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early trafficking steps. Moreover, this provides further evidence to consider macromolecular complex ion channel interplay in the development of antiarrhythmic therapeutic strategies to treat ATS and BrS.

## PO05-83

### SMALL CONDUCTANCE CALCIUM ACTIVATED K CURRENTS IN RABBIT PURKINJE CELLS

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**Introduction:** Purkinje cells (PCs) are sources of automaticity and triggered activity (TA) in the ventricles. We hypothesize that PCs express abundant small conductance calcium activated K (SK) currents to maintain repolarization reserve, and blocking the SK current is proarrhythmic in normal PCs.
**Methods:** We isolated PCs and ventricular myocytes (VMs) from pseudotendons of 3 normal rabbit ventricles. PCs are rod-shaped, possessed finger-like projections at either end and are larger than VMs. Cells which maintained a resting membrane potential below -75mV were used for current clamp and for gap-free recordings. Action potential duration was measured to 80% repolarization (APD80). Apamin and UCL-1684 (UCL), specific SK channel blockers, were used to block SK currents. The p-value is calculated based on a linear mixed-effects model where each cell is treated as random.
**Results:** Apamin (100 nM) prolonged APD80 of PCs (n=5) from 228±12 to 244±17 ms (6.63% increase) at 2000 ms pacing cycle length (PCL), from 203±18 to 226± 3 ms (9.22% increase) at 1000 ms PCL, from 190±26 to 199±16 ms (5.58% increase) at 600 ms PCL and from 171±16 to 177±25 ms (2.2% increase) at 500 ms PCL. There is a large variation of apamin-response among PCs. The range of delta APD80 was 0 to 24 ms at 2000 ms PCL, 0 to 41 ms at 1000 ms PLC, 0 to 22 ms at 600 ms PCL and 0 to 12 ms at 500 ms PCL. Delta APD (difference of APD before and after apamin) is larger for longer PCL (p=0.006). In comparison, APD80 of VMs (n=3) changed by -0.59% at 2000 ms PCL and 1.32% at 1000 ms PCL after apamin. The delta APD is higher in PCs than in VM (p=0.01). UCL (100nM) increased early and delayed afterdepolarizations (EADs and DADs) and TA in 1 nondriven PC cell. In the absence of drug (baseline and washout), the cell spends 38.64%±0.073 of time below -75mV. However, due to frequent EAD and TAs, the cell spent only 5.67%±0.001 during UCL administration (p=0.009, two-sample T test).
**Conclusion:** SK current blockade heterogeneously prolongs APD of the PCs and induces EAD, DAD and TA in normal PCs. APD prolongation was more apparent at long rather than short PCL. These data indicate that, at slow activation rate, SK current is important in maintaining repolarization reserve and in preventing TAs in normal PCs.

## PO05-84

### A NOVEL C-TERMINAL TRUNCATION OF NAV1.5 CHANNELS ASSOCIATED WITH IDIOPATHIC VENTRICULAR FIBRILLATION

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**Introduction:** We report a novel heterozygous frameshift mutation in the SCN5A gene encoding cardiac Na+ channels (Nav1.5) found in a proband diagnosed with idiopathic ventricular fibrillation that also presented bradycardia. Genetic and electrophysiological testing of the family showed that PR and QRS intervals in the mutation carriers (n=8) were significantly prolonged compared to healthy relatives (n=7). Two of the mutation carriers displayed a positive response with development of type 1 BrS pattern after flecainide challenge. The mutation (p.D1816VfsX7) resulted in a severe truncation (200 residues) of the C terminus of Nav1.5.
**Methods:** WT and mutated Nav1.5 channels together with hNavβ1 were expressed in CHO cells and currents were recorded at room temperature using the whole-cell patch-clamp. To analyze trafficking, WT and mutated green fluorescent protein (GFP)-tagged-Nav1.5 channels were localized by confocal microscopy.
**Results:** Expression of p.D1816VfsX7 alone resulted in a marked reduction in peak Na+ current density (69.8±24.7 pA/pF) compared to WT channels (-691±105 pA/pF; P<0.01). Peak-current density generated by p.D1816VfsX7+WT channels (-350±119 pA/pF) was approximately 50% of the WT value, indicating that the mutation did not produce a dominant negative effect. p.D1816VfsX7 shifted positively both the activation and inactivation curves, leading to a significant reduction of the window current. Moreover, the mutation accelerated current activation and reactivation kinetics and increased the fraction of channels that developed slow inactivation with prolonged depolarizations. p.D1816VfsX7 produced a marked reduction in channel trafficking toward the membrane, that was not restored by decreasing incubation temperature during cell culture or by incubation with 300 μM mexiletine and 5 mM 4-phenylbutirate.
**Conclusion:** Despite a severe truncation of the C-terminus, the resulting mutated channels generate currents, albeit with reduced amplitude and altered biophysical properties, demonstrating the key role of the C-terminal domain in the expression and function of the cardiac Na+ channel.

