Characterization Of Interventricular Septal Development and Perinatal Mortality in Mice Lacking Natriuretic Peptide Receptor A (NPRA)

DALHOUSIE UNIVERSITY

Cindy Liu, Supervisor: Dr. Kishore Pasumarthi
Department of Pharmacology, Dalhousie University, Halifax, NS, Canada

DALHOUSIE UNIVERSITY

Background

Natriuretic peptide receptor A (NPRA) is a high-affinity receptor for cardiac hormone **atrial natriuretic peptide (ANP),** and it is known to play an essential role in embryonic heart development and the maintenance of normal cardiac function.

Previous research has shown that NPRA Knockout (KO) mice exhibit **perinatal mortality**, and recent findings from our lab show that stillborn neonatal NPRA KO mice display a **ventricular septal defect** (VSD) phenotype.

VSD is the most prevalent type of congenital heart disease (CHD), which is the most common type of birth defect worldwide. Despite advancements in clinical interventions treating VSDs**, the cellular mechanism responsible for VSD are still unclear.**

Hypothesis: Loss of NPRA in the developing interventricular septum of neonatal mice **disrupts cardiac cell fate** and **proliferation**, **metabolic status,** leading to VSD, increased cardiac hypertrophy, fibrosis, and ultimately contributing to perinatal mortality and cardiac abnormalities.

Methods

Experimental animals: Neonatal NPRA-KO mice and WT mice as controls for the study.
Cryosection: Neonatal heart tissues were cryoprotected in 30% sucrose, embedded in OCT, and cryosectioned at 10 µm.
Immunohistochemistry: Heart cryosections were immunostained using primary and fluorescent secondary antibodies, counterstained with Hoechst nucleus stain, and imaged on a Leica TCS SP8 Confocal microscope.
Histology: Heart cryosections were stained following standard fixation, hematoxylin and eosin protocols. Fibrosis staining was also performed using Sirius red and fast green staining.
Statistical analysis: All datasets were presented as mean ± standard error of the mean. Between-group comparisons were performed using a two-tailed unpaired t-test or one-way analysis of variance (ANOVA). Significance for all statistical analyses was assigned at P<0.05.

Acknowledgements

DALHOUSIE UNIVERSITY FACULTY OF MEDICINE
nova scotia health
Heart&Stroke
QEII HEALTH SCIENCES CENTRE FOUNDATION

Results

- **NPRA KO stillborn neonatal mice show a VSD phenotype (Figure 1).**

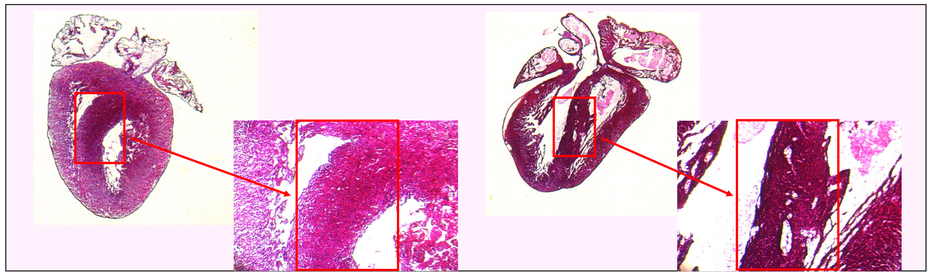
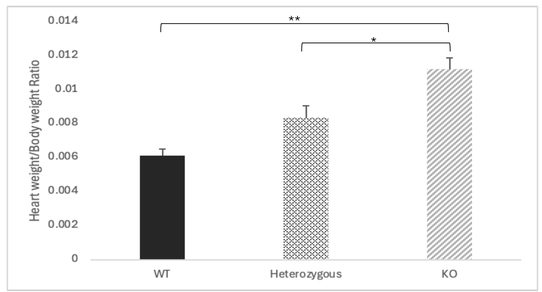


Figure 1. Histology H&E staining of neonatal heart sections (left- WT, right-KO)



- **Neonatal KO mice exhibit significantly higher heart/body weight ratios Vs. WT and heterozygous mice, suggesting cardiac hypertrophy in KO.**

Figure 2. Heart weight/body weight ratio in WT, heterozygous and KO mice. Values are mean ± SEM, *P<0.05, **P<0.005, n=3-6 mice per group, ANOVA, Turkey's HSD.

