Influence of ScaI and Natriuretic Peptide (NP) Clearance Receptor Polymorphisms of the NP System on NP Concentration in Chronic Heart Failure

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Background: Genetic variants related to the natriuretic peptide (NP) system [ScaI mutated allele (A1) of the atrial NP (ANP) gene and the C variant of the natriuretic peptide clearance receptor (NPRC) gene] have been identified as independent risk factors for cardiovascular morbidity and mortality. Despite the importance of NPs in heart failure (HF), the role of these polymorphisms in HF has not been evaluated.

Methods: We screened 124 HF patients [mean (SD), age 66 (12) years, 100 men, ejection fraction 32% (10%), New York Heart Association (NYHA) class I–II 65, III–IV 59] for NP concentrations [ANP, brain NP (BNP) and amino-terminal pro-BNP (NT-proBNP)] and for the ScaI and NPRC variants.

Results: ScaI polymorphism had no effect on NP concentration in the NYHA I–II subgroup. Conversely, in severe HF, A1 carriers had higher ANP (P <0.05), BNP (P <0.01), and NT-proBNP (P <0.01) than A2A2 patients. After multivariate adjustment, A1 presence remained an independent predictor for increased NP. Regarding NPRC polymorphism in mild HF, higher ANP (P<0.05) and BNP (P <0.05) were observed in CC than A allele carriers. After multivariate adjustment, however, this association did not remain significant. In severe HF, the NPRC polymorphism had no effect on NP.

Conclusions: The ScaI polymorphism of the ANP gene might be an important additive genetic factor influencing neurohormonal activation and disease progression in severe HF. The NPRC polymorphism is not an independent determinant of NP concentration in HF.

Heart failure (HF) pathophysiology and clinical evolution are greatly influenced by neurohormonal activation of the adrenergic and renin-angiotensin-aldosterone systems (1). Cardiac endocrine function is widely recognized to play essential roles in maintaining salt-water homeostasis, regulating hemodynamics, and counteracting the remodeling process at the cardiac and vascular level (2). The production and secretion of A- and B-type natriuretic peptides (NPs) reflect the severity of the disease, and their plasma concentrations have diagnostic and prognostic value (3). Recent evidence indicates that some genetic variants related to the NP system [ScaI polymorphism of the atrial NP (ANP)4 gene, transition T2238C (formerly A1)] and the natriuretic peptide clearance receptor (NPRC) gene polymorphism C(−55)A might be independent risk factors for hypertension, cerebrovascular events, and myocardial infarction (4–7). Surprisingly, however, in view of the importance of NPs in the management of patients with HF (8–10), the possible role of these polymorphisms as alternative and/or additive markers in HF disease has still not been evaluated. In the present study we investi-
gated the effect of these 2 genetic variants of the NP system on concentrations of NPs [ANP, brain NP (BNP), amino-terminal pro-BNP (NT-proBNP)] in patients with chronic HF.

Materials and Methods

Study Participants
We recruited patients with a diagnosis of HF according to criteria described (11). We enrolled 124 consecutive HF patients admitted to the department of cardiovascular medicine of our institution between October 2003 and November 2005, all with echocardiographic evidence of impaired left ventricular systolic function [ejection fraction (EF) <50%] (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue11). HF severity was evaluated according to the New York Heart Association (NYHA) classification. Exclusion criteria were myocardial infarction or a history of unstable angina within 6 months before the examination and severe renal disease. All patients were on stable (>1 month) optimal pharmacologic treatment (see Table 1 in the online Data Supplement).

