Plasma Soluble Corin in Patients with Heart Failure

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Abstract

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 (AMI). Soluble corin levels in plasma were measured by an ELISA method. In healthy adults (n=198), plasma corin levels were 690 (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 to that of healthy controls (365 (SD 259) pg/mL, *p*<0.001). The reduction in plasma corin levels appeared to correlate with the severity of HF. In patients of New York Heart Association (NYHA) classes II, III and IV, plasma corin levels were 450 (SD 281) (n=69), 377 (SD 270) (n=132), and 282 (SD 194) (n=90) pg/mL, respectively (*p*<0.001 class II *vs.* IV; *p*<0.05 class III *vs.* IV). In contrast, plasma corin levels in patients with AMI (n=73) were similar to that of healthy controls (678 (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 AMI. 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.

Keywords

corin; natriuretic peptides; heart failure; biomarker

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[1]. The mechanisms underlying HF are complex, involving a variety of structural and biological alterations that directly or indirectly impair cardiac function[2-4]. 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.

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 pro-atrial

natriuretic peptide (pro-ANP) and pro-brain natriuretic peptide (pro-BNP) 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 vasodialation[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 African Americans[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 two frizzled-like domains, eight LDL receptor repeats, one 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 matriptases[25, 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 (unpublished data). We hypothesized, therefore, that shed corin may enter into 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 three 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 re-hospitalized for acute decompensation. The average value of ejection fraction in these HF patients 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 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 echocardiogram. 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 based on the NYHA classification standards. Most HF patients were treated with diuretics, ACE inhibitors, angiotensin receptor blockers and β-blockers, according to clinical management guidelines.

For AMI patients, the diagnosis was based on at least two of the following three 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 elevation of serum cardiac enzymes. A troponin test also was done 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 3,000 g for 10 min. Aliquots of samples were prepared, stored at -80°C, and used within 12 weeks. Samples from HF, AMI and healthy control groups were stored in similar lengths of time. In a pilot study, soluble corin appeared 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, MN) 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 min. The reaction was visualized by adding a horseradish peroxidase substrate (3,3′,5,5′-tetramethylbenzidine, TMB) and the optical density was monitored with a spectrometer at wavelength of 450 nm.

Statistical Analysis

The analysis was done using the MedCalc software (version 10.4.0.0; Mariakerke, Belgium) and the Statistical Analysis Software (version 9.0; SAS Institute, Cary, NC). Data are presented as mean value and standard deviation (SD) in parenthesis. Comparisons of plasma corin levels in healthy controls and patient groups were performed by ANOVA followed by Tukey's post test. Multiple linear regression analysis was performed to identify independent predictors for plasma corin levels in HF patients. 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 non-normal distribution of corin levels in HF patients. 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 two-tailed and p values less than 0.05 were considered as statistically significant.

Results

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

Next we measured plasma soluble corin in HF patients. The base-line characteristics of these patients and healthy controls are shown in Table 1. The mean age of the HF patients was 67.5 (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 HF patients (365 (SD 259) pg/mL, *p*<0.001) compared to that of healthy controls (Fig. 1). In HF patients, corin levels in males (n=170) appeared to be higher than that of females (n=121) (387 (SD 272) *vs.* 335 (SD 225) pg/mL), but the difference was not statistically significant (*p*=0.092).

We also measured plasma soluble corin in AMI patients. The mean age of this group of patients was 64.5 (SD 11.6) years. There were 58.9% men and 41.1% women (Table 1). Interestingly, plasma corin levels in AMI patients did not differ significantly from that of the control group. The values were 678 (SD 285) pg/mL (n=73, *p*>0.05 *vs.* control; *p*<0.001 *vs.* HF) (Fig. 1). Like in the healthy control group, corin levels in males (n=43) were higher than that in females (n=30) with AMI (799 (SD 272) *vs.* 534 (SD 135) pg/mL, *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 addition, the reduction in plasma corin levels in HF patients appeared to correlate with the severity of the disease. The levels of plasma corin were lower progressively in patients with more severe HF. In patients of NYHA classes II (n=69; 56.5% male), III (n=132; 59.1% male) and IV (n=90; 58.9% male), the plasma soluble corin levels were 450 (SD 281), 377 (SD 270), and 282 (SD 194) pg/mL, respectively (*p*<0.001 class II *vs.* class IV; *p*<0.05 class III *vs.* class IV) (Fig. 2).

