Plasma Soluble Corin in Patients With Heart Failure

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Background—Corin is a transmembrane protease that processes natriuretic peptides in the heart. Like many membrane proteins, corin is shed from the cell surface.

Methods and Results—In this study, we obtained plasma samples from healthy controls and patients with heart failure (HF) and acute myocardial infarction. Soluble corin levels in plasma were measured by an ELISA method. In healthy adults (n=198), plasma corin levels were 690 pg/mL (SD, 260 pg/mL). The corin levels did not differ significantly among different age groups. In patients with HF (n=291), plasma corin levels were significantly lower compared with that of healthy controls (365 pg/mL [SD, 259]; P<0.001). The reduction in plasma corin levels seemed to correlate with the severity of HF. In patients of New York Heart Association classes II, III, and IV, plasma corin levels were 450 pg/mL (SD, 281 pg/mL; n=69), 377 pg/mL (SD, 270 pg/mL; n=132), and 282 pg/mL (SD, 194 pg/mL; n=90), respectively (P<0.001 class II vs class IV; P<0.05 class III vs class IV). In contrast, plasma corin levels in patients with acute myocardial infarction (n=73) were similar to that of healthy controls (678 pg/mL [SD, 285 pg/mL]; P>0.05).

Conclusions—Soluble corin was detected in human plasma. Plasma corin levels were reduced significantly in patients with HF but not in those with acute myocardial infarction. Our results indicate that corin deficiency may contribute to the pathogenesis of HF and that plasma corin may be used as a biomarker in the diagnosis of HF. (Circ Heart Fail. 2010;3:207-211.)

Key Words: corin • natriuretic peptides • heart failure • biomarker • diagnosis • hypertension

Heart failure (HF) is a major disease that affects ∼5.7 million Americans.1 The disease has a high mortality, and its annual costs exceed $37 billion in the United States.7 The mechanisms underlying HF are complex, involving a variety of structural and biological alterations that directly or indirectly impair cardiac function.9 When HF progresses to an end stage, medical options are limited. Thus, timely diagnosis and early intervention are important for managing this life-threatening disease.

Clinical Perspective on p 211

Corin is a transmembrane protease that regulates blood pressure and cardiac function.5,6 The enzyme is expressed primarily in cardiomyocytes, where it converts inactive proatrial natriuretic peptide and probrain natriuretic peptide to active peptides. Under high blood pressure or volume overload, the production of the natriuretic peptides in the heart is increased to promote natriuresis, diuresis, and vasodilation.7–9 In HF, these natriuretic peptide–mediated actions serve as an important compensatory mechanism to lower blood volume/pressure and improve cardiac function. In mice, corin deficiency leads to hypertension, cardiac hypertrophy, and impaired cardiac function.10 In humans, corin gene variants have been associated with an increased risk for hypertension and cardiac hypertrophy in blacks,11,12 a population known for their high prevalence of cardiovascular disease.13,14

Many membrane proteins are shed from the cell surface in a regulated manner and can be detected in plasma.15,16 Topologically, corin belongs to the type II transmembrane serine protease family.17,18 Corin has a cytoplasmic tail and a single-span transmembrane domain near the N-terminus. In its extracellular region, there are 2 frizzled-like domains, 8 low-density lipoprotein receptor repeats, 1 scavenger receptor–like domain, and a trypsin-like protease domain.6,19 The transmembrane domain anchors corin to the cell surface but is not required for its catalytic activity.20,21 Within the family of type II transmembrane serine proteases, several members such as enteropeptidase,22,23 hepsin,24 and matriptases25,26 were shown to be shed from the cell surface. We also detected soluble corin in cell culture medium, indicating that corin is shed from the cells (authors’ unpublished data). We hypothesized, therefore, that shed corin may enter into the blood circulation and that plasma corin levels may reflect cellular homeostasis within the heart. To test this hypothesis, we measured plasma corin in healthy controls and patients with heart disease.
Methods

Study Population
This study was approved by the local ethics committees, and participants gave informed consent. A total of 291 patients with HF from 3 hospitals in Jiangsu Province, China, were included. These patients were hospitalized for symptoms of HF such as fatigue, shortness of breath, and edema at rest or with exercise (New York Heart Association [NYHA] functional classes II, III, or IV). Some patients were previously diagnosed with HF and rehospitalized for acute decompensation. The mean value of ejection fraction in these patients with HF was 51.9% (SD 16.9%). Patients with chronic obstructive lung disease, congenital heart disease, and cancer were excluded. In addition, 73 patients with acute myocardial infarction (AMI) and 198 healthy subjects, who underwent routine medical check-ups at the hospitals and had no medical history of cardiovascular disease, were also included. All participants were ethnic Han Chinese, which is the predominant population in that region of China.

