SWACSM Abstract

Genetic drivers of cardiac remodeling in health and disease in female mice

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ABSTRACT

PURPOSE: Sex differences in cardiac metabolism and cardiometabolic disease susceptibility are well documented. However, the mechanisms underlying sexual dimorphism and the role estrogens play in cardiac physiology aren't well understood, especially in aging women when cardiometabolic disease susceptibility is heightened. The purpose of the current study was to determine key genetic drivers of healthy vs. pathogenic cardiac remodeling and determine the impact of estrogen action on cardiomyocellular function.

METHODS: The UCLA Exercise Hybrid Mouse Diversity Panel (ExcHMDP), comprised of ~100 strains of inbred mice, was leveraged to interrogate genetic drivers of cardiac remodeling in response to exercise training. Female mice from the ExcHMDP remained sedentary (SED) or preformed volitional exercise (TRN) by in cage wheel running (30d). Heart samples (4 SED and 4 TRN mice per strain) harvested following a 6h fast, 30h after the last bout of exercise, were subjected to RNA sequencing. A similar analysis was performed on hearts from 91 strains of female mice treated with the cardiac remodeling drug isoproterenol (ISO). Estrogen action related to cardiac remodeling was studied in female mice with a conditional cardiac-specific deletion of estrogen receptor alpha (encoded by Esr1). Integrated informatic assessment of these transcriptomic data sets identified pathways driving healthy versus pathogenic cardiac remodeling.

RESULTS: Heart weight was increased following exercise training in 85 of 100 strains studied. Cardiac enrichment analysis of differentially expressed transcripts and candidate gene identification analyses revealed 5 potential regulatory genes associated with healthy cardiac remodeling in response to exercise training. We contrasted these findings with the genetic architecture of two mouse models of cardiac hypertrophy-associated heart failure, the ISO-HMDP and cardiac-specific Esr1 knockout. Mitochondrial function and calcium homeostasis emerged as key pathways of regulation related to cardiac hypertrophy.

CONCLUSION: Our studies provide important insight into the genetic architecture and key genetic drivers of cardiac remodeling in females. The goal of our research is to identify cardiac-specific transcripts and pathways that can be targeted therapeutically to preserve cardiac function during aging in women.

Genetic Drivers of Cardiac Remodeling in Health and Disease in Female Mice

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BACKGROUND

Cardiovascular Disease (CVDs) impacts more than 17 million deaths annually, making CVDs the number one cause of mortality in both men and women worldwide[1]. Evidence suggests sex biasing of cardiac disease risk and outcomes, and estrogens are shown to play a role in protection against cardiac pathology. Importantly the incidence of cardiometabolic disease increases in post-menopausal women however the mechanisms underlying this loss of cardioprotection remain inadequately understood[2,3]. Estradiol exerts its effects through both genomic and non-genomic actions regulating cardiovascular physiology primarily by binding and activating estrogen receptor alpha (ERα)[4]. ERα binds to chromatin as dimers at specific [5]DNA sequences known as estrogen response elements (EREs); or may activate other transcription factors to regulate downstream gene expression. Although the protective effects of E_2 in regulating cardiac fibrosis, oxidative stress, mitochondrial function and hypertrophy, the selective effects of ERα in the regulation of E_2 mediated outcomes are unknown. We find in other metabolic tissues that Esr1 exerts strong regulatory control over mitochondrial function and indeed mitochondrial dynamics and function are implicated in the pathobiology of cardiomyopathies including fibrosis, diabetic cardiomyopathy, and ischemic reperfusion injury.[14-16] The heart is a high energy demand organ that consumes ATP derived from the mitochondria and to meet the demand of cardiomyocytes, mitochondria are shown to occupy more than one-third of myocytes by volume.[10] Similar to estradiol, exercise is shown to exert cardioprotective effects however the molecular drivers of this protection are unknown.

