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 HYPOMORPHIC 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 transcriptional 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\textsuperscript{Gata\_delet}), using CRISPR/Cas9 gene editing and assessed clinical phenotypes. Cardiovascular defects in Nkx2-5\textsuperscript{Gata\_delet} mice were physiologically assessed using electrocardiography (ECG) and echocardiography (TTE). Cardiac morphological defects were characterized using histology and scanning electron micrography. Altered expression of genes involved in OFT and RBB development were assessed.

Results: Nkx2-5\textsuperscript{Gata\_delet} 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 severe conotruncal abnormalities and hypoplasia of the right trabecular system. Nkx2-5 expression is reduced selectively in the RV and OFT in mutant hearts. Factors implicated in conotruncal septation (Sema3c and Rapo3) and ventricular trabeculation (Anpt1) were significantly reduced.

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\textsubscript{1} 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\textsubscript{1} 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-2\textsubscript{1} overexpression in human iCMs resulted in ~12.5% larger nuclear area compared to controls (p<0.001). SYNE2 KD or nesprin-2\textsubscript{1} overexpression led to 57.5% or 33.2% decreases, respectively, in nuclear stiffness compared to controls (p<0.001). Nesprin-2\textsubscript{1} 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 SYNE2\textsubscript{1} in the human LAA. SYNE2\textsubscript{1} 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)
between atrial tissue samples from well-characterized patients with persistent AF and patients without a history of AF in two independent patient cohorts.

**Methods:** Poly-A tailed RNA molecules, extracted from total RNA, from left and right atrial appendage tissue samples from independent discovery and replication cohorts CATCH ME (n=142) and RACE V (n=82) were sequenced, and analyzed according to patient AF history. Analyses were performed stratified by atrial side, adjusting for age, sex, heart failure and a combination of eleven clinical characteristics determined by principal component analysis. Transcripts were considered DE in CATCH ME if their fold change reached transcriptome-wide significance (false discovery rate (FDR) < 0.05). DE transcripts were replicated in RACE V with a concordant direction of effect and a within-set FDR < 0.05.

**Results:** Persistent AF was associated with 184 left atrial DE transcripts in CATCH ME of which 85 (46%) were replicated in RACE V, and with 208 right atrial DE transcripts in CATCH ME of which 86 (41%) were replicated in RACE V. Overall, 26 transcripts were discovered and replicated in both atria. Non-replicated transcripts often exhibited concordant direction of effect (left: 78%, right: 83%). Replicated transcripts consisted of protein coding genes, antisense and non-coding RNAs. Protein coding genes showed involvement in pathways linking persistent AF to cardiomyocyte structure, conduction properties, fibrosis, inflammation, molecule trafficking, and endothelial dysfunction.

**Conclusion:** RNA sequencing of human atrial tissue samples identified many transcripts associated with persistent AF in left and/or right atria, discovered and replicated using two independent cohorts. These consistent findings of AF-induced changes provide a starting point for targeted proteomic analysis and single-nucleus sequencing to further unravel the molecular mechanisms underlying AF and the progression to persistent AF, and biomarker development to quantify AF progression and enable precision medicine in individual patients.

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**ABSTRACT CE-541: Arriving at a better understanding of COVID-19 and arrhythmias**

**Saturday, April 30, 2022**

**1:00 PM - 2:00 PM**

**CE-541-01**

**OUTCOMES IN PATIENTS WITH COVID-19 COMPLICATED BY HIGH GRADE ATRIOVENTRICULAR BLOCK**

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**Background:** There is growing evidence showing that arrhythmias are one of the major complications of COVID-19. However, there are currently only a few case reports of high-grade atrioventricular block (AVB). We sought to describe a large case series of AVB as a complication of COVID-19.

**Objective:** The purpose of the current study is to describe a large case series of AVB as a complication of COVID-19.

**Methods:** We included a series of twenty-five (25) consecutive patients with confirmed COVID-19, who developed advanced AVB in a prospective observational multi-center study. Patients underwent clinical, laboratory evaluation, Holter, telemetry, Echocardiogram, Chest X-Ray, chest CT scan and cardiac MRI.

**Results:** Of the 25 patients 13 were male with a mean age of 62±13 years. 19 developed complete AVB, one a 3:1 AVB and five 2:1 AVB. None of the patients had a history of cardiac arrhythmia. AVB was not related to medication or intubation. Eighteen patients developed AVB during their hospitalization for COVID-19 and 7 after the first month as a late sequela. Five patients were asymptomatic, 6 presented syncope, seven dyspnea and seven dizziness. Eleven patients presented reverse AVB early by a high dose of corticosteroid in all of them, and...