Optogenetic sensors in zebrafish hearts as novel in vivo electrophysiological readout tools to study cardiac arrhythmogenesis

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Background and Rationale
To study underlying mechanisms causative for cardiac arrhythmogenesis, suitable experimental animal models are needed. Zebrafish (Danio rerio) hearts have a surprisingly close electrophysiological similarity towards the human heart. We generated two zebrafish lines, expressing a genetically encoded voltage indicator (VSFP chimera13 Butterfly) or a calcium indicator (GCaMP6f) in the heart. This in order to verify whether cardiac electrical impulse propagation and intracellular Ca²⁺ homeostasis can be studied accurately in the intact embryonic zebrafish, making use of the introduced optogenetic sensors.

Methods and Results
High speed fluorescence microscopy was used for detailed analysis of in vivo spatiotemporal patterning of electrical activation and intracellular Ca²⁺ homeostasis, in embryonic zebrafish hearts. Injection of a silent heart morpholino successfully prevented motion artefacts during imaging. Ratiometric image analysis of Citrine and Cerulean signals from the VSFP sensor provided spatial information of action potential (AP) configuration (characteristics presented in Figure 1a) and propagation of electrical activation. By extracting and linearizing the myocardial wall, and plotting the ratiometric signals as line versus time (similar to a line scan), we obtained a clear depiction of electrical impulse propagation throughout the entire heart (Figure 1b). GCaMP6f signal intensity allowed examination of speed in intracellular Ca²⁺ release and reuptake of Ca²⁺. Pharmacological agents, targeting cardiac ion channels and the autonomic nervous system, where used to validate functionality and sensitivity of the sensors. Isoproterenol (100 µM) significantly (p=0.02) increased AP frequency in VSFP (n=15) and increased (p=0.35) frequency of Ca²⁺ transients in GCaMP6f (n=11) fish. Propranolol (100 µM) significantly (p=0.00003) decreased AP frequency in VSFP (n=12) and frequency of Ca²⁺ transients (p=0.00006) in GCaMP6f (n=10) fish. Nifedipine (100 µm) caused total blockade of Ca²⁺ transients in GCaMP6f fish, in a dose-responsive (1-100 µM) matter, reversible after washout. Nifedipine (100 µM) significantly reduced AP frequency (p=0.0024), increased APD10 (p=0.003), APD20 (p=0.01) and APD50 (p=0.03) in VSFP fish (n=11), not affecting APD90. E4031 (500 µM) induced a significant reduction of AP frequency (p=0.05) and increased APD50 (p=0.04) and APD90 (p=0.05) in VSFP fish (n=10).

Conclusion
By introducing the optogenetic sensors VSFP chimera13 Butterfly and GCaMP6f in zebrafish we successfully generated a minimal invasive in vivo electrophysiological readout tool for the embryonic zebrafish heart. Parallel use showed the ability to study heart rate, cardiac AP configuration, spatiotemporal patterning of electrical
activation and intracellular Ca2+ homeostasis, presenting promising new research tools that can help to unravel underlying electrophysiological disease mechanisms in arrhythmogenic cardiomyopathy.