PURPOSE

Sex differences in cardiac metabolism and cardiometabolic disease susceptibility are documented. The mechanisms underlying sexual dimorphism and the role estrogens play in cardiac physiology aren't well understood, especially in aging women when cardiometabolic disease susceptibility is heightened. The purpose of the current study was to identify key genetic drivers of healthy vs pathogenic cardiac remodeling and determine the impact of estrogen action on cardiomyocellular function.

DESIGN and METHODS

Animals

ExcHMDP and ISO-HMDP. All studies were approved by the Institutional Animal Care and Use Committee (IACUC) and the Animal Research Committee (ARC) at the University of California, Los Angeles (UCLA). Female mouse strains of the ExcHMDP were acquired from The Jackson Laboratory (Bar Harbor, ME, USA) or through the University of Tennessee Health Science Center. Mice were maintained on a strict 12h light/dark cycle with *ad libitum* access to standard rodent chow (Teklad 8604, Envigo, Indianapolis, IN, USA) and water. Sedentary mice were housed 1-4 animals per cage. Exercised mice were individually housed with access to an in cage running wheel monitored by VitalView® Activity Software (Starr Life Sciences, Oakmont, PA, USA). Mice were given access to the running wheel for about 30 days. After the 30 days, running wheels were locked between 6-9am local time. 24-hours post, cages were replaced, and chow removed from all animals for six hours. Animals were sacrificed between 12-4pm local time.

Daily running distance was calculated as the average running distance per day over the experiment timeframe. Average running speed was calculated by normalizing all 15 second intervals with values > 0 relative to 1 second. Percent of time running was calculated by dividing the sum of 15 second intervals > 0 by the sum of all 15 second intervals.

To interrogate the genetic architecture of isoproterenol (ISO)-induced heart failure, female mice (age 10 weeks) of a second HMDP cohort were treated with ISO for 21 days (30mg/kg per day) by ALZET osmotic mini-pump as previously described (Rau et al. 2015b). Animals will euthanized and hearts excised and prepared for RNAsequencing as described below.

Genetically engineered mice. Control f/f animals were bred with a transgenic line expressing Cre recombinase driven by a tamoxifen-inducible alpha-MHC-MerCreMer (Jackson: 005657) promoter to generate animals with a cardiomyocyte- and time-specific deletion of *Esr1*. Animals were aged to 12 weeks and then injected with a single dose of tamoxifen to induce ERα deletion. Animals were studied 4-6 weeks after tamoxifen washout. All animal experiments were conducted following the guidelines and protocols approved by the University of California, Los Angeles Institutional Animal Care and Use Committee (IACUC).

RNA Isolation, Library Preparation, and Sequencing

ExcHMDP. Whole heart was pulverized in liquid nitrogen. Tissue was homogenized in Trizol (Invitrogen, Carlsbad, CA, USA), RNA was isolated using the RNeasy Isolation Kit (Qiagen, Hilden, Germany), and then tested for concentration and quality with samples where RIN > 7.0 used in downstream applications. Libraries were prepared using KAPA mRNA HyperPrep Kits and KAPA Dual Index Adapters (Roche, Basel, Switzerland) per manufacturer's instructions. A total of 800-1000 ng of RNA was used for library preparation with settings 200-300 bp and 12 PCR cycles. The resultant libraries were tested for quality. Individual libraries were pooled and sequenced using a HiSeq 3000 or NovaSeq 6000 S4 UCLA Technology Center for Genomics and Bioinformatics (TCGB) following in house established protocols.

ExcHMDP Genome Wide Association Analyses

Genome wide association analyses were conducted as described previously (Norheim *et al.*, 2019). Quantitative trait loci (QTLs) were considered distinct between groups if the significant locus was more than 20 Mb from a locus in the other group below the suggestive significant threshold ($P<4.1x10^{-5}$).

ExcHMDP Trait by Trait Correlations

Biweight midcorrelation was calculated for pairwise trait correlations within each group using the WGCNA package in R. The sedentary and exercised trait correlation matrix was visualized using the 'ComplexHeatmap' package (Gu et al., 2016) in R. For trait by trait correlations involving a group difference (sedentary value subtracted from trained value for each strain, or trait delta), a random pairing method was used.

