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Salt-Sensitive Hypertension and Cardiac Hypertrophy in Transgenic Mice Expressing a Corin Variant Identified in African Americans

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Abstract

African Americans represent a high risk population for salt-sensitive hypertension and heart disease but the underlying mechanism remains unclear. Corin is a cardiac protease that regulates blood pressure by activating natriuretic peptides. A corin gene variant (T555I/Q568P) was identified in African Americans with hypertension and cardiac hypertrophy. In this study, we test the hypothesis that the corin variant contributes to the hypertensive and cardiac hypertrophic phenotype *in vivo*. Transgenic mice were generated to express wild-type or T555I/Q568P variant corin in the heart under the control of α -myosin heavy chain promoter. The mice were crossed into a corin knockout background to create KO/TgWT and KO/TgV mice that expressed WT or variant corin, respectively, in the heart. Functional studies showed that KO/TgV mice had significantly higher levels of pro-atrial natriuretic peptide in the heart compared with that in control KO/TgWT mice, indicating that the corin variant was defective in processing natriuretic peptides *in vivo*. By radiotelemetry, corin KO/TgV mice were found to have hypertension that was sensitive to dietary salt loading. The mice also developed cardiac hypertrophy at 12–14 months of age when fed a normal salt diet or at a younger age when fed a high salt diet. The phenotype of salt-sensitive hypertension and cardiac hypertrophy in KO/TgV mice closely resembles the pathological findings in African Americans who carry the corin variant. The results indicate that corin defects may represent an important mechanism in salt-sensitive hypertension and cardiac hypertrophy in African Americans.

Keywords

cardiac hypertrophy; corin; natriuretic peptide; mouse models; salt-sensitive hypertension

Hypertension is a major risk factor for cardiovascular disease such as stroke and myocardial infarction. The prevalence of hypertension is particularly high in African Americans, but the underlying mechanism is unclear.^{1, 2} Environmental, socioeconomic and genetic factors may all contribute to the disease.^{3–6} Genome-wide linkage analyses indicate several chromosomal loci that may influence blood pressure in African Americans.^{7, 8} Genetic

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Disclosures

None.

variants in enzymes in epinephrine synthesis and the renin-angiotensin-aldosterone system also are associated with hypertension in this population.^{9–11}

Natriuretic peptides are important for maintaining salt-water balance and normal blood pressure.¹² Corin is a serine protease highly expressed in cardiac myocytes.^{13, 14} It activates natriuretic peptides, thereby regulating blood pressure and cardiac function.^{15, 16} In mice, corin deficiency prevented atrial natriuretic peptide (ANP) activation and caused salt-sensitive hypertension.^{17, 18} Corin-deficient mice had cardiac hypertrophy and poor cardiac function.^{17, 19, 20}

Single nucleotide polymorphisms (SNPs) (T555I/Q568P) in the *CORIN* gene were identified in African Americans with hypertension and cardiac hypertrophy.²¹ These SNPs are located in exon 12 of a minor *CORIN* allele that is more common in African Americans than Caucasians (~12% vs. <0.2% with one or two copies of the allele).^{21, 22} In patients with heart failure, individuals with this minor *CORIN* allele had impaired natriuretic peptide processing and worse clinical outcomes compared with those without this allele.²³ Biochemical studies showed that recombinant corin variant T555I/Q568P had a reduced biological activity, indicating that the SNPs may alter corin protein structure and function.²⁴ The results suggested that corin variant T555I/Q568P may contribute to hypertension and cardiac hypertrophy in African Americans.

To test this hypothesis, we generated transgenic (Tg) mice expressing the corin variant in a corin null background and examined corin variant function *in vivo* and its effect on blood pressure and cardiac morphology. Here we report that the Tg mice had impaired ANP processing in the heart and developed hypertension and cardiac hypertrophy, a phenotype similar to that in African Americans with the *CORIN* variant allele. Our results indicate that defects in the corin-ANP pathway may be an important contributing factor in hypertension and heart disease in humans, especially in African Americans.

