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Genomic-based diagnosis of arrhythmia disease in a personalized medicine era

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Abstract

Introduction—Although thousands of potentially disease-causing mutations have been identified in a handful of genes, the genetic heterogeneity has led to diagnostic confusions, stemming directly from the limitations in our arsenal of genetic tools.

Areas covered—We discuss the genetic basis of cardiac ion channelopathies, the gaps in our knowledge and how Next-generation sequencing technology (NGS) and can be used to bridge them, and how induced pluripotent stem cell (iPSC) derived-cardiomyocytes can be used for drug discovery.

Expert commentary—Univariate, arrhythmogenic arrhythmias can explain some congenital arrhythmias, however, it is far from a comprehensive understanding of the complexity of many arrhythmias. Mutational screening is a critical step in personalized medicine and is critical to the management of patients with arrhythmias. The success of personalized medicine requires a more efficient way to identify a high number of genetic variants potentially implicated in cardiac arrhythmogenic diseases than traditional sequencing methods (eg, Sanger sequencing). Next-generation sequencing technology provides us with unprecedented opportunities to achieve high-throughput, rapid, and cost-effective detection of congenital arrhythmias in patients. Moreover, in personalized medicine era, iPSC derived-cardiomyocytes can be used as ‘cardiac arrhythmia in a dish’ model for drug discovery, and help us improve management of arrhythmias in patients by developing patient-specific drug therapies with target specificity.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Keywords

Precision Medicine; Next generation sequencing; Cardiac arrhythmia; Personalized Medicine; Long QT Syndrome; Brugada Syndrome; Catecholaminergic Polymorphic Ventricular Tachycardia; Short QT syndrome; Acquired Long QT syndrome

1. Introduction

Our understanding of cardiac arrhythmias has undergone a remarkable revolution in the last two decades. Many of these advances owe the existence to the genome-wide studies of several diseases. As the subject of genomics has advanced, it has enabled us to understand the complexity of several hereditary diseases. For example, mutations within the same gene such as SCN5A have been implicated in several different congenital cardiac ion channel disorders [1–4], challenging the often-covered single-factor, one-arrhythmia paradigm of cardiac dysrhythmias. While the monogenic view of cardiac channelopathies may account for some congenital arrhythmias, it falls far short of a comprehensive understanding of the complexity of these arrhythmias, as demonstrated by Mango et al. [1], mainly due to limitations in our diagnostic tools. The diagnostic limitations of traditional sequencing methods obviated the need for more powerful sequencing tools.

The traditional Sanger sequencing used in many cases, for instance, is limited to discover small insertions, deletions and substitutions. Others rely heavily on analysis of dedicated assays such as fluorescence *in situ* hybridization (FISH), or comparative genomic hybridization (CGH) microarrays to detect microdeletions.

Until recently, massively parallel DNA sequencing platforms known as high-throughput genome sequencing technologies or next-generation sequencing (NGS), were largely being utilized in research laboratories. However, given their rapid turnaround time, affordability and relative ease of use, their utilization is starting to expand into clinical medicine.

2. Next-generation sequencing and conventional Sanger sequencing

NGS or high-throughput sequencing is a term used to describe a number of different technologies which allow for rapid sequencing of large numbers of DNA and RNA molecules simultaneously. All the NGS machines share common features despite their technical details. For instance, they all require a sample library (collection of randomly sized DNA fragments representing the sample input), a sequencing machine, and data output. They differ by the specific sequencing method used. Irrespective of the machine, NGS technologies can be used to sequence an entire human genome (whole genome sequencing; WGS) within a single day. It can be constrained to only coding genes (whole exome sequencing; WES) or even small numbers of individual genes (targeted gene sequencing; TGS) using MiSeq technology, which allows small genome sequencing and assembly, and enables detection of target variants with unmatched accuracy, especially within homopolymer regions, and has the shortest sample-to-data workflow. WGS provides a richer data set, captures information across genomic polymorphisms, and potentially captures genomic rearrangements while WES covers only protein-coding exomes, which are only a

small portion of the whole genome, and not include noncoding regions which are important for regulating gene expression [2]. Targeted gene sequencing covers a panel of a select set of genes or gene regions that have known or suspected associations with the disease or phenotype identified to date, TGS produces a smaller and more manageable data set, therefore, TGS is a fast and cost-effective method in the clinical genetic screening. Unlike Sanger sequencing, NGS does not rely on plasmid cloning, a major hindrance in rapid sequencing. A recent study by Pua CJ et al. [3] shows that TGS using MiSeq system can achieve 100% sensitivity and precision for single nucleotide variants and indels across the protein-coding target, however, WGS and WES had poorer performance across the inherited cardiac condition target.

NGS technologies provide an unprecedented opportunity to screen for genetic variations underlying many congenital cardiac arrhythmias. Understanding the genetic heterogeneity of cardiac arrhythmias can help the clinician not only from a diagnostic standpoint, but may also help to guide prognostication and therapeutic options for affected patients in this era of personalized medicine. Future studies using NGS technologies, be it whole genome sequencing or whole exome sequencing, have the potential to profoundly change the practice of medicine.

Next generation will soon replace conventional sequencing in diagnostics of inherited arrhythmia syndromes, and Ware JS et al. [4] reported that the initial tests with NGS arrhythmia diagnostics achieved up to 89 % sensitivity at a fraction of current costs. The challenge of NGS technologies is the interpretation of these gene variations, for example, how to determine which of the many sequence variants in the patients are truly pathogenic, and how to identify their relationship with complex phenotypes of hereditary diseases. Therefore, NGS data need locus-specific database containing accurate information about pathogenic mutations. Consequently, it requires rigorous laboratory follow-up to confirm mutant pathogenicity [5].

