HHS Public Access

Author manuscript

Heart Rhythm. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Heart Rhythm. 2015 May ; 12(5): 1062–1070. doi:10.1016/j.hrthm.2015.01.011.

Next Generation Sequencing for the Diagnosis of Cardiac Arrhythmia Syndromes

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Abstract

Inherited arrhythmia syndromes are collectively associated with substantial morbidity, yet our understanding of the genetic architecture of these conditions remains limited. Recent technological advances in DNA sequencing have led to the commercialization of genetic testing now widely available in clinical practice. In particular, next generation sequencing allows the large-scale and rapid assessment of entire genomes. Although next generation sequencing represents a major technological advance, it has introduced numerous challenges with respect to the interpretation of genetic variation, and has opened a veritable floodgate of biological data of unknown clinical significance to practitioners. In this review, we discuss current genetic testing indications for inherited arrhythmia syndromes, broadly outline characteristics of next generation sequencing techniques, and highlight challenges associated with such testing. We further summarize future directions that will be necessary to address to enable the widespread adoption of next generation sequencing in the routine management of patients with inherited arrhythmia syndromes.

Keywords

Sequencing; mutation; arrhythmia; genetics

Inherited arrhythmia syndromes (**Table 1**) are collectively associated with substantial risks of morbidity and sudden cardiac death. Over the past three decades, recognized familial aggregation and traditional genetic mapping efforts have substantiated the genetic basis of these arrhythmia syndromes. Yet for many inherited arrhythmia syndromes, our understanding of the genetic architecture and causal mechanisms remains limited.

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Conflicts of interest: None

Recent technological advances in DNA sequencing have enabled insights into human biology and disease that were not previously feasible, and have led to the commercialization of genetic testing now widely available in clinical practice. In particular, high-throughput sequencing techniques (also referred to as next generation sequencing) have been developed that allow large-scale and rapid assessment of entire genomes. Although next generation sequencing represents a major technological advance, it has introduced numerous challenges with respect to the interpretation of genetic variation, and has unleashed a flood of biological data of unknown clinical significance to practitioners.

In this review, we discuss current genetic testing indications for inherited arrhythmia syndromes, broadly outline characteristics of next generation sequencing techniques, and highlight challenges associated with such testing. We also discuss future directions relevant to the application of genetic testing in the clinical management of patients with inherited arrhythmia syndromes.

Genetic basis of arrhythmia syndromes

Over the past three decades, causal genes have been identified for most recognized inherited arrhythmia syndromes (**Table 1**). Discovered mutations in ion channel subunits governing cardiac electrical function, in sarcomeric proteins critical for cardiac contractile and structural integrity, and in other genes have informed our understanding of cardiac development and physiology. Yet currently recognized disease susceptibility genes, summarized elsewhere,[1-8] represent merely a partial list of causal genes underlying these conditions. Indeed, conventional genetic testing for known genes underlying these conditions has been associated with incomplete diagnostic yield (**Figure 1**).[9]

Genetic mapping of inherited arrhythmia syndromes is associated with limited power owing to the incomplete penetrance, variable expressivity, and locus heterogeneity characteristic of these conditions. For example, in Brugada syndrome, not all *SCN5A* mutation carriers in an affected family manifest the disorder (incomplete penetrance), and often the disorder does not manifest until adulthood (age-dependent penetrance),[10] making it difficult to understand whether a genetic variant is truly disease-causing or not. Mutations in some genes, such as those in *SCN5A*, may manifest with a wide array of phenotypes (variable expressivity) such as long QT syndrome, Brugada syndrome, cardiomyopathy, atrial fibrillation, and conduction system disease,[11] which similarly complicates efforts to discern whether a variant is truly pathogenic. Some inherited conditions may be caused by genetic variation in any number of genes (locus heterogeneity), as is the case with dilated cardiomyopathy.[12] Furthermore, large and multigenerational families with inherited arrhythmia syndromes affecting multiple individuals are rare, making it challenging to robustly associate a given genetic locus segregating with disease.

As such, knowledge of the genetic underpinnings of many arrhythmia syndromes remains incomplete. Genetic testing for inherited arrhythmia syndromes is therefore imperfect, with current tests generally having limited sensitivity for the detection of disease causing variants underlying these conditions.