Blood Sample Collection and Biochemical Assays
Blood was collected between 8 and 9 AM, after study participants had fasted and rested in a supine position for 20 min. Blood samples (10 mL) were put into ice-chilled polypropylene tubes containing EDTA (1 g/L of plasma) and aprotinin (500 IU/L blood, respectively) for NPs. Plasma was rapidly separated by centrifugation (1500g) for 15 min at 4 °C and stored at −20 °C until analysis. We measured creatinine by use of standard laboratory methods from plasma samples and ANP and BNP by use of 2-site IRMAs (Shionogi), as described in detail (12). We measured NT-proBNP concentration by use of an automated electrochemiluminescent immunoassay (Roche Diagnostics) (11). The total imprecision (CV%), tested by repeated measurement of selected plasma samples with different hormone concentrations, was 11.4% (mean 22.6 ng/L, n = 16) and 10.7% (mean 25.6 ng/L, n = 12) for ANP, 11.0% (mean 5.10 ng/L, n = 10) and 9.0% (mean 58.7 ng/L, n = 10) for BNP, and 4.0% (mean 103.8 ng/L, n = 20) and 3.8% (mean 601.7 ng/L, n = 20) for NT-proBNP.

Gene Polymorphisms
We isolated leukocytes from peripheral blood and extracted DNA according to standard techniques (13). The ScaI and NPRC genotypes were determined as described (5, 7). In brief, for the analysis of ScaI polymorphism, we performed a PCR amplification (GeneAmp PCR System 2400, Applied Biosystems) with the primers 5′-GGG AAG AAG CAG GTG GTC AGT ACT CAA GTT CAG AGG ATG GGC-3′ and 5′-CAC AAC TCC ATG GCA ACA AGA TGA CAC AAA TGC-3′ (7). In a reaction mix of 50 μL, 1 μg DNA was amplified in presence of 200 μmol/L deoxyribonucleoside triphosphates, 50 pmol of each primer, 1.5 mmol/L MgCl2, and 1.25 units thermostable Taq DNA polymerase. After pretreatment at 96 °C for 120 s, 62 °C for 120 s, and 72 °C for 120 s, 35 cycles of denaturation (94 °C, 45 s) annealing (58 °C, 30 s), and extension (72 °C, 30 s) were carried out. After that, a digestion with the ScaI restriction enzyme was performed, and the products of the digestion process were highlighted by electrophoresis on a 3% agarose gel and stained with ethidium bromide. The studied transition leads to the loss of one of these restriction sites, generating 3 fragments corresponding to sizes 117, 96, and 2 bp for the A2 allele, vs the 2 fragments of 213 and 21 bp observed for the A1 allele. Moreover, the loss of the site leads to extension of the ANP by 2 additional arginine residues, so the original peptide is extended from 28 to 30 amino acids.

We performed PCRs for amplification of the NPRC genotype by use of described conditions and primers (5). A single, 2-allele polymorphism was detected by digestion with Hgal restriction enzyme, and fragments obtained after the digestion process were size-separated by gel electrophoresis on a 3% agarose gel; detection was evidenced with ethidium bromide. In the presence of the polymorphic site, 3 fragments corresponding to sizes 177, 105, and 88 bp were generated; in the absence of the site, 2 fragments of 265 and 105 bp were observed.

Statistical Analysis
Assuming an allele frequency of 20% for NPRC and ScaI variants (14–16), we calculated that a sample size of 120 patients was needed for detecting a significant 40% increase of NP with a minimum power of 80% (α = 0.05) in carriers compared with patients with wild-type genotypes.

Because neurohormonal values are not gaussian distributed, natural logarithmic transformation of data was used for statistical analysis. The results are expressed as mean (SE), unless otherwise stated. We used χ2 and unpaired t tests to evaluate differences between 2 groups. Differences between independent groups were analyzed by ANOVA followed by Scheffe post hoc test. We analyzed continuous relationships between variables by univariate regression analysis and then performed multiple regression analysis to identify the variables that independently predicted the relationships. P <0.05 was considered significant.

Results
Patient demographic and clinical features associated with concentrations of NP are reported in Table 1. When the ScaI polymorphism was considered, a trend toward higher BNP and NT-proBNP concentrations was observed in the A1 carriers. Moreover, in patients carrying the A variant of the NPRC gene, ANP and BNP values were lower than those found in CC patients (Table 1). As expected, aging, EF, creatinine, and NYHA class were significantly associated with NP concentration, and multivariate analysis revealed age and EF as significant
NPRC polymorphism

ScaI

NYHA class

Sex

Creatinine, mmol/L

r

EF, %

Table 1. Demographic, clinical, and genetic parameters according to NP concentrations.

