have identified more than twenty gain-of-function mutations. These mutants provide information about specific loci in Orai1 that are crucial in keeping the channel closed and highlight the potential importance of reversible interactions between the TMs that play a role in Orai1 channel gating.

2653-Pos Board B260
Contribution of Orai1 to Sex-Based Differences in Cardiac Excitation-Contraction Coupling
Fiona Bartoli[1], Baptiste Rode[2], David J. Beech[2], Ana Maria Gomez[1], Jean Pierre Benitah[1], Jessica Sabourin[1].
[1]Faculty of Pharmacy, Inserm UMR-S1180, Châtenay-Malabry, France, [2]School of Medicine, Leeds Institute of Cardiovascular and Metabolic Medicine, Leeds, United Kingdom.
The Orai1-dependent Ca^{2+} entry is the major mechanism of Ca^{2+} entry in non-excitable cells but its role in cardiac Ca^{2+} homeostasis is unclear. Although a role in cardiac pathological processes such as arrhythmias, hypertrophy and heart failure is increasingly recognized, the contribution of Orai1 to normal cardiac function remains elusive. Using a mouse model with cardiac specific expression of a dominant negative Orai1^{R91W} mutation (dn-Orai1^{R91W}), we assessed the Orai1 contribution to cardiac function with special emphasis on sex differences.
At 2/3-months of age, dn-Orai1^{R91W} Tg mice show normal cardiac function evaluated by echocardiography and ECG telemetry under physiological conditions compared to WT littermates.
Using Mn^{2+}-quenching method, we observed on freshly isolated adult ventricular myocytes similar level of store-operated Ca^{2+} entry activated by Ca^{2+} stores depletion in male and female WT myocytes that is reduced by 50% in dn-Orai1^{R91W} Tg myocytes. Interestingly, immunostaining data illustrate a T-tubular network of Orai1 in female cardiomyocytes whereas Orai1 pattern in male is preferentially at the sarcolemma.
Analysis of Ca^{2+} homeostasis using confocal microscopy on Fluo-4 loaded myocytes did not reveal any obvious differences between male WT and dn-Orai1^{R91W} Tg mice, whereas alterations were observed in female. Female WT myocytes have smaller $[Ca^{2+}]_i$ transients amplitude associated with lower sarcoplasmic reticulum (SR) Ca^{2+} content and prolonged $[Ca^{2+}]_i$ transients decay time than male with similar cell shortening. The Ca^{2+} sparks frequency is unchanged but sparks amplitude is smaller in female cardiomyocytes, which is consistent with lower SR Ca^{2+} content. In comparison, female dn-Orai1^{R91W} myocytes have increased $[Ca^{2+}]_i$ transients amplitude associated with larger SR Ca^{2+} load, faster decay time, increased cell shortening and Ca^{2+} sparks frequency compared to female WT cells.
In conclusion, our findings highlighted the possible involvement of Orai1 in cardiac excitation-contraction coupling in sex-based differences.

2654-Pos Board B261
Orai1 Mutations with Distinct Channel Gating Defects cause Tubular Aggregate Myopathy
Monica Bulla Didier, Maud Frieden, Nicolas Demaurex.
Cell Physiology and Metabolism, Faculty of Medicine of Geneva, Geneva, Switzerland.
Skeletal muscle contraction and differentiation is controlled by cytosolic Ca^{2+} signals relying partly on store-operated Ca^{2+} entry (SOCE), a Ca^{2+} homeostatic mechanism mediated by STIM and ORAI proteins that links the depletion of endoplasmic/sarcoplasmic (ER/SR) Ca^{2+} stores to the activation of membrane Ca^{2+}-permeable channels. Gain of function mutations in STIM1 or ORAI1 cause tubular aggregates myopathy (TAM), a skeletal muscle disorder with muscular pain, weakness and cramping with exercise. Here, we characterize two new mutations in the *ORAI1* gene associated with TAM. Mutation V107M is located near the channel selectivity filter, and mutation T184M in the third transmembrane domain. Clinical features differed between patients carrying the two different mutations, prompting us to test the regulation and Ca^{2+} permeability of the mutated channels. When ectopically expressed in HEK cells, both mutated channels formed plasma membrane clusters, conveyed high basal Ca^{2+} entry and increased SOCE, indicative of constitutive activation. Electrophysiological recordings revealed that currents carried by the V107M channel were of larger amplitude, reduced Ca^{2+} selectivity, and increased fast Ca^{2+}-dependent inactivation; while slow Ca^{2+}-dependent inactivation was preserved in both mutants. Preliminary results suggests that the T184M mutation alters the sensitivity of the channel to ROS, suggesting that this mutation might hinder the accessibility of a reactive nearby cysteine residue. Molecular dynamics simulations will explore this possibility and test whether substitution V107M enlarges the first portion of the channel pore, increasing its permeability to ion and displacing the residues responsible for Ca^{2+} selectivity. By characterizing new mutations of *ORAI1*, we hope to improve our understanding of the channel permeation and regulatory properties. This will allow us to identify new targets for the development of therapies against diseases with gain of function mutations in ORAI1.