Establishing the rationale for performing genetic testing

Broadly, genetic testing is used for diagnostic, predictive, therapeutic, pharmacogenetic, preimplant testing, newborn screening, and forensic applications. In adult cardiovascular medicine, genetic testing for inherited arrhythmia syndromes is most often reserved for diagnostic, predictive, or therapeutic applications.

The clinical utility of genetic testing for inherited arrhythmia syndromes is most relevant for confirmation of a suspected condition, or cascade screening in relatives of probands (index affected family members) that carry a disease-causing mutation. Some examples exist whereby mutation characteristics may influence prognosis, as in the case of mutations in pore forming (*KCNH2*)[13] and transmembrane spanning regions (*KCNQ1*)[14] in long QT syndrome. However, in aggregate, the prognostic features of specific genotypes currently remain limited. There are some data that suggest treatment may differ based on a particular genotype,[15-18] most notably in the context of long QT syndrome type 3, where mutations leading to persistent late inward sodium current (I_{Na}) may warrant treatment with a sodium channel blocker.[15] Future research may expand our understanding of the prognostic and therapeutic implications of specific genetic testing findings.

Inherited arrhythmia conditions for which genetic testing is indicated

In recent years consensus guidelines have emerged that provide recommendations for genetic testing for suspected inherited arrhythmia syndromes.[1,2] Whereas these guidelines provide general consensus as to when testing may or may not be medically indicated, it is acknowledged that a variety of disease-specific, patient-level, and external factors must be weighed to determine the appropriateness of genetic testing in any given individual.[9,19] In **Table 2**, we have summarized current indications for genetic testing in the context of inherited arrhythmia syndromes.

Overview of genetic sequencing techniques

The human genome is comprised of about 3 billion nucleotide pairs, and contains about 20,000 known genes.[20-22] Each gene is comprised of both protein coding (exons) and noncoding (e.g., introns, promoter region, etc.) sequences. Protein-coding regions comprise about 1% of the human genome. Despite widespread and increasing recognition that non-coding portions of the genome have important regulatory properties and may participate in disease pathogenesis,[23,24] clinical genetic testing for inherited arrhythmia syndromes classically is targeted and focuses on protein-coding regions.

Commercial genetic sequencing techniques have changed in recent years as a result of high-throughput technology. These changes have impacted the numbers of genes available for interrogation when ordering genetic testing. To provide the reader with an understanding of the scope of sequencing available when ordering tests today, we briefly discuss the technical properties of traditional sequencing and contrast it with high-throughput sequencing. Although additional methods for genetic variant detection exist, we will focus our discussion by contrasting Sanger and next generation sequencing techniques, which are most commonly employed for genetic testing in inherited arrhythmia syndromes.

Sanger sequencing

Causal gene discovery was traditionally performed using a combination of linkage mapping in affected families followed by candidate gene sequencing within an identified disease susceptibility locus. This approach has been a successful mainstay of causal variant and gene discovery for inherited arrhythmia syndromes for the past several decades. The ability to accurately decipher the coding sequences of genes within a genomic locus of interest has largely relied on advances described in 1977 by Allan Maxam and Walter Gilbert,[25] and by Frederick Sanger.[26] These methods were described about 25 years after Watson and Crick reported on the double-stranded helical structure of DNA.[27]

Sanger sequencing involves the competitive process of both synthesis and termination of DNA templates of interest using 2'deoxynucleotides (dNTPs) and 2'3'-dideoxynucleotides (ddNTPs) using DNA polymerase, respectively. The process of synthesis and termination creates a population of sequences of different lengths, which can be separated by size using gel electrophoresis. Typically, the ddNTPs are tagged with a fluorescent dye specific to that nucleotide, which produce detectable and specific emissions in response to excitation by a laser within the sequencer. The specific emissions thus reflect the individual nucelotides in the sequenced templates, and after transformation and processing, yield the sequences of the DNA templates.

Desirable qualities of Sanger sequencing include the high quality and accuracy of generated reads. Indeed, Sanger sequencing was used to create the initial genome in the Human Genome Project[20,21] and remains the standard for definitive confirmation of single genetic variants. Nevertheless, Sanger sequencing has several limitations, including those related to inefficient excitation of the ddNTP fluorescent dyes, and ineffective detection or discrimination of the emitted signals.[28] Sanger sequencing is time-consuming and expensive, which has traditionally relegated its application to targeted sequencing, rather than to high-throughput whole exome or whole genome sequencing. While Sanger sequencing is reasonably priced when performed at a small scale such as screening a limited set of genes, it remains far too slow and expensive when scaled to exomes or genomes.

