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CORRECTION OF MUTATION IN ATRIAL FIBRILLATION SUSCEPTIBILITY GENE *NFATc1* using CRISPR-Cas9 systems

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Atrial fibrillation (AF) is the most common type of irregular heartbeat. In patients with AF, the upper chambers (atria) of the heart quiver, creating an abnormal rhythm which does not allow blood to pass through the heart efficiently and often causes blood clots. When these clots break off and block blood flow through arteries, the patient undergoes a stroke.

The Tristani lab has identified a novel candidate AF-susceptibility gene, Nuclear Factor of Activated T-Cells (*NFATc1*), which codes for a transcription factor that is expressed in the heart and plays a role in cardiac development. The *NFATc1* mutation we identified is missense mutation M527L, which has been found only in affected AF patients and not sibling controls within a high-risk Utah family. To test whether *NFATc1* is indeed an AF susceptibility gene, we have generated induced pluripotent stem cells (iPSCs) from patients with AF who carry the M527L mutation and their unaffected (control) siblings. This project explores the use of CRISPR-Cas9 gene editing systems to repair the *NFATc1* M527L point mutation by homologous recombination and testing whether correcting the genetic mutation grants normal function to iPSCs differentiated into cardiomyocytes.

The first thing to do was determine the optimal iPSC electroporation protocol to be used with the transfection system to maximize the efficiency of introducing DNA of interest. This was done using a control plasmid that contained a gene for green fluorescent protein (GFP). 3 different programs were tested, based off of suggestions from the manufacturer. The efficiency of these programs were then analyzed by viewing the transformed cells under a GFP microscope. The brighter the image was, the more cells had been transformed, and therefore the more successful the program was.

The CRISPR gRNA, Cas9 protein, and donor DNA molecule were then electroporated into the iPSCs using the most effective settings, as determined in the previous step. The iPSCs were then sorted in order to isolate single cells, using fluorescence-activated cell sorting cytometry (FACS). DNA was extracted from these cells, then sequenced using two methods: TA cloning and Next Generation Sequencing. Results from the two sequencing methods were used to confirm one another. Results revealed that although the desired point mutation was not introduced, deletions in the *NFATc1* gene were introduced. This should result in premature termination of the transcription factor *NFATc1* codes for, which we hypothesize will result in a nonfunctional protein.

Future steps of this project will involve differentiating the mutant iPSCs into beating cardiomyocytes and examining their electrophysiological properties. These will be compared to

the properties of control cardiomyocytes differentiated from iPSCs which exhibit normal *NFATc1* expression. In addition to this, further electroporation experiments will be carried out until the M527L mutation is corrected in the *NFATc1* gene.