## PO05-85

### NAV1.5 DIFFERENTIALLY REGULATES EXPRESSION OF KIR2.1 AND KIR2.3 CHANNELS

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**Introduction:** Cardiac inward rectifying K+ current (IK1) plays a key role in the control of resting membrane potential and action potential duration. IK1 is mainly carried by Kir2.1 channels in ventricular cells, whereas relative contribution of Kir2.2 and Kir2.3 seems to be greater in atrial cells. It has been described that an increase in the expression of sodium channels (Nav1.5) increases Kir2.1 expression, leading to an enhanced cardiac excitability. This modulation involves synapse-associated protein 97 (SAP97), which binds to the PDZ domain of the channels composed by the last 3 C-terminal residues. The PDZ domain of Kir2.x channels is highly conserved (Ser-X-Ile), where X is a glutamic acid in Kir2.1 (Glu426) and alanine in Kir2.3 (Ala444).
**Methods:** We have analyzed whether Kir2.1 and Kir2.3 channel activities are modulated differentially by the presence of Nav1.5. Kir2.x currents (IKir2.x) were recorded by using the patch-clamp technique in CHO cells transiently transfected with wild-type

(WT) and mutant Kir2.x and Nav1.5 channels (1:1 ratio).
**Results:** In the presence of WT Nav1.5, IKir2.1 density was significantly increased compared to that in the absence of Nav1.5, both at negative (-211±22 vs -126±12 pA/pF at -120 mV, P<0.01) and positive (20.0±4.4 vs 11.4±2.0 pA/pF at -50 mV) potentials to the reversal potential for K+. Conversely, cotransfection of Nav1.5 and Kir2.3 did not modify IKir2.3 density (-25.8±4.0 pA/pF at -120 mV, P>0.05). Importantly, when Kir2.1 Glu426 was replaced by Ala (p.E426A), the increase induced by coexpression with Nav1.5 was abolished at all membrane potentials. Coexpression of Nav1.5 with p.A444E Kir2.3 channels markedly increased IKir2.3 density (-44.0±5.8 pA/pF at -120 mV, P<0.01). The increase of Kir2.1 and p.A444E Kir2.3 density was not observed when they were cotransfected with either p.D1690N or p.D1816VX7 Nav1.5 two Brugada syndrome-associated SCN5A mutations that severely impair channel trafficking.
**Conclusion:** IKir2.1, but not IKir2.3, is increased by coexpression with Nav1.5. The specificity of the effect is determined by Glu426 in the PDZ domain of Kir2.1 and could lead to differential electrophysiological effects on atrial and ventricular cells on patients carrying SCN5A mutations.

## PO05-86

### AGE-RELATED PITX2 EXPRESSION IN RATS WITH SPONTANEOUS ATRIAL TACHYARRHYTHMIAS

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**Introduction:** The time-course of Pitx2 down-regulation and the associated molecular pro-arrhythmic changes in the left atria (LA) of atrial fibrillation (AF) patients are unknown. We aimed to assess atrial transcriptomic profile associated with atrial arrhythmogenicity in a rat model of spontaneous atrial arrhythmias and to evaluate Pitx2 changes that precede the onset of arrhythmias in these rats.
**Methods:** LA sampling was performed in 3 groups of young, adult, and aging spontaneously hypertensive rats (SHR; n = 4 each), and 3 groups of age-matched Wistar-Kyoto (WKY) rats. LA mRNA expressions of Pitx2
and other 89 pro-arrhythmogenic genes were studied using TaqMan Low-Density Array.
**Results:** No difference was found between LA Pitx2 expression in young or adult SHRs compared to age-matched WKY rats (P> 0.05), while aging SHRs presented significantly lower LA Pitx2 expression compared to age-matched WKY rats (P = 0.02). Among SHRs, Pitx2 expression showed an age-dependent decrease (34.9 ± 6.7 in young, 17.1 ± 3.6 in adult, and 10.7 ± 1.7 in aging SHRs, P = 0.04) and was significantly negatively correlated with age (r = -0.86, P< 0.01). Of the 89 studied genes, 6 were differentially expressed between aging SHRs and WKY rats, showing decreased ICa,L, connexin-43, and desmocollin 2 densities, and increased collagen VI.
**Conclusion:** Similarly to what has been found in AF patients, we found reduced LA Pitx2 expression in aging SHRs with spontaneous atrial arrhythmias. Reduced ICa,L, desmocollin 2, and connexin-43, and increased collagen VI densities were also observed. The age-dependency of Pitx2 changes suggests that a timely appropriate therapy in hypertensive patients may prevent AF onset in this population.

