus, and β-blocker use. In the placebo arm, with multivariable analysis adjusting for all covariates except Ln(BNP), the corin I555(P568) minor allele remained significantly associated with an increased risk for the composite end point of death or heart failure hospitalization (relative risk 2.33; 95% CI, 1.07 to 5.05; P=0.03). Multivariable analysis including log-transformed BNP demonstrated that participants heterozygous for the corin I555(P568) minor allele were at a significantly increased risk for the composite end point (relative risk 3.49; 95 CI, 1.45 to 8.39; P=0.005). However, in the group of participants randomized to treatment with FDC H/I (BiDil) the corin I555(P568) allele was not associated with adverse outcomes in multivariable analysis regardless of the inclusion or exclusion of BNP levels.

Association of Corin I555(P568) Allele With Baseline BNP Values

The absolute and log-transformed baseline plasma measures of both BNP and pro-BNP were lower in the corin variant compared with nonvariant groups (Table 4). When we calculated the Pearson correlation coefficient, the corin allele was significantly associated with lower BNP values in univariate analysis (P=0.007) and multivariable analysis that adjusted for age, gender, body mass index, ischemic etiology, and use of β-blockers. Likewise, when calculating the Spearman correlation coefficient, the corin I555(P568) allele was associated with adverse outcomes in multivariable analysis regardless of the inclusion or exclusion of BNP levels.
Ratio of Pro-BNP to BNP Is Increased in Carriers of the Corin I555(P568) Allele

As an index of impaired natriuretic peptide processing, we compared the ratio of pro-BNP to BNP-32 using the values measured from plasma samples obtained at the baseline visit in carriers versus noncarriers of the corin I555(P568) allele. We reasoned that a higher ratio would be indicative of greater impairment in BNP processing. The mean pro-BNP/BNP-32 ratio in the corin variant group was 1.92 compared with 1.41 in the nonvariant group (37% higher). The median pro-BNP/BNP ratio in the corin variant group was 1.64 compared with 1.21 in the nonvariant group (36% increase) (Figure 2). The pro-BNP/BNP-32 ratios were not normally distributed, so we used nonparametric testing to compare the ratio between corin genotype groups. The pro-BNP/BNP-32 ratio was significantly higher in the corin I555(P568) group (Van der Waerden test \( P = 0.045 \)) as compared with corin wild-type group.

Discussion

We examined the association of the corin I555(P568) allele with clinically relevant outcomes in blacks with moderate-to-severe systolic heart failure and identified a pharmacogenomic interaction between the presence of the corin I555(P568) allele and the administration of FDC I/H on the risk for death or heart failure hospitalization. We report that blacks with systolic heart failure heterozygous for the corin I555(P568) allele who are not on FDC I/H but are receiving standard neurohormonal blockade of the renin-angiotensin-aldosterone and sympathetic nervous systems with angiotensin-converting enzyme-I/angiotensin-receptor blockers, aldosterone, and \( \beta \)-adrenergic receptor blockers are at significantly increased risk of heart failure progression as evidenced by a significant increased risk for the composite end point of death or heart failure hospitalization. This survival difference for the composite end point was largely driven by an increased risk for heart failure hospitalization. In contrast, in the arm randomized to treatment with FDC I/H (BiDil), no increase in death or hospitalization for heart failure was observed in carriers of the corin I555(P568) allele, as compared with noncarriers.

Moreover, using an immunoassay for processed BNP\(_{77} \) to BNP\(_{108} \) with a known 20% cross-reactivity to pro-BNP and a highly specific novel immunoassay for unprocessed BNP\(_{1} \) to BNP\(_{32} \), we report in the black carriers of the corin I555(P568) allele a higher pro-BNP/BNP ratio consistent with greater impairment in BNP processing. There is no reason to suspect that
the magnitude of cross-reactivity (≈19%) between the Biosite Triage immunoassay and BNP1 to 108 would differ between the corin variant as compared with the corin nonvariant groups. Therefore, a comparison of the BNP1 to 108/BNP77 to 108 ratio represented a reasonable method of estimating differences in BNP processing efficiency between corin genotypes. However, the pro-BNP1 to 108/BNP-32 ratio serves as an approximate estimate of impaired processing, and future studies are needed to confirm our finding of an increased ratio of unprocessed to processed BNP associated with the corin I555(P568) allele. Interestingly, despite the increased risk for death or first hospitalization in the corin I555(P568) variant group, the BNP-32 (Biosite) levels were lower in this group, a paradoxical finding given the prognostic importance of an increased BNP in heart failure. In fact, adjusting for the BNP levels increased the risk ratio for the composite end point in the corin variant group receiving placebo, which is consistent with our hypothesis that impaired processing of brain (and potentially other natriuretic peptides) may be the mechanistic link between the presence of the corin I555(P568) allele and disease progression in congestive heart failure.

