Commentary

Paradigm shifts in the genetics of inherited arrhythmias: Using next-generation sequencing technologies to uncover hidden etiologies

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Long QT syndrome (LQTS), a rare inheritable arrhythmia first described by Romano and Ward et al. in the 1960s, is characterized by the prolongation of the QT interval on surface ECGs and an increased risk of potentially fatal ventricular arrhythmias, especially torsade de pointes [1]. In 1995, after years of extensive clinical investigation and linkage analysis, several research groups, including Keating et al., successfully identified three distinct LQTS phenotypes (LQT1, LQT2, and LQT3) associated with mutations in genes encoding plasma membrane ion channels (KCNQ1, KCNH2, and SCN5A, respectively) [2–4]. These seminal studies motivated further extensive genetic screening in LQTS patients using a candidate gene approach and functional analyses of the mutant genes. These efforts provided evidence that ion channel genes represent the genetic basis for several other arrhythmogenic syndromes that occur in the structurally intact heart, often referred to as idiopathic ventricular fibrillation. At present, 13 genes responsible for LQTS have been identified. Approximately 90% of the genotyped LQTS subjects belong to the three major subtypes (LQT1-3) [5] in which numbers of distinct genotype-specific clinical characteristics have been demonstrated, including T-wave morphology [6], triggers for cardiac events [7], response to the epinephrine provocation test [8] and drug therapy [9]. Genetic testing for known arrhythmia susceptibility genes has become the standard-of-care for a number of disorders, including LQTS. Considering the remarkable progress of research in this area, there is no doubt that 1995 was the year in which genetic technologies experienced a paradigm shift with respect to both the understanding and clinical management of inherited arrhythmias. However, it should be noted that despite the rapid progress in understanding the genetic basis, the etiology still remains unknown in approximately 20% of LQTS conditions [10]. Therefore, additional studies are needed to reveal the missing heritable factors associated with these syndromes.

Traditionally, DNA sequence information has been elucidated using Sanger sequencing, but this method is limited by the amount of DNA that can be processed at a given time and by the read length (average 800 base pairs). Although the Human Genome Project completed the sequencing of the entire human genome in 2001 using Sanger sequencing, it took 13 years of effort at an estimated cost of $2.7 billion. Now, next-generation sequencing (NGS), a revolutionary new genetic technology, has enabled whole genomes to be sequenced over a period of a few days at projected costs of less than $10,000. This technology involves massively parallel sequencing of clonally amplified or single DNA molecules that are spatially separated in a flow cell.

To date, the genes responsible for disease in more than 3000 Mendelian disorders have yet to be identified. NGS opens up exciting new possibilities of discovering the genes associated with these monogenic disorders. Classical strategies involve linkage analysis in families with known shared genetic heritage by identifying candidate genomic regions encompassing the gene

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with the causative mutation. After narrowing the interval with additional families/probands, a candidate gene approach or an approach of systematically sequencing the genes located within the interval can be implemented. Genome-wide association studies (GWAS), which use single nucleotide polymorphisms (SNPs), can significantly expedite the linkage analysis by narrowing the regions of interest for further directed sequencing. GWAS have been applied in the cardiac electrophysiological field and over 100 traits have been identified for common diseases such as atrial fibrillation, or ECG parameters such as PR, QT, and RR intervals and QRS durations [11]. These conventional approaches are costly and time-consuming, and their success in identifying the causative genetic variants has been variable, mainly because of the small numbers of affected individuals for a given Mendelian disease and also possibly due to locus heterogeneity. However, a recent multicenter GWAS study involving our group has shown that common genetic variation can have a strong impact on the predisposition of rare diseases such as Brugada syndrome [12].