<table>
<thead>
<tr>
<th></th>
<th>ANP</th>
<th>P</th>
<th>BNP</th>
<th>P</th>
<th>NT-proBNP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>r = 0.38</td>
<td>&lt;0.001</td>
<td>r = 0.43</td>
<td>&lt;0.001</td>
<td>r = 0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF, %</td>
<td>r = −0.37</td>
<td>&lt;0.001</td>
<td>r = −0.51</td>
<td>&lt;0.001</td>
<td>r = −0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>r = 0.23</td>
<td>&lt;0.01</td>
<td>r = 0.32</td>
<td>&lt;0.001</td>
<td>r = 0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Female</td>
<td>136.0 (23.7)</td>
<td>0.8</td>
<td>255.8 (66.6)</td>
<td>0.4</td>
<td>2399.0 (268.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>Male</td>
<td>145.7 (13.6)</td>
<td></td>
<td>312.0 (40.2)</td>
<td></td>
<td>2923.2 (460)</td>
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<tr>
<td>NYHA class</td>
<td></td>
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<tr>
<td>I−II</td>
<td>113.6 (13.6)</td>
<td>0.0027</td>
<td>164.0 (19.9)</td>
<td>0.0011</td>
<td>1699.2 (300.8)</td>
<td>0.0069</td>
</tr>
<tr>
<td>III−IV</td>
<td>177.1 (19.2)</td>
<td></td>
<td>440.1 (65.3)</td>
<td></td>
<td>4058.7 (844.5)</td>
<td></td>
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<tr>
<td>ScaI polymorphism genotypes</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>A1 allele</td>
<td>156 (18)</td>
<td>0.24</td>
<td>369 (72)</td>
<td>0.07</td>
<td>3587 (955)</td>
<td>0.06</td>
</tr>
<tr>
<td>A2A2</td>
<td>138 (15)</td>
<td></td>
<td>261 (38)</td>
<td></td>
<td>2471 (474)</td>
<td></td>
</tr>
<tr>
<td>NPRC polymorphism genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CC</td>
<td>151 (13)</td>
<td>0.04</td>
<td>321 (42)</td>
<td>0.05</td>
<td>2800 (479)</td>
<td>0.16</td>
</tr>
<tr>
<td>A allele</td>
<td>126 (24)</td>
<td></td>
<td>234 (64)</td>
<td></td>
<td>2873 (977)</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as mean (SE), unless otherwise stated. NP concentrations in ng/L. r, regression coefficient.

determinants of ANP concentrations, whereas age, EF, and creatinine were independent determinants of BNP and NT-proBNP.

The effect of ScaI genotypes on NP concentration was then assessed separately in patients with mild (NYHA class I–II) and severe (III–IV) clinical forms of HF. ScaI polymorphism had no effect on NP in the NYHA class I–II patients (see Table 2 in the online Data Supplement). In patients with NYHA class III–IV, those carrying the A1 variant (ScaI gene) had significantly higher concentrations of ANP (P <0.05), BNP (P <0.01), and NT-proBNP (P <0.01; Fig. 1). After adjusting for age, creatinine, and EF at the multivariate analysis, the presence of the A1 variant remained an independent predictor for increased BNP (standard coefficient 0.245, P <0.05) and NT-proBNP (standard coefficient 0.24, P <0.05) in this subgroup. In mild HF, CC homozygote patients (NPRC gene) showed significantly higher concentrations of ANP (P <0.05) and BNP (P <0.05) than those carrying the A allele (Fig. 2). However, after adjustment for age, creatinine, and EF at the multivariate analysis, the NPRC genotype did not independently affect NP concentration. Carrying NPRC different alleles did not affect NP circulating concentrations in patients with severe HF (see Table 2 in the online Data Supplement).

