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Polymorphisms in cardiac arrhythmias

Impact of polymorphisms on cardiac event risk in long QT syndrome

Cardiovascular disease is a leading cause of mortality worldwide. Approximately half of all cardiac deaths can be classed as sudden, and are due to severe disturbances in cardiac rhythm (ventricular tachycardia or fibrillation) that lead to a fatal cardiac arrhythmia.

Long QT syndrome (LQTS) is a rare hereditary cardiac disease characterized by a delay in the ventricular repolarisation of the heart, with a detectable prolongation of the QT interval on the electrocardiogram (ECG), leading to a risk of syncope and sudden death. The majority of patients carry heterozygous mutations in the genes *KCNQ1* (LQT1, 40-50%) and *KCNH2* (LQT2, 35-45%), which encode the alpha subunits of cardiac potassium ion channels responsible for cardiac repolarisation.

While many mutation carriers have syncope and are at risk of sudden death by ventricular fibrillation, others remain asymptomatic. Variable penetrance is a common theme for many LQTS mutations, and suggests an important role for other modifying or triggering factors.

We aimed to investigate if specific single base-pair DNA changes, (single nucleotide polymorphisms, or SNPs) associated with a lengthened QTc (QT interval corrected for heart rate) in the normal population, can influence the course of disease in patients who have previously described causative mutations in ion channel genes.

We genotyped 25 selected SNPs in 112 family duos, consisting of one affected and one asymptomatic family member, both with the same LQT1 or LQT2 genotype. Population characteristics (age, gender, ethnicity, ECG parameters, treatment and history of cardiac events) were collected. Our goal was to determine whether SNP analysis could have a prognostic value for patient follow-up.

We are currently in the process of analyzing our results, however, initial analysis suggests that only one coding SNP was significantly more frequent in the symptomatic group than in asymptomatic patient. Even if increased QTc appears to be linked to cardiac events, the combination of several SNPs previously reported to induce a small increase in QT duration through GWAS studies in the normal population do not appear to play a major role in influencing patient symptoms. We conclude so far that only the genotyping of *KCNE1* D85N should be performed in all patients and could influence their risk of syncope and sudden death.

This research demonstrates progress in the field of SNP studies, and we envisage that with larger cohorts and faster and more efficient genotyping techniques, non-invasive ECG measurements coupled with detailed genetic SNP profiles will be able to increase the accuracy of risk assessment for cardiac events. This will aid in the prospective identification of those patients who carry protective or risk-enhancing SNPs. Availability of this information will make it possible to improve patients' long term outcome via medication, pacemakers or defibrillator implantation.

Exploration of microRNAs in Brugada Syndrome

Brugada syndrome (BrS) is a dominant inherited cardiac arrhythmia, also associated with ventricular fibrillation and sudden death. About 20% of patients have loss of function mutations in the cardiac sodium channel Nav1.5, encoded by the *SCN5A* gene. Mutations in other ionic channel genes have been reported and may cause another ~10% of cases, however, the genetic origin of BrS in the majority of patients remains unknown.

We aimed to explore if rare variants in the *SCN5A* 3' untranslated region (3'UTR) can be pathogenic in a cohort of BrS patients, by creating novel, or by enhancing existing targets of microRNAs.

MicroRNAs are short single stranded RNAs molecules. They act post-translationally to negatively regulate protein expression, by targeting specific sequences often located in the 3'UTR of messenger RNA transcripts.

We sequenced the entire *SNC5A* 3'UTR (2259 bp) in 97 BrS patients of various ethnic origins. Patients were selected if they had a typical BrS ECG, and they did not carry mutations in the coding region of *SCN5A*, or other tested genes. Two novel 3'UTR heterozygous variants, c.*539 A>G, and c.*1677 G>A, were identified in two sporadic patients, and were not found in >300 control chromosomes. *In silico* analysis of the two variants was performed using on-line programs which predict microRNA targeting to a given nucleotide sequence. MicroRNAs reported to be expressed in the heart and predicted to bind to the variants were also assessed with programs requiring the input of specific target:microRNA pairs. Several predictions for variant c.*539 were confirmed, however we excluded the possibility that variant c.*1677 creates a new target site for microRNAs. Candidate microRNA expression was tested in human heart RNA, and one microRNA was moderately expressed. We are now testing to see if this microRNA can differentially interact with the WT and c.*539 alleles using a vector containing a luciferase reporter gene fused to the *SCN5A* 3'UTR. In addition with this vector, we were able to show *in vitro* that endogenous microRNAs in several cell lines are able to down-regulate the expression of the reporter gene linked to the *SCN5A* 3'UTR. It would be fascinating to determine which microRNAs are likely to target the wild-type sequence, as little is known about the normal microRNA regulation of this sodium channel.

As for mis-targeting microRNAs being a new cause of BrS, we detected only two novel 3'UTR changes in the BrS patient cohort, indicating that if the c.*539 variant is responsible for the mis-targeting of a microRNA, this mechanism is not likely to be a frequent cause of disease. However, the 3'UTR of *SCN5A* had a large number of known SNPs, and perhaps these variants, in different combinations, may influence disease severity or penetrance, either in BrS or in other arrhythmias, via the action of microRNAs.

Can one specific mutation alone cause two different phenotypes?

Loss-of-function mutations in the gene *KCNQ1* encoding a potassium channel cause long QT syndrome type 1 (LQT1), whereas gain-of-function mutations are associated with short QT syndrome as well as familial atrial fibrillation (FAF). However, a single *KCNQ1* mutation causing both LQT1 and FAF, has not previously been demonstrated. We studied the genotype– phenotype relationships for six R231C mutation carrying families, five of which have LQTS and one family with four R231C-positive individuals affected by lone AF. The AF proband was screened for mutations in ten other genes linked to AF, as well *KCNH2* and *SCN5A*, and no other mutations were found. Twenty-four members from all six R231C families were genotyped for 21 SNPs, described as being at risk or protective for AF. These analyses may have identified a specific allele, *NKX2-5*, that discriminates AF versus LQTS patients. The cardiac homeobox Nkx2-5 protein is essential in cardiac development, and mutations in *NKX2-5* cause various congenital heart malformations. Interestingly, Nkx2.5 regulates the transcription of *NPPA*, a gene in which mutations cause AF. Additional study will be necessary to confirm the role of this specific allele. In addition, the R231C mutation was expressed in a heterologous cell line, and the results suggested that the mixed functional properties of the R231C *KCNQ1* mutation may predispose some families to LQT1, FAF, or both.

Overall impact

In performing these studies, we have increased the understanding of these disorders, which we hope will aid others towards the treatment and prevention of cardiac disease, contributing to the reduction of the burden of this disease on families and society.

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