Below, we discuss some well-established pathogenic variants in the major congenital cardiac ion channel abnormalities, and limitations to our understanding of their genetic basis. Using these dataset, missense changes in ion channel genes in which the genomic changes are readily interpretable, by doing so, we shed light on the utility of NGS technology as an essentially bedside diagnostic tool for clinicians to provide personalized management of congenital cardiac arrhythmias based on the patient's genomic profile.

3. Pathogenic variants in patients with congenital cardiac ion channel abnormalities

3.1. Long QT syndrome (LQTS)

LQTS is a congenital repolarization abnormality that encompasses an array of ion channel abnormalities with both autosomal dominant and recessive inheritance patterns [6] (Table 1). It bears the risk of syncope and sudden cardiac death due to ventricular tachycardias [7]. More than 1400 mutations have been identified in at least 15 distinct genes [6,8]. An additional 20% of clinically confirmed LQTS does not have detectable pathogenic variants

in one of the known LQTS causing genes [9]. There is significant variability in the spectrum of phenotypic manifestations of these mutations. Some of the phenotypic variability is accounted for by incomplete penetrance, reduced expressivity, and phenotypic overlap [6,10]. Others are attributed to a host of different processes including the role of other genes, leading to the expressed phenotype [10,11]. The location and type of mutations (e.g. missense, frameshift, and splice site) are equally important in the degree of ion channel dysfunction and phenotypic manifestation [12–16]. For instance, mutations that affect the transmembrane portion of the ion channels lead to more severe electrophysiologic phenotypes, tend to manifest at younger age, and carry greater risk of adverse cardiac events [12,13,16,17], independent of traditional clinical risk factors. Mutations involving the C-terminus of the ion channel, on the other hand, tend to carry the lowest risk of cardiac events [12]. Individuals with compound mutations, that is simultaneous mutations in the same gene or in different long-QT related genes, appear to have a more severe phenotype and earlier onset of cardiac events [18,19].

The considerable genetic heterogeneity has created some diagnostic confusion. In an attempt to simplify the process, in 2010 Allegue et al. [20] reported the development of a new diagnostic approach to screen for LQTS and BrS, based on the three most common disease-causing genes KCNQ1, KCNH2 and SCN5A, using the Sequenom MassArray system. This system employs matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry detection system [21]. However, NGS is perhaps even more sensitive than this and other certain traditional sequencing methods as reported by Asadi et al. [22]. It can allow for comprehensive analysis of many arrhythmogenic mutations simultaneously and help to distinguish pathogenic variants from genetic background noise [23]. In both of these instances, NGS was used to detect a mutation that was either not detected on gene sequence analysis alone, or identified as a novel variant in established disease causing genes, highlighting the importance of high-throughput technology. More recently, Millat G et al. [24] used NGS to sequence the five most prevalent cardiomyopathy-causing genes (KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2) on a cohort of 30 previously studied patients to evaluate the sensitivity, specificity and practicability of NGS. They found that the NGS is a highly efficient, fast, and cheap high-throughput mutation detection compared to previously used mutation detection methods, and this technology is ready to be deployed in clinical laboratories.

3.2. Brugada syndrome (BrS)

Brugada syndrome, first described in 1992, is a clinical diagnosis of genetic disorders characterized by ST segment abnormalities in leads V1-V3 on surface ECG, and carries a high risk of ventricular arrhythmias and sudden cardiac death [25,26] (Table 2). It affects various cardiac ion channels with an autosomal dominant inheritance pattern, and exhibits variable expressivity, incomplete penetrance and mixed phenotypes [26–28]. One proposed mechanism for BrS cites the accentuated difference in repolarization timing between the epicardium and endocardium, particularly in the right ventricle [29]. So far, mutations in at least 16 different genes have been identified to manifest phenotypically as BrS [30]. Since these account for approximately 25–30% of the pathogenic variants, many more genetic mutations are likely to be identified, as demonstrated by Juang et al. [31] who reported *de*

nov mutations in patients with nonfamilial BrS. Understandably, many of the genes involved in the pathogenesis of BrS have also been implicated in various other congenital arrhythmias since the underlying cellular mechanisms involve variable expression and function of the same cardiac ion channels. This mixed phenotype manifestations, that is combination of BrS with other cardiac arrhythmias, have been shown with SQTS [27,32], cardiac conduction disease [32], LQTS [33] and other arrhythmias [34].

In a large proportion of clinically confirmed BrS cases, no genetic mutations have yet been identified. NGS can therefore be used in such cases not only for diagnostic and prognostic purposes, but also to screen for mutations in relatives of the afflicted individuals. For instance, in a cohort of 45 genetically undiagnosed patients with clinical diagnosis of BrS, Allegue et al. used NGS to detect novel candidate genes associated with BrS [35]. More recently, NGS has been used to identify new potential genetic associations between BrS and other cardiac disorders [1,36].

