Results

LQTS-associated CaM mutants differentially altered Ca2+-bound, but not Ca2+-free, CaM structure, with an up to 30% reduction in $\alpha$-helical content. Interaction with NSCaTE and IQ peptides were also affected, with mutant A increasing affinity for the IQ domain by almost 2-fold, whilst mutant B exhibited a greater than 3-fold weaker binding to NSCaTE. Electrophysiological examination of Cav1.2 function revealed that CaM mutations dramatically impaired channel CDI, without significantly affecting the voltage dependence of activation and inactivation.

Conclusions

Together, these results demonstrate that disease-associated CaM mutations severely impact the structure-function relationship of CaM and its regulation of Cav1.2, through mechanisms unique to each CaM variant. This provides a crucial insight into the molecular factors contributing to CaM-mediated arrhythmias. This work is funded by the Wellcome Trust PhD Studentship (to NG) and the British Heart Foundation Intermediate Basic Science Research Fellowship FS/17/56/32925 (to NH).

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 absent (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.

BS14 FIRST DEMONSTRATION OF A NOVEL EXPERIMENTAL MODEL OF ATHEROSCLEROSIS. THE EX VIVO ON-ECMO AMPUTATED 'LIVING' HUMAN LIMB MODEL
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Introduction Translational modelling for the study of atherosclerosis has been traditionally centred around the use of small animal models, translating to large animal models prior to first in man studies. We propose to disrupt this paradigm by designing an ex vivo pump perfused 'live' limb model, to enable researchers to study molecular targeting of atherosclerosis.

Purpose To develop and test a novel experimental model of atherosclerosis, based on perfusion of an amputated human limb, to reduce time spent in translational pipelines and reliance on animal preclinical atherosclerotic research.

Methods The novel model consists of taking a freshly amputated limb and incorporating it into an ex-situ ECMO system. A custom-made operating table was designed that facilitates the flow of venous blood back into the circuit. The outflow of the table is connected to an oxygenator and then a pulsatile pump in series. The oxygenator is supplied with a sweep gas of 40% oxygen: air mixture. A parallel circuit warms the system to 37°C using a thermocirculator. The circuit outflow is connected to a cannula that is sutured into the proximal end of an amputated limb artery, which permits the passage of an introducer sheath and guiding catheter for intravascular imaging and x-ray angiography (Figure 1A). The pump is set to provide an output of 15ml per stroke (to represent approximately 10% of clinical stroke volume), with an average pump rate of 70 per minute (equating to a heart rate of 70 beats per minute). Thus, total pump output, or 'cardiac output' to the limb is estimated at 1,050ml/min. Regular monitoring is performed using arterial blood gas analysis, with correction of pH, oxygenation, haemoglobin, lactate and electrolytes. All participants provided written informed consent, and ethical permission was granted by the Imperial College Healthcare Tissue Bank (REC Wales 17/WA/0161; subcollection CAR_RK_17_070)

Results The model has been successfully performed (n=3), maintaining oxygen saturations >99% for the length of perfusion (up to 6 hours). X-ray angiography (Figure 1B), intravascular ultrasound (Volcano, Phillips) (Figure 1C) and optical coherence tomography (Dragonfly Optis, Abbott) (Figure 1D) was performed. In one limb, indocyanine green, a near-infrared fluorescent probe that localises to atherosclerotic plaque, was injected into the system (2mg/kg) and left to circulate for 90 minutes. The arterial tissue was then dissected (Figure 1E), and fluorescence reflectance imaging performed (790nm) on the extracted tissue (Figure 1F). This confirmed indocyanine green uptake in areas of calcific atherosclerotic plaque on intravascular ultrasound and optical coherence tomography.

Conclusions This is the first demonstration of this novel ex vivo on ECMO ‘living’ limb experimental model of atherosclerosis, showing promise for future use in translational interventional imaging and targeting studies.