



# Conditional cardiomyocyte-restricted Corin KO mice demonstrate increased cardiac hypertrophy and pro-fibrotic gene activation in response to TAC and PL-3994-a novel selective GC-A receptor peptide agonist rescues the phenotype

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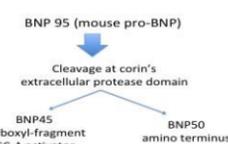
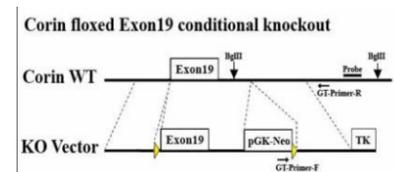
## INTRODUCTION

Corin is a type II trans-membrane serine protease expressed by cardiomyocytes capable in vitro of proANP/proBNP cleavage activation. In vitro, unprocessed proBNP demonstrates reduced binding to and activation of its cognate receptor, guanylate-cyclase A (GC-A). The loss-of function Corin I555(P568) haplotype carried in the heterozygous state by ~13% of the U.S. Black population and is associated with an increased risk for left ventricular hypertrophy (LVH) after adjusting for differences in blood pressure, suggesting that genetic Corin deficiency enhances the cardiac hypertrophic response to pressure-overload. However, these were cross-sectional data and therefore we could not exclude the possibility that association of the Corin I555(P568) haplotype with risk for LVH was caused by residual confounding from a greater severity or duration of antecedent HTN in the Corin variant group. Global Corin knockout (KO) mice have higher blood pressure than controls and develop LVH, creating similar difficulties in interpretation. Thus, to directly test the hypothesis that genetic Corin moderates the cardiac response to equal magnitudes of pressure-overload, we developed a novel conditional, cardiomyocyte-restricted Corin KO mouse. Our hypothesis was that genetic Corin deficiency disrupts the autocrine/paracrine actions of the cardiac NPS that attenuate cardiac hypertrophy by reducing natriuretic peptide activation resulting in reduced GC-A activation.

## Methods

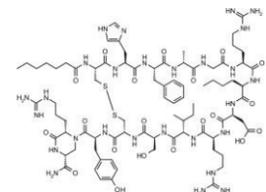
### Conditional Corin KO Mouse Construct

### Murine proBNP95 Processing by Corin



Corin exon 19<sup>fllox/fllox</sup> mice were crossed with Myh6-cre mice [129-Tg(Myh6-cre/Esr1)1Jmk/J] obtained from Jackson lab to produce Corin exon19<sup>fllox/fllox</sup>/ Myh6-Cre mice. This allows temporal gene deletion under control of tamoxifen to induce nuclear translocation and the  $\alpha$ -MHC promoter to achieve cardiomyocyte restriction of excision of lox P flanked DNA sequences.

**PL-3994:** This is a selective GC-A receptor peptide agonist. PL-3994, developed by Palatin Technologies Inc., Cranbury, NJ, is resistant to neutral endopeptidase degradation.



### Peptide GC-A Activator

### Compound PL-3994\*

**Pilot PL-3994 dosing experiments:** Demonstrated that PL-3994 infused via an osmotic infusion pump at 0.01 mg/kg/min resulted in minimal decreases in BP but significant increases in cardiac cGMP and activated protein kinase G (PKG) consistent with myocardial GC-A activation.

**Study Design:** Utilized a standard model of thoracic aortic banding (TAC).

**PL-3994 group:** exon19<sup>Fllox/Fllox</sup>/ Myh6-Cre male mice, 10 weeks age (N=10)

**Corin KO group:** exon19<sup>Fllox/Fllox</sup>/ Myh6-Cre male mice, 10 weeks age (N=10)

**WT (control) group:** exon19<sup>WT/WT</sup>/ Myh6-Cre male mice, 10 weeks age (N=10)

**Protocol:** 5 days prior to TAC surgery at age 10 weeks tamoxifen was administered IP for 5 consecutive days. This was immediately followed by TAC surgery and simultaneous placement of osmotic infusion pumps that infused PL-3994 at 0.01 mg/kg/min in the PL-3994 group, and saline in the other 2 groups. After 4 weeks, TAC echocardiograms were performed and mice were euthanized, hearts explanted and weighed and plasma collected for BNP measurements.

**Mouse BNP Processing:** We have developed and validated ELISAs that are specific for murine BNP45 and BNP95.

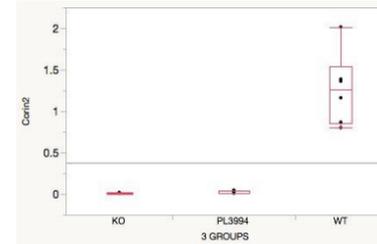
**cGMP-dependent Protein kinase G (PKG):** active PKG was determined by measuring the serine/threonine kinase activity of PKG in left-ventricular (LV) apical cardiac tissue.