3.3. Catecholaminergic polymorphic ventricular tachycardia (CPVT)

CPVT characteristically manifests with bidirectional VT, polymorphic VT and ventricular fibrillation in the setting of high adrenergic state and no structural heart disease [42–44]. The genes involved in CPVT mainly involve Ca^{2+} homeostasis in the sarcoplasmic reticulum of cardiac myocytes [45–48]. However, mutations in K^{+} ion channel gene *KCNJ2* have also been reported [49,50]. Inheritance patterns vary largely depending on the gene involved. Mutations in *CASQ2* display mostly an autosomal recessive inheritance pattern [51,52], although autosomal dominant inheritance has also been reported [53]. Mutations in *RYR2* gene are generally autosomal dominant [54–56]. Postma et al. showed reduced penetrance [54], while Jabbari et al. suggested that some of the previously reported monogenic mutations may, in fact, be due to polygenic variants [57].

Calcium homeostasis in the sarcoplasmic reticulum of myocytes is maintained by complex interactions between ryanodine receptor, calmodulin, triadin, calsequestrin, and Ca^{2+} itself, among others. Therefore, mutations in any component of this complex would expectedly lead to abnormalities in Ca^{2+} homeostasis and potentially cardiac arrhythmias [47,52–63]. An example is the *TRDN* gene, which encodes triadin, a protein that helps to anchor calsequestrin (*CASQ2*) to RyR2 [64,65] in the sarcoplasmic reticulum. Since triadin is a necessary component of the protein complex responsible for Ca^{2+} homeostasis, mutations in *TRDN* have been implicated in CPVT, suggesting an autosomal recessive mode of inheritance [60,66].

The inward rectifier potassium channel Kir2.1, encoded by *KCNJ2*, has also been implicated in CPVT. Tester et al. reported that CPVT caused by mutations in *KCNJ2* was due to molecular mimicry of Andersen–Tawil Syndrome/LQT7 [67].

Recently, Jimenez-Jaimez et al. [68] studied causes of unexplained cardiac arrest in a cohort of 35 patients from nine different Spanish centers using NGS. Among other cardiac channelopathies, they made a firm diagnosis of CPVT in five, SQTS in one, BrS in seven of these patients. These examples emphasize the need for NGS technologies to discover

unsuspecting common or rare genetic variants that can have vastly different phenotypic manifestations.

3.4. Short QT syndrome (SQTS)

SQTS was first associated with atrial fibrillation and sudden cardiac death [69] (Table 3). The abnormally short QT interval generally results from gain-of-function mutations in genes that affect cardiac potassium channels [70–74] or loss-of-function mutations in genes encoding calcium ion channels [27]. The resultant net outward current due to either reduction in inward sodium or calcium ions, or augmentation of outward potassium ions, or a combination of these will tend to favor early repolarization that lead to shortening of the action potential duration and the QT interval [75]. Short QT syndromes appear to be inherited in an autosomal dominant fashion [76]. Genetic studies have been less robust in SQTS compared to other channelopathies like LQTS and BrS. This is another area where NGS technologies can provide a powerful means for expanding our understanding of the genetic basis of SQTS [68].

3.5. Acquired long QT syndrome (aLQTS)

Acquired long QT syndrome is characterized by prolongation of the QT interval in response to a nongenetic stimulus that can lead to torsades de pointes ventricular tachycardia. The triggering stimulus can be certain drugs [77–79], hypokalemia [80], and bradycardia [81] among many others. Most cases of aLQTS are attributed to mutations in *KCNH2* and *KCNQ1* with over 60% of true aLQTS attributed to *KCNH2* [82]. Itoh et al. also report mutations in other genes such as *SCN5A*, *KCNE1/KCNE2* as causing a smaller percentage of overall aLQTS. It is interesting to note that many of the drugs that precipitate aLQTS appear to affect *HERG* or *KCNH2* K⁺ ion channels [83–85].

Genes responsible for even more rare forms of this disorder will likely continue to emerge as our diagnostic tools continue to evolve and newer generations of NGS technologies continue to become more readily available, affordable and easier to use.

4. Expert commentary and five-year view

The goal of personalized medicine in cardiac arrhythmia diagnosis, prevention and treatment is to take account of the individual variability in genes for each patient [86]. The traditional sequencing approach is still applied to diagnosis of certain cardiac arrhythmia, however, its low-coverage for defined regions remains a significant bottleneck in the diagnosis of complex cardiac arrhythmia. The recent developments of high-throughput sequencing technologies and bioinformatics have drastically changed the diagnosis of cardiac arrhythmia. NGS technologies can be constrained to only coding genes (whole exome sequencing) or even small numbers of individual genes. There are several NGS platforms with key differences; however, they all have relatively fast turnaround time [87,88] compared to traditional sequencing methods like Sanger sequencing, and a single sequencing for entire human genome can take in one day. Thus, NGS technologies provide an unprecedented opportunity to screen for genetic variations underlying many congenital cardiac arrhythmias in a fast, accurate and cost-effective manner. It has even been used in

postmortem case analyses of a cohort of autopsy-negative sudden unexplained deaths to reveal the likely culprit congenital cardiac channel mutations [89]. The potential for this technology is immense. It continues to evolve as we have witnessed from popular platforms of Roche 454, *AB SOLiD*, Illumina to newer platforms such as Ion Torrent, PacBio RS, Oxford Nanopore etc. NGS technologies can be applied at scale of whole genome sequencing to exome sequencing, target resequencing, RNA-sequencing and so on [90,91]. It has even been suggested that exome sequencing may be used as an additive tool to predict the pathogenicity of known variants of certain congenital arrhythmias [92].