Methods

Generation of Tg mice

Plasmid encoding mouse corin variant T623I/Q636P, corresponding to human corin variant T555I/Q568P, was made by mutagenesis. To generate Tg mice with heart specific corin expression, corin wild-type (WT) and variant cDNAs were inserted into a plasmid driven by the mouse α -myosin heavy chain (MHC) promoter (Supplemental Figure S1A).²⁵ The plasmids were used for pronuclear microinjection to produce Tg mice, which were crossed with corin knockout (KO) mice to generate KO/Tg mice expressing WT or variant corin in the heart in a null background. Heterozygous mice with one null allele and one WT or variant transgene allele were studied. The animal procedures were approved by the IACUC of the Cleveland Clinic. Detailed methods for making Tg mice are described in Online Supplemental Methods.

Western Blotting and ELISA

To analyze corin protein in hearts, tissues were homogenized in a buffer containing 50 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl, 1% Triton X-100 (vol/vol), and a protease inhibitor cocktail (1:100 dilution, Sigma). Proteins were analyzed by SDS-PAGE and Western blotting with a polyclonal antibody (Berlex Biosciences).²⁶ Cardiac pro-ANP expression was analyzed by Western blotting with a polyclonal antibody (Santa Cruz). Plasma levels of N-terminal (NT)-pro-ANP were measured by ELISA (Alpco Diagnostics).

Heart Membrane Fractions and Pro-ANP Processing Assay

Cell membrane fractions from hearts were prepared by ultracentrifugation.²⁶ Cell membrane pellets were resuspended in an NP-40 buffer and protein concentrations were determined by a Bradford method (Bio-Rad). Recombinant pro-ANP from transfected HEK293 cells was added to the heart membranes and incubated at 37°C over time. Pro-ANP conversion to ANP was analyzed by immunoprecipitation and Western blotting.^{27, 28} Detailed methods are described in Online Supplemental Methods.

Blood Pressure Measurement

Blood pressure was monitored continuously by radiotelemetry in conscious and unrestrained mice.²⁵ Detailed methods for radiotelemetry are described in Online Supplemental Methods.

Effects of Dietary Salt on Blood Pressure

Mice were fed normal (0.3% NaCl), or high (4% and 8% NaCl) salt diets (Harlan Teklad) for three weeks. Blood pressure was monitored by radiotelemetry before, during and after different salt diets.

Histological Analysis of Hearts

Hearts were isolated, weighed and fixed with 10% formalin. Longitudinal and transversal sections (5 µm in thickness) were stained with hematoxylin and eosin (H&E). Computer-assisted measurement (Measure IT, Olympus) at a high magnification (×400) was used to determine the diameter of ~100 individual cardiac myocytes in 5 randomly selected fields in left ventricular (LV) sections. The analysis was done in a blind fashion.

Statistical Analysis

Data were analyzed using the Prism software (Graph-Pad) and presented as means ± SD. Comparisons between two groups were made using the Student's *t* test. Three or more groups were compared using ANOVA followed by *post hoc* least significant difference. A *P* value of <0.05 was considered to be statistically significant.

Results

Generation of Corin Tg Mice

To test corin variant function *in vivo*, we generated Tg mice with cardiac-specific expression of the corin variant and WT control. Transgene copy numbers in founder lines were determined by Southern blotting (Supplemental Figure S1B). WT and corin variant founders with similar transgene copy numbers were selected to cross with corin KO mice to create KO/Tg mice expressing WT or corin variant (V) in the heart in a null background (Supplemental Figure S1C). The tissue specificity of Tg corin expression was verified by RT-PCR (Supplemental Figure S1D). Similar levels of heart-specific corin protein expression were confirmed by Western analysis (Supplemental Figure S1E and F).

Impaired Pro-ANP Processing in Corin KO/TgV Mice

Previously, corin variant T555I/Q568P was found to have a reduced pro-ANP processing activity in cell-based assays.²⁴ To determine if the variant had an impaired activity *in vivo*, pro-ANP levels in hearts from KO/TgWT and KO/TgV mice were analyzed by Western blotting. An ~20-kDa band, representing pro-ANP, was found to be stronger in intensity in corin KO and KO/TgV mice compared to that in WT and KO/TgWT mice (Figure 1A). Quantitatively, pro-ANP levels in WT and KO/TgWT mice were comparable (*p*>0.05, n=6), whereas the levels in corin KO and KO/TgV mice were ~3-fold higher than that of WT or

KO/TgWT mice ($p < 0.01$, $n = 6$) (Figure 1B). On Western blots, ANP was not detected, indicating that it was secreted from the heart once activated from pro-ANP.

