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patient-derived native cardiomyoctes. Mutagenesis of beta-2-syntrophin showed that the cardiac sodium current was reduced due to a defective functional SCN5A - beta-2-syntrophin interaction.

P5836 | BENCH
A mutation in the gene encoding the tbx5 transcription factor is associated with the Brugada Syndrome

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Introduction: Loss-of-function mutations in SCN5A, the gene encoding cardiac Nav1.5 channels, are associated with primary arrhythmogenic syndromes such as the Brugada syndrome. Strikingly, many patients with Brugada Syndrome do not carry SCN5A mutations, pointing to the implication of mutations in other genes affecting expression and/or function of Nav1.5 channels. The transcription factor Tbx5, encoded by the TBX5 gene, plays a key role in cardiac development. Moreover, it has been described that it drives SCN5A expression in the adult mouse heart. In a proband diagnosed with Brugada syndrome, in whom screening for mutations in all described Brugada Syndrome genes was negative, next generation sequencing identified a missense mutation in TBX5 encoding for p.F206L Tbx5. This variation was confirmed by Sanger, predicted as pathogenic and was not previously annotated.
Purpose: We aimed to study the effects of p.F206L Tbx5 on the cardiac sodium current (INa) to unravel whether it can be associated to Brugada syndrome.
Methods: Human native (WT) and mutated Tbx5 tagged with GFP were transfected in HL-1 cells or included in lentiviral particles for infecting human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Peak and late INa (INaL) were recorded using the whole-cell patch-clamp at room temperature. Luciferase reporter assays were conducted to determine the effects of this mutation on Nav1.5 channel promoter activity.
Results: Transfection of HL-1 cells with WT Tbx5 significantly increased the peak INa density (from -37.5±5.1 to -62.6±8.2 pA/pF, $n \geq 6$, $P < 0.05$), whereas it did not modify the kinetics or voltage-dependence of activation and inactivation of the INa. Conversely, p.F206L Tbx5 strongly reduced the peak INa density (-6.7±0.2 pA/pF; n=6; $P < 0.01$) compared to cells transfected or not with Tbx5 WT. However, p.F206L Tbx5 did not modify time- and voltage-dependent properties of the current. Neither WT nor p.F206L Tbx5, modified the INaL density (-1.9±0.7 pA/pF at -20 mV; $P > 0.05$). The effects produced by Tbx5 either WT or mutated on HL-1 cells were completely reproduced in hiPSC-CM. Indeed, in hiPSC-CM, WT Tbx5 increased (-27.6±1.9 pA/pF; n=7), while p.F206L Tbx5 decreased (-9.5±1.9 pA/pF) the peak INa compared to non-infected cells (-19.4±2.8 pA/pF; n=10; $P < 0.05$), leaving the time- and voltage-dependent properties of the current unaffected. Luciferase reporter assays demonstrated that WT Tbx5 doubled the activity of the human SCN5A minimal promoter, whereas p.F206L completely suppressed Tbx5 pro-transcriptional activity over SCN5A.
Conclusions: The p.F206L mutation disables the remarkable Tbx5 pro-transcriptional activity over human SCN5A. Therefore, loss-of-function TBX5 mutations could be associated with the Brugada syndrome.
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P5837 | BENCH
Optogenetic cardioversion terminates atrial fibrillation in wild-type mice after gene transfer