The NGS has advantages of revealing multiple variants of about 19,000 coding genes at the same time, therefore, it changes the diagnostic paradigm [93]. Neveling et al. [94] did a *post hoc* comparison of sanger sequencing and NGS sequence for the diagnosis of heterogeneous diseases, and found that NGS sequencing has a much higher diagnostic yield than Sanger sequencing, and the larger number of genes captured by the NGS could lead to a clearly superior diagnostic yield at a fraction of the cost. As the NGS technology continues to improve and the cost of whole exome or genome sequencing reduces, NGS will become the mainstream tools for diagnosis of cardiac arrhythmia diseases and for rational design of personalized medicine (Figure 1).

The challenge of NGS technologies in diagnosis of cardiac arrhythmia is the interpretation of high-throughput data. To avoid the errors in interpreting the sequence variants from NGS data, we need follow the recent published guidance from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [95]. Physicians should obtain accurate and complete clinical information, including patients and their family history, physical examination, and previous laboratory tests, and then testing the genetic variants of patients and their family members to distinguish mutations leading to arrhythmias and those benign or accidental variants, which are unique to the individual [95]. To further identify patient population with genotypic or phenotypic segmentation, cardiologists should also consult with experts in statistical or population genetics to avoid wrong conclusion of the relationship of the gene mutation to the cardiac arrhythmia, and make sure the family history is consistent with the inheritance disease mode.

In the era of personalized medicine, induced pluripotent stem cell (iPSC) technology is revolutionizing medical science, allowing exploration of disease mechanisms and new therapeutic molecular targets, as well as providing opportunities for drug discovery and proof-of-concept study drug development [96]. As the technologies to differentiate iPSC into cardiomyocytes become mature, we can use the discovery of a personalized antiarrhythmia drug with target specificity by generating iPSC-derived cardiomyocytes carrying the genotype of patients. Different types of the long-QT syndrome have been studied by analyzing the phenotype of cardiomyocytes generated from patient-specific iPSC lines [97,98]. We can use iPSC-cardiomyocytes as 'cardiac arrhythmia in a dish' model for drug testing, and to improve management of arrhythmias in patients in the future by developing patient-specific drug therapies with target specificity.

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References

1. Mango R, Luchetti A, Sangiuolo R, et al. Next generation sequencing and linkage analysis for the molecular diagnosis of a novel overlapping syndrome characterized by hypertrophic cardiomyopathy and typical electrical instability of Brugada syndrome. *Circ J Off J Jpn Circ Soc.* 2016; 80:938–949.
2. Meynert AM, Ansari M, FitzPatrick DR, et al. Variant detection sensitivity and biases in whole genome and exome sequencing. *BMC Bioinf.* 2014; 15:247.
3. Pua CJ, Bhalshankar J, Miao K, et al. Development of a comprehensive sequencing assay for inherited cardiac condition genes. *J Cardiovasc Transl Res.* 2016; 9:3–11. [PubMed: 26888179]
4. Ware JS, John S, Roberts AM, et al. Next generation diagnostics in inherited arrhythmia syndromes: a comparison of two approaches. *J Cardiovasc Transl Res.* 2013; 6:94–103. [PubMed: 22956155]
5. Tucker EJ, Mimaki M, Compton AG, et al. Next-generation sequencing in molecular diagnosis: NUBPL mutations highlight the challenges of variant detection and interpretation. *Hum Mutat.* 2012; 33:411–418. [PubMed: 22072591]
6. Alders, M., Christiaans, I. Long QT Syndrome. In: Pagon, RA, Adam, MP, Ardinger, HH., et al., editors. *GeneReviews(R)*. Seattle (WA): University of Washington; 1993.
7. Moss AJ. Long QT syndrome. *JAMA.* 2003; 289:2041–2044. [PubMed: 12709446]
8. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation.* 2000; 102:1178–1185. [PubMed: 10973849]
9. Medeiros-Domingo A, Iturralde-Torres P, Ackerman MJ. Clinical and genetic characteristics of long QT syndrome. *Rev Esp Cardiol.* 2007; 60:739–752. [PubMed: 17663859]
10. Giudicessi JR, Ackerman MJ. Determinants of incomplete penetrance and variable expressivity in heritable cardiac arrhythmia syndromes. *Transl Res: J of Lab and Clin Med.* 2013; 161:1–14.
11. Viswanathan PC, Benson DW, Balser JR. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. *J Clin Invest.* 2003; 111:341–346. [PubMed: 12569159]
12. Shimizu W, Moss AJ, Wilde AA, et al. Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol.* 2009; 54:2052–2062. [PubMed: 19926013]
13. Moss AJ, Shimizu W, Wilde AA, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation.* 2007; 115:2481–2489. [PubMed: 17470695]
14. Newton-Cheh C, Eijgelsheim M, Rice KM, et al. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet.* 2009; 41:399–406. [PubMed: 19305408]
15. Pfeufer A, Sanna S, Arking DE, et al. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. *Nat Genet.* 2009; 41:407–414. [PubMed: 19305409]
16. Moss AJ, Zareba W, Kaufman ES, et al. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. *Circulation.* 2002; 105:794–799. [PubMed: 11854117]
17. Thomas D, Khalil M, Alter M, et al. Biophysical characterization of KCNQ1 P320 mutations linked to long QT syndrome 1. *J Mol Cell Cardiol.* 2010; 48:230–237. [PubMed: 19540844]
18. Itoh H, Shimizu W, Hayashi K, et al. Long QT syndrome with compound mutations is associated with a more severe phenotype: a Japanese multicenter study. *Heart Rhythm: Off J Heart Rhythm Soc.* 2010; 7:1411–1418.

