**High-throughput identification of specific QT interval modulating enhancers at the *SCN5A* locus**

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**Objective***:*Genome-wide association studies (GWAS) have indicated that sequence variation in cis-regulatory elements (CREs) plays important roles in common disease risk and trait variation. However, identification of these causal variants has remained the major challenge in complex trait genetics. Here, we performed reporter assays for all common variants at the QT interval associated *SCN5A* GWAS locus, with the goal of identifying the underlying causal variants.

**Methods***:*A target region of ~500kb at *SCN5A* was defined based on recombination hotspots (rate>10cM/Mb; estimated from HapMap) flanking the 5 independent QT interval GWAS hits. Within the target region, all common variants (minor allele frequency >5%), from the 1000 Genomes European ancestry populations in moderate linkage disequilibrium (r2>0.3) with any of the 5 independent GWAS hits, were selected for reporter assays. Both alleles of these variants were amplified along with flanking sequences and cloned upstream of a minimal promoter driven firefly luciferase gene in pGL4.23. Cardiomyocyte cells, AC16 (human) and HL1 (mouse), were transfected with test constructs and Renilla luciferase vector (for transfection normalization) in triplicate and luciferase assays were performed 24h later. All cloning and reporter assays were performed in 96- and 24-well plates.

**Results***:*Of a total 121 variants selected, 112 variants in 104 amplicons passed primer design (amplicon size 256-617bp; median 397bp), and we successfully cloned both alleles for 106 variants in 98 amplicons. In reporter assays across the two cell lines, compared to empty vector, 37 amplicons showed enhancer activity (z-score>99 %tile), with a concordance rate of ~70%. Of these 37 enhancer CREs, 9 overlapped open chromatin regions (DNase-seq peaks) observed in adult human heart tissue, largest among all human tissues evaluated by the RoadMap Epigenomics project. Of these 37 enhancer CREs, 12 showed allelic difference in reporter activity (P<0.05), thus identifying at least one enhancer CRE variant in high-to-moderate LD with each of the 5 sentinel hits. Using GTEx heart left ventricle (n=190) gene expression data, we showed correlation between *SCN5A* expression and the number of QT interval prolonging alleles across the 5 index SNPs.

**Conclusions***:*Independent of the publicly available epigenomic data, which are of limited cell-type relevance, an unbiased *in vitro* reporter screen for CREs overlapping all common variants associated with QT interval at the *SCN5A* GWAS locus identified 12 common cis-regulatory variants that map to cardiac open chromatin regions and correlate with *SCN5A* cardiac expression.