The T555I and Q568P amino acid changes in corin are located in conserved amino acids within corin’s second cysteine-rich, frizzled domain.4 This domain is involved in protein-protein interactions, and previous work had demonstrated this domain to be required for corin catalytic activity.22 Recently, in vitro studies using both human embryonic kidney 293 cells and murine HL-1 cardiomyocytes have shown that the corin variant gene product containing both the I555/P568 amino acid substitutions had a reduced activity for processing pro-ANP (38% to 7%, P < 0.01) and pro-BNP (44% to 15%, Table 4. Baseline Ln(BNP) and Ln(ProBNP) Values in Corin Variant (I555/P568) Compared With Nonvariant Groups

<table>
<thead>
<tr>
<th>Corin</th>
<th>BNP, pg/mL</th>
<th>ProBNP</th>
<th>Ln(BNP), pg/mL</th>
<th>Ln(ProBNP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corin++/++</td>
<td>339.8±429.6</td>
<td>505.1±773.7</td>
<td>5.04±1.44</td>
<td>5.22±1.63</td>
</tr>
<tr>
<td>Corin+/-</td>
<td>190.4±249.7</td>
<td>236.1±403.0</td>
<td>4.37±1.50</td>
<td>4.29±1.59</td>
</tr>
</tbody>
</table>

BNP and proBNP measures were taken using Biosite and Bio-Rad immunoassays, respectively. Corin++/++ indicates wild type; Corin+/-, heterozygous for corin I555(P568) allele.

Table 3. Impact of Corin Variant on Event-Free Survival (Death or First Hospitalization for Heart Failure) in GRAHF (Substudy of A-HeFT)

<table>
<thead>
<tr>
<th>Group</th>
<th>Corin++/++, n (%)</th>
<th>Corin+/-, n (%)</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td>1.499</td>
<td>0.816 to 2.752</td>
<td>0.192</td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td>1.644</td>
<td>0.851 to 3.177</td>
<td>0.139</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td>1.475</td>
<td>0.791 to 2.751</td>
<td>0.222</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td>2.266</td>
<td>1.068 to 4.806</td>
<td>0.033</td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td>3.485</td>
<td>1.447 to 8.394</td>
<td>0.0054</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td>2.326</td>
<td>1.072 to 5.045</td>
<td>0.033</td>
</tr>
<tr>
<td>Bidil</td>
<td></td>
<td></td>
<td>0.842</td>
<td>0.293 to 2.420</td>
<td>0.749</td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td>0.698</td>
<td>0.226 to 2.159</td>
<td>0.532</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td>0.639</td>
<td>0.211 to 1.931</td>
<td>0.427</td>
</tr>
<tr>
<td>Adjusted w/o Ln(BNP)</td>
<td></td>
<td></td>
<td>1.39 (85%)</td>
<td>24 (15%)</td>
<td>0.639</td>
</tr>
</tbody>
</table>

Adjusted analysis included the following covariates: sex, ejection fraction, systolic blood pressure, age, Ln(BNP), ischemic cause (yes or no), diabetes mellitus, β-blocker use. Corin++/++ indicates wild type; Corin+/-, heterozygous for corin I555(P568) allele.
Compared with that of wild type.5 This study also demonstrated that the mechanism explaining the reduced natriuretic processing capacity of the mutant I555(P568) corin to be impaired mutant corin zymogen activation, not a reduction in corin catalytic capacity per se. Interestingly, the presence of each mutation individually did not significantly reduce the biological activity of corin. This is intriguing because within the black populations we have studied, the single nucleotide polymorphisms causing the T555I and Q568P amino acid changes are in complete linkage disequilibrium so that both amino acid changes are indeed transcribed into the same corin molecule in subjects that are heterozygous for the corin I555(P568) allele.