19. Westenskow P, Splawski I, Timothy KW, et al. Compound mutations: a common cause of severe long-QT syndrome. *Circulation*. 2004; 109:1834–1841. [PubMed: 15051636]
20. Allegue C, Gil R, Sanchez-Diz P, et al. A new approach to long QT syndrome mutation detection by Sequenom MassARRAY system. *Electrophoresis*. 2010; 31:1648–1655. [PubMed: 20486126]
21. Oeth P, Del Mistro G, Marnellos G, et al. Qualitative and quantitative genotyping using single base primer extension coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MassARRAY). *Methods in Mol Biol*. 2009; 578:307–343. [PubMed: 19768603]
22. Asadi M, Foo R, Samienasab MR, et al. Genetic analysis of Iranian family with hereditary cardiac arrhythmias by next generation sequencing. *Adv Biomed Res*. 2016; 5:55. [PubMed: 27110552]
23. Campuzano O, Sarquella-Brugada G, Mademont-Soler I, et al. Identification of Genetic Alterations, as Causative Genetic Defects in Long QT Syndrome, Using Next Generation Sequencing Technology. *Plos One*. 2014; 9:e114894. [PubMed: 25494010]
24. Millat G, Chanavat V, Rousson R. Evaluation of a new high-throughput next-generation sequencing method based on a custom AmpliSeq library and ion torrent PGM sequencing for the rapid detection of genetic variations in long QT syndrome. *Mol Diagn Ther*. 2014; 18:533–539. [PubMed: 24687331]
25. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol*. 1992; 20:1391–1396. [PubMed: 1309182]
26. Brugada R, Campuzano O, Sarquella-Brugada G, et al. Brugada syndrome. *Methodist Debakey Cardiovasc J*. 2014; 10:25–28. [PubMed: 24932359]
27. Antzelevitch C, Pollevick GD, Cordeiro JM, et al. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation*. 2007; 115:442–449. [PubMed: 17224476]
28. Crotti L, Marcou CA, Tester DJ, et al. Spectrum and prevalence of mutations involving BrS1-through BrS12-susceptibility genes in a cohort of unrelated patients referred for Brugada syndrome genetic testing: implications for genetic testing. *J Am Coll Cardiol*. 2012; 60:1410–1418. [PubMed: 22840528]
29. Antzelevitch C. The Brugada syndrome: ionic basis and arrhythmia mechanisms. *J Cardiovasc Electrophysiol*. 2001; 12:268–272. [PubMed: 11232628]
30. Nielsen MW, Holst AG, Olesen SP, et al. The genetic component of Brugada syndrome. *Front Physiol*. 2013; 4:179. [PubMed: 23874304]
31. Juang JM, Lu TP, Lai LC, et al. Disease-targeted sequencing of ion channel genes identifies de novo mutations in patients with non-familial Brugada syndrome. *Sci Rep*. 2014; 4:6733. [PubMed: 25339316]
32. Rossenbacker T, Carroll SJ, Liu H, et al. Novel pore mutation in SCN5A manifests as a spectrum of phenotypes ranging from atrial flutter, conduction disease, and Brugada syndrome to sudden cardiac death. *Heart Rhythm: Off J Heart Rhythm Soc*. 2004; 1:610–615.
33. Makita N, Behr E, Shimizu W, et al. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. *J Clin Invest*. 2008; 118:2219–2229. [PubMed: 18451998]
34. Brugada, R., Campuzano, O., Sarquella-Brugada, G., et al. Brugada Syndrome. In: Pagon, RA, Adam, MP, Ardinger, HH., et al., editors. *GeneReviews(R)*. Seattle (WA): University of Washington; 1993.
35. Allegue C, Coll M, Mates J, et al. Genetic analysis of arrhythmogenic diseases in the era of NGS: the complexity of clinical decision-making in Brugada syndrome. *Plos One*. 2015; 10:e0133037. [PubMed: 26230511]
36. Di Resta C, Pietrelli A, Sala S, et al. High-throughput genetic characterization of a cohort of Brugada syndrome patients. *Hum Mol Genet*. 2015; 24:5828–5835. [PubMed: 26220970]
37. Modell SM, Lehmann MH. The long QT syndrome family of cardiac ion channelopathies: a HuGE review. *Genet Med: Off J Am Coll Med Genet*. 2006; 8:143–155.
38. Boczek NJ, Best JM, Tester DJ, et al. Exome sequencing and systems biology converge to identify novel mutations in the L-type calcium channel, CACNA1C, linked to autosomal dominant long QT syndrome. *Circ Cardiovasc Genet*. 2013; 6:279–289. [PubMed: 23677916]