## PO05-87

### HIGH RESOLUTION MULTIDIMENSIONAL PROTEOMICS DETECTS CANDIDATE ARRHYTHMIA BIOMARKERS

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**Introduction:** Although biomarkers can predict prognosis, studies are limited to small panels, chosen a priori. Proteomic techniques can powerfully demonstrate candidate proteins in an unbiased fashion. We aimed to identify candidates associated with arrhythmia by utilising a novel whole proteome method.
**Methods:** Consecutive patients attending device clinic were recruited. Stored baseline sera were later pooled according to death or arrhythmia occurrence. Samples underwent iTRAC isotope labelling, fractionation and immunodepletion, followed by tandem mass spectroscopy (MS) and identification of proteins. Technical validation was achieved by detection of B type natriuretic peptide (BNP) using MS and standard ELISA.
**Results:** 243 patients (54% male, age 71±6) were included in the analysis. During follow up of 40 months, there were 8 cardiovascular deaths. 25 experienced VT>182bpm or VF, whilst 48 never experienced VT at any rate/VF (controls).
634 proteins were identified by this method. When compared to the control group, proteins had significant differential expression if 2-fold up- or down-regulated. Overall, 94 proteins were differentially expressed in those who died or experienced VT>182 or VF. 15 proteins were associated with the arrhythmia endpoint but not death (table 1). BNP was detected by both MS and ELISA, and had greater up-regulation in patients who died, but was not discriminative for arrhythmia occurrence.
**Conclusion:** This study provides proof of principle that proteomic techniques can identify candidate proteins for use as biomarkers of arrhythmia risk. Further investigation is needed to select proteins with potential for clinical application before testing in a prospective setting.

| Differential protein expression (value:control) | | | |
|---|---|---|---|
| Protein | Death (n=8) | VT/VF (n=25) | No VT/VF (n=48) |
| Semaphorin-6B | 0.728 | 7.064 | 1 |
| Tropomyosin alpha-3 chain | 1.354 | 3.367 | 1 |
| Heat shock cognate | 1.849 | 3.048 | 1 |
| Ubiquitin hydrolase | 1.686 | 2.175 | 1 |
| F-box only protein 36 | 1.751 | 2.132 | 1 |
| Apolipoprotein C-III | 1.629 | 2.095 | 1 |
| Histone H2A type 1-H | 1.928 | 2.091 | 1 |
| Hepatocyte nuclear factor 6 | 1.179 | 0.333 | 1 |
| Proteasome alpha 1 | 0.859 | 0.344 | 1 |
| Dynein heavy chain 17 | 0.726 | 0.355 | 1 |
| Keratin, type I cytoskeletal | 1.981 | 0.437 | 1 |
| Collagen alpha-1(XVIII) | 1.741 | 0.440 | 1 |
| Ig heavy chain V-II region | 0.706 | 0.489 | 1 |
| DnaJ homolog C | 1.177 | 0.496 | 1 |
| TIR containing adapter | 0.811 | 0.498 | 1 |
| Natriuretic peptides B | 4.374 | 1.764 | 1 |

## PO05-88

### A MUTATION IN CALM1 ENCODING CALMODULIN CAUSES SUDDEN CARDIAC DEATH IN CHILDHOOD AND ADOLESCENCE

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**Introduction:** Although sudden cardiac death in childhood and adolescence is rare, it frequently presents as the first clinical manifestation of an underlying inherited arrhythmia syndrome.

Genetic testing therefore plays an important role in identifying individuals at risk.
**Methods:** N/A
**Results:** We characterized a Moroccan family presenting with a history of ventricular tachycardia and sudden death. Of seven siblings, two died suddenly at the ages of 9 and 10 years, and another two were resuscitated from out-of-hospital cardiac arrest (OHCA) at the ages of 10 and 16, respectively. ICDs were implanted in the latter two individuals; one of these individuals had recurrent self-limiting episodes of ventricular tachycardia and there were three ICD discharges for sustained episodes of VT/VF. Physical stress precipitated all the OHCA events. ECGs, exercise tests and 24-hour Holter monitoring did not reveal ectopic beats or arrhyth-mias but did show evidence of QTc interval prolongation at frequencies > 110 bpm (e.g. QTc = 501 ms at 143 bpm). We performed exome sequencing in the two siblings with aborted cardiac arrest. This identi-fied 75 novel heterozygous non-synonymous and splice site substitutions that were shared among the two siblings and which were not present in any of the publicly available variant databases. We subsequently overlayed these variants with a comprehensive list of cardiac candidate genes and identified a missense mutation affecting a highly conserved amino acid in the CALM1 gene (p.Phe90Leu) encoding calmodulin. This variant was absent in 100 individuals of Moroccan descent. The mutation was inherited from the asymptomatic mother who displayed a somewhat prolonged QT-interval. DNA was available for one of the two sibs who had died suddenly; this individual also carried the p.Phe90Leu mutation in CALM1.
**Conclusion:** We here identified a novel missense mutation in CALM1 associated with exercise-induced QT prolongation, ventricular tachycardia and sudden cardiac death in childhood.