**Gene expression:** RNA extracted from LV apex tissue was converted to cDNA. Real time PCR was performed using Taqman chemistry for genes of interest. Expression was compared to control mice of same genetic background not undergoing TAC and normalized to 18s ribosomal RNA.

**Statistics:** We employed **non-parametric statistical testing** throughout. Global difference in mean between 3 groups was compared using the non-parametric Wilcoxon test. When this global test was significant ( $p \leq 0.05$ ) we then utilized the Wilcoxon non-parametric comparison for each pair that accounts for multiple comparisons to reduce overall type I error to  $\leq 5\%$ .

## RESULTS

### A. Corin gene expression in 3 groups

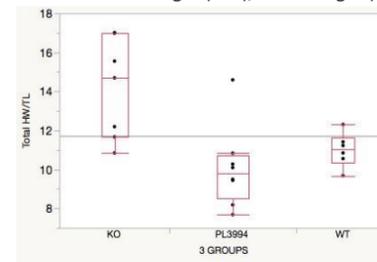


### Absent Corin protein (western) in KO hearts



### B. Increased cardiac hypertrophy in Corin KO compared to controls is rescued by PL-3994

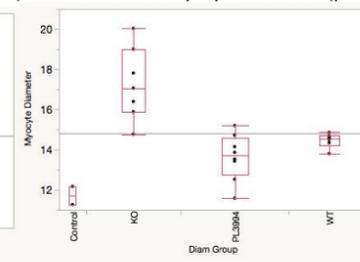
#### Total Heart Weight (HW)/Tibial Length (TL)



KO vs. WT,  $p = 0.03$ ; KO vs. PL-3994,  $p = 0.005$ ;

PL-3994 vs. WT,  $p = 0.08$

#### Mean Cardiomyocyte Diameter ( $\mu$ m)

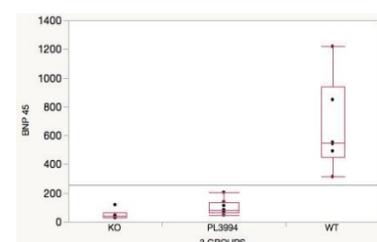


KO vs. WT,  $p = 0.005$ ; KO vs. PL-3994,  $p = 0.002$ ;

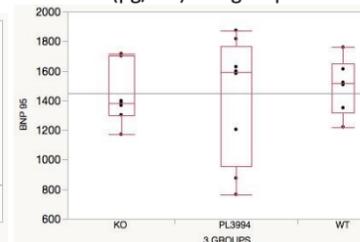
PL-3994 vs. WT,  $p = 0.17$

### C. Impaired ProBNP processing in Corin KO mice

#### Post 4-week TAC Plasma BNP45 and BNP95 levels (pg/mL) in 3 groups

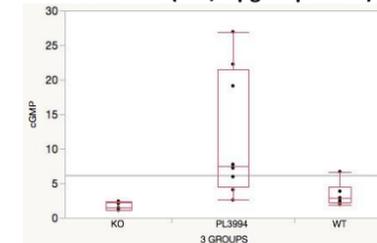


KO vs. WT,  $p = 0.03$ ; KO vs. PL-3994,  $p = 0.005$ ; PL-3994 vs. WT,  $p = 0.08$



Global Wilcoxon  $p = n.s.$

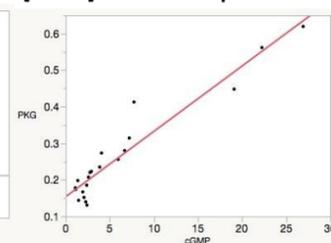
### D. Cardiac cGMP (nM/4 $\mu$ g LV protein)



KO vs. WT,  $p = 0.02$ ; KO vs. PL-3994,  $p = 0.002$ ;

PL-3994 vs. WT,  $p = 0.02$

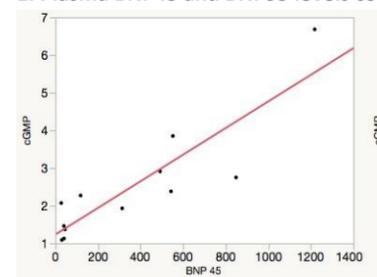
### [cGMP] vs. cGMP-dependent PKG activity



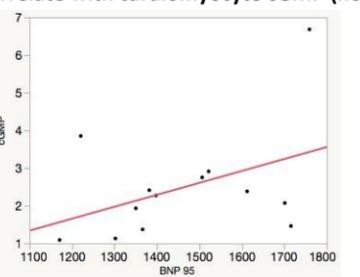
$R^2 = 0.92$ ;  $\beta$  coefficient  $p < 0.001$