39. Juang JJ, Horie M. Genetics of Brugada syndrome. *J Arrhythmia*. 2016; 32:418–425.
40. Gourraud JB, Barc J, Thollet A, et al. The Brugada syndrome: a rare arrhythmia disorder with complex inheritance. *Front Cardiovasc Med*. 2016; 3:9. [PubMed: 27200363]
41. Maluli HA, Meshkov AB. A short story of the short QT syndrome. *Cleve Clin J Med*. 2013; 80:41–47. [PubMed: 23288944]
42. Leenhardt A, Lucet V, Denjoy I, et al. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation*. 1995; 91:1512–1519. [PubMed: 7867192]
43. Femenia F, Barbosa-Barros R, Sampaio SV, et al. Bidirectional ventricular tachycardia: a hallmark of catecholaminergic polymorphic ventricular tachycardia. *Indian Pacing Electrophysiol J*. 2012; 12:65–68. [PubMed: 22557844]
44. Kawata H, Ohno S, Aiba T, et al. Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) associated with ryanodine receptor (RyR2) gene mutations- long-term prognosis after initiation of medical treatment. *Circ J: Off J Jpn Circ Soc*. 2016; 80(9):1907–1915.
45. Kawamura M, Ohno S, Naiki N, et al. Genetic background of catecholaminergic polymorphic ventricular tachycardia in Japan. *Circ J: Off J Jpn Circ Soc*. 2013; 77:1705–1713.
46. Okuda S, Yano M. New data on catecholaminergic polymorphic ventricular tachycardia in Japan: from the bench to the bedside. *Circ J: Off J Jpn Circ Soc*. 2013; 77:1684–1686.
47. Priori SG, Chen SR. Inherited dysfunction of sarcoplasmic reticulum Ca²⁺ handling and arrhythmogenesis. *Circ Res*. 2011; 108:871–883. [PubMed: 21454795]
48. Knollmann BC, Chopra N, Hlaing T, et al. Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca²⁺ release, and catecholaminergic polymorphic ventricular tachycardia. *J Clin Invest*. 2006; 116:2510–2520. [PubMed: 16932808]
49. Barajas-Martinez H, Hu D, Ontiveros G, et al. Biophysical and molecular characterization of a novel de novo KCNJ2 mutation associated with Andersen-Tawil syndrome and catecholaminergic polymorphic ventricular tachycardia mimicry. *Circ Cardiovasc Genetics*. 2011; 4:51–57.
50. Zhang L, Benson DW, Tristani-Firouzi M, et al. Electrocardiographic features in Andersen-Tawil syndrome patients with KCNJ2 mutations: characteristic T-U-wave patterns predict the KCNJ2 genotype. *Circulation*. 2005; 111:2720–2726. [PubMed: 15911703]
51. Liu N, Denegri M, Dun W, et al. Abnormal propagation of calcium waves and ultrastructural remodeling in recessive catecholaminergic polymorphic ventricular tachycardia. *Circ Res*. 2013; 113:142–152. [PubMed: 23674379]
52. Lahat H, Pras E, Olender T, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet*. 2001; 69:1378–1384. [PubMed: 11704930]
53. Gray B, Bagnall RD, Lam L, et al. A novel heterozygous mutation in cardiac calsequestrin causes autosomal dominant catecholaminergic polymorphic ventricular tachycardia. *Heart Rhythm: Off J Heart Rhythm Soc*. 2016; 13:1652–1660.
54. Postma AV, Denjoy I, Hoorntje TM, et al. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. *Circ Res*. 2002; 91:e21–26. [PubMed: 12386154]
55. Laitinen PJ, Brown KM, Piippo K, et al. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*. 2001; 103:485–490. [PubMed: 11157710]
56. Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001; 103:196–200. [PubMed: 11208676]
57. Jabbari J, Jabbari R, Nielsen MW, et al. New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. *Circ Cardiovasc Genet*. 2013; 6:481–489. [PubMed: 24025405]
58. Knollmann BC. New roles of calsequestrin and triadin in cardiac muscle. *J Physiol*. 2009; 587(Pt 13):3081–3087. [PubMed: 19451205]
59. Lanner JT, Georgiou DK, Joshi AD, et al. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb Perspect Biol*. 2010; 2:a003996. [PubMed: 20961976]