We did multiple linear regression analysis to identify variables that may independently predict plasma corin levels (Table 2). The results showed that hypertension (*p*=0.0012) and NYHA classes (*p*=0.0016 for II; *p*<0.0001 for III and IV) were strong independent predictors for low soluble corin levels in HF patients. Sex also was an independent predictor (*p*<0.0001). In contrast, age, coronary artery disease, left ventricular ejection fraction and diabetes did not appear to be independent predictors for plasma corin levels in HF patients.

Discussion

Corin is a cardiac membrane protease that activates pro-natriuretic 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.

To test this hypothesis, we measured plasma corin in HF and AMI patients. Our results showed that corin levels were significantly lower in HF patients compared to 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 (41.2 (SD18.8) *vs.* 67.5 (SD 13.2)). It appears, however, plasma corin levels did not change significantly over age. In healthy subjects, interestingly, plasma corin levels appeared to be higher in males than females but the reason for such an apparent gender difference is unknown. In HF patients, however, this difference between males and females did not reach statistical significance.

In contrast to the reduced levels in HF patients, plasma corin levels did not differ significantly in AMI patients compared to that of healthy controls (Fig. 1). The data suggest that low plasma corin levels were associated more closely with pathological changes in HF than that in acute ischemic cardiac injury. In our study, the samples from AMI patients were collected within 12 hours of disease onset. Further studies are needed with samples from more time points to determine if plasma corin levels vary over a longer course after AMI. Previously, other shed membrane proteins, such as tumor necrosis factor α (TNF-α) and interleukin-1 receptors, also were identified in HF patient plasma[29, 30]. Unlike plasma corin, however, these soluble proteins

were increased in both HF and AMI[29-32], 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 extra-cardiac tissues remains to be determined. The low levels of plasma corin observed in HF patients 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 down-regulation of corin shedding that was associated with failing hearts.

In multiple linear regression analysis, hypertension and NYHA class were two 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 ANP-mediated pathway also has a local anti-hypertrophic 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 (SNPs) in the corin gene, which were present in patients with hypertension and cardiac hypertrophy[11, 12]. In cell-based studies, these SNPs 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 pro-ANP and pro-BNP are highly elevated in patients with severe HF[44-50], indicating that processing these natriuretic peptides becomes rate-limiting as the disease progresses. It appears, therefore, that low plasma corin levels in HF patients 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 of in cardiovascular disease. At this time, we do not know which enzyme(s) shed corin from cells. It remains to be determined if and how corin shedding is regulated under physiological and pathological conditions. Our finding of low plasma corin levels in HF patients 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 if plasma corin levels are altered in HF patients over a longer period after acute decompensation and/or following medical treatment. Such studies shall help to understand the role of corin in heart disease and to define the diagnostic and prognostic values of plasma corin for HF.

