atria dimensions. Thus, the determination of peri-atrial adipose tissue improves the predictor value of epicardial fat in AF. These findings stablish the local interaction of epicardial fat with dysfunctional cardiac structures. Several authors have paid attention on EAT-released proteins as mediators of AF substrate. In this way, the fibrotic, inflammatory and metabolic activity of this tissue can alter the atrial myocardium structure with consequences on the electrical conduction wave. Besides, EAT also shelters ganglionated plexuses and cholinergic and adrenergic nerves wich might be regulating EAT activity and propitiating an AF substrate. The principal cholinergic neurotransmitter is acetylcholine (ACh). ACh reduces the action potential duration through muscarinic receptors. Recent data showed that the inhibition of ACh release on epicardial fat reduced AF presence on patients after coronary artery bypass grafting. In another study, the authors showed that the EAT transcriptome was dependent on localization. Some authors select EATv for studying EAT contribution to AF while others suggest the knowledge of adipose tissue closer to the disorder.

Purpose: We wanted to compare the secretome between peri-atrial EAT (EATa) and peri-ventricular EAT (EATv) and its differential regulation by acetylcholine (ACh).

Methods: EATa and EATv from 11 patients underwent open heart surgery were splitted in 100 mg pieces and cultured. After 24 hours washing, tissue proteins were separated by 2-Dimensional electrophoresis. Secretome proteins were separated by SDS-polycarilamide electrophoresis gel, quantified by an analysis software and identified by mass spectrometry. Muscarinic receptor type's expression was analyzed by real time polymerase chain reaction or western blot. Then ACh and acetylcholinesterase (AChE) activities were determined by colorimetric assays.

Results: Our results showed high similarities between EATa and EATv regarding to their protein and secretome profiles. Thus, 282 common proteins were identified in both tissues. EATa and EATv contained muscarinic receptor type 3 (mAChR 3), wich is increased in adipogenesis-induced cells. Despite AChE activity was higher in EATa (128 [17–543 mU/mg tissue]) than in EATv (43 [8–142 mU/mg tissue]); $p<0.05$, both tissues modified their released protein profile after ACh treatment (Figure 1).

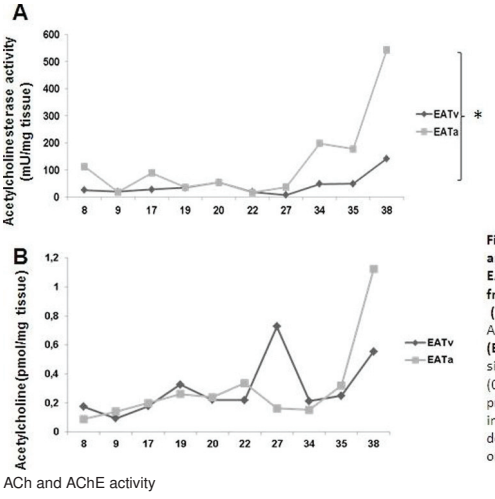

Figure 1. AChE activity and ACh in 10 EATv and EATa explants obtained from the same patients. **(A)** The mean activity of AChE was lower in EATv. **(B)** Ach levels were similar in EATv and EATa (0,29±0,06 *vs.* 0,30±0,09 pmol/mg tissue). The individual analysis determined differences in only two patients.

ACh and AChE activity

Conclusions: The similarity between the released proteins from EATa and EATv and its regulation by ACh makes them an appropriate preclinical model to clarify the interplay among EAT, AF and autonomic dysfunction.

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P2839
Significance of cell-specific precise computer simulation using new mathematical models of human induced pluripotent stem cell derived cardiomyocyte in drug testing

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Background: Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) are anticipated to be a useful tool for conducting proarrhythmia risk assessments of drug candidates, because hiPSC-CMs have not only human human ether-a-go-go-related gene (hERG) potassium channel but other cardiac ion channels in addition. However, they show different action potential (AP) morphology among cells and cell lines, and electrophysiological reaction for drug must be different among each cell (Figure A). Some in-vitro studies showed cell-to-cell differences when E4031 was used. In order to understand those phenomenon, some mathematical models has been constructed, but they could not fully precisely recapitulated AP morphologies.

Purpose: To construct precise model that is compatible for drug testing and molecular disease prediction.