Deep resequencing of all human genes for the discovery of allelic variants could potentially identify genes whose dysfunction underlies any given rare monogenic disease when a shared genetic heritage is not readily available. Protein-coding exons represent only approximately 1% of the entire 30-Mbp human genome but are estimated to harbor approximately 85% of the mutations that cause largely govern the expression of disease-related traits. Indeed, most Mendelian disorders are thought to be attributable to exonic mutations or splice-site mutations that alter the amino acid sequence of the affected gene. Therefore, whole exon sequencing (WES) or exome sequencing using NGS platforms will allow vastly higher sequence coverage with considerably less raw sequence and lower cost than whole genome sequencing. Since its first application in 2008, a number of previously unidentified monogenic traits have been discovered using WES techniques [13–15]. These results have been achieved frequently using a limited number of patients with genetic defects in both autosomal dominant [14] and recessive [15] disorders. The implementation of NGS technology in cardiology is expected to bring a second paradigm shift in how clinical cardiologists diagnose patients and how researchers investigate both common and rare disorders. Potentially, all causative variants and genes and their relation to phenotypes in Mendelian disorders will be uncovered in the very near future. Such technologies should also enable us to identify all the variants in an individual’s personal genome and, in particular, to highlight clinically relevant alleles. Whole genome sequencing is expected to produce a major shift in clinical practice in terms of diagnosis and understanding of cardiovascular diseases, which will ultimately enable personalized medicine based on an individual’s genome.

Crotti et al. recently identified two novel disease-causing genes responsible for sudden unexplained death syndrome using WES [16]. They studied two unrelated infants with recurrent cardiac arrest and dramatically prolonged QTC intervals who were both born to healthy parents. The probands were confirmed in advance to be negative for mutations in major LQTS genes KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2. Applying WES to the two unrelated probands and their healthy parents, they searched for de novo genetic variants and found de novo mutations in either CALM1 or CALM2, which encode calmodulin. To validate these WES results, they performed follow-up genetic screening of the calmodulin genes (CALM1, CALM2, and CALM3) on an independent cohort of 82 subjects who had congenital LQTS without a known genetic cause. They identified two individuals from this cohort who also displayed variations in calmodulin; one individual harbored the same mutation in CALM1 as that seen in proband 1, and the other individual had a novel missense mutation in CALM1. The mutations altered residues in, or adjacent to, critical calcium binding loops in the calmodulin carboxyl-terminal domain. To determine the functional consequences of calmodulin mutations, they measured the Ca$^{2+}$ binding activities of the recombinant calmodulin proteins in bacteria and confirmed that three mutations have significantly reduced Ca$^{2+}$ affinity in the C domain.

The discovery of pathologic variations in calmodulin has raised a variety of interesting and novel research questions. First, the observations by Crotti et al. illustrate the genotypic and phenotypic heterogeneity of calmodulin mutations. The mutant carriers in the study by Crotti et al. presented with early-onset life-threatening cardiac arrhythmias, prolonged QT intervals, and neurodevelopmental delay. Studies by other groups showed that mutations in CALM1 may be linked to catecholaminergic polymorphic ventricular tachycardia with [17] or without [18] prolonged QT intervals. Furthermore, although the three calmodulin genes (CALM1, CALM2, and CALM3) are dispersed in the genome and encode identical calmodulin polypeptides, the phenotype variants exhibit considerable differences in severity of clinical phenotype. This phenotypic variability may suggest that the cellular distribution, gene dosage, or function of each calmodulin gene may not be equivalent. Although the authors did not address this issue, it will be intriguing to identify the molecular targets of calmodulin in the heart that underlie the action potential prolongation, as well as the predisposition to lethal arrhythmias. Clearly, more extensive experimental work, including studies of genetically engineered animals, is required to address these questions, to refine current disease classifications, and to learn more about the genomic origins of these and other diseases.

NGS technologies have the potential to uncover the hidden etiologies of many diseases; however, such technologies are a “double edged sword” because investigators leading clinical sequencing efforts may face serious ethical issues. They may unintentionally find a life-threatening genetic variation in a patient who is unrelated to the project, or they may question the decision whether they should provide the genetic information to family members in the event that a patient dies.

Conflict of interest

There is no conflict of interest related to this article.

References