Candidate Gene Identification

Candidate genes in GWAS loci were prioritized based on known biologic function or correlation in co-expression with a specific trait. In particular, genes whose *cis*- regulation was correlated with the trait were considered as highly likely candidate genes. Calculations were computed as described previously (Gusev et al., 2016). Briefly, when only exercise trained animals were used, SNPs within 1 Mb (*cis*-acting) of a gene with a *cis*-eQTL ($p < 1E^{-4}$) were identified. The median of the allele specific expression for each SNP of that gene was calculated and those values were then correlated with a particular trait. For candidate genes identified using both sedentary and exercised animals, sedentary gene expression was subtracted from trained gene expression giving the exercise induced change in gene expression.

RESULTS

Molecular Response to Exercise Training in Heart

Exercise is reproducibly shown to improve cardiovascular function (Ades et al., 1996; Hellsten and Nyberg, 2015). Because heart weight was increased in TRN vs. SED animals (increased in 85% of strains), we conducted cardiac transcriptomics. Enrichment analysis of DEG displayed mitochondrial function, inflammatory and immune processes (leukocyte regulation, macrophage activation, and *TNFα* and other cytokine products), calcium signaling and regulation, muscle growth and development, and angiogenesis (FDR<0.05). These biological processes overlap with those identified in skeletal muscle. Candidate gene identification analysis of exercise-induced cardiac hypertrophy identified five potential regulatory genes associated with healthy cardiac

remodeling: *IL31ra*, *Fam167b*, *Tafa5*, *Crip3*, and *Nanos1* (P<0.01). We have identified two of these targets as E_2-responsive with EREs in their proximal promoters.

***ERα* is required for normal cardiac function and protection against fibrosis.**

Female mice showed a significant increase in heart weight (10% increase; p<0.02) 4 weeks after *Esr1* gene deletion compared with f/f control. To assess the cardioprotective impact of ERα we performed echocardiography on the hERαKO model as described[20]. hERαKO and f/f control (n=5) were subjected to three echo evaluations: baseline prior to gene deletion, and 2 and 4-weeks after gene deletion. hERαKO mice presented with a reduced left ventricular ejection fraction (LVEF) reaching significance by four weeks when compared controls. A similar finding was observed for left ventricular fractional shortening (LVFS) and heart rate. Each of these functional changes are indicative of cardiomyopathy further solidifying the significance of ERα in overall heart functional capacity. Collectively, these structural and functional changes in ejection fraction, fractional shortening, heart rate and left ventricular end-systolic diameter in hERαKO mice substantiate the cardioprotective effects of ERα/E2 in rodents and human subjects. Previous studies have shown that E_2 supplementation in ovariectomized (OVX) rodents reduces cardiac fibrosis after transverse aortic contrition (TAC)[23-25]. Hearts from hERαKO and f/f control were harvested from 4-month-old mice, four weeks after conditional *Esr1* gene deletion. Masson's trichrome staining showed a marked increase in fibrotic area in hERαKO compared to control.

DISCUSSION

Postmenopausal women with low circulating E_2 have a substantially increased risk of cardiometabolic dysfunction. Interventions to prevent morbidity and delay mortality as a consequence of cardiometabolic decline are needed. We leveraged large transcriptomics data sets to better understand the pathobiology of heart failure and the healthy vs. pathogenic underpinnings of cardiac hypertrophy. Using transcriptomic analyses to mine for gene drivers of these biological

processes allowed us to formulate and test new hypotheses related to cardiac function. We used mouse genetics to test hypotheses related to gene targets of interest controlled by Esr1. The cardiomyocyte-specific estrogen receptor alpha knockout model allowed us to illustrate the importance of ERα expression selectively in cardiomyocytes in the regulation of mitochondrial metabolism, fibrosis, and cardiac function.

CONCLUSIONS Our studies provide important insight into the genetic architecture and key genetic drivers of cardiac remodeling in females with the overarching goal to identify cardiac-specific transcripts and pathways that can be targeted therapeutically to preserve cardiac function during aging in women.

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