Next generation sequencing

Recent technological advances have enhanced the scale of sequencing, allowing for the assessment of nucleotide variants across the genome with rapidity and relatively low cost. Next generation sequencing describes a number of different techniques, which collectively generate massive amounts of sequence reads that are aligned to a backbone reference genome for subsequent variant assessment. A full review of the chemistry and sequencing approaches is beyond the scope of this review, but excellent resources describing various sequencing approaches have been summarized elsewhere.[29,30] Below, we have described one specific next generation sequencing technology as an illustration.

Next generation sequencing (**Figure 2**) approaches involve a combination of DNA template preparation, sequencing, and data analysis. First, DNA template preparation involves the fragmentation of genomic DNA into small segments, in contrast to Sanger sequencing in which specific templates of interest are often amplified using the polymerase chain reaction.

Second, after fragmentation of genomic DNA, DNA templates are immobilized to a solid-state medium to enable multiple simultaneous sequencing reactions. In some cases, the DNA templates are clonally amplified, whereas other approaches rely on single molecule templates generated from the fragmentation step. Sequencing technologies vary substantially, but broadly involve the extension of a DNA template with detection of the added nucleotides through an imaging mechanism that is specific to the individual nucleotides. These imaging mechanisms may rely on the detection of dye or fluorescently-labeled nucleotides, detection of light energy released from pyrophosphates during nucleotide incorporation, or other approaches. Reactions are often repeated 50 or more times to generate sufficient coverage to provide confidence in the observed alignments and reads later performed. Third, data analysis involves the computational assembly of the many short DNA sequence reads by aligning them to a reference genome backbone, and subsequently determining the presence of genomic variation. This latter step requires considerable computational effort.[31]

Major advantages of next generation sequencing include the capacity and efficiency of the approach, which has enabled the application of the technology to families and large study samples at a massive scale. Next generation sequencing can be applied to targeted panels of genes (e.g., 5-15 genes for conditions such as arrhythmogenic cardiomyopathy or long QT syndrome), comprehensive panels (e.g., 20-80 genes for comprehensive arrhythmia or cardiomyopathy tests), whole exomes, and even whole genomes.

Despite the promise, numerous limitations of the next generation sequencing exist. For example, exome sequencing for genetic diagnostics faces several challenges,[32] some of which are summarized here. By definition, many genomic regions of interest are not included in commercially available capture methods (e.g., promoter regions, intronic enhancers, other functional noncoding regions). False negative findings may also occur because of technical limitations resulting in errant alignment of reads, insertions or deletions, or poor capture or sequencing over a particular gene or region. Indeed, relatively lower depth of coverage, or the number of times that a particular region of interest is interrogated, has been observed in genes such as *KCNH2* with exome sequencing.[33] Higher coverage results in more reliable discovery of genetic variation. Variability in sequencing coverage throughout the genome results from the facts that probes designed to amplify or immobilize specific exons do not have uniform specificity, and sequencing efficiency is not uniform across all DNA templates. Furthermore, capture probes are designed only for regions of the genome for which we have adequate templates, yet many segments of the genome remain difficult to ascertain.

Interpretation of results

Although interpretation of sequencing results is challenging irrespective of the approach chosen, the massive amount of data generated from next generation sequencing poses a specific challenge unto itself. Sequencing of an exome or the protein coding region of the genome typically identifies ~200 novel protein altering, single nucleotide variants per individual,[34] which leads to major challenges with respect to data manipulation and interpretation. Many of these variants are of unknown significance, underscoring the

tradeoff between test sensitivity and intepretability that occurs when more comprehensive sequencing panels, such as whole exomes, are employed (**Figure 3**).

