Corin Overexpression Improves Cardiac Function, Heart Failure, and Survival in Mice With Dilated Cardiomyopathy

Inna P. Gladysheva,* Dong Wang,* Rachel A. McNamee, Aiilyan K. Houn, Almois A. Mohamad, T. Michael Fan, Guy L. Reed

Abstract—Heart failure, caused by dilated cardiomyopathy and other cardiac disorders such as hypertension, is a major public health problem with high morbidity and mortality. Corin, a cardiac enzyme that cleaves natriuretic peptides, is a promising biomarker of cardiomyopathy and heart failure, but its functional role in these processes is not understood. We evaluated the potential effects of corin in mice with a well-characterized model of dilated cardiomyopathy. Mice with dilated cardiomyopathy developed heart failure, reduced contractile function, cardiac fibrosis, and accelerated mortality in the setting of low corin expression. In wild-type mice, transgenic, cardiac-targeted, overexpression of corin enhanced cyclic guanosine monophosphate and blood pressure responses to pro-atrial natriuretic peptide, but did not affect heart size, contractility, body weights, survival, and blood pressure. In mice with dilated cardiomyopathy, corin overexpression significantly reduced the development of myocardial fibrosis (P<0.05). Corin overexpression also enhanced heart contractile function (fractional shortening and ejection fraction; P<0.01) and it significantly reduced heart failure as assessed by lung water (P<0.05) and alveolar congestion (P<0.001). Consistent with these observations, corin overexpression significantly prolonged life in mice with dilated cardiomyopathy (P<0.0001). These results provide the first experimental evidence that corin expression plays a role in cardiomyopathy by modulating myocardial fibrosis, cardiac function, heart failure, and survival. (Hypertension. 2013;61:00:00.) • Online Data Supplement

Key Words: corin • dilated cardiomyopathy • heart failure • natriuretic peptides

Heart failure (HF) is a syndrome of abnormal salt and water retention that frequently occurs in the setting of reduced cardiac function or cardiomyopathy. HF is a leading cause of morbidity and mortality; it affects >5.7 million Americans, and ~670 000 new cases are diagnosed each year. Despite improvements in treatment, HF is a progressive process and nearly half of patients die within 5 years.1 The factors that modulate HF development and progression in patients with cardiomyopathy are still poorly understood.

Corin is a potential biomarker of HF and cardiomyopathy.3–5 Polymorphisms in corin are linked to more severe hyperten-
sion.6 Corin is a transmembrane serine protease expressed by cardiomyocytes that cleaves natriuretic pro-atrial natriuretic peptide (ANP) to generate ANP; there is increasing evidence that it may also cleave pro-brain natriuretic peptide (BNP).7–14 The natriuretic peptides (NPs) play a critical role in maintaining normal salt and water balance and arterial blood pressure; they are also important diagnostic and prognostic biomarkers for patients with HF.15 ANP and BNP interact with the NP receptor-A to regulate cGMP levels, vasodilation, natriuresis, fibrosis, etc.9,16 As such the corin-NP system should protect against the development of progressive HF in patients with reduced systolic function.4

One of the most common causes of progressive HF, cardiac transplantation, and mortality is dilated cardiomyopathy (DCM).17 DCM has several genetic and environmental causes in humans and mice.17,18 One of the best characterized models of DCM in mice is caused by a phophorylation-resistant cAMP response element-binding protein (CREB) mutant transgene (DCM−).17–21 Mice with DCM− develop HF with features similar to human DCM including biventricular dilation, elevated NP levels, fibrosis, electrophysiologic abnormalities as well as progressive edema, dyspnea, hepatic congestion, and early demise.19–23

Through its positive effects on natriuresis, fibrosis, and vascular resistance, the corin-NP system should delay the progression of DCM and HF. However, we and others have found that blood levels3–5,24 and cardiac transcripts for corin25 are paradoxically reduced in patients with severe DCM. Similar to humans, mice with DCM− have reduced systolic function, enhanced cardiac fibrosis, elevated NP levels, and accelerated mortality, all in the setting of decreased cardiac corin.

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expression. Still, the contribution of corin to HF development remains controversial and poorly understood.3–5,7,24,26–28 To examine this we genetically overexpressed corin in the hearts of mice with DCMc.