By ELISA, plasma NT-pro-ANP was measured. The levels in WT mice were 1.13 ± 0.58 nmol/L ($n = 11$) but undetectable in KO mice ($n = 8$) (Figure 1C). In corin KO/TgV mice, the levels were significantly lower than that of WT and KO/TgWT mice (0.57 ± 0.35 vs. 1.13 ± 0.58 and 1.33 ± 0.87 nmol/L, respectively; both p values < 0.05 , $n = 8-10$). There was no significant difference between WT and KO/TgWT mice (Figure 1C). The results indicated that detected plasma NT-pro-ANP represented cleaved N-terminal pro-ANP fragments and that pro-ANP processing was impaired in corin KO/TgV mice.

Pro-ANP Processing by Heart Membranes

Corin is a membrane protein.^{28, 29} To determine corin activity in hearts, we prepared heart membrane fractions from Tg mice and measured pro-ANP processing activity. The activity was detected in a time-dependent manner in WT but not KO mice (Figure 2A and B). A similar activity was observed in KO/TgWT mice (Figure 2C). The activity was significantly reduced in KO/TgV mice (Figure 2D and E).

Hypertension in Corin KO/TgV Mice

Corin variant T555I/Q568P was associated with hypertension and cardiac hypertrophy in African Americans.^{21, 30} We measured blood pressure in corin KO/TgWT and KO/TgV mice. On a normal salt (0.3% NaCl) diet, corin KO mice had higher blood pressures than WT mice [systolic (SBP) 124 ± 4 vs. 112 ± 3 mmHg, $p < 0.01$; diastolic (DBP) 91 ± 4 vs. 83 ± 3 mmHg, $p < 0.01$] (Figure 3). In KO/TgWT mice, both SBP and DBP were restored to normal levels (SBP 113 ± 4 vs. 112 ± 3 mmHg in WT; DBP 83 ± 3 vs. 83 ± 3 mmHg in WT, both p values > 0.05) (Figure 3). In KO/TgV mice, blood pressures remained high (SBP 121 ± 4 mmHg; DBP 88 ± 2 mmHg, $p < 0.01$ vs. WT or KO/TgWT) (Figure 3).

Salt-sensitive Hypertension in Corin KO/TgV Mice

We next tested effects of high-salt diets on blood pressure. On a 4% NaCl diet, SBP in KO/TgV mice increased within a week from 121 ± 2 to 129 ± 1 mmHg ($p < 0.01$) (Figure 4A). Similarly, DBP also increased in these mice (data not shown). Similar salt-sensitive hypertension occurred in corin KO mice (Figure 4A). In contrast, blood pressure did not increase significantly in WT or KO/TgWT mice (Figure 4A). When the mice were switched to the normal salt diet, blood pressure in KO and KO/TgV mice remained high for 2 more weeks (Figure 4A).

When the mice were fed with an 8% NaCl diet, blood pressure in KO/TgV mice increased further (SBP from 120 ± 2 to 137 ± 10 mmHg, $p < 0.01$), which was similar to that in KO mice (Figure 4B). On this high salt diet, blood pressure in WT and KO/TgWT mice also increased (Figure 4B). When the mice were switched to the normal salt diet, blood pressure in WT and KO/TgWT mice quickly returned to normal levels whereas that in KO and KO/TgV mice remained high for 3–4 weeks (Figure 4B).

Cardiac Hypertrophy in Corin KO/TgV Mice

Previous studies showed that corin KO mice developed cardiac hypertrophy at ~12 months of age.¹⁷ Consistently, no apparent cardiac hypertrophy was observed in 4-month old KO/TgV mice (Figure 5A). LV wall thickness increased significantly by 12–14-months in these mice (Figure 5B). Such a change was not observed in KO/TgWT mice (Figure 5B). In 12–14-month old KO/TgV mice, LV muscle fibers were much thicker with an average diameter of 20.1 ± 1.5 μ m, significantly greater than that in KO/TgWT mice (14.6 ± 1.3 μ m, $p < 0.01$) (Figure 5B). The ratio of heart weight to body weight or tibia length was significantly

greater in 12–14-month old KO/TgV mice compared to that in KO/TgWT mice of similar age (Supplemental Figure S2A and B).

