Online Supplemental Data

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

Wei Wang, Yujie Cui, Jianzhong Shen, Jingjing Jiang, Shenghan Chen, Jianhao Peng, Qingyu Wu

From the Departments of Molecular Cardiology, Nephrology and Hypertension (W.W., Y.C., J.S., J.J., S.C., J.P., Q.W.), Lerner Research Institute, Cleveland Clinic, Cleveland, OH; the Cyrus Tang Hematology Center (Q.W.), Jiangsu Institute of Hematology, First Affiliated Hospital, Soochow University, Suzhou, China

Current address: Department of Cardiology, Peking Union Medical College, Beijing (W.W., J.S.)

Running Title: Transgenic Mice Expressing Corin Variant

Corresponding to: Qingyu Wu, Cleveland Clinic, 9500 Euclid Ave., Cleveland 44195, OH; Tel: 216-444-4351; Fax: 216-445-8204; Email: wuq@ccf.org

Supplemental Methods

Generation of Tg mice

Plasmid expressing mouse corin variant T623I/Q636P was made by mutagenesis using mouse wild-type (WT) corin plasmid as a template. Corin WT and variant cDNAs were inserted into a plasmid with the mouse α-myosin heavy chain (MHC) promoter and a 3' human growth hormone poly(A) site (Figure S1A).[1, 2] 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.

Tg founder mice were analyzed by Southern blotting. Genomic DNA was extracted from tissues using the DNeasy kit (Qiagen), digested with *Hin*dIII endonuclease, separated in agarose gels and transferred onto nylon membranes, which were hybridized with a digoxigenin-dUTP-labeled probe. The transgene copy number was estimated by comparing with copy number standards.

To examine tissue specific transgene expression, total RNAs were isolated from tissues using TRIzol reagents (Invitrogen) to synthesize first strand cDNAs by SuperScript III reverse transcriptase (Invitrogen). RT-PCR was done using oligonucleotide primers specific for the corin transgene: sense 5'-AAG CCT ATC CCT AAC CCT CTC-3' and antisense 5'-ACA GGA ATA ACA CCA GGC ACT C-3'. Primers for the mouse β-actin gene were used as controls. PCR products were analyzed on 1% agarose gels.

Pro-ANP Processing Assay

Plasmid expressing human pro-ANP was transfected in HEK cells using Lipofectamine 2000 (Invitrogen), as described previously.[3] Cells were cultured at 37°C for 24-48 h. Conditioned medium containing recombinant human pro-ANP was collected, added to the heart membranes, and incubated at 37°C over time. Pro-ANP and ANP in the medium were analyzed by immunoprecipitation and Western blotting. Western blots were developed using enhanced chemiluminescent (ECL) reagents (Denville Scientific) and exposed to X-ray films. The optical density of bands representing pro-ANP and ANP was measured by densitometry, and the

percentage of pro-ANP to ANP conversion was calculated using computer software (Bio-Rad), as described previously.[4]

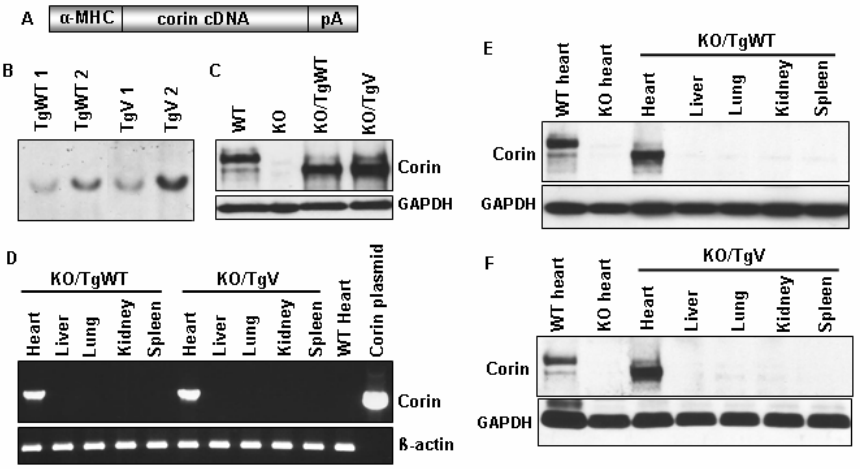
Blood Pressure Measurement

Blood pressure was monitored by radiotelemetry in conscious and unrestrained mice.[1] Mice were anesthetized with ketamine and xylazine on a 37°C warming pad. A TA11PA-C10 telemetry device (Data Science International) was inserted into the left common carotid artery under a microscope. After the surgery, mice were singly caged and fed with standard diet and water *ad libitum* for ~7 days. Blood pressure was recorded by telemetry receivers (model RPC-1) and the Dataquest System (Data Science International).