Methods

We analyzed corin-transgenic (Tg) and DCMc mice in vivo and ex vivo. Experimental details are found in the online-only Data Supplement.

Statistical Analysis

Survival was analyzed by the Kaplan-Meier method. Other statistical analyses were performed using nonparametric methods (unless otherwise indicated). Differences were considered to be significant if the 2-tailed \( P<0.05 \). The number of animals (n) is indicated in the figure legends or results. Data are reported as mean±SEM.

Results

Reduced Corin Expression in DCMc Mice

Mice with cardiac Tg expression of the CREB mutant develop a DCMc accompanied by frank HF with edema, ascites, and shortened survival.19,23 Although the promoter of the corin gene does not contain CREB binding sites,29 the DCMc mice showed reduced levels of corin transcripts (Figure 1A) and protein (Figure 1B–1D) versus wild-type (WT) littermates. DCMc mice had higher ANP (2.1-fold; \( P<0.05 \); Figure S1 in the online-only Data Supplement) and BNP transcripts (3.3-fold; \( P<0.01 \); Figure S2).

Corin-Transgenic Mice

To examine whether corin modulates HF, corin-Tg mice were backcrossed with DCMc mice on the same strain background. Female littermates were examined at 14 to 15 weeks. There was no significant difference in body weight or body:heart weight ratios (Figure S4). CREB Tg transcript levels did not change after backcrossing (\( P=0.41 \)). Corin transcripts were higher in DCMc, corin-Tg mice than in DCMc mice (Figure 4A). Enhanced expression of corin protein was also found (Figure 4B–4D). Higher blood levels of soluble corin were detected in DCMc, corin-Tg mice than DCMc mice (\( P<0.05; n=4–5 \) each group). Transcripts for ANP (1.7-fold; \( P<0.05 \); Figure S5) and BNP (1.4-fold; \( P<0.001 \); Figure S6) were higher in DCMc, corin-Tg than in DCMc mice. Consistent with this observation, levels of cGMP were significantly higher in DCMc, corin-Tg mice (Figure 7; \( P<0.05 \)). DCMc, corin-Tg mice had reduced interstitial and perivascular cardiac fibrosis (54% lower; \( P<0.05; n=4–5 \) each group; Figure 4E and 4F) by Masson trichrome staining. Transcripts for collagen I (\( P<0.01 \)) and collagen III (\( P<0.05 \)) were lower in DCMc, corin-Tg mice (Figures S8 and S9). There was a trend to lower transforming growth factor-\( \beta \) levels, but chymase 1, matrix metalloproteinases 9, and furin transcripts were higher in DCMc, corin-Tg versus WT mice (\( P=0.06 \) and 0.03, respectively).

Enhanced Corin Activity in Corin-Tg Mice

The cleavage of pro-ANP to ANP enhances cellular generation of cGMP and lowers blood pressures. In corin-Tg mice cGMP levels were slightly higher than in WT mice (Figure 3A), but there were no significant differences in mean arterial pressure (Figure 3B) or heart rate. There was enhanced cleavage of recombinant pro-ANP by hearts from corin-Tg mice (Figure S3). Bolus injection of pro-ANP increased cGMP levels in both corin-Tg and WT mice (Figure 3A). In response to pro-ANP injection, but not saline, mean arterial pressure dropped significantly in corin-Tg but not WT mice (Figure 3B; \( P<0.05 \)).
were not different between the 2 groups (Figures S10–S13). DCMc, corin-Tg mice had better contractile function with a higher ejection fraction % (P<0.01; Figure 4G) and fractional shortening (23.0±2.4% versus 12.9±1.3%; P<0.01) than DCMc mice despite similar left ventricle internal dimensions (Figure S14). HF was significantly reduced in DCMc, corin Tg versus DCMc mice as assessed by reduced alveolar edema and congestion (Figure 4H and 4I; P<0.001) and reduced lung water (lung wet:dry ratio, P<0.05). Most importantly, the survival of DCMc, corin-Tg mice was significantly longer than the survival of DCMc mice (Figure 4J; P<0.0001).