We next tested the effect of high salt diet on cardiac hypertrophy. When 4-month old WT, KO, KO/TgWT and KO/TgV mice, which did not have LV hypertrophy, were fed with an 8% NaCl diet for 3 weeks, LV wall thickness and ratio of heart weight to body weight or tibia length all increased in KO and KO/TgV mice (Figure 6A–D and Supplemental Figure S3A–B). In contrast, these changes were not observed in WT and KO/TgWT mice (Figure 6A–D and Supplemental Figure S3A–B).

Discussion

Hypertension occurs in all ethnic groups but its high prevalence in African Americans is striking.^{1, 2} Corin is a cardiac protease that regulates blood pressure. Genetic studies have identified corin variant T555I/Q568P in African Americans who had hypertension and cardiac hypertrophy.^{21, 30} In biochemical studies, recombinant corin variant T555I/Q568P had an impaired natriuretic peptide processing activity,²⁴ suggesting that genetic variations in the *CORIN* gene may reduce corin activity *in vivo*, thereby contributing to hypertension in African Americans.

Mouse models are useful tools to study human genetic variants.^{31, 32} To test our hypothesis, we generated KO/TgWT and KO/TgV mice that had comparable cardiac corin levels in a corin null background. We found similarly low levels of pro-ANP in hearts from WT and KO/TgWT mice, whereas the levels were much higher in corin KO and KO/TgV mice (Figure 1A and B). Consistently, comparable plasma levels of NT-pro-ANP fragments were detected in WT and KO/TgWT mice, whereas the levels were low in KO/TgV mice and undetectable in KO mice (Figure 1C). The results show that pro-ANP processing was restored in the heart in KO/TgWT but not KO/TgV mice, supporting that the corin variant was defective *in vivo*.

In our previous *in vitro* studies,²⁴ the corin variant exhibited impaired zymogen activation and hence reduced activity. Because of lacking a suitable antibody that recognizes the activated corin protease fragment, we were unable to directly determine corin zymogen activation in mouse hearts. To circumvent this problem, we developed an assay measuring corin activity in heart membrane fractions. The results showed that corin activity was significantly lower in KO/TgV mice than that in KO/TgWT mice (Figure 2). Since corin protein levels were similar in KO/TgWT and KO/TgV mouse hearts (Supplemental Figure S1E and F), reduced corin activity in KO/TgV mouse hearts was probably due to impaired corin zymogen activation, consistent with our previous *in vitro* findings. Recent studies indicated that impaired corin zymogen activation and reduced corin activity may be important in the pathogenesis of heart failure in patients.^{26, 33, 34}

Corin is essential for maintaining normal blood pressure.³⁵ We found elevated SBP and DBP in corin KO/TgV mice (Figure 3). Moreover, blood pressure in KO/TgV mice was highly sensitive to dietary salt loading, a phenotype similar to that of corin KO mice (Figure 4). Recent studies in mice showed that corin deficiency caused sodium retention in an ENaC-dependent mechanism, which may underlie salt-sensitive hypertension.^{17, 18, 36, 37} African Americans are known for high prevalence of salt-sensitive hypertension.^{3–5} Population studies show that corin variant T555I/Q568P allele was more common in African Americans than Caucasians.²¹ It is possible, therefore, that the corin variant may contribute to high prevalence of salt-sensitive hypertension in African Americans.

Natriuretic peptides are shown to have a direct anti-hypertrophic function in the heart.^{38–40} Mice lacking either corin or ANP developed cardiac hypertrophy.^{17, 19, 20, 41} In African

Americans, corin variant T555I/Q568P was associated with severe cardiac hypertrophy.³⁰ In this study, we showed that mice carrying the corin variant developed significant cardiac hypertrophy either at an older age when on a normal salt diet or at a younger age when on a high salt diet (Figures 5 and 6). Thus, the results from our mouse model studies helped to establish a link between the corin variant and the cardiac phenotype *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Perspectives

The results from this study showed that Tg mice expressing the corin variant identified in African Americans developed hypertension and cardiac hypertrophy. The phenotype mimics the clinical features in African Americans who carry the *CORIN* variant allele. The results provide direct experimental evidence that this *CORIN* allele is defective *in vivo*, suggesting that the corin variant may contribute to hypertension and heart disease in African Americans. Previously, SNPs in the genes coding for ANP or its receptor also were reported in patients with hypertension and cardiac hypertrophy.^{42, 43} Together, these data suggest that defects in the corin-ANP pathway may be an important mechanism in hypertension and cardiac hypertrophy in patients. Most recently, corin and ANP have been found to act locally in the pregnant uterus to regulate spiral artery remodeling, which is critical for preventing pregnancy-induced hypertension.²⁵ Our findings should encourage more genetic studies to determine if additional corin gene variants or mutations may play a role in hypertensive disease in patients.

