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. 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. Polymorphisms in corin are linked to more severe hypertrophy. 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). 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. ANP and BNP interact with the NP receptor-A to regulate cGMP levels, vasodilation, natriuresis, fibrosis, etc. As such the corin-NP system should protect against the development of progressive HF in patients with reduced systolic function.

One of the most common causes of progressive HF, cardiac transplantation, and mortality is dilated cardiomyopathy (DCM). DCM has several genetic and environmental causes in humans and mice. One of the best characterized models of DCM in mice is caused by a phosphorylation-resistant cAMP response element-binding protein (CREB) mutant transgene (DCM™). 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.

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 levels and cardiac transcripts for corin 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.\textsuperscript{3-5,7,24,26-28} To examine this we genetically overexpressed corin in the hearts of mice with DCM\textsuperscript{c}.

**Methods**

We analyzed corin-transgenic (Tg) and DCM\textsuperscript{c} 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 DCM\textsuperscript{c} Mice**

Mice with cardiac Tg expression of the CREB mutant develop a DCM\textsuperscript{c} accompanied by frank HF with edema, ascites, and shortened survival.\textsuperscript{19,23} Although the promoter of the corin gene does not contain CREB binding sites,\textsuperscript{29} the DCM\textsuperscript{c} mice showed reduced levels of corin transcripts (Figure 1A) and protein (Figure 1B–1D) versus wild-type (WT) littermates. DCM\textsuperscript{c} mice had higher ANP (2.1-fold; \( P<0.05 \); Figure S1 in the online-only Data Supplement) and BNP transcripts (not shown), thus we focused our studies on the Tg line expressing the highest corin levels. Three corin-Tg lines were identified that displayed 1.3 to 13.5-fold increased levels of corin transcripts versus WT mice (Tg1=1.3±0.1-fold; Tg2=6.9±0.5-fold; Tg3=13.5±2.5-fold assessed by Northern blot). Corin protein was increased in the heart (Figure 2B) and blood (1.4-fold; \( P<0.05 \); n=3 each group). There was no difference between WT mice and corin-Tg littermates in survival (n=701; Figure 2C) or in ANP ratios (WT 296.4±12.8 versus 300.5±22.5; n=11–17 each group; Figure 2D); male mice were also similar to each other (11–20 each group). Indeed no differences in body weight were observed in mice up to 500 to 600 days old. There were no significant differences between WT and corin-Tg mice of the same gender in baseline systolic, diastolic, or mean arterial blood pressure (Figure 2E), heart rate or fractional shortening (31.9±1.2 versus 32.4±2.3).

**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; \( P<0.05 \)).

**Corin Modulates HF in Mice With DCM\textsuperscript{c}**

To examine whether corin modulates HF, corin-Tg mice were backcrossed with DCM\textsuperscript{c} 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 (WT 296.4±12.8 versus 300.5±22.5; n=11–17 each group). There was no difference in body weight or body:heart weight ratios (WT 296.4±12.8 versus 300.5±22.5; n=11–17 each group). There was no significant difference in baseline systolic, diastolic, or mean arterial blood pressure (Figure 2E), heart rate or fractional shortening (31.9±1.2 versus 32.4±2.3).

**Figure 1.** Corin expression is reduced in the hearts of mice with DCM\textsuperscript{c}. A, Relative corin expression in dilated cardiomyopathy (DCM) and wild-type (WT) mice assessed by quantitative reverse transcription polymerase chain reaction analysis. Transcripts are means of averages of triplicate measures in 7 mice. B, Corin protein expression assessed by Western blotting under reducing conditions with anti-corin antibodies in WT and DCM\textsuperscript{c} mice (upper panel). Densitometry analysis of corin expression relative to wild-type mice (bottom graph). C and D, Corin protein expression assessed by immunohistochemical staining and densitometry analysis vs wild-type mice. Representative staining of left ventricular sections (n=2 per group) with anti-corin antibodies (40×, bar=50 μm). Corin expression from image analyses of immunohistochemical staining (D). \( *P<0.05 \); \( **P<0.01 \).
were not different between the 2 groups (Figures S10–S13). DCM<sup>c</sup>, 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 DCM<sup>c</sup> mice despite similar left ventricle internal dimensions (Figure S14). HF was significantly reduced in DCM<sup>c</sup>, corin Tg versus DCM<sup>c</sup> 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 DCM<sup>c</sup>, corin –Tg mice was significantly longer than the survival of DCM<sup>c</sup> 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.<sup>1,5</sup> Cardiac transcripts<sup>25</sup> and circulating levels of corin<sup>1,5,24</sup> are reduced in patients with HF and DCM but not in all cardiac conditions, particularly those involving hypertrophy.<sup>7,26,28</sup> Still, the functional role of corin in DCM has not been established. In a well-characterized model of HF and DCM,<sup>19,23</sup> 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.<sup>27</sup> Restoration of corin levels in DCM<sup>c</sup> 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 DCM<sup>c</sup>, corin-Tg versus DCM<sup>c</sup> mice. HF was also improved in DCM<sup>c</sup>, 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 DCM<sup>c</sup>, corin –Tg mice versus DCM<sup>c</sup> 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.<sup>5,30</sup> ANP increases salt and water excretion which should reduce the salt and water retention of HF.<sup>3,26</sup> 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.<sup>3,4</sup> Indeed, corin deficiency reduces sodium excretion in response to high-salt diets.<sup>32</sup> Our data show that overexpression of corin increases physiologic responses to pro-ANP, increases cGMP levels, and reduces fluid retention in
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 (Natrecon/Nesiritide) therapy in HF patients is controversial, and a large-scale clinical trial showed no significant effect 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 KitW-sh/W-sh mice developed rapidly progressive cardiac dilatation 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 where enhanced expression 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
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.

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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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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: ctggaaggttggagag and acgctctctgtcctctca for corin; tcatagaggggtctcc and gctttggaaggggtgta for BNP; cagctctggtggtccagaga and cccatcctctacggtcctc for ANP; ggaggctggacggtttaa and gcatctccactctgtggtt for CREB; catggtcagttgctcc and gcagtctgacgttagt for collagen 1; gcagctctcctcagctc for TGFbeta 1; gcagctgacctcatac and gctgtgccacagttg for MMP-9; cggagcatctcttcttcg and cttgagccgcttcttctc for collagen 3; tggagcagacattta and cagcagcggtgtca for TGFbeta1; tgcctctctcctct for CMA1; gcgctctctctctc for CMA1; gcagcatctcagttg for MMP-9; cttgcctgctc and gctgtgccacagttg 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 described 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.). Immuno-
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. 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. 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). Immuno-precipitated 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).

**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. 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


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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 DCM<sup>c</sup> and DCM<sup>c</sup>, corin-Tg female, 14-15 weeks mice littermates (n=10-12 in each group).

Figure S5. Cardiac expression of ANP 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 S6. Cardiac expression of BNP 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<0.001.
Figure S7. cGMP level in plasmas of DCMc and DCMc, 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 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<0.01.

Figure S9. Cardiac expression of collagen III 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 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 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. \( p=0.42 \)

Figure S14. Heart LV internal dimensions, LVID for DCMc (n=6) and DCMc, corin-Tg (n=7). \( p=0.84 \).