A full-length review of variant interpretation is beyond the scope of this article but can be found elsewhere.[35] Here we briefly summarize general features of variants that suggest pathogenicity. Specific features utilized to infer deleteriousness include rarity, occurrence of variants at nucleotide or amino acid positions that are conserved across species, deleterious classifications (as in the case of nonsense mutations), and location within functional domains of proteins. In addition to these and many other bioinformatic considerations, pathogenicity is supported by the presence of segregation with disease within a family, and by evidence of a functional effect in model systems (although functional characterization of variants is not feasible in the clinical diagnostic settings). Inferring pathogenicity based on the integration of these diverse data elements is a somewhat subjective process,[36] which introduces potential bias in the interpretation of observed variants. The extent to which subjectivity contributes to differences in interpretation of variants as pathogenic between research or clinical laboratories is unknown.

Defining and annotating variant pathogenicity is a major problem for inherited arrhythmia syndromes. Whereas repositories of patients with inherited arrhythmia syndromes have contributed to our understanding of the distribution of variation in these conditions,[37-39] recent publications have called into question the pathogenicity of variants that previously were thought to be causally related to arrhythmia syndromes.[40-42] As such, pathogenicity is subject to changes and modifications over time as more knowledge is gained. How that information will be communicated to clinicians, and what the clinician's responsibility is with respect to periodically updating the annotation information, is unclear at the moment.

In summary, determining pathogenicity of identified variants is best performed in the context of information demonstrating reliable co-segregation of a particular variant with disease in a family. Furthermore, robust functional data supporting the pathogenicity of specific variants, while rare and often infeasible in clinical settings, lends credence to the deleterious nature of a variant. In the future, large and well-annotated databases of deleterious genetic variation will facilitate the interpretation of observed genetic variation generated from sequencing, whether performed in the clinical setting or research laboratory.

Practical genetic testing in the clinic

The advent of high-throughput sequencing has produced a number of commercially available sequencing options that include a) targeted panels that range from a handful or more genes, b) broad panels containing dozens of genes for a class of traits such as pan-cardiomyopathy or pan-arrhythmia, c) whole exomes, and d) whole genomes. Additional commercially available options for testing include insertion or deletion tests, as well as targeted single variant testing (which is typically reserved for testing whether a relative of an affected carrier also carries a variant of interest). The advantage of the more comprehensive tests is the potential for increased sensitivity of variant detection. The major drawbacks of more comprehensive testing include potentially increased costs, increased detection of genetic variants of unknown clinical significance, and decreased interpretability (**Figure 3**).

It is also worth mentioning that comprehensive testing may be associated with a variety of incidental findings, ranging from variants that potentially affect drug response to variants that increase risk of non-cardiovascular diseases (e.g., dementia, breast cancer).[43-45] As such, targeted testing currently remains the cornerstone of testing in the clinical setting.[46]

Determining which of the many commercially available targeted testing options to choose from depends on the purpose of the test, pretest probability of disease, and the specific test characteristics. Since the yield of genetic testing varies by the suspected inherited arrhythmia syndrome (**Figure 1**), having a high pretest probability for a specific condition and understanding the genetic basis of that particular condition are essential. For example, initial testing for conditions with relatively high diagnostic yields such as long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, and hypertrophic cardiomyopathy need not include a broad sequencing panel, considering that most affected individuals have variation in only one of a small number of known genes. In contrast, more comprehensive testing may be appropriate for diseases with substantial locus heterogeneity, such as dilated cardiomyopathy, though this approach requires sufficient expertise to interpret variants of unknown significance and provide proper counseling to patients. In some cases, the specific genes included in a panel may vary from one laboratory to another, and may influence decisions about which test to order (**Table 3**).

Here we would like to emphasize that the decision to perform genetic testing can be complex, and extends beyond the technical aspects of different sequencing panels. Prior to performing testing, it is advised that pre-test counseling be performed to articulate such issues as the goals, potential consequences, and limitations of genetic testing, as well as address how the results will specifically alter each individual's care. Similarly, after test results have been returned, it is important to summarize with patients, and often their relatives, how the findings may impact them and what the next steps are in light of the knowledge gained. Given these complexities, it is recommended that genetic testing be performed in centers with experience in the counseling of patients and interpretation of genetic testing results.

Future directions / challenges

Technological innovations have improved the extent to which we can now probe the genome, both in research and clinical settings. However several obstacles remain with respect to the appropriate use of next generation sequencing and other technologies in clinical settings. Among these challenges are cost, technological hurdles, determination of variant pathogenicity, and legal and ethical conundrums. The utility of genetic sequencing in the clinic, particularly in the area of complex or polygenic diseases, is of unclear utility. Existing challenges and potential