Clinical Diagnosis
To confirm the diagnosis of HF, paper or electronic medical records were reviewed to obtain information on medical history, clinical examination, ECG, echocardiography, chest X-ray, and other laboratory tests of the patients. Cardiac arrhythmia was confirmed by an ECG or 24-hour Holter monitoring. Valvular heart disease was confirmed by echocardiography. As part of routine practice, all patients underwent evaluation for HF diagnosis and determination of disease severity by clinical history and laboratory tests including ECG. On the basis of available data, the diagnosis of HF and the underlying pathogenesis were determined by experienced cardiologists who cared for the patients but were blinded to the study. The HF functional class for each patient was assessed by HF specialists on basis of the NYHA classification standards. Most patients with HF were treated with diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and β-blockers, according to the clinical management guidelines.

For AMI patients, the diagnosis was based on at least 2 of the following 3 criteria: (1) a clinical history of characteristic chest pain; (2) serial electrocardiographic tracings indicative of myocardial infarction, such as ST-segment elevation, new left bundle-branch block, ST-segment depression, and T-wave inversion; and (3) characteristic serial electrocardiographic tracings indicative of myocardial infarction, such as ST-segment elevation, new left bundle-branch block, ST-segment depression, and T-wave inversion; and (3) characteristic elevation of serum cardiac enzymes. A troponin test was also performed in some patients for the diagnosis of AMI. Blood samples were collected within 12 hours of the ischemic onset to measure soluble corin.

Measurement of Plasma Soluble Corin
Blood samples were collected into tubes containing EDTA as an anticoagulant. Plasma samples were obtained by centrifugation at 3000g for 10 minutes. Aliquots of samples were prepared, stored at −80°C, and used within 12 weeks. Samples from HF, AMI, and healthy control groups were stored for similar lengths of time. In a pilot study, soluble corin seemed to be stable in plasma samples frozen at −80°C after several cycles of freezing and thawing. The results were consistent with the findings from a recent report.27 We used an ELISA kit (R&D Systems, Minneapolis, Minn) to measure soluble corin levels in plasma. In brief, microtiter plates were coated with an anti-corin antibody. Plasma samples or recombinant human corin protein standards were added and incubated at room temperature for 2 hours. The plates were washed with a buffer, and a biotinylated anti-human corin antibody was added and incubated for 2 hours. After washing, peroxidase-conjugated streptavidin was added and incubated at room temperature for 20 minutes. The reaction was visualized by adding a horseradish peroxidase substrate (3,3′,5,5′-tetramethylbenzidine), and the optical density was monitored with a spectrophotometer at a wavelength of 450 nm.

Statistical Analysis
The analysis was performed by using the MedCalc software (version 10.4.0; Mariakerke, Belgium) and the Statistical Analysis Software (version 9.0; SAS Institute, Cary, NC). Data are presented as mean value (SD). Comparisons of plasma corin levels in healthy controls and patient groups were performed by using ANOVA followed by a Tukey post test. Multiple linear-regression analysis was performed to identify independent predictors for plasma corin levels in patients with HF. Variables in the analysis included sex, age, hypertension, coronary artery disease, left ventricular ejection fraction, cardiomyopathy, cardiac arrhythmia, diabetes, and NYHA classification. A residual analysis of the regression model indicated a nonnormal distribution of corin levels in patients with HF. A square root transformation resulted in normally distributed data. The standard errors as percentages of the coefficients, however, remained essentially the same with or without the transformation. The final results presented were from the analysis of the transformed data. All probabilities were 2-tailed, and probability values <0.05 were considered statistically significant.