Novelty and Significance

What Is New?

- This study shows that Tg mice expressing the corin variant identified in African Americans developed salt-sensitive hypertension and cardiac hypertrophy.
- The data provide direct experimental evidence that this *CORIN* variant allele is defective *in vivo*.

What Is Relevant?

- African Americans are a high risk population for salt-sensitive hypertension and heart disease.
- The *CORIN* variant gene allele is associated with African Americans with hypertension and cardiac hypertrophy but its contribution to the disease was unknown.

Summary

These data indicate that corin gene defects may be an important mechanism in salt-sensitive hypertension and cardiac hypertrophy in patients, especially in African Americans.

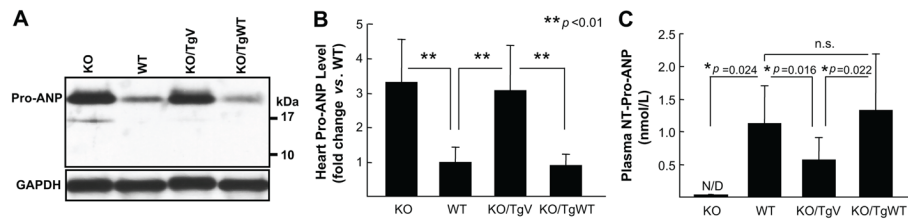


Figure 1. Pro-ANP expression and processing

(A) Western analysis of pro-ANP in hearts from corin KO, WT, KO/TgWT and KO/TgV mice. (B) Quantitative data of Western blots from 6 independent experiments. (C) Plasma levels of NT-pro-ANP in corin KO, WT, KO/TgWT and KO/TgV mice. n = 8 per group. N/D, not detectable; n.s., not significant.

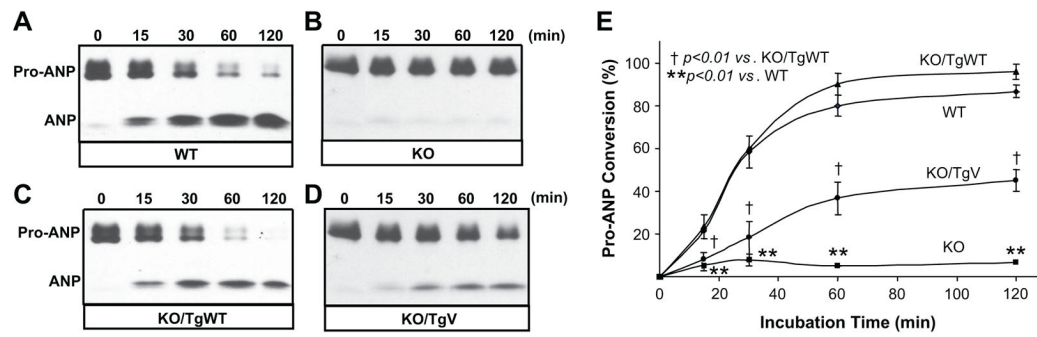


Figure 2. Pro-ANP processing activity in Tg mouse hearts

(A–D) Pro-ANP processing activity was assayed using membrane fractions from corin WT, KO, KO/TgWT and KO/TgV mouse hearts. Conversion of pro-ANP to ANP was analyzed by immunoprecipitation and Western blotting. (E) Quantitative data of Western blots from 3 independent experiments.