Acknowledgments

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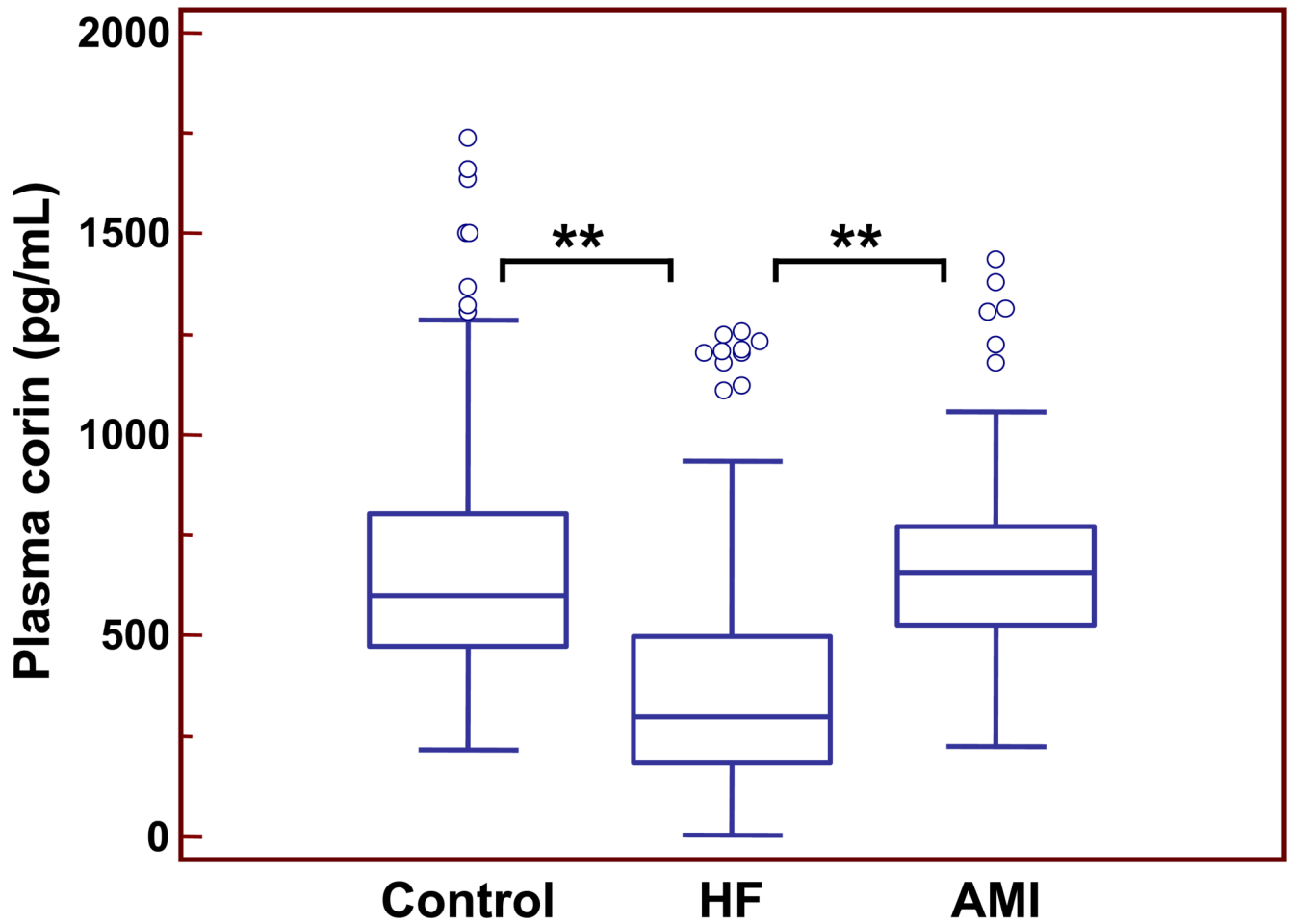
Figure 1.
Plasma corin levels in controls and patients with HF and AMI. Results were from 198 healthy controls, 291 HF and 73 AMI patients. Each box represents the median and interquartile range (IQR) values. The outliers that are >1.5 times of IQR are indicated by open circles. The vertical I bars indicate the non-outlier minimum and maximum. **$p<0.001$ *vs.* control or AMI.
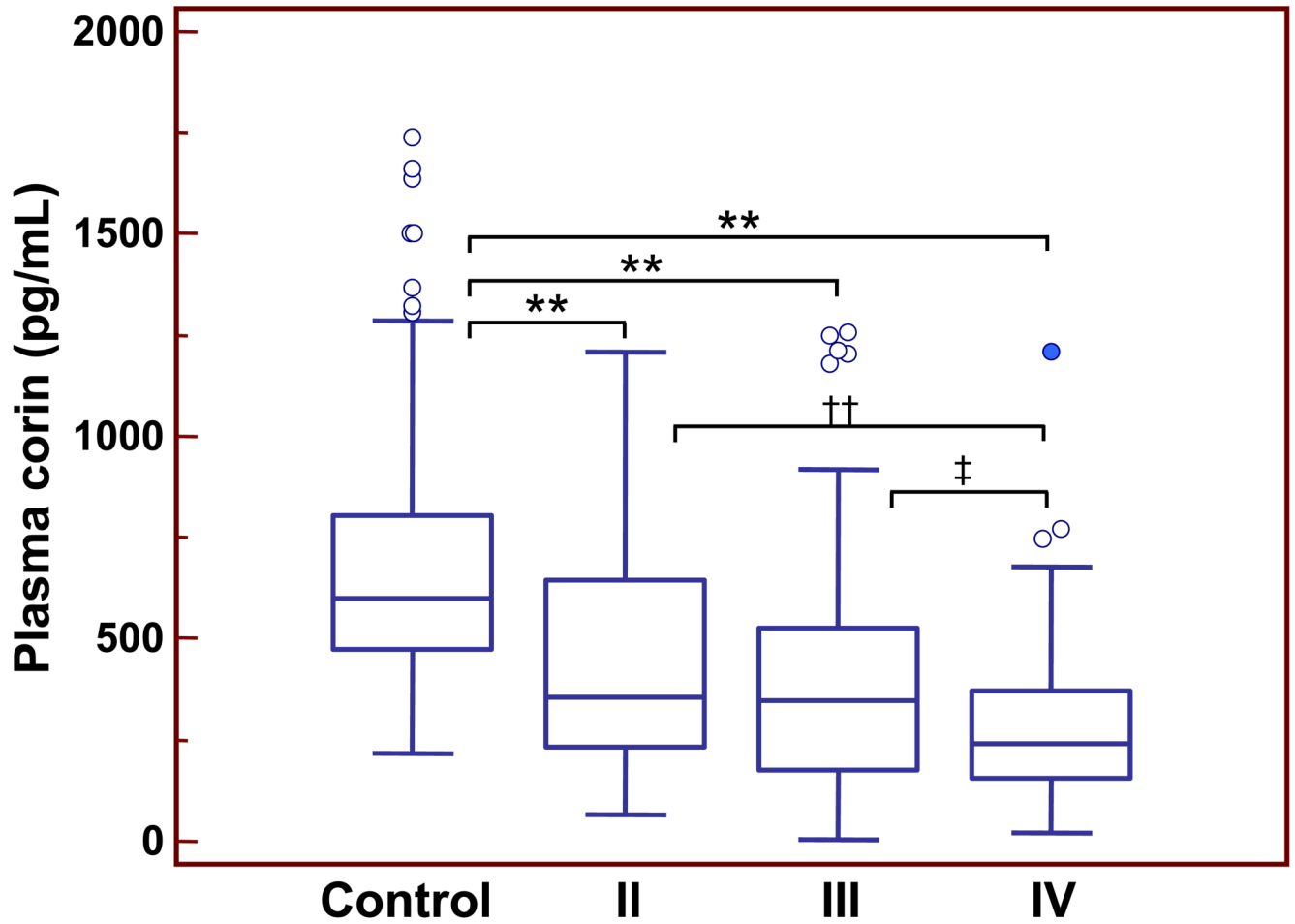
Figure 2.
Plasma corin levels in controls and HF patients of NYHA classes II-IV. Results were from 198 normal controls, 69 HF patients of class II, 132 HF patients of class III, and 90 HF patients of class IV. Each box represents the median and IQR values. The outliers that are >1.5 and >3 times of IQR are indicated by open and filled circles, respectively. The vertical I bars indicate the non-outlier minimum and maximum. **$p<0.001$ *vs.* control; ††$p<0.001$ *vs.* IV; ‡$p<0.05$ *vs.* IV.