**Discussion**

In patients with DCM, progressive HF is a major cause of morbidity and mortality with high social costs. As such, there is a critical need to discover mechanisms that regulate HF development and progression to create new diagnostic, treatment, and prevention strategies. Corin’s cardiac-selective expression and its key role in regulating the NP system make it a potential biomarker of acute HF in the setting of diminished systolic function.1,5,6 Cardiac transcripts25 and circulating levels of corin3–5,24 are reduced in patients with HF and DCM but not in all cardiac conditions, particularly those involving hypertrophy.7,26,28 Still, the functional role of corin in DCM has not been established. In a well-characterized model of HF and DCM,19–23 we confirmed that myocardial corin transcripts (and protein levels) were reduced. Similar reductions in corin expression were observed in a model of HF induced by arterial venous shunting.27 Restoration of corin levels in DCMc mice markedly reduced development of cardiomyopathy and HF. There were significant reductions in myocardial fibrosis and improvements in contractile indices (fractional shortening, ejection fraction) in DCMc, corin-Tg versus DCMc mice. HF was also improved in DCMc, corin-Tg mice as assessed by objective indices of lung water and congestion. Perhaps the most compelling finding was that restoration of corin levels significantly increased the survival of DCMc, corin–Tg mice versus DCMc mice.

There are several potential mechanisms through which corin and the NP system may modulate the development of HF. Corin cleaves pro-ANP to ANP, which has enhanced physiologic effects.5,30 ANP increases salt and water excretion which should reduce the salt and water retention of HF.26,31 We found low levels of circulating corin and impaired pro-ANP cleavage in patients with acute decompensated HF suggesting that low corin levels might contribute to this syndrome of salt and water retention in some patients.3,4 Indeed, corin deficiency reduces sodium excretion in response to high-salt diets.32 Our data show that overexpression of corin increases physiologic responses to pro-ANP, increases cGMP levels, and reduces fluid retention in

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Comparison of wild-type and corin-transgenic (Tg) mice. A, Corin-Tg and wild-type female littermates and hearts are similar in appearance (15 weeks old, bar=2 mm). B, Corin protein expression is increased in the heart of corin-Tg mice (female, 15-week-old) assessed by Western blotting under reducing conditions with anti-corin antibodies (upper panel). Quantitation of corin expression (vs. Glut-4 expression, n=4 each group). C, Survival in lines of corin-Tg mice and wild-type mice is similar (n=701). D, Comparison of body weight (B), heart weight (H), and B:H values between corin-Tg and wild-type mice littermates (n=11–20 in each group; female data are shown). E, Similar systolic (SBP), diastolic (DBP), or mean arterial blood pressure (MAP) in corin-Tg (n=5 females, 15 males) and wild-type (n=6 females, 16 males) mice.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Enhanced corin activity and mean arterial blood pressure (MAP) in corin-transgenic (Tg) female mice. A, Plasma levels of cGMP in corin-Tg and wild-type (WT) female mice before (open bars) and after (filled bars) bolus pro-atrial natriuretic peptide (ANP; 10.5 ng/200 μL PBS) injection (n=4–7 age matched per group). B, MAP in corin-Tg (n=6) and WT mice (n=6) before (open bars) and after (filled bars) bolus pro-ANP containing medium injection. MAP was recorded using a Millar catheter and Power Lab software. *P<0.05.
Corin overexpression in DCMc mice reduces fibrosis, heart failure (HF), and increases survival. A, Cardiac expression of corin transcripts in DCMc and DCMc, corin-transgenic (Tg) mice assessed by quantitative reverse transcription polymerase chain reaction analysis, relative to wild-type mice. Transcripts are means of averages of triplicate measures in 7 mice. B and C, Corin protein expression assessed by immunohistochemical staining. Representative immunoperoxidase-stained heart sections (n=2 per group) probed with anti-corin antibody (40× magnification, bar=50 um). Quantification of corin expression by image analyses. D, Cardiac protein expression in heart assessed by Western blotting under reducing conditions with anti-corin and anti-actin antibodies. Relative corin levels normalized to actin. E and F, Cardiac fibrosis in representative heart ventricles sections (n=4–5 each group) stained with Masson trichrome (E, 40× magnification, bar=50 um). Quantification of fibrosis by image analyses. G, Cardiac fibrosis in representative formalin-fixed lung sections (H, 40× magnification, bar=50 um) stained by hematoxylin and eosin from female DCMc and DCMc, corin-Tg mice. Bar graph (I) of total alveolar area free of edema and congestion per 20× field. Results are means of averages of 10 randomly selected fields from 6 to 7 mice of each group. J, Kaplan-Meier survival curves of DCMc (n=56) and DCMc, corin-Tg (n=46) mice. *P<0.05, **P<0.01, ***P<0.001.