Results
By ELISA, we detected corin protein in human plasma. In 198 plasma samples from healthy volunteers, soluble corin levels were 690 pg/mL (SD, 260 pg/mL; Figure 1). The corin level did not seem to change in different age groups. When this control cohort was divided into 3 age groups, 16 to 25 years (n = 40), 26 to 50 years (n = 100), and >50 years (n = 58), the levels of soluble corin in these 3 groups were 619 pg/mL (SD, 251 pg/mL), 676 pg/mL (SD, 286 pg/mL), and 730 pg/mL (SD, 302 pg/mL), respectively. No statistical significant difference was found among these groups (P = 0.17). Plasma corin levels in men (n = 104) seemed to be higher than that in women (n = 94; 798 pg/mL [SD, 285 pg/mL] vs 551 pg/mL [SD, 224 pg/mL]; P < 0.001).

Next, we measured plasma soluble corin in patients with HF. The baseline characteristics of these patients and healthy controls are shown in Table 1. The mean age of the patients with HF was 67.5 years (SD, 13.3 years). There were 170 men (58.4%) and 121 women (41.6%). The results showed that plasma corin levels were significantly lower in this cohort of patients with HF (365 pg/mL [SD, 259 pg/mL]; P < 0.001) compared with that of healthy controls (Figure 1). In patients with HF, corin levels in men (n = 170) seemed to be higher than that of women (n = 121; 387 pg/mL [SD, 272 pg/mL] vs 335 pg/mL [SD, 225 pg/mL]), but the difference was not statistically significant (P = 0.092).

We also measured plasma soluble corin in patients with AMI. The mean age of this group of patients was 64.5 years (SD, 11.6 years). There were 58.9% men and 41.1% women...
was detectable in human plasma, indicating that corin was shed from cells and later entered the circulation. The results suggested that plasma corin may serve as a biomarker to indicate the status of cardiomyocytes and, possibly, pathological conditions in the heart.

To test this hypothesis, we measured plasma corin in patients with HF and AMI. Our results showed that corin levels were significantly lower in patients with HF compared with that of healthy controls. Moreover, the reduction closely correlated with the severity of the disease. In our study, the mean age of the healthy group was younger than that of the HF group. Therefore, the reduction of plasma corin levels associated with the severity of the disease. In our study, the mean age of the healthy group was younger than that of the HF group (41.2 years vs 67.5 years; P<0.001). These results suggest that the reduction of plasma corin levels, as measured under our current experimental conditions, may be related more closely to the pathological changes associated with HF than that of AMI.

In contrast to the reduced levels in patients with HF, plasma corin levels in patients with AMI did not differ significantly from that of the control group. The values were 678 pg/mL (SD, 285 pg/mL; n=73; P<0.001 vs HF; Figure 1). Like in the healthy control group, corin levels in men (n=43) were higher than that in women (n=30) with AMI (799 pg/mL [SD, 272 pg/mL] vs 534 pg/mL [SD, 135 pg/mL]; P<0.001 vs HF; Figure 1). Like in the healthy control group, corin levels were higher in men than in women, but the reason for such an apparent difference between the sexes is unknown. In healthy subjects, interestingly, plasma corin levels seemed to be higher in men than in women, but the reason for such an apparent difference between the sexes is unknown. In healthy subjects, interestingly, plasma corin levels seemed to be higher in men than in women, but the reason for such an apparent difference between the sexes is unknown.

In contrast to the reduced levels in patients with HF, plasma corin levels did not change significantly with age. In healthy subjects, interestingly, plasma corin levels seemed to be higher in men than in women, but the reason for such an apparent difference between the sexes is unknown.

We performed multiple linear-regression analysis to identify variables that may independently predict plasma corin levels (Table 2). The results showed that hypertension (P<0.001) and NYHA classes II (P<0.001 vs class II; P<0.0001 for classes III and IV) were strong independent predictors for low soluble corin levels in patients with HF. Sex also was an independent predictor (P<0.0001). In contrast, age, coronary artery disease, left ventricular ejection fraction, and diabetes did not seem to be independent predictors for plasma corin levels in patients with HF.

**Discussion**

Corin is a cardiac membrane protease that activates pronatriuretic peptides.28 To date, the role of corin in pathological conditions such as HF remains unclear. In this study, we showed that corin was detectable in human plasma, indicating that corin was shed from cells and later entered the circulation. The results suggested that plasma corin may serve as a biomarker to indicate the status of cardiomyocytes and, possibly, pathological conditions in the heart.

