A Novel *Pkp2* Mouse Model of Genetic Arrhythmogenic Right Ventricular Cardiomyopathy and Its Rescue by Gene Therapy

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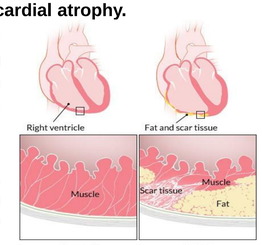
Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac disorder affecting 1 in 5000 individuals worldwide [5] and an estimated 70,000 patients in the U.S. Previous work has demonstrated that heterozygous mutations in the PKP2 gene, encoding plakophilin-2, are the most common cause of ARVC (Brenda Gerull, et al). Lack of plakophilin-2 degrades the structural integrity of the desmosomes, resulting in increased electrical instability, fibrofatty muscle replacement and myocardial atrophy.

PKP2-associated ARVC is a progressive condition whose symptoms include palpitations, lightheadedness, fainting and a decline in ventricular function. It typically presents in young adults (<40yr) and places patients at increased risk of sudden cardiac arrest [2-4].



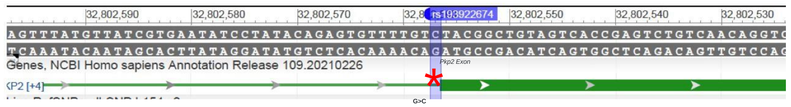
Our previous data has demonstrated that AAV:hPKP2 (mTN-401) has showed efficacy in our *Pkp*2 cKO mouse model [1]. But the *Pkp2* cKO model cannot truly represent the human condition in clinical practice, so we are trying to develop a new and better model to simulate clinical patients.

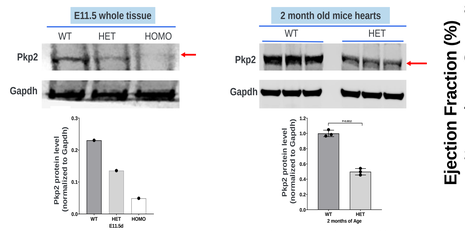
The current objective was to report the different approaches by work with three murine models: (1) A *PKP2* knock-in (KI) heterozygous (het) model of human gene with chow, (2) KI of human gene + high fat diet (HFD) induction model, (3) KI of human gene model crossed with *Pkp2* cKO mouse. The mechanisms of ARVC development and a potential ARVC treatment strategy using a novel mouse model have also been investigated.

Methodology

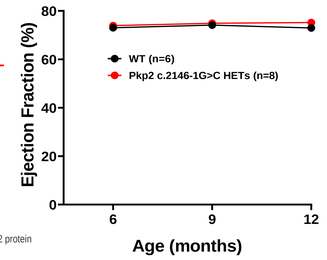
A knock-in (KI) mouse model with a point mutation corresponding to a human *PKP2* mutation, a disease-causing allele of c.2146-1G>C, was generated via CRISPR/Cas9 technology. Several approaches were investigated to trigger ARVC phenotypes in the *Pkp2* KI heterozygous (het) mice. First, we evaluated the heart function at different ages in response to regular chow. As a second approach, high fat diet (HFD) was used to induce the ARVC phenotype in the *Pkp2* KI het mice. The *Pkp2* KI het mice were crossed with a cardiac-specific *Pkp2* cKO mouse to generate a tamoxifen inducible model (KIxKO). Cardiac function was assessed by echocardiography and arrhythmia burden by electrocardiogram (ECG). Protein expression level was examined by Western Blot. The KIxKO animals received AAV:hPKP2 (mTN-401) via retro-orbital (RO) injection.

Model #1:*Pkp2* KI het mice on normal chow had slow and mild ARVC development



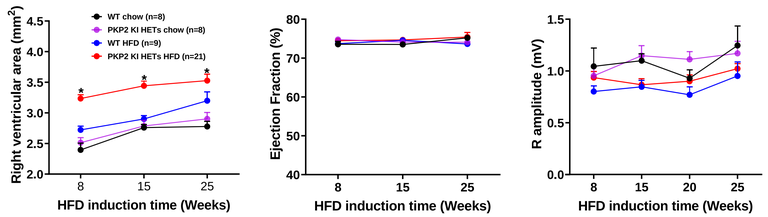


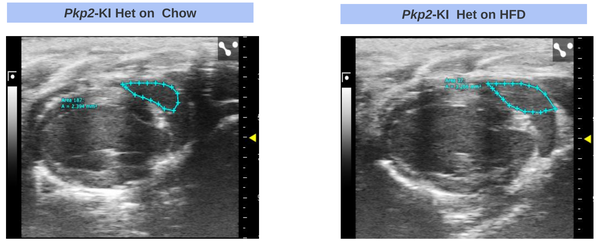
Heterozygous showed ~50% reduction of wildtype Pkp2 protein