Corin overexpression in DCMc mice reduces fibrosis, heart failure (HF), and increases survival. In addition, the DCMc, corin-Tg mice had increased corin levels, cGMP levels, and ANP and BNP transcripts. ANP and BNP inhibit collagen synthesis and proliferation of cardiac fibroblasts, which in turn inhibits cardiac fibrosis in vivo. Thus, the reduced fibrosis seen in DCMc, corin-Tg mice may be attributable to increased activity of the NP system and may contribute to the improved ventricular function seen in these mice.

In summary, consistent with findings in humans with HF and DCM, we find that corin expression is significantly reduced in experimental DCMc and HF. In a recently published study, corin-deficient Kitl<sup>α<sub>1</sub>B<sub>4</sub>/ab<sub>W<sub>sh</sub></sub> mice developed rapidly progressive cardiac dilation and loss of cardiac function after aortic banding. These findings, in addition to the cardiac-selective expression of corin and its role as regulator of the NP system, make corin an attractive biomarker for DCM and HF. Beyond its potential diagnostic value, corin appears to play a key functional role in DCM and HF, and high expression is associated with heart failure, which is associated with reduced myocardial fibrosis, enhanced contractility, prevention of HF, and prolongation of life. Further studies of corin in other types of HF and cardiomyopathies, for instance, hypertensive heart disease and chronic myocardial infarction, will be necessary to determine the value of corin as a biomarker and potential therapeutic agent.

**Perspectives**

Corin is a key regulator of the NP system, which modulates salt and water balance in HF. However, levels of corin mice with DCMc. Although the relative contributions of cardiac and circulating corin to NP cleavage are still unknown, patients with HF respond to ANP infusions with increased cGMP levels and improved long-term prognosis. ANP also enhances vasodilation, which can increase cardiac output in the presence of reduced cardiac function.

There is increasing evidence that corin also may cleave pro-BNP to BNP. Recent studies have linked a hypofunctional polymorphism in corin to diminished pro-BNP cleavage and worse outcomes. Some patients with chronic HF appear to have abnormal processing of pro-BNP to BNP fragments with diminished biologic activity. Still, the therapeutic value of BNP (Natreco/Nesiritide) therapy in HF patients is controversial, and a large-scale clinical trial showed no significant improvement in symptoms or mortality.

In addition to natriuretic and vasodilatory effects, ANP and BNP also affect apoptosis, inflammation, and cardiac fibrosis—each of these mechanisms may affect the progression of cardiomyopathies. Indeed, deletion of the receptor for ANP and BNP (natriuretic peptide receptor A) accelerated mortality in mice with DCM. Cardiac fibrosis is significant in all DCMc mice by 8 weeks of age though no significant apoptosis or inflammation was appreciated. Cardiac fibrosis was also seen in knockout mice lacking ANP, BNP. Cardiac fibrosis affects diastolic and systolic dysfunction and contributes to the development of HF. When analyzed at 14 to 15 weeks of age, hearts from DCMc, corin-Tg mice showed significantly less interstitial and perivascular ventricular fibrosis than DCMc mice.
are unexpectedly reduced in humans and mice with DCM. Increasing cardiac corin expression in mice with DCM enhances ANP and BNP expression, improves cardiac function, reduces cardiac fibrosis, and prolongs survival. Thus in addition to its value as a potential biomarker, strategies for increasing corin levels in DCM may mitigate the progression of cardiac fibrosis, HF, systolic dysfunction, and death.

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Disclosures

None.

References

4. Ibebuogu UN, Gladysheva IP, Houng AK, Reed GL. Decompensated heart failure is associated with reduced corin levels and decreased cleavage of pro-atrial natriuretic peptide. Circ Heart Fail. 2011;4:114–120.


