BS13 Genome-wide association study coupled with promoter interactomic data identifies neuroplastin (NPTN) as a potential novel gene regulating heart rate

Mun-Kit Choy, Yingjuan Liu, Simon Williams, James Eales, Bernard Keavney

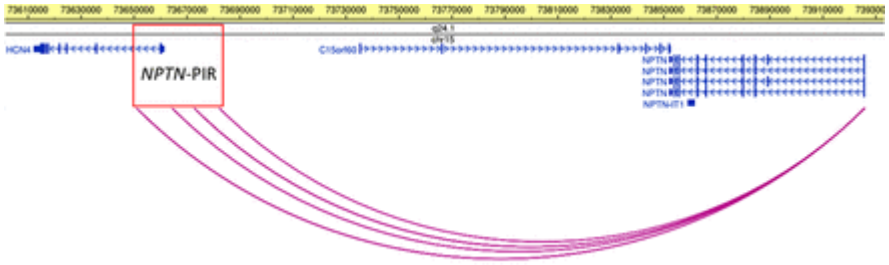
Abstract

Introduction Long-range chromosomal interactions bring distal regulatory elements to gene promoters to influence gene expression. Previously, we mapped the promoter interactome of cardiomyocytes derived from human embryonic stem cells (hESC-CMs) and contrasted these with undifferentiated hESCs. The promoter interacting regions in hESC-CMs (cPIRs) overlapped significantly with GWAS signals associated with heart rate. One such locus located upstream of the HCN4 gene was identified (Figure 1). However this risk locus was found to maintain a promoter interaction with neuroplastin (NPTN) gene ~200kb away, rather than with HCN4, which is a key cardiac ion channel. In this study, we investigate the possible role of this NPTN promoter interacting region (NPTN-PIR) in cardiac rhythm.

Methods First, we conducted data-mining using publicly available databases to identify the gene expression pattern of NPTN, cardiac expression quantitative trait loci (eQTLs) in the region, and phenotypes of NPTN-knock out mice. Second, we deleted the promoter interacting region (NPTN-PIR) in human embryonic stem cells (hESCs) to assess its effect on gene expression using CRISPR.

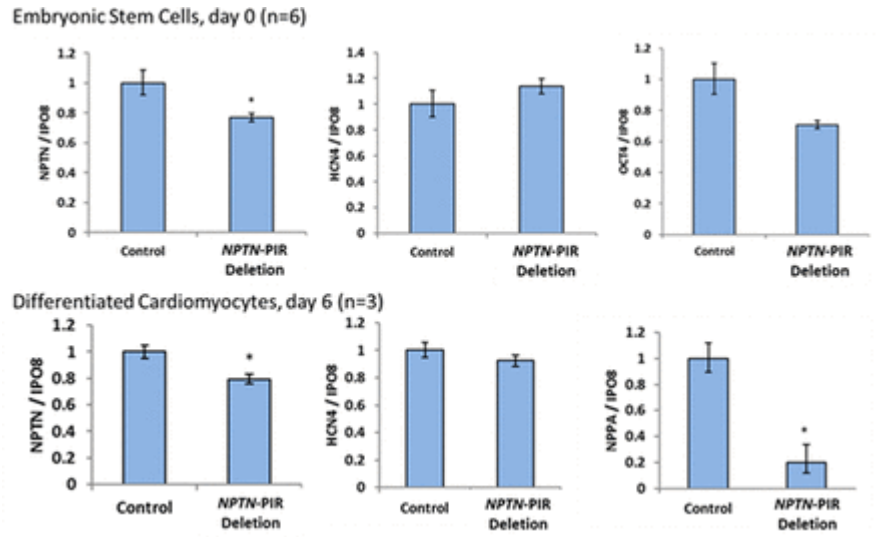
Results Although NPTN is predominantly expressed in the brain, the gp55 (2.2kb) transcript of NPTN was expressed in the heart. Specifically, atrial appendages tend to have higher expression of NPTN than ventricular tissue. Cardiac eQTLs in the NPTN-PIR were significantly associated with NPTN but not HCN4 expression, supporting the occurrence of promoter interaction between the NPTN gene and the NPTN-PIR. Phenotypically, mice with NPTN gene knockout showed a significant difference in QT dispersion, an indication of arrhythmia risk. By deleting the NPTN-PIR in hESCs, we observed that the expression of NPTN was downregulated but HCN4 was unchanged (Figure 2). Upon cardiomyocyte differentiation, marker genes for atrial cardiomyocytes, such as NPPA, were downregulated in the differentiated cells without the NPTN-PIR, suggesting that atrial differentiation may be perturbed when the NPTN-PIR is absent and NPTN is downregulated.

Conclusions We identified a novel candidate gene, NPTN, that may play an important role in regulating the heart rhythm. The study of promoter interactions in hESC-CMs is of potential utility in functional investigation of GWAS-associated regions.



Abstract BS13 Figure 1

Promoter interactions between NPTN gene and a risk locus for heart rate located upstream of HCN4 gene (NPTN-PIR).



Abstract BS13 Figure 2

Gene expression studies when NPTN-PIR was deleted by CRISPR. OCT4 is a stem cell marker gene, NPPA an atrial marker gene and IPO8 a control housekeeping gene. * $p < 0.05$.