2655-Pos Board B262
The Role of EHD2 in Orai1-STIM1 Interaction
Dora Bodnar.
Molecular Physiology and Therapeutics Branch, NIH, NIDCR, Bethesda, MD, USA.
Ca^{2+} is a second messenger whose cytoplasmic concentration is determined by Ca^{2+} pumps and channels. Ca^{2+} enters non-excitable cells though store operated channels (SOC). The main SOC is Orai1, responsible for the Ca^{2+}-release-activated (CRAC) current. Orai1 is activated in response to Ca^{2+} release from the ER and is gated by the ER Ca^{2+} sensor STIM1. Store depletion results in Orai1-STIM1 clustering in ER/PM junctions. However formation of junctions and its affect on the Orai1-STIM1 complexes is not well understood. The C-terminal Eps15 homology domain-containing protein 2 (EHD2) is an ATPase that oligomerizes/localizes to caveolae in the PM inner leaflet and associate with $PI(4,5)P_2$. Caveolae regulate membrane turnover, many signaling processes. EHD2 confines caveolae to the plasma membrane by providing a link to actin filaments. Endogenous EHD2 is enriched in ER/PM junctions positive for endogenous caveolin1. Here we asked whether EHD2 role in the ER/PM junctions affect Orai1-STIM1 clustering and current. We used combination of techniques such as whole cell patch clamp, calcium imaging, Co-IP, FRET. When co-expressed with Orai1 and STIM1, EHD2 markedly increases while the knockdown of EHD2 dramatically reduces the Orai1 current size. Measurements of $[Ca^{2+}]_i$ corroborated the current measurements. The EHD2 enhanced Orai1 current rapidly inactivated, but the effect of EHD2 was independent of SARAF, which mediates the slow Ca^{2+}-dependent inactivation of Orai1. To determine which property of EHD2 is responsible for the increased current size, different mutants have been used. The K327 mutant, which reduces $PI(4,5)P_2$ interaction has no effect; the F322 mutant that eliminates localization of EHD2 at the ER/PM junctions and I157Q that forces EHD2 into puncta by increasing its ATPase activity both decreased the current size by acting as dominant negatives. Co-IP results showed strong interaction between EHD2 and STIM1 either in the presence or the absence of Orai1 that is enhanced by store depletion. These findings suggest that EHD2 may play an important role in formation of stabilization of the ER/PM junctions in Caveolar sites to modulate the Orai1-STIM1 complexes.

2656-Pos Board B263
Role of Zinc Transporters in Mammalian Heart under Physiological and Pathological Conditions
Aysegul Durak[1], Yusuf Olgar[1], Erkan Tuncay[1], Verda C. Bitirim[1], Evren Ozcinar[2], Mustafa Bahadır Inan[2], Kamil Can Akcali[1], Semir Ozdemir[3], Ahmet Ruchan Akar[2], **Belma Turan**[1].
[1]Biophysics, Ankara University Faculty of Medicine, Ankara, Turkey, [2]Cardiovascular Surgery, Ankara University Faculty of Medicine, Ankara, Turkey, [3]Biophysics, Akdeniz University Faculty of Medicine, Antalya, Turkey.
Cellular free Zn^{2+}-changes, including those in either sarco(endo)plasmic reticulum [S(E)R] or sarcolemma, are primarily coordinated by Zn^{2+}-transporters. Both role of Zn^{2+}-transporters in the heart and association between defective Zn^{2+}-homeostasis and cardiovascular dysfunction in mammalians is not known yet. Since Zn^{2+}-homeostasis is regulated through Zn^{2+}-transporters, any imbalance in Zn^{2+}-homeostasis via these transporters maybe associated with several chronic diseases. Although it has been mentioned that ZIP8 and ZIP14 mostly localize in sarcolemma while others including ZIP7, ZnT7 and ZnT8 localize to subcellular compartments, however, very little is known about their roles and subcellular localizations in mammalian cardiac cells. In this regard, we first examined the expression levels of these transporters in left ventricle of failing human heart. For further confirmation, we used either rat model mimicing heart failure via transverse aortic constriction or rat ventricular cell line (H9c2 cells) mimicking heart failure via adriamycin incubation. Protein expression level of ZIP8 and ZnT7 were decreased while ZIP14, ZIP7 and ZnT8 were increased in all cardiac preparations, significantly. Additionally, high intracellular free Zn^{2+} level, high ER-stress marker proteins, GRP78 and Gadd153, high apoptotic status marker such as expression of Bax/Bcl-2 were observed in all failing heart samples. Our confocal imaging examinations for subcellular localizations in H9c2 cells showed that ZIP8, ZIP14 and ZnT8 were localized on the sarcolemme as about over 60% and into the S(E)R as about 40-50% due to calculated Pearson's coefficients. Overall data provide an impression on possible contribution of differential expression of these Zn^{2+}-transporters as one of the crucial signs of failing heart and they may provide important information on their role for the new therapeutic strategy against heart failure (*Supported through Tubitak SBAG-113S466*).