It is also important to recognize that although the magnitude of impaired BNP processing was greater in the corin variant group, impaired BNP processing is evident in noncarrier participants with moderate-to-severe congestive heart failure. Several recent reports have demonstrated that the presence of immature natriuretic peptide precursors is common in patients with congestive heart failure.14,23,24 However, the molecular mechanisms underlying this phenomenon remain undetermined. The initial report describing the highly specific immunoassay for pro-BNP used in our study identified increasing levels of BNP1 to 108 in patients with congestive heart failure and a correlation with New York Heart Association functional class.14 Furthermore, this study and an early investigation of circulating BNP forms in humans report substantial interindividual variability in the relative proportions of the various forms of natriuretic peptides.14,25 Whether processing efficiency of natriuretic peptides or some other physiological mechanism can account for differences in the relative proportions of natriuretic peptides remains to be determined. We suspect that genetic determinants, such as the corin I555(P568) allele and others yet to be discovered, may explain some of the variability in the processing of natriuretic peptides.

There is a plausible biological hypothesis to account for the observed pharmacogenomic interaction in this study. The natriuretic peptides stimulate cGMP production by activating the particulate guanylate cyclase (pGC) domain, which is a domain contained within the intracellular region of the type A natriuretic peptide receptor. FDC I/H (BiDil) increases intracellular cGMP by activation of soluble GC (sGC). Some investigators report that these 2 cGMP pools are compartmentalized and activate distinct signaling cascades.26 On the other hand, there is also evidence for significant reciprocal regulation of the nitric oxide (NO)-sGC and the ANP/BNP-pGC cGMP pools in both type A natriuretic peptide receptor27 and endothelial NO synthase −/− knockout models implicating cross-talk between the NO-sGC and the NP-pGC pathways in the regulation of cGMP-dependent vasodilatation and pressure overload induced cardiomyocyte hypertrophy. Assuming that the corin I555(P568) allele reduces BNP processing, especially under conditions of decompensation when BNP transcription substantially increases, this would phenotypically be expected to have the same effect as any of the genetic models that perturbed type A natriuretic peptide receptor function; namely, an enhanced response to exogenously administered NO donors. Indeed, although the sample size was small, our data suggest an enhanced treatment effect the group of I555(P568) carriers—the corin variant group randomized to FDC I/H (BiDil) demonstrated 64% improvement in event-free survival (HR = 0.36, P = 0.078), as compared with the reported 37% increase in event free
survival (HR = 0.67, P < 0.0001) in the overall cohort of 1050 A-HeFT patients who were randomized to FDC I/H as compared with placebo.18

We recognize the limitations of this analysis. The sample size carrying the corin I555(P568) allele is small, and we have not replicated these results. We recognize that replication is essential to avoid type I error in genetic association studies. Unfortunately, there are limited clinical trials in heart failure with an adequate black sample to permit replication. Moreover, the design of A-HeFT was unique and, therefore, it may not be possible to replicate the pharmacogenomic interaction observed. Another concern is the potential for confounding from population stratification. We did not genotype random ancestry-informative markers to allow us to adjust for confounding from population stratification. However, the previous work related to the corin I555(P568) allele is reassuring in this regard. First, in our previous studies of the corin I555(P568) allele with risk for hypertension and concentric cardiac remodeling, adjustment for population stratification using >1000 ancestry-informative markers did not reduce the association of the corin I555(P568) allele with our phenotypes of interest.4 Second, recent experiments have convincingly demonstrated the functional effects of the 2 amino acid substitutions on corin; specifically, an ~70% reduction in the ability of mutant corin (I555/P568) to process either pro-ANP or pro-BNP. Third, we are reassured by the biomarker data that support the hypothesis of impaired BNP processing in the patients heterozygous for the corin I555(P568) allele. Considered together, these points argue against the results of this study being explained by confounding from population stratification.

