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OPTIMIZATION OF NFATc1 IMMUNOSTAINING IN ZEBRAFISH HEARTS

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Atrial Fibrillation is the most common type of cardiac arrhythmia and is usually presented as a progressive disease in the elderly (> 65 yrs.). It is characterized as a very fast atrial rate causing an increased risk of stroke, complications, and mortality. We have identified a family with young-onset of atrial fibrillation where the phenotype segregates with a missense mutation on the cardiac transcription factor NFATc1. While this gene has been proven to be essential for cardiac development its role in AF has not been determined. To study this mechanism we used CRISPR/Cas9 genomic editing to introduce a 31bp deletion in exon 2 of *nfatc1* gene in zebrafish. This deletion is predicted to cause a premature stop codon and loss of NFATc1 function. The purpose of the present study is to confirm the NFATc1 deletion at the protein level in a nfatc1$^{-/-}$ zebrafish model that we developed. We explanted hearts from wild type (WT) and nfatc1$^{-/-}$ adult zebrafish, fixed them and sectioned them using a vibratome (Leica VT1200S). To optimize our immunostaining protocol we tested different section thickness, blocking solution composition, mounting agent, and concentration/incubation of the primary and secondary antibodies. We used two antibodies against NFATc1 (TA345589, OriGene or AAS10311C, Antibody Verify), a cTnT antibody (RVC2, DSHB) and DAPI to mark the nucleus. We scanned the slices with a confocal microscope (Leica SPE) and processed the images with ImageJ software. Using our optimized protocol, we found a positive NFATc1 staining on the hearts from the WT zebrafish. In contrast, we did not find any signal in the NFATc1 channel when scanning the hearts from the nfatc1$^{-/-}$ adult zebrafish. This confirms that our nfatc1$^{-/-}$ zebrafish do not express NFATc1 protein. This is in agreement with the reduced levels of NFATc1 mRNA that we found previously. Future experiments include immunostainings of other proteins of interest (ion channels) as well as structures present in the heart (fibroblast, collagen).