Methods: We developed novel hiPSC-CMs mathematical models based on HuVEC model, adopting experimental data of ionic channels. We recorded APs from 50 hiPSC-CMs and corrected liquid junction potential, and recapitulated all AP morphologies simulationally by changing conductance of each ion current. After that, in-silico IKr-blocking test was performed.

Results: All 50 AP morphologies were successfully recapitulated in ±5% error range of each morphological parameters (APD10, APD30, APD70, APD90, mean beating rate, mean maximum diastolic potential (MDP), mean Peak voltage, and so on, Figure B). In simulational IKr-blocking test, AP duration (APD) prolongation was observed in 34 cells (64%). In 16 cells, APD shortening and rising of maximum diastolic potential (MDP) was observed. All APD shortening cell has morphological character of MDP > -63.0mV (Figure C, D).

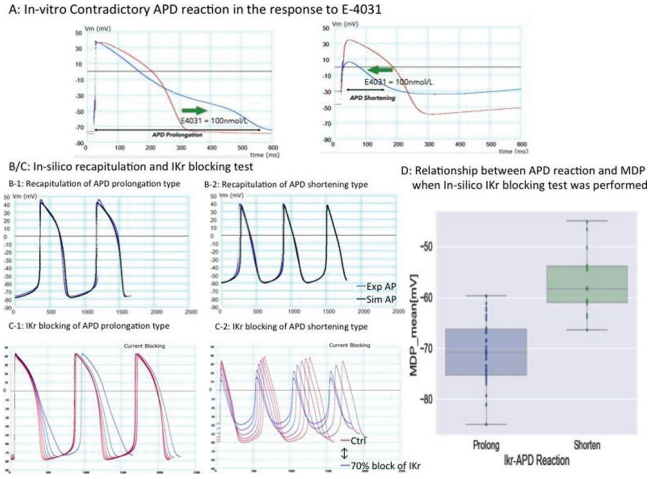

Figure 1

Conclusion: To interpret the results of hiPSC-CMs drug testing appropriately, cell-specific computer simulation using our new mathematical models is very useful.

P2840
Inhibition of Ca2+-calmodulin dependent protein kinase II has protective effect on hypertension and bradycardia in hypertensive heart disease

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Background: In heart failure, there is sinus node dysfunction and bradyarrhythmic deaths are common. Hypertension is the most important risk factor for the development of heart failure and is the most common cardiovascular disease in the modern world affecting more than 40% of the world population. Ca2+-calmodulin dependent protein kinase II (CaMKII) has been shown to be involved in the sinus node dysfunction caused by angiotensin II (ANGII)-induced hypertension. Here the effect of CaMKII on hypertension and sinus node function (in particular, ion channel expression) in hypertensive heart disease was investigated.

Methods and results: Wild–type (WT) mice and AC3-I male mice aged 10–12 weeks received ANGII, 2.5 mg/kg/day, via a subcutaneous mini-osmotic pump, while littermate controls were left untreated. AC3-I mice are genetically modified animals and have reduced CaMKII activity specifically in the heart. After four weeks of infusion with ANGII, blood pressure, measured by tail-cuff, was elevated in the WT ANGII-treated animals by 34% in systole ($P<0.0001$) and 30% in diastole ($P<0.0001$). This increase in blood pressure was halved in the AC3-I ANGII-treated animals: there were 15% and 17% increases in systole and diastole, respectively. Echocardiography showed no changes in fractional shortening, ejection fraction or left ventricular posterior wall thickness in ANGII-treated animals (both WT and AC3-I). However, it did show an increase in interventricular septal thickness in ANGII-treated animals (similar in both WT and AC3-I animals). Consistent with this, ANGII infusion caused a similar degree of hypertrophy measured by heart weight to body weight ratio in WT and AC3-I animals. The heart rate (measured using an ECGenie) in the conscious mouse was unchanged in ANGII-treated animals (both WT and AC3-I). However, the intrinsic heart rate measured in ex-vivo sinus node preparations was decreased in WT ANGII-treated animals (bradycardia of 17%, $P<0.001$). In the sinus node of WT ANGII-treated animals, there was a significant downregulation in the mRNA level of two Ca2+ channels, Cav1.2 and Cav1.3 (measured by qPCR), and this could be involved in the bradycardia. In AC3-I ANGII-treated animals, there was no downregulation of the Ca2+ channels and no bradycardia.

Conclusion: This study shows that ANGII (at a level similar to that in hypertensive