60. Roux-Buisson N, Cacheux M, Fourest-Lieuvin A, et al. Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. *Hum Mol Genet.* 2012; 21:2759–2767. [PubMed: 22422768]
61. Liu Z, Liu X, Yu H, et al. Common variants in TRDN and CALM1 are associated with risk of sudden cardiac death in chronic heart failure patients in Chinese Han population. *Plos One.* 2015; 10(7):e0132459. [PubMed: 26196381]
62. Nyegaard M, Overgaard MT, Sondergaard MT, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. *Am J Hum Genet.* 2012; 91:703–712. [PubMed: 23040497]
63. di Barletta MR, Viatchenko-Karpinski S, Nori A, et al. Clinical phenotype and functional characterization of CASQ2 mutations associated with catecholaminergic polymorphic ventricular tachycardia. *Circulation.* 2006; 114:1012–1019. [PubMed: 16908766]
64. Gyorke I, Hester N, Jones LR, et al. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys J.* 2004; 86:2121–2128. [PubMed: 15041652]
65. Kobayashi YM, Alseikhan BA, Jones LR. Localization and characterization of the calsequestrin-binding domain of triadin 1. Evidence for a charged beta-strand in mediating the protein-protein interaction. *J Biol Chem.* 2000; 275:17639–17646. [PubMed: 10748065]
66. Rooryck C, Kyndt F, Bozon D, et al. New family with catecholaminergic polymorphic ventricular tachycardia linked to the triadin gene. *J Cardiovasc Electrophysiol.* 2015; 26:1146–1150. [PubMed: 26200674]
67. Tester DJ, Arya P, Will M, et al. Genotypic heterogeneity and phenotypic mimicry among unrelated patients referred for catecholaminergic polymorphic ventricular tachycardia genetic testing. *Heart Rhythm: Off J Heart Rhythm Soc.* 2006; 3:800–805.
68. Jimenez-Jaimez J, Peinado R, Grima EZ, et al. Diagnostic approach to unexplained cardiac arrest (from the FIVI-Gen Study). *Am J Cardiol.* 2015; 116:894–899. [PubMed: 26189708]
69. Gussak I, Brugada P, Brugada J, et al. Idiopathic short QT interval: a new clinical syndrome? *Cardiology.* 2000; 94:99–102. [PubMed: 11173780]
70. Brugada R, Hong K, Dumaine R, et al. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation.* 2004; 109:30–35. [PubMed: 14676148]
71. Bellocq C, van Ginneken AC, Bezzina CR, et al. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. *Circulation.* 2004; 109:2394–2397. [PubMed: 15159330]
72. Hong K, Piper DR, Diaz-Valdecantos A, et al. De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. *Cardiovasc Res.* 2005; 68:433–440. [PubMed: 16109388]
73. Priori SG, Pandit SV, Rivolta I, et al. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. *Circ Res.* 2005; 96:800–807. [PubMed: 15761194]
74. Sun Y, Quan XQ, Fromme S, et al. A novel mutation in the KCNH2 gene associated with short QT syndrome. *J Mol Cell Cardiol.* 2011; 50:433–441. [PubMed: 21130771]
75. Gollob MH, Redpath CJ, Roberts JD. The short QT syndrome: proposed diagnostic criteria. *J Am Coll Cardiol.* 2011; 57:802–812. [PubMed: 21310316]
76. Patel C, Yan GX, Antzelevitch C. Short QT syndrome: from bench to bedside. *Circ Arrhythm Electrophysiol.* 2010; 3:401–408. [PubMed: 20716721]
77. Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med.* 2004; 350:1013–1022. [PubMed: 14999113]
78. Wysowski DK, Bacsanyi J. Cisapride and fatal arrhythmia. *N Engl J Med.* 1996; 335:290–291. [PubMed: 8657260]
79. Yap YG, Camm AJ. Drug induced QT prolongation and torsades de pointes. *Heart.* 2003; 89:1363–1372. [PubMed: 14594906]
80. Berthet M, Denjoy I, Donger C, et al. C-terminal HERG mutations: the role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. *Circulation.* 1999; 99:1464–1470. [PubMed: 10086971]
81. Kurita T, Ohe T, Marui N, et al. Bradycardia-induced abnormal QT prolongation in patients with complete atrioventricular block with torsades de pointes. *Am J Cardiol.* 1992; 69:628–633. [PubMed: 1536113]

82. Itoh H, Crotti L, Aiba T, et al. The genetics underlying acquired long QT syndrome: impact for genetic screening. *Eur Heart J*. 2016; 37:1456–1464. [PubMed: 26715165]
83. Sanguinetti MC, Bennett PB. Antiarrhythmic drug target choices and screening. *Circ Res*. 2003; 93:491–499. [PubMed: 14500332]
84. Mitcheson JS, Chen J, Lin M, et al. A structural basis for drug-induced long QT syndrome. *PNAS*. 2000; 97:12329–12333. [PubMed: 11005845]
85. Fernandez D, Ghanta A, Kauffman GW, et al. Physicochemical features of the HERG channel drug binding site. *J Biol Chem*. 2004; 279:10120–10127. [PubMed: 14699101]
86. Heinzelmann E. 7th waedenswil day of chemistry personalized medicine: full speed ahead! *Chimia*. 2015; 69:491–493.
87. Quail MA, Smith M, Coupland P, et al. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics*. 2012; 13:341. [PubMed: 22827831]
88. Frey KG, Herrera-Galeano JE, Redden CL, et al. Comparison of three next-generation sequencing platforms for metagenomic sequencing and identification of pathogens in blood. *BMC Genomics*. 2014; 15:96. [PubMed: 24495417]
89. Farrugia A, Keyser C, Hollard C, et al. Targeted next generation sequencing application in cardiac channelopathies: Analysis of a cohort of autopsy-negative sudden unexplained deaths. *Forensic Sci Int*. 2015; 254:5–11. [PubMed: 26164358]
90. Mardis ER. A decade's perspective on DNA sequencing technology. *Nature*. 2011; 470:198–203. [PubMed: 21307932]
91. Loman NJ, Constantinidou C, Chan JZ, et al. High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. *Nat Reviews Microbiol*. 2012; 10:599–606.
92. Risgaard B, Jabbari R, Refsgaard L, et al. High prevalence of genetic variants previously associated with Brugada syndrome in new exome data. *Clin Genet*. 2013; 84:489–495. [PubMed: 23414114]
93. Thiffault I, Lantos J. The challenge of analyzing the results of next-generation sequencing in children. *Pediatrics*. 2016; 137(Suppl 1):S3–S7. [PubMed: 26729700]
94. Neveling K, Feenstra I, Gilissen C, et al. A post-hoc comparison of the utility of Sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. *Hum Mutat*. 2013; 34:1721–1726. [PubMed: 24123792]
95. Richards S, Aziz N, Bale S, et al. Committee ALQA. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015; 17:405–424. [PubMed: 25741868]
96. Inoue H, Yamanaka S. The use of induced pluripotent stem cells in drug development. *Clin Pharmacol Ther*. 2011; 89:655–661. [PubMed: 21430656]
97. Sinnecker D, Goedel A, Dorn T, et al. Modeling long-QT syndromes with iPS cells. *J Cardiovasc Transl Res*. 2013; 6:31–36. [PubMed: 23076501]
98. Wang Y, Liang P, Lan F, et al. Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. *J Am Coll Cardiol*. 2014; 64:451–459. [PubMed: 25082577]