In contrast to the reduced levels in patients with HF, plasma corin levels did not differ significantly in patients with AMI and controls, 69 patients with HF of class II, 132 with HF of class III, and 90 with HF of class IV. Each box represents the median and interquartile range values. The outliers that are >1.5 and >3 times the interquartile range are indicated by open and filled circles, respectively. The vertical I bars indicate the nonoutlier minimum and maximum. *P<0.001 versus control; ††P<0.001 versus class II; †††P<0.001 versus class IV; ‡‡P<0.05 versus class IV.

Table 1. Characteristics of Controls and Patients With HF and AMI

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=198)</th>
<th>HF (n=291)</th>
<th>AMI (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>41.2 (18.8)*</td>
<td>67.5 (13.3)†</td>
<td>64.5 (11.6)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>104 (52.5)</td>
<td>170 (58.4)</td>
<td>43 (58.9)</td>
</tr>
<tr>
<td>Female</td>
<td>94 (47.5)</td>
<td>121 (41.6)</td>
<td>30 (41.1)</td>
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<tr>
<td>Medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0 (0)</td>
<td>175 (60.1)</td>
<td>36 (49.3)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0)</td>
<td>65 (23.3)</td>
<td>10 (13.7)</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>0 (0)</td>
<td>42 (14.4)</td>
<td>NA</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>0 (0)</td>
<td>46 (15.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>0 (0)</td>
<td>95 (32.6)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>0 (0)</td>
<td>37 (12.7)</td>
<td>NA</td>
</tr>
<tr>
<td>Others</td>
<td>0 (0)</td>
<td>58 (19.9)</td>
<td>32 (43.8)</td>
</tr>
</tbody>
</table>

*P<0.001, control versus HF or AMI.
†P<0.05, HF versus AMI.
‡Sex distributions were not statistically different among 3 groups.

Table 2. Multiple Linear Regression to Predict Plasma Corin Levels in HF

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex*</td>
<td>−2.62</td>
<td>0.56</td>
<td>−4.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>−0.01</td>
<td>0.02</td>
<td>0.46</td>
<td>0.6471</td>
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<tr>
<td>Hypertension</td>
<td>−2.59</td>
<td>0.79</td>
<td>−3.27</td>
<td>0.0012</td>
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<tr>
<td>Coronary artery disease</td>
<td>0.21</td>
<td>0.85</td>
<td>0.25</td>
<td>0.8021</td>
</tr>
<tr>
<td>LV ejection fraction</td>
<td>0.00</td>
<td>0.02</td>
<td>0.16</td>
<td>0.8744</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.31</td>
<td>0.94</td>
<td>0.33</td>
<td>0.7394</td>
</tr>
<tr>
<td>NYHA class II</td>
<td>−4.00</td>
<td>1.26</td>
<td>−3.17</td>
<td>0.0016</td>
</tr>
<tr>
<td>NYHA class III</td>
<td>−6.64</td>
<td>1.27</td>
<td>−5.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NYHA class IV</td>
<td>−8.65</td>
<td>1.39</td>
<td>−6.22</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*The coding for dummy variables was as follows: sex (male, 0; female, 1), hypertension, coronary artery disease, and diabetes (yes, 1; no, 0). NYHA classes II (DN1, 1; DN2, 0; DN3, 0), III (DN1, 0; DN2, 1; DN3, 0), and IV (DN1, 0; DN2, 0; DN3, 1) with the normal controls being the reference.
closely with pathological changes in HF than in acute ischemic cardiac injury. In our study, the samples from patients with AMI were collected within 12 hours of disease onset. Further studies are needed with samples from more time points to determine whether plasma corin levels vary over a longer course after AMI. Previously, other shed membrane proteins, such as tumor necrosis factor-α and interleukin-1 receptors, also were identified in plasma from patients with HF.29,30 Unlike plasma corin, however, these soluble proteins were increased in both HF and AMI29–32 suggesting that the shedding of these cytokine receptors may be a part of the inflammatory response to heart damage or stress.