(combined data for all 3 groups)

### E. Plasma BNP45 and BNP95 levels correlate with cardiomyocyte cGMP (no PL3994 hearts)



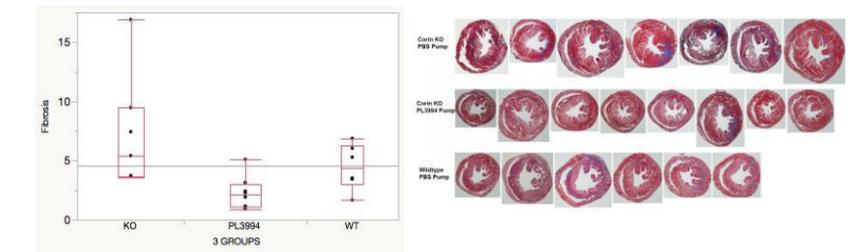
$R^2 = 0.78$  ( $\beta$  coefficient  $p = 0.0001$ )



$R^2 = 0.2$  ( $\beta$ -coefficient  $p = 0.21$ )

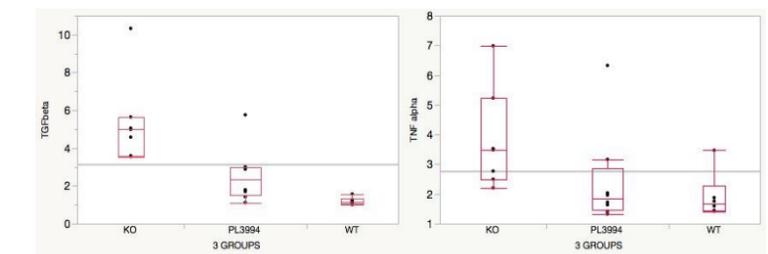
## RESULTS

### F. PL-3994 (4 week TAC protocol) demonstrates reductions in cardiac fibrosis



KO vs. WT  $p = 0.17$ ; KO vs. PL-3994  $p = 0.005$ ; PL-3994 vs. WT  $p = 0.03$

### G. TGF- $\beta$ and TNF- $\alpha$ gene expression in 3 groups

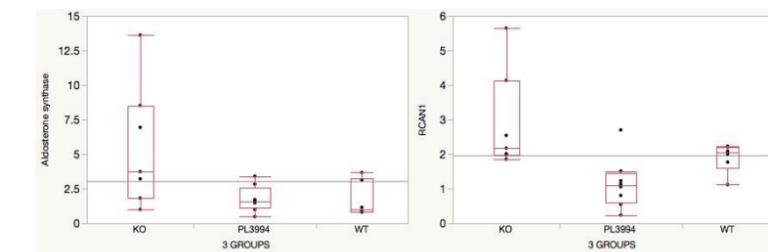


KO vs. WT,  $p = 0.01$ ; KO vs. PL-3994,  $p = 0.03$ ;

PL-3994 vs. WT,  $p = n.s.$

$p = n.s.$

### H. Aldosterone synthase and Regulator of Calcineurin 1 gene expression in 3 groups



KO vs. WT,  $p = 0.04$ ; KO vs. PL-3994,  $p = 0.04$

PL-3994 vs. WT,  $p = n.s.$

KO vs. WT,  $p = 0.28$ ; KO vs. PL-3994,  $p = 0.009$

PL-3994 vs. WT,  $p = 0.06$

\* BNP expression was significantly increased in the PL-3994 (4.9 fold), KO (6.2 fold) and WT (8.4 fold) but the ANOVA p-value for group differences was non-significant ( $p = 0.36$ ).

## CONCLUSION

1. Cardiomyocyte Corin deficiency increases cardiac hypertrophy and pro-fibrotic signaling pathways. Given the direction of pro-fibrotic gene expression changes a longer period of pressure-overload likely would have enhanced disparities in fibrosis (pathological hypertrophy) between Corin KO and control mice.
2. The molecular mechanism(s) whereby cardiomyocyte-restricted Corin deficiency disrupts the cardiac autocrine/paracrine that oppose cardiac hypertrophy and fibrosis is reduced GC-A activation resulting in part from impaired proBNP processing.
3. In vivo PL-3994 is a potent peptide activator of cardiac GC-A and significantly increases cardiomyocyte cGMP, activates cGMP-dependent PKG, and reduces pro-hypertrophic and pro-fibrotic signaling pathways. These pharmacological properties make it an attractive agent to provide cardiac protection from pathological remodeling in response to hypertension and a potentially novel pharmacogenomic approach to the 13% (~ 5.2 million) U.S Blacks heterozygous for the loss-of-function Corin I555(P568) haplotype, especially given the high prevalence of HTN and resistant HTN in the Black community.