There are intriguing clinical implications from our study if it can be confirmed in future analyses. The pharmacogenomic interaction suggest that treatment with FDC I/H may attenuate the adverse prognosis associated with the corin I555(P568) allele in the setting of moderate-to-severe heart failure. However, many would argue that based on the A-HeFT results from the main trial, there is sufficient rationale to treat all blacks fitting the enrollment criteria with BiDiL. However, if this pharmacogenomic interaction can be confirmed and further elucidated, it suggests the possibility of tailored treatment of blacks heterozygous for the corin I555(P568) allele with American Heart Association/American College of Cardiology Stage A or B heart failure. In our previous work, untreated and hypertensive blacks heterozygous for the corin I555(P568) allele were at increased risk for concentric cardiac remodeling.3 Perhaps, restoration of cGMP pools with phosphodiesterase inhibitors or FDC I/H would be of particular benefit in ameliorating adverse hypertensive cardiac concentric remodeling in these patients. Another potential clinical implication relates to the observation of the decoupling of plasma BNP levels and prognosis in blacks heterozygous for the corin I555(P568) allele, found in ~13% of blacks at the population level. In the overall A-HeFT cohort, plasma BNP remained a powerful prognostic indicator (data not shown), but in the placebo arm, the corin I555(P568) allele carriers demonstrated an increased risk of heart failure hospitalization, despite having a lower baseline BNP as compared with noncarriers of the corin allele. If our hypothesis is correct, a low Biosite BNP value in these patients may reflect impaired natriuretic peptide processing and increased risk for heart failure progression, rather than clinical stability and decreased risk for adverse outcomes. Our study also suggests the possibility that simultaneous measurement of BNP using an assay relatively specific for BNP-32 coupled with simultaneous ascertainment of BNP, to 108 in blacks with systolic heart failure may improve risk stratification and the capacity of natriuretic peptides to prognosticate in patients with chronic ambulatory congestive heart failure.

In conclusion, we report that in blacks with moderate-to-severe systolic heart failure, the functional corin I555(P568) allele is associated with an increased risk for heart failure progression in the absence of treatment with FDC I/H. Furthermore, we have identified increased impairment of BNP processing with lower plasma BNP in the corin I555(P568) carriers and hypothesize that this pharmacogenomic interaction might be explained by an amelioration of the adverse prognostic impact of the corin variant allele with FDC I/H by virtue of cross-talk between the soluble and pGc cGMP signaling systems. These results are hypothesis generating and require additional confirmation in independent cohorts and animal models. Future studies should focus on further determination of the mechanism and clinical significance of this pharmacogenomic interaction in the management of moderate-to-severe heart failure and an understanding of the physiological basis of the relative proportions of the various forms of natriuretic peptides in human heart failure.

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Disclosures

References


**CLINICAL PERSPECTIVE**

The endogenous natriuretic peptide system, characterized by the ability of the heart to secrete atrial and brain natriuretic peptide (BNP) into the circulation on increased cardiac filling pressures, exerts essential compensatory actions in heart failure. Activation of the natriuretic peptide system helps restore volume status and antagonizes adverse neurohormonal systems. To function properly, pro–atrial natriuretic peptide and pro-BNP must first be cleaved into biologically active peptide hormones by corin, a transmembrane protease expressed by cardiomycocytes. The corin I555(P568) allele results from 2 amino acid-changing single nucleotide polymorphisms (T555I and Q568P) that are in complete linkage disequilibrium in blacks. These 2 mutations in vitro impair the ability of corin to process pro–atrial natriuretic peptide/BNP efficiently into biologically active peptides. Blacks carrying this minor corin allele are at increased risk for heart failure hospitalization in the absence of treatment with BiDil. They also demonstrate evidence of less efficient BNP processing. Inefficient processing of natriuretic peptides would reduce activation of the natriuretic peptide receptor and thereby reduce generation of cyclic GMP by the particulate guanylate cyclase moiety of the natriuretic peptide receptor. We hypothesize that by augmenting intracellular cGMP pools independently of the NPR-A receptor, BiDil may ameliorate the adverse prognosis caused by impaired proBNP processing in the presence of the corin I555(P568) allele in blacks with moderate-to-severe systolic heart failure.
Dysfunctional Corin I555(P568) Allele Is Associated With Impaired Brain Natriuretic Peptide Processing and Adverse Outcomes in Blacks With Systolic Heart Failure: Results From the Genetic Risk Assessment in Heart Failure Substudy

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