**Novelty and Significance**

**What Is New?**

In a model of heart failure we found:

- Reduced heart function and measurable heart failure
- High levels of natriuretic peptides atrial natriuretic peptide and brain natriuretic peptide
- Reduced levels of corin, a novel heart protein

Increasing corin in the heart of normal mice:

- Reduced blood pressure in response to pro-atrial natriuretic peptide
- Increased cGMP which regulates blood pressure

Increasing the level of corin in mice with enlarged hearts:

- Reduced heart scarring
- Prevented heart failure
- Increased heart function
- Saved lives

**What Is Relevant?**

- Hypertension often causes heart failure
- Corin activates natriuretic peptides to reduce blood pressure
- Corin polymorphisms may cause heart problems in patients with hypertension
- Increasing corin levels in heart failure prevents loss of heart function, fluid retention, heart scarring, and early death

**Summary**

Corin is an attractive biomarker for heart failure and cardiomyopathy. These results also provide the first experimental evidence that corin expression may reduce heart scarring, improve heart function, prevent heart failure, and increase survival.
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ONLINE SUPPLEMENT

CORIN IMPROVES CARDIAC FUNCTION, HEART FAILURE AND SURVIVAL IN MICE WITH DILATED CARDIOMYOPATHY

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Methods

Assessment of corin function in vivo. Anesthesia was induced with 3% followed by 1.5% isoflurane. Mice were mechanically ventilated as described. Body temperature was maintained at 37°C by a warming pad. Mice (age-matched females) were randomly assigned to receive intravenous pro-ANP in PBS or PBS alone. Hemodynamics were measured as we have described. Retro-orbital blood was collected ≥ 1 week before and immediately after hemodynamic measurement into EDTA (2ul, 500mM) siliconized (AquaSil, Pierce, Rockford, IL) tubes (Natelson, Fisher Scientific, Pittsburgh, PA) and spun at 1460 x g at 4°C for 20 mins. Plasma was stored at -20 ºC for assay.

Pro-ANP production. Pro-ANP was expressed recombinantly with a C-terminal V5 tag in human embryonic kidney HEK293 cells in serum-free conditioned medium as described, lyophilized and dissolved in sterile PBS. Pro-ANP was characterized by immunoprecipitation followed by Western blot and ELISA as described.

Enzyme immunoassay for mouse corin and cGMP in plasma. Plasma cGMP and corin levels were measured in duplicate or triplicate by immunoassays (Assay Designs, Inc., Ann Arbor, MI and USCN Life Science Inc., China).

Northern analysis and DNA probes. Total mouse heart RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. RNA (10 µg) electrophoresed on 1.0% formaldehyde-agarose gels and transferred to nitrocellulose. After pre-hybridization (30 min.) Northern blots were hybridized (ExpressHyb, BD Biosciences, San Diego, CA) for 60 minutes with a corin-(32P)dCTP probe (2 x 106 cpm/ml) labeled with Ready-To-Go DNA labeling beads, Amersham Biosciences, Piscataway, NJ). After washing blots were visualized by a Storm 840 (Amersham Biosciences). Results were normalized to GAPDH control.

Real-time polymerase chain reaction (RT-PCR). Total RNA was extracted from whole hearts using the RNeasy® Mini Kit (Qiagen). First strand cDNA synthesis was performed with 1 µg of total RNA (Transcriptor First Strand cDNA Synthesis Kit, Roche). Quantitative real-time PCR (qRT-PCR) was performed using the LightCycler® 480 System following the manufacturer’s protocol. Specific primers were: ctggaaggatgcttgggag and aecgtctctgtctgctcta for corin; tccatatcaggggtcaca and gcccattgaaggtggtta for BNP; cacagatctcattacagcag for ANP; ggaaggatcaccaggtaaa and gcatctcactgtagt for CREB; cagatcaggtgcctacac and gcagcggctgcaagcag for collagen 1; gcacatcaggtgcctacac and gcagcggctgcaagcag for collagen 3; tggagcaacaggtcagact and gcagcggctgcaagcag for collagen 3; tggagcaacaggtcagact and gcagcggctgcaagcag for TGFbeta1; gcagcggctgcaagcag and gcagcggctgcaagcag for CMA1; gcagcggctgcaagcag and gcagcggctgcaagcag for MMP-9; vcggagaccctctctcagtgccttcttccag and gcagcggctgcaagcag for furin. PCR was performed at: 95°C for 5 min, followed by 40 cycles of 95°C (10 s), 60°C (30 s), and 72°C (10 s). PCR products were confirmed by melting curve analysis using the Lightcycler Software 4.0 and samples normalized to a β-actin control. Experiments were performed in triplicate and the qRT-PCR was subjected to log transformation as recommended to achieve a normal distribution.