Corin is most abundantly expressed in the heart.19,33 In Northern analysis with multiple human tissues, corin mRNA was detected only in the heart.19 By other more sensitive methods, low levels of mouse or rat corin mRNA were detected in other tissues, including scar myofibroblasts, developing kidneys, chondrocytes, lung cancer cells, and certain regions of the brain.19,34–36 Recently, corin mRNA and protein also were detected in mouse skin hair follicles.37 The function of corin in these extracardiac tissues remains to be determined. The low levels of plasma corin observed in patients with HF are likely to reflect either the chronic loss of cardiomyocytes, reduced corin production either in the heart or other tissues, accelerated clearance of plasma corin, and/or downregulation of corin shedding that was associated with failing hearts.

In multiple linear-regression analysis, hypertension and NYHA class were 2 strong independent predictors for low plasma corin levels. The primary corin function is to regulate blood pressure by activating natriuretic peptides, which in turn promote natriuresis, diuresis, and vasodilation. The atrial natriuretic peptide–mediated pathway also has a local antihypertrophic function in the heart, which is independent of its systemic blood pressure–lowering function.38–40 Consistently, knockout mice lacking corin developed hypertension and cardiac hypertrophy.10 A similar cardiac hypertrophy phenotype was reported in a naturally occurring mutant mouse strain, in which the corin gene was disrupted by genetic inversion.41 Corin-knockout mice also had reduced ejection fractions.10 These data indicate that corin is important in maintaining normal blood pressure and cardiac function in vivo.

The human corin gene is on chromosome 4p12-13, which has 22 exons and spans >200 kb in length.42 Population genetic studies identified single-nucleotide polymorphisms in the corin gene, which were present in patients with hypertension and cardiac hypertrophy.11,12 In cell-based studies, these single-nucleotide polymorphisms were found to alter corin protein structure and impair its biological activity.43 The results suggest that corin defects may contribute to hypertension and heart disease in humans. Plasma levels of unprocessed proatrial natriuretic peptide and probrain natriuretic peptide are highly increased in patients with severe HF,44–50 indicating that processing these natriuretic peptides becomes rate-limiting as the disease progresses. It seems, therefore, that low plasma corin levels in patients with HF may reflect the underlying disease mechanism in the heart.

Corin is a newly identified protease that is essential for processing natriuretic peptides in the heart.6,10 We have much to learn about the biology of this new enzyme and its role in cardiovascular disease. At this time, we do not know which enzymes shed corin from cells. It remains to be determined whether and how corin shedding is regulated under physiological and pathological conditions. Our finding of low plasma corin levels in patients with HF suggests that corin may play an important role in the development and/or progression of HF in patients and that plasma corin may be used as a biomarker for HF diagnosis. At this time, our study has its limitations because of its retrospective nature and a relatively small set of patient samples from a single ethnic group. Our data, however, should encourage designing future prospective studies with larger cohorts of patients with acute and chronic HF from different ethnic populations. Additional studies are important to determine whether plasma corin levels are altered in patients with HF over a longer period after acute decompensation and/or after medical treatment. Such studies shall help investigators to understand the role of corin in heart disease and to define the diagnostic and prognostic values of plasma corin for HF.

Acknowledgments
We thank Robert S. Butler for helping with the statistical analysis.

Sources of Funding
This work was supported in part by grants from the Ohio Third Frontier Program, the Ralph Wilson Medical Research Foundation, and the National Institutes of Health (R01HL089298, R01HL089298-S1 to Q.W.).

Disclosures
None.

References
Heart failure (HF) is a major disease, and its underlying mechanisms are complex. Corin is a membrane serine protease that activates natriuretic peptides in the heart. In mouse models, lack of corin causes hypertension and cardiac hypertrophy, indicating the importance of corin in regulating blood pressure and cardiac function in vivo. In humans, corin gene polymorphisms are associated with an increased risk for hypertension and adverse outcomes in blacks with HF, suggesting that corin may play a role in cardiovascular disease in patients. Membrane proteins are shed from the cell surface and can be detected in blood. In this study, we measured corin levels in human plasma. We found that the corin level was decreased in patients with HF and the reduction was correlated with the severity of the disease. The data indicate that corin deficiency may contribute to the pathogenesis of human HF. The data also suggest a possibility to develop a corin-based assay as a biomarker for the diagnosis of HF.
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Circ Heart Fail. 2010;3:207-211; originally published online January 8, 2010; doi: 10.1161/CIRCHEARTFAILURE.109.903849

Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

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