- **Cell proliferation immunostaining suggests reduced cell proliferation in the NPRA KO mice, with only minimal change in apoptosis.**

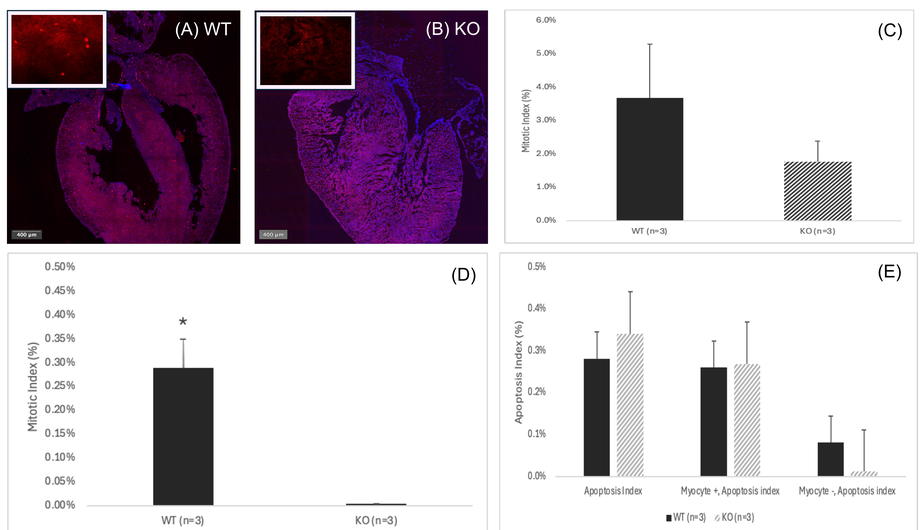


Figure 3. Quantification of cell proliferation and apoptosis levels: (A & B) Representative low and high-power images of phosphor-Histone H3 signal in KO and WT hearts; (C & D) Mitotic index (%) determined by immunostaining of phospho-Histone H3 (C) and Aurora Kinase B (D) in the septal cells of WT and KO hearts; (E) Apoptosis index for septal cardiomyocytes and non-myocytes populations determined by immunostaining for activated Caspase-3 and sarcomeric myosin staining. Values are mean ± SEM, *p<0.05, n=3 mice per group.

Results

- **Representative images suggest a notable reduction of metabolic marker p-PDH in KO hearts (Figure 4).**

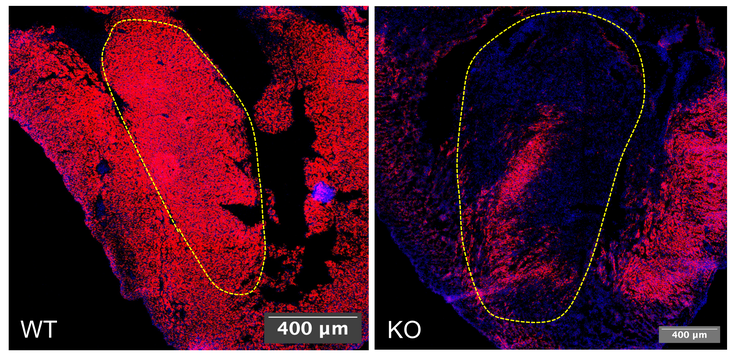
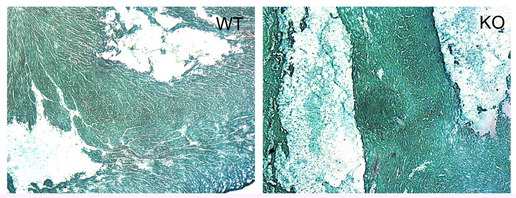


Figure 4. Immunolocalization of phospho-pyruvate dehydrogenase (p-PDH, red) merged with the Hoechst nuclear stain (blue) in the septum of the neonatal hearts.



- **Absence of collagen accumulation in the septal region of neonatal WT and KO hearts (Figure 5).**

Figure 5. Fibrosis staining (Sirius red and Fast green) of neonatal heart sections under 10x magnification (left– WT, right – KO)

Conclusions

- VSD in the neonatal stillborn NPRA KO shows septum malformation during cardiac development.
- KO mice exhibit a significantly higher heart weight/body weight ratio compared to WT (P= 0.001)
- Immunostaining experiments revealed a significant reduction in septal cardiomyocyte proliferation in KO Vs WT hearts, as assessed by Aurora kinase B (P= 0.04), with a clear trend in reduced phospho-histone H3 staining, and no significant changes in apoptosis levels.
- Additionally, immunostaining of metabolic marker p-PDH revealed a reduced signal in KO Vs WT.
- Histological fibrosis staining revealed no significant differences in collagen accumulation between the two groups.
- Future experiments will quantify P-PDH signal intensity to confirm metabolic alterations and increase the number of animals analyzed for Histone H3 to determine statistical significance.
- CHD remain the leading cause of mortality from birth defects worldwide. This work may help identify new molecular targets and point us towards potential drug targets.