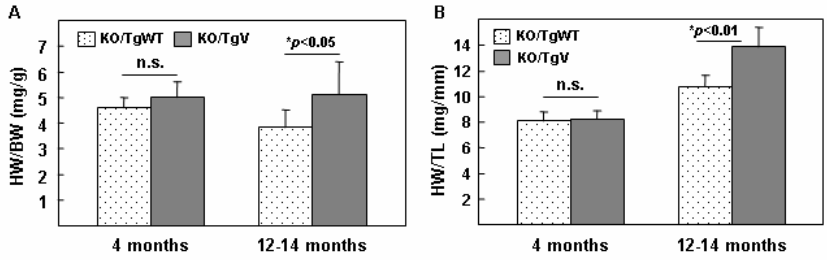
Supplemental References

1. Cui Y, Wang W, Dong N, Lou J, Srinivasan DK, Cheng W, Huang X, Liu M, Fang C, Peng J, Chen S, Wu S, Liu Z, Dong L, Zhou Y, Wu Q. Role of corin in trophoblast invasion and uterine spiral artery remodelling in pregnancy. *Nature.* 2012;484:246-250.
2. Robbins J. Remodeling the cardiac sarcomere using transgenesis. *Annu Rev Physiol.* 2000;62:261-287.
3. Yan W, Wu F, Morser J, Wu Q. Corin, a transmembrane cardiac serine protease, acts as a pro-atrial natriuretic peptide-converting enzyme. *Proc Natl Acad Sci U.S.A.* 2000;97:8525-8529.
4. Qi X, Jiang J, Zhu M, Wu Q. Human corin isoforms with different cytoplasmic tails that alter cell surface targeting. *J Biol Chem.* 2011;286:20963-20969.

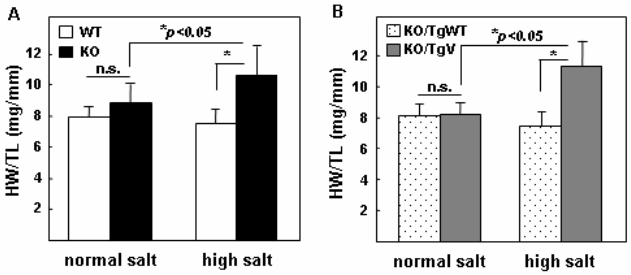
Supplemental Figures and Legends



Supplemental Figure S1. Generation of corin Tg mice. (**A**) Plasmid expressing mouse corin transgene contained a 5' α-MHC promoter and a 3' human growth hormone poly (A) (pA) site. (**B**) Southern analysis of corin transgene in founder mice. WT and corin variant founders with similar transgene copy numbers (TgWT1 and TgV1) were selected to cross with corin KO mice. (**C**) Western analysis of corin protein in hearts from corin WT, KO, KO/TgWT and KO/TgV mice. (**D**) RT-PCR analysis of specific corin transgene expression in the heart. Negative (WT heart) and positive (corin plasmid) controls and β-actin control were included. (**E**, **F**) Western analysis of WT and variant corin proteins in hearts from KO/TgWT and KO/TgV mice. GAPDH control was included. On Western blots, recombinant WT and corin variant migrated slightly faster than endogenous corin (**E**, **F**) due to differences in protein glycosylation (data not shown).



Supplemental Figure S2. **Cardiac hypertrophy in corin KO/TgV mice.** Ratios of heart weight (HW) to body weight (BW) (**A**) or tibia length (TL) (**B**) were calculated in KO/TgWT and KO/TgV mice on a normal salt diet at 4 and 12-14 months of age. Data were from 8-10 mice per group. n.s., not significant.



Supplemental Figure S3. Cardiac hypertrophy in corin KO/TgV mice on high salt diet. Corin WT, KO, KO/TgWT and KO/TgV mice at 4-months of age were on normal or 8% NaCl diets. Ratios of heart weight (HW) to tibia length (TL) (**A, B**) were calculated from 8-10 mice per group. n.s., not significant.