Immunohistological Analysis. Frozen mouse hearts were embedded in OCT (Sakura Finetek U.S.A. Inc., Torrance, CA) and cut into 5 µm cryosections. Slides were fixed (10% formalin, 20 min), rinsed in PBS and incubated with 0.3% H2O2 for 30 min. Non-specific antibody binding was blocked with 5% goat serum for 30 min. Corin was detected with rabbit polyclonal anti-mouse corin protease domain antibodies (1:700 dilution in PBS containing 5% goat serum) for 60 min at 21°C followed by biotinylated goat anti-rabbit secondary antibody (Vector Lab., Burlingame, CA) and avidin horseradish peroxidase (Vector Lab.).
reactivity was demonstrated by 3,3'-diaminobenzidine (DAB, Vector Lab.). Nuclei were identified by hematoxylin counterstaining.Slides were scanned (Aperio's ScanScope) and images were taken using ImageScope software (MAN-0001, revision G). In control sections primary antibodies were replaced with pre-immune rabbit serum. Images (10 random fields from each mouse) were analyzed using ImagePro Plus 6.2 software (Media Cybernetics, Bethesda, MD).

Masson’s trichrome stain was used to detect fibrosis. Both perivascular and interstitial fibrosis were measured in 5 hearts from each group. The digital images of ten random ventricular fields (40 x) were analyzed using ImagePro Plus 6.2 software and the mean fibrosis for each mouse was determined (Media Cybernetics, Bethesda, MD).

**Lung Edema and Lung Water Retention Analysis.** Formalin-fixed lung sections were stained by hematoxylin and eosin. Images (10 random fields from each mouse) were analyzed using ImagePro Plus 6.2 software (Media Cybernetics, Bethesda, MD) to determine the percent of total alveolar area free of congestion in each field (20 x) by comparison to normal wild-type controls as a reference. Lung edema was also assessed by wet/dry lung weight ratios as described 7. Right and left lungs were excised and rapidly weighed (wet weight) and then were oven dried (65°C; Fisher Isotemp, Fisher Scientific) for 72 h to a stable dry lung weight. Data are presented as the ratio of right+left lung wet weight/ right+left lung dry weight.

**Western blot analysis.** Equivalent amounts of protein extracted from whole frozen heart samples (n=2-4 per group) were subjected to reducing SDS-PAGE, electroblotted to Immobilon-P transfer membrane (Millipore Corp., Bedford, MA) and probed with rabbit polyclonal anti-corin stem or protease domain antibodies as described 2, 6. Protein loading was normalized by α-actin (polyclonal anti-actin antibody, Santa Cruz) or Glut4 (Glug 4 mouse monoclonal antibody sc-53566, Santa Cruz).

**Pro-ANP processing by mouse heart tissue.** Frozen hearts were homogenized in 10 mM Tris-HCl, pH 7.4 buffer. The homogenate was centrifuged at 10,000 g for 30 min at 4 °C. Pellets were washed with ice-cold 10 mM Tris-HCl, pH 7.4 buffer and centrifuged. Washed pellets were combined with pro-ANP medium containing 1 mM EDTA, 20 mM CaCl2, 50 µM soybean trypsin inhibitor, 0.1% Triton X100, proceeded by ultra-sound for 10 inputs, and incubated for 5.5 h at 37 °C. The pH of reaction mixture was adjusted by 3 M Tris-HCL, pH 8.0. ANP was immunoprecipitated from the conditioned medium by a mouse monoclonal anti-V5 tag antibody (Invitrogen, Carlsbad, CA) coupled to protein A –Sepharose (Pierce, Rockford, IL) 6. Immunoprecipitated proteins were solubilized in SDS-PAGE sample buffer and analyzed by Western blotting under reducing conditions with an anti-V5 tag rabbit polyclonal antibody (Immunology Consultants Laboratory, Inc., Newberg, OR) followed by incubation with an alkaline phosphatase-conjugated goat anti-rabbit/or goat anti-mouse antibody, and detection by ECF substrate (Amersham Biosciences, Piscataway, NJ) 6.