Key issues

- Arrhythmias are the leading cause of morbidity and mortality in patients with congenital heart disease.
- Mutational screening is a critical step in personalized medicine and is critical to the management of patients with arrhythmias.
- Univariate, arrhythmogenic arrhythmias can explain some congenital arrhythmias, however, it is far from a comprehensive understanding of the complexity of many arrhythmias.
- The success of personalized medicine requires a more efficient way to identify a high number of genetic variants potentially implicated in cardiac arrhythmogenic disease than traditional sequencing methods (eg, Sanger sequencing). Next-generation sequencing technology provides us with unprecedented opportunities to achieve high-throughput, rapid, and cost-effective detection of congenital arrhythmias in patients.
- iPSC-cardiomyocytes can be used as ‘cardiac arrhythmia in a dish’ model for drug testing to identify patient-specific drug with target specificity.

Figure 1.
Summary – Precise medicine vs Traditional medicine in genetic diagnosis of cardiac arrhythmia.

Table 1

Genetic basis of LQTS [37,38].

Type	Gene name	Locus	Ion current affected	Effect of mutation
LQT1	<i>KCNQ1</i>	11p15.5–15.4	I _{Ks}	Loss of function
LQT2	<i>KCNH2</i>	7q36.1	I _{Kr}	Loss of function
LQT3	<i>SCN5A</i>	3p22.2	I _{Na}	Gain of function
LQT4	<i>ANK2</i>	4q25–q26	Na ⁺ /Ca ²⁺ , Na/K ATPase, InsP3	Loss of function
LQT5	<i>KCNE1</i>	21q22.12	I _{Ks}	Loss of function
LQT6	<i>KCNE2</i>	21q22.11	I _{Kr}	Loss of function
LQT7	<i>KCNJ2</i>	17q24.3	I _{K1}	Loss of function
LQT8	<i>CACNA1C</i>	12p13.33	I _{CaL}	Gain of function
LQT9	<i>CAV3</i>	3p25.3	I _{Na}	Gain of function
LQT10	<i>SCN4B</i>	11q23.3	I _{Na}	Gain of function
LQT11	<i>AKAP9</i>	7q21.2	I _{Ks}	Loss of function
LQT12	<i>SNTA1</i>	20q11.21	I _{Na}	Gain of function
LQT13	<i>KCNJ5</i>	11q24.3	I _K	Loss of function
LQT14	<i>CALM1</i>	14q32.11	I _{Ca}	Loss of function
LQT15	<i>CALM2</i>	2q21	I _{Ca}	Loss of function

Table 2

Genetic basis of Brugada syndrome [39,40].

BrS subtype	Associated gene	Locus	Ion channel	Net effect
BrS1	<i>SCN5A</i>	3p22.2	I _{Na}	Loss of function
BrS2	<i>GPD1-L</i>	3p22.3	I _{Na}	Loss of function
BrS3	<i>CACNA1C</i>	12p13.33	I _{Ca-L}	Loss of function
BrS4	<i>CACNB2b</i>	10p12.31	I _{Ca-L}	Loss of function
BrS5	<i>SCN1B</i>	19q13.11	I _{Na}	Loss of function
BrS6	<i>KCNE3</i>	11q13.4	I _{to}	Gain of function
BrS7	<i>SCN3b</i>	11q24.1	I _{Na}	Loss of function
BrS8	<i>KCN4</i>	11p14.1	I _K	Gain of function
BrS9	<i>KCND3</i>	1p13.2	I _{to}	Gain of function
BrS10	<i>KCNJ8</i>	12p12.1	I _{KATP}	Gain of function
BrS11	<i>CACNA2D1</i>	7q21.11	I _{Ca-L}	Loss of function
BrS12	<i>KCNE5</i>	Xq23	I _{to}	Gain of function
BrS13	<i>RANGRF</i>	17p13.1	I _{Na}	Loss of function
BrS14	<i>KCND2</i>	7q31.31	I _{to}	Gain of function
BrS15	<i>TRPM4</i>	19q13.33	I _{Na}	Loss of function
BrS16	<i>SCN2B</i>	11q23.3	I _{Na}	Loss of function
BrS17	<i>PKP2</i>	12p11.21	I _{Na}	Loss of function
BrS18	<i>ABCC9</i>	12p12.1	I _{KAPT}	Gain of function
BrS19	<i>SLMAP</i>	3p14.3	I _{Na}	Loss of function
BrS20	<i>KCNH2</i>	7q36.1	I _{Kr}	Gain of function

Table 3

Genetic basis of short QT syndrome [41].

Type	Gene	Locus	Ion channel	Net effect
SQT1	<i>KCNH2/HERG</i>	7q36.1	I _{Kr}	Gain of function
SQT2	<i>KCNQ1</i>	11p15.5–4	I _{Ks}	Gain of function
SQT3	<i>KCNJ2</i>	17q24.3	I _{K1}	Gain of function
SQT4	<i>CACNA1C</i>	12p13.33	I _{Ca-L}	Loss of function
SQT5	<i>CACNB2b</i>	10p12.33–31	I _{Ca-L}	Loss of function