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Introduction: Atrial fibrillation (AF) is the most common cardiac arrhythmia with considerable morbidity and mortality. Restoration and maintenance of sinus rhythm is the primary aim in the treatment of patients with symptomatic AF. Electrical cardioversion using high amplitude electrical current is very painful and therefore can only be performed under analgo-sedation and cardioversion by implantable devices is not accepted by patients.
Expression of the light-gated cation channel Channelrhodopsin2 (ChR2) in cardiomyocytes enables cardiac pacing of atria and ventricles and optogenetic defibrillation of ventricular arrhythmia.
Purpose: The aim of our study was to show AF termination by light in wild-type mice after adeno associated virus (AAV) mediated gene transfer. This would open a translational approach for shockless and pain-free optogenetic cardioversion.
Methods: For gene transfer of ChR2 in fusion with mCherry, the AAV9-CAG-hChR2(H134R)-mCherry virus (Penn Vector Core), consisting of AAV9 capsid and AAV2 DNA, was injected systemically via the internal jugular vein of 10-weeks old female CD1 wild-type mice. 6–8 months later hearts were explanted and perfused in Langendorff configuration with low K+ Tyrode's solution (2 mM) supplemented with the atrial KATP-channel activator Diazoxide (300 μM). AF was induced by epicardial electrical burst stimulation of the right atrium (5 s, 30–100 Hz, 2–10 mA). ChR2-mCherry expression was analyzed by fluorescent images taken from the epicard or after dissociation of the right atrium and in histological sections of the left atrium.
Results: We found bright atrial mCherry signals indicating stable long-term ChR2 expression. Expression of mCherry was equally distributed throughout the whole atrial wall and localized within the membrane of cardiomyocytes. Histological analysis revealed no obvious side effects, such as wall thinning or infiltration of immune cells. Induced AF episodes lasted reliably longer than 5 s and epicardial illumination of the atria with focused blue light terminated AF in 6 of 7 mice. The overall efficacy of a single light pulse (470 nm, 1 s, 5 mW/mm^2, 100 mm^2) was 74.7±9.1% (n=7), which was significantly higher (p=0.016) than the spontaneous conversion rate (8.0±3.8%, n=7) analyzed in the same time frame. The percentage of ChR2 expressing cardiomyocytes correlated well with the success rate of cardioversion and importantly suggested that failure to terminate AF in one heart was because of the low expression (8%).
Conclusion: In summary, we provide the first evidence for optogenetic termination of atrial tachyarrhythmia in hearts from wild-type mice after AAV-mediated gene transfer as absolute requirement for future clinical applicability. This report lays out the foundation for the development of implantable devices for pain-free termination of AF.

P5838 | BENCH
Local epicardial light pulse terminates ventricular arrhythmias in the adult rat heart upon optogenetic modification: towards biological shockless defibrillation

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Background: Current treatment options for ventricular tachyarrhythmias (VTs) rely on modulation of cardiac electrical function through drugs, ablation or electroshocks, which are all non-biological and rather unspecific, irreversible or traumatizing interventions. Optogenetics, in contrast, is a novel biological technique allowing electrical modulation in a specific, reversible and trauma-free manner using light-gated ion channels.
Purpose: The purpose of this study was to evaluate the feasibility and requirements for optogenetic termination of monomorphic and polymorphic VTs in the whole heart.
Methods: Cardiotropic adeno-associated virus (AAV) vectors encoding the light-gated ion channel red-activatable channelrhodopsin (ReaChR) were systemically injected in the tail vain of adult Wistar rats (n=11). Four weeks later, VTs were induced in the optogenetically modified hearts by electrical burst pacing in a Langendorff setup, followed by programmed, local epicardial LED illumination.
Results: Systemic delivery of AAV vectors encoding ReaChR resulted in cardiomyocyte-restricted transgene expression with an average ventricular transduction rate of 93±4%. A single 470-nm light pulse (1000 ms, 2.97 mW/mm^2), illuminating 125 mm^2 of the ventricular surface, terminated 96% of monomorphic and 52% of polymorphic VTs vs 0% without illumination, as assessed by electrocardiogram recordings. Optogenetic termination rate of polymorphic VTs increased to 89% (p=0.029) when the ventricular illumination area was enlarged to 250 mm^2, while light pulse intensity and duration remained unchanged. Optical mapping recordings showed significant prolongation of the last voltage signal just before arrhythmia termination. Pharmacological shortening of the action potential duration (APD) almost fully inhibited light-induced arrhythmia termination, indicating an important role for APD in this process.
Conclusions: Depolarizing endogenous photocurrents generated by the optogenetically modified heart, evoked by a single epicardial light pulse, were sufficient to terminate both monomorphic and polymorphic VTs in an effective and repetitive manner. Optical termination efficiency of polymorphic VTs significantly improved by increasing the ventricular illumination area, thereby indicating an important difference in illumination requirements for terminating different types of VTs. Optogenetic arrhythmia termination could open the way to pain-free cardiac defibrillation, as it would make extraneous high-voltage shocks obsolete.
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P5839 | BENCH
Patient-specific iPSC-derived cardiomyocytes reveal a disease-causing role of an ACTN2 mutation in HCM and an unexpected LQT phenotype

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Background: Hypertrophic cardiomyopathy (HCM) is not only a genetic struc-