interactions and expression dysregulation of PITX2. We describe a new molecular mechanism implying a yet unidentified non-coding regulatory element of PITX2 and responsible for a complex electrical and developmental cardiac syndrome.

**BS-515-02**

DELETION OF A GATA-RESPONSIVE NKX2-5 ENHANCER CAUSES CYANOTIC CONGENITAL HEART DISEASE AND HYPMORPHIC RIGHT PURKINJE NETWORK

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**Background:** Cardiac progenitor cells of the second heart field (SHF) give rise to the right ventricle (RV), outflow tract (OFT), and right bundle branch (RBB). Perturbation of the SHF contribution during development results in congenital heart disease involving the OFT and RBB. Although critical transcripional programs, such as Gata and Nkx2-5, are important for SHF development, little is known about how these factors function in a gene regulatory network. Prior work has shown that a putative Gata-dependent cardiac enhancer enriches expression of Nkx2-5 in the SHF, however validation studies confirming relevance for cardiac morphogenesis is lacking.

**Objective:** To study the importance of this cardiac enhancer on heart development, we generated Nkx2-5 enhancer knockout mice and characterized the impact on SHF-derived structures.

**Methods:** We generated mice harboring a 226-nucleotide deletion of the GATA-responsive Nkx2-5 cardiac enhancer (Nnk2-5) using CRISPR/Cas9 gene editing and assessed clinical phenotypes. Cardiovascular defects in Nkx2-5 mice were physiologically assessed using electrocardiography (ECG) and echocardiography (TTE). Cardiac morphological defects were characterized using histology and scanning electron microscopy. Altered expression of genes involved in OFT and RBB development were assessed.

**Results:** Nkx2-5 mice exhibit neonatal lethality due to cyanotic heart disease. Congenital heart defects include persistent truncus arteriosus, double-outlet right ventricle, and D-transposition of the great arteries. Homozygous mutants display marked hypoplasia of the right Purkinje network with corresponding right ventricular conduction delay seen as QRS prolongation by ECG and interventricular dys synchrony by TTE. Cardiac morphological defects were characterized using histology and scanning electron microscopy. Altered expression of genes involved in OFT and RBB development were assessed.

**Conclusion:** Our results reveal that a single cardiac enhancer is essential for major cardiac developmental events, such as outflow tract septation and RBB formation.

**BS-515-03**

ATRIAL FIBRILLATION ASSOCIATED COMMON INTRONIC RISK VARIANTS IN SYNE2 LEAD TO LOWER EXPRESSION OF NESPRIN-2A1, INCREASED NUCLEAR STIFFNESS AND EARLY AFTER DEPOLARIZATIONS IN CARDIOMYOCYTES

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**Background:** Atrial fibrillation (AF) genome-wide association studies (GWAS) identified significant associations with rs1152591 and linked variant rs1152595 in the SYNE2 gene, encoding the nesprin-2 protein that connects the nuclear membrane with the cytoskeleton.

**Objective:** To determine the effects of the AF-associated SNPs on SYNE2 expression and investigate the mechanisms for their association with AF.

**Methods:** RNA sequencing was performed on 234 AF human left atrial appendage (LAA) tissues. Human induced pluripotent stem cell-derived cardiomyocytes (iCMs) were used to study the cellular effects of SYNE2 knockdown (KD) or GFP-Syne2 overexpression. Reporter gene vectors was used to study the regulatory roles of the SNPs. Nuclear size and stiffness were measured by immunofluorescent microscopy and atomic force microscopy. Calcium transient assay was performed using epifluorescence microscopy and Fura-2 AM.

**Results:** RNA sequencing of LAA tissues indicated that rs1152591 and rs1152595 were significantly associated with the expression of short SYNE2 isoform, without affecting the expression of the full-length SYNE2 mRNA. Risk vs. reference alleles of rs1152591 and rs1152595 had decreased promoter or enhancer activity. SYNE2 siRNA KD or nesprin-2a1 overexpression in human iCMs resulted in ~12.5% larger nuclear area compared to controls (p<0.001). SYNE2 KD or nesprin-2a1 overexpression led to 57.5% or 33.2% decreases, respectively, in nuclear stiffness compared to controls (p<0.001). Nesprin-2a1 overexpression rescued the effects of SYNE2 siRNA KD on calcium transient, significantly decreasing the intracellular calcium concentration (p<0.001) and the incidence of early afterdepolarizations (EADs) (p<0.001).

**Conclusion:** AF-associated SNPs rs1152591 and rs1152595 downregulate the expression of SYNE2a1 in the human LAA. SYNE2a1 has a dominant-negative effect on the nucleus, decreasing nuclear-cytoskeletal connectivity and nuclear stiffness that may protect iCMs from repetitive motion stress, and also has a gain of function activity on the calcium cycling, decreasing EADs that may protect against atrial arrhythmia.

**BS-515-04**

REPLICATED GENE EXPRESSION CHANGES IN PATIENTS WITH PERSISTENT ATRIAL FIBRILLATION

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**Background:** Little is known about changes in the atrial transcriptome associated with persistent atrial fibrillation (AF).

**Objective:** To identify major molecular mechanisms in persistent AF, we determined consistent differential expression (DE)