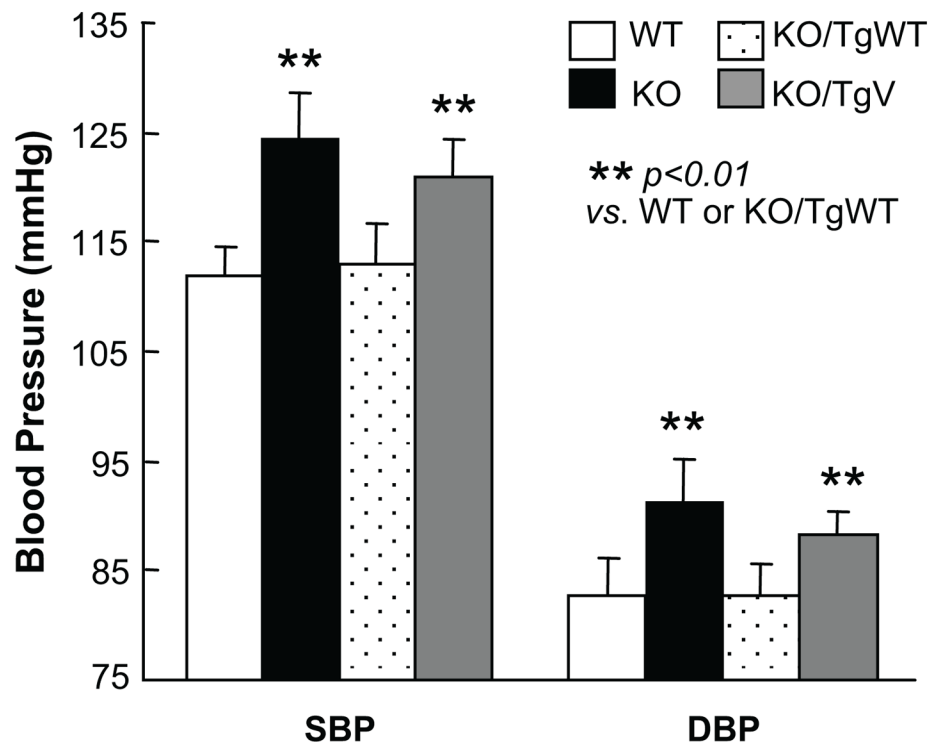


Figure 3. Hypertension in corin KO/TgV mice

SBP and DBP in Tg mice on a normal salt (0.3% NaCl) diet were measured by radiotelemetry. Data are mean \pm SD from at least 6 mice per group. ** $p < 0.01$ vs. WT or KO/TgWT mice by two-way ANOVA.

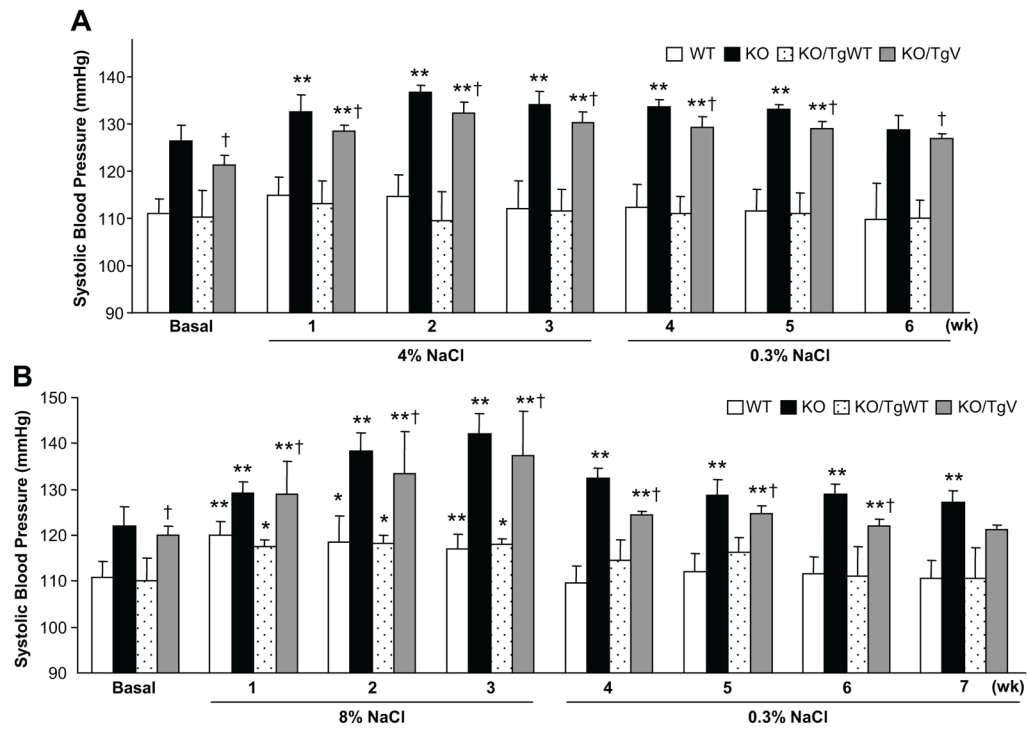


Figure 4. Salt-sensitive hypertension in corin KO/TgV mice

(A) Tg mice on a 0.3% NaCl diet (basal) were switched to a 4% NaCl diet for 3 weeks (wk) and then back to the 0.3% NaCl diet. SBP data from 6–10 mice per group are shown. Corin WT and KO mice were included as controls. (B) Similar studies were conducted in the Tg mice on an 8% NaCl diet. SBP data from 4–6 mice per group are shown. * $p < 0.05$ or ** $p < 0.01$ vs. basal of the same genotype; † $p < 0.01$ vs. KO/TgWT of the same group by two-way ANOVA.