Table 1

Characteristics of Controls and Patients with HF and AMI

Characteristics	Control	HF	AMI
Age	41.2 (SD 18.8)[*] (n=198)	67.5 (SD 13.3)[†] (n=291)	64.5 (SD 11.6) (n=73)
Sex (%)[‡]			
Male	104 (52.5)	170 (58.4)	43 (58.9)
Female	94 (47.5)	121 (41.6)	30 (41.1)
Medical History (%)			
Hypertension	0 (0)	175 (60.1)	36 (49.3)
Diabetes	0 (0)	65 (22.3)	10 (13.7)
Valvular heart disease	0 (0)	42 (14.4)	NA
Cardiomyopathy	0 (0)	46 (15.8)	0 (0)
Coronary artery disease	0 (0)	95 (32.6)	73 (100)
Cardiac arrhythmia	0 (0)	37 (12.7)	NA
Others	0 (0)	58 (19.9)	32 (43.8)

HF, heart failure; AMI, acute myocardial infarction;

[*] $p<0.001$ control *vs.* HF or AMI;

[†] $p>0.05$ HF *vs.* AMI;

[‡] Sex distributions were not statistically different among three groups; NA, not available.

Table 2

Multiple Linear Regression to Predict Plasma Corin Levels in HF[*]

Variable	Coefficient	Standard Error	t	p
Sex[†]	-2.62	0.56	-4.72	<0.0001
Age	-0.01	0.02	0.46	0.6471
Hypertension	-2.59	0.79	-3.27	0.0012
Coronary artery disease	0.21	0.85	0.25	0.8021
LV ejection fraction	0.00	0.02	0.16	0.8744
Diabetes mellitus	0.31	0.94	0.33	0.7394
NYHA class II	-4.00	1.26	-3.17	0.0016
NYHA class III	-6.64	1.27	-5.22	<0.0001
NYHA class IV	-8.65	1.39	-6.22	<0.0001

[*] HF, heart failure; LV, left ventricular; NYHA, New York Heart Association. The response variables were analyzed using square root transformed data. The R-square = 0.32.

[†] The coding for dummy variables: sex (male=0, female=1), hypertension, coronary artery disease, and diabetes (yes=1, no=0), NYHA class 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.