- **Animals were fed with chow, no additional induction were performed; Then echo/ECG were performed monthly;**
- **No arrythmias or premature ventricular complex (PVCs) were observed at 12 months of age in regular chow feeding *Pkp2* KI het mice; mild increased right ventricular (RV) size was observed; no animals were found dead; 50% PKP2 protein decrease in *Pkp2* KI mice.**

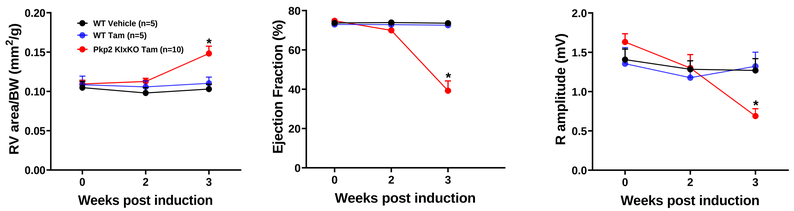
Model #2: HFD accelerated and worsened RV dilation in *Pkp2* KI het mice





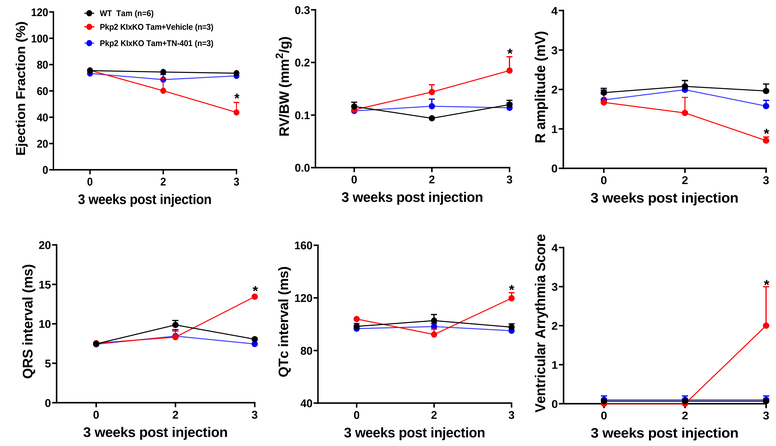
- ***Pkp2* KI het mice were induced with HFD from 8 weeks of age, echo/ECG performed monthly;**
- **8wks HFD induction hastened and worsened the RV dilation without affecting LV function, RV size was significantly increased compared with control;**
- **Some PVCs and arrythmias were observed in both of HFD induced Wild Type (WT) and *Pkp2 KI* het groups, but not enough to significantly differ between groups.**

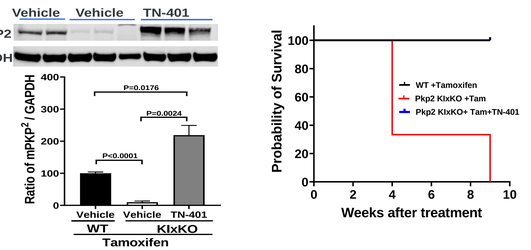
Model #3: *Pkp2* KIxKO mice rapidly exhibited a severe ARVC phenotype



- ***Pkp2* KI hets were bred with αMyHC-Cre ER(T2); *Pkp2* fl/fl mice to obtain αMyHC-Cre ER(T2);*Pkp2* fl/c.2146-1G>C pups (Pkp2 KIxKO mice);**
- **80 mg/kg tamoxifen induced the KIxKO mice starting from 8 wks of age; Echo/ECG were performed weekly;**
- **KIxKO mice exhibited >90% decrease in PKP2 protein expression and a correspondingly severe ARVC phenotype starting from 3 weeks Tamoxifen induction (*P<0.05);**
- **A high arrhythmia burden, RV area dilation, decreased LV ejection fraction and 100% mortality were observed (*P<0.05);**

mTN-401 gene replacement therapy prevented ARVC phenotypes in *Pkp2* KIxKO mice





Summary

- **Treatment with mTN-401 was shown to restore heart function in *Pkp2* KIxKO mice, including improved ejection fraction level and increased R amplitude; recovery of PKP2 protein expression to wild-type levels within 3 weeks following administration of gene therapy (*P<0.05);**
- **9 weeks following gene therapy treatment decreased RV size, QRS and QTc interval, arrythmia score and mortality with a stable durability (*P<0.05);**
- **The current results demonstrated that AAV:PKP2 gene therapy may be a promising therapeutic approach to treat ARVC patients with *PKP2* mutations resulting in haploinsufficiency;**
- **mTN-401, Tenaya's AAV9-based gene therapy for PKP2-associated ARVC is being advanced into clinical trials.**

References

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