Discussion

ANP is a cardiac hormone that plays an important role in a variety of clinical conditions associated with an increase in cardiac pressure and/or volume overload (3). Thus, the ANP gene has long been on the list of candidate genes for susceptibility to cardiovascular diseases (additionally, some polymorphisms have been identified at this locus). More studied is the transition T2238→C in the ANP precursor gene, which has been found to be associated with higher circulating concentrations of ANP in salt-sensitive essential hypertension, with nonfatal myocardial infarction, extent of coronary artery disease and stroke, and left ventricular mass in essential hypertension, although no data are available regarding its role in the HF disease (7, 14). Moreover, results of these association studies are often controversial, essentially because of differences in the heterogeneity of study designs, endpoints considered, and data computing and analysis (7, 14, 15, 17–22). The functional significance of this polymorphism is even more unclear. In fact, although it is necessary to establish whether a plausible biological explanation exists for the observed statistical relationship between gene variants and pathological phenotypes, generally ANP levels are not measured. To overcome this limitation in the present study, we measured concentrations of not only ANP but also BNP and NT-proBNP, which are also increased in HF (10). Thus, although the relatively low number of study participants may represent a limitation, the importance of our results is reinforced by strict criteria used to select our population, homogeneity of pharmacologic treatment, and strengthened by the clinical phenotype and circulating NP correlates.

Results show a trend toward higher concentrations of all NP related to A1 variant in the overall population. However, it is well known that HF, a complex disease, is the product of complex interplay between genetic susceptibility and other causative determinants. Moreover, different hemodynamics, renal clearance, and neurohormonal patterns characterize different disease stages, markedly deregulated in severe chronic HF forms and more preserved in less severe stages of this condition. Accordingly, circulating concentrations of NP are markedly increased in NYHA III–IV patients compared with those at less severe stages (Table 1). As concerns the ScaI polymorphism, when patients were divided according to their symptom severity, results indicated that the A1 variant seems independently related to NP concentration.
only in the subgroup including NYHA III–IV patients. It is possible that the relative importance of this genetic factor varies over the course of the disease, having a role when a marked cardiac dysfunction and NP overproduction is established, with a limited influence in milder disease stages. It is otherwise plausible that the presence of the genetic variant might represent an additive factor influencing disease progression and prognosis in severe HF, although our results need further confirmation.

The NPRC polymorphism is located in a region that contains elements that bind nuclear factors and contains an additional transcription starting point that may be functional in specific cell types (23). Consequently, the presence of one or the other NPRC allele might influence cell type–specific NPRC gene expression. Only a few studies, all from the same research group and none conducted in HF patients (5, 16, 24), have evaluated this polymorphism in association studies. Our results indi-
cated that patients carrying the A allele had significantly lower ANP and BNP concentrations than noncarriers in the NYHA I–II subgroup. This observation suggests that NPRC polymorphism might represent an additional contributing factor for NP concentrations in mild-to-moderate HF. Conversely, the lack of association of NP concentrations with NPRC genotypes in NYHA III–IV patients might imply that other mechanisms have a major role in determining neurohormonal abnormalities in patients with more severe disease. However, after the multivariate adjustment in the NYHA I–II subgroup, the association between NPRC polymorphism and NP did not remain statistically significant, revealing a confounding effect of other determinants in the crude analysis.

The main value of this study is that it represents the 1st attempt to evaluate the effect of 2 genetic variants related to the NP system, one pertaining to the ANP and the other to the NPRC gene, on NP concentrations in patients with HF. ScI polymorphism appears to be independently related to NP concentrations in the NYHA III–IV subgroup. Conversely, the NPRC polymorphism did not result in a significant independent risk determinant that affects NP concentrations in HF patients. These observations encourage additional studies in this area, which may add insights into how genes related to the NP system fit into the complex network of factors that determine susceptibility and course of disease in HF.

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References