## PO05-89

### ATRIAL FIBRILLATION RISK IS INCREASED WHEN CAVEOLIN-1 MRNA EXPRESSION IS DECREASED

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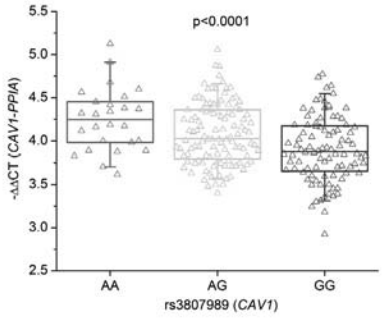
**Introduction:** Genome wide association studies have identified 9 genetic loci associated with risk of atrial fibrillation (AF). One locus is intronic in caveolin-1 (CAV1) on chromosome 7q.31. CAV1 is a scaffolding protein that affects the localization and activity of numerous membrane receptors, ion channels and enzymes. CAV1 is reported to be expressed in atrial but not ventricular myocytes, and highly expressed in fibroblasts and endothelial cells. The impact of this locus on atrial CAV1 mRNA and protein expression is not yet reported. We evaluated the relationship of CAV1 mRNA abundance to genotype of the index CAV1 SNP, rs3807989, and to clinical variables.
**Methods:** In a dataset of 239 left atrial (LA) tissue specimens obtained from cardiac surgery patients with/without a history of AF, CAV1 mRNA levels and genotype were evaluated using Illumina HT12 microarrays and qPCR.
**Results:** Illumina HT-12 arrays include 2 CAV1 probes. Ilmn_2149226 was significantly associated with rs3807989 genotype. This relationship was confirmed using qPCR (Figure) which showed that CAV1 mRNA abundance was decreased with each copy of the risk (G) allele (consistent with Ilmn_2149226). Further, with both array and qPCR, CAV1 mRNA levels were lower in patients presenting for surgery in AF, and in patients with a history of CAD.
**Conclusion:** The risk allele of AF-associated SNP rs3807989 is associated with decreased CAV1 mRNA abundance. Thus, CAV1 appears to protect the atria from the development of AF. We are currently studying how rs3807989 regulates CAV1 mRNA expression, and using western blot and confocal microscopy studies to assess the relationship of rs3807989 genotype to CAV1 protein abundance and cellular distribution.




## PO05-90

### MIR-21 DECREASES ICAL AND INCREASES IK1 IN HL-1 CELLS

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**Introduction:** microRNAs are small non-coding RNA that regulate expression of target mRNA by binding to their 3'-untranslated regions (3'-UTR). Recent evidence has demonstrated that miR-21 expression increases in left atrial appendages and plasma of patients with atrial fibrillation (AF). Moreover, it has been suggested that miR-21 could promote AF by increasing the fibrotic substrate of the atria. The main objective of the present study was to determine whether miR-21 could also play a role in the shortening of the atrial action potential duration produced in patients with chronic AF (electrical remodeling) by affecting two of the main atrial ion currents that are remodeled by AF, L-type calcium (ICaL) and inward rectifier K+ (IK1) currents.
**Methods:** ICaL and IK1 were recorded at room temperature in HL-1 cells transiently transfected with a miR-21 precursor by using the whole-cell patch-clamp. ICaL was recorded by using Ba2+ as charge carrier.
**Results:** miR-21 did not modify cell size as assessed by cell capacitance measurement (17.3±3.0 vs 22.9±3.2 pF; P>0.05). miR-21 markedly decreased peak ICaL density at potentials between +10 and +60 mV (from -6.2±1.4 to -3.4±0.6 pA/pF at +20 mV; P<0.01). Moreover, miR-21 significantly modified both voltage dependence of activation and inactivation. Indeed, the midpoint of ICaL activation was shifted by ≈5 mV to more negative potentials. On the contrary, the midpoint of ICaL inactivation was shifted by ≈10 mV to more positive potentials. Bioinformatic algorithms such as miRanda and TargetMiner predicted that 3 UTR of both α1C (CACNA1C) and β2 (CACNB2), but not α2δ (CACNA2D), subunits can be targeted by miR-21, through target bindings which are highly conserved in different species, including rat, mouse, dog and humans. On the other hand, miR-21 also increased IK1. Indeed, IK1 density at -120 mV reached -1.8±0.9 pA/pF and -2.4±0.9 pA/pF in control conditions and in the presence of miR-21, respectively (P<0.05).
**Conclusion:** These results suggest that miR-21 could participate in the development of electrical remodeling, by means of a reduction of ICaL and an increase of IK1.