**Echocardiography.** Transthoracic echoes were performed by an echocardiographer blinded to genotype with a VisualSonic Vevo 2100 Imaging System (VisualSonic Inc. Toronto, Canada) as we previously described with some modifications 8. Briefly, female 3.5-month-old mice were sedated with 1.5% inhaled isoflurane; the hemithorax of each mouse was carefully shaved, and two-dimensional and M-mode images of LV at the long axis were recorded. M-mode images were analyzed using Vevo software; left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) were measured at least 6 times and averaged for each mouse. All measurements were performed using edge-to-edge convention adopted by the American Society of Echocardiography. The %FS and ejection fraction (%EF) were calculated.
Supplemental References


4. Ibebuogu UN, Gladysheva IP, Houng AK, Reed GL. Decompensated heart failure is associated with reduced corin levels and decreased cleavage of pro-atrial natriuretic peptide. *Circ Heart Fail*. 2011;4:114-120.


6. Gladysheva IP, Robinson BR, Houng AK, Kovats T, King SM. Corin is co-expressed with pro-anp and localized on the cardiomyocyte surface in both zymogen and catalytically active forms. *J Mol Cell Cardiol*. 2008;44:131-142.


**Figure S1.** Relative cardiac ANP expression in DCM and wild-type (WT) assessed by qRT-PCR analysis. Transcripts are means of averages of triplicate measures in 7 mice. *p<0.05.

**Figure S2.** Relative cardiac BNP expression in DCM and wild-type (WT) assessed by qRT-PCR analysis. Transcripts are means of averages of triplicate measures in 7 mice. **p<0.01.

**Figure S3.** Analyses of corin activity in the wild-type and corin Tg hearts by cleavage of recombinant pro-ANP with a carboxy-terminal V5-tag. The serum free conditioned medium containing pro-ANP was incubated with the pelleted heart tissue (membrane fraction) of wild-type and corin Tg mice (n=3 per group). Cleaved ANP was immunoprecipitated and analyzed by Western blot analysis with the use of anti-V5 tag antibody (top). The blots were subjected to image analysis (NIH Image Quant program). The percent cleavage of pro-ANP to ANP was calculated.
Figure S4. Comparison of heart weight (HW), body weight (BW) and BW:HW values between DCMc and DCMc, corin-Tg female, 14-15 weeks mice littermates (n=10-12 in each group).

Figure S5. Cardiac expression of ANP transcripts in DCMc and DCMc, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. *$p<0.05$.

Figure S6. Cardiac expression of BNP transcripts in DCMc and DCMc, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. ***$p<0.001$. 
**Figure S7.** cGMP level in plasmas of DCM<sup>c</sup> and DCM<sup>c</sup>, corin-Tg mice assessed by ELISA. Plasma levels are means of averages of duplicate measures in 7 mice of each group. *p<0.05*, unpaired t-test.

**Figure S8.** Cardiac expression of collagen 1 transcripts in DCM<sup>c</sup> and DCM<sup>c</sup>, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. **p<0.01**.

**Figure S9.** Cardiac expression of collagen III transcripts in DCM<sup>c</sup> and DCM<sup>c</sup>, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. *p<0.05.*
Figure S10. Cardiac expression of TGF beta transcripts in DCMc and DCMc, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. $p=0.056$.

Figure S11. Cardiac expression of CMA1 transcripts in DCMc and DCMc, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. $p=1.00$

Figure S12. Cardiac expression of MMP-9 transcripts in DCMc and DCMc, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. $p=0.84$
**Figure S13.** Cardiac expression of furin transcripts in DCM<sup>c</sup> and DCM<sup>e</sup>, corin-Tg assessed by qRT-PCR analysis, relative to wild-type. Transcripts are means of averages of triplicate measures in 7 mice. \( p=0.42 \)

**Figure S14.** Heart LV internal dimensions, LVID for DCM<sup>c</sup> (n=6) and DCM<sup>e</sup>, corin-Tg (n=7). \( p=0.84 \).