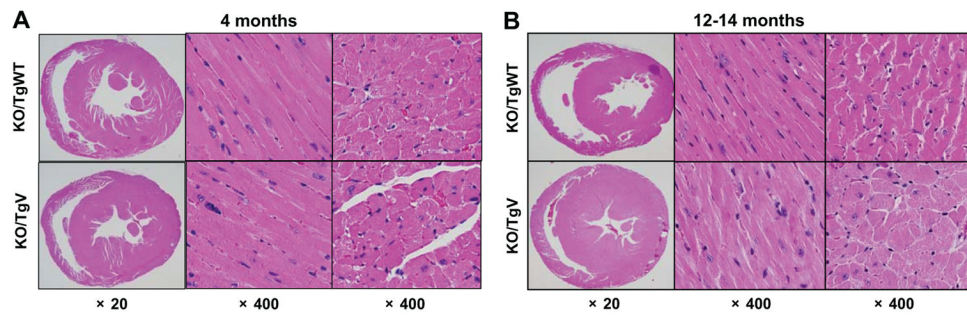


Figure 5. Cardiac hypertrophy in corin KO/TgV mice on normal salt diet
H&E-stained heart sections from corin KO/TgWT and KO/TgV mice at 4-months (**A**) or 12–14-months (**B**) of age on a 0.3% NaCl diet were shown at low ($\times 20$) and high ($\times 400$) magnifications.

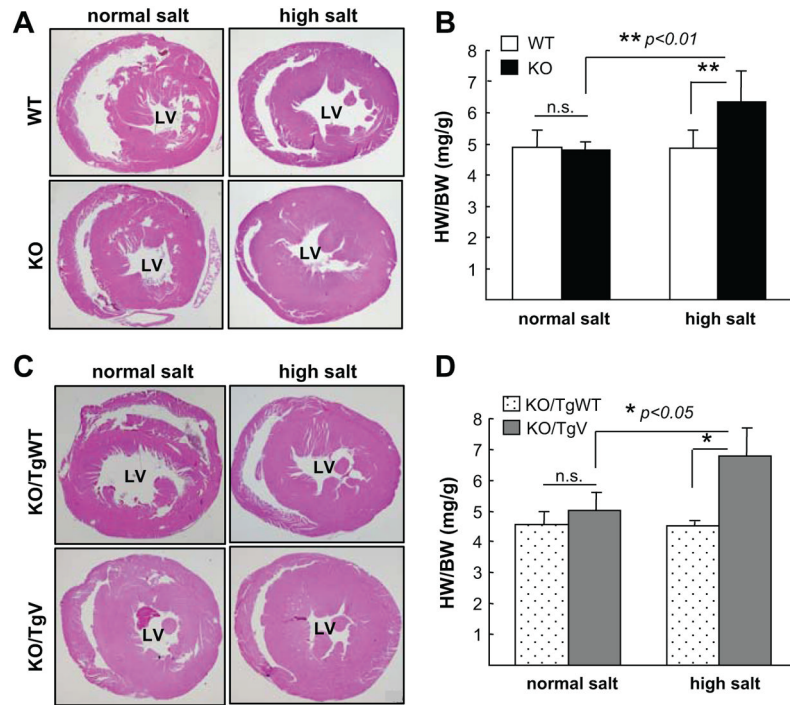


Figure 6. Cardiac hypertrophy in corin KO/TgV mice on high salt diet
H&E-stained heart sections from corin WT and KO (A) or KO/TgWT and KO/TgV (C) mice at 4-months of age on normal and 8% NaCl diets were shown at a lower ($\times 20$) magnification. Ratios of heart weight (HW) to body weight (BW) (B, D) were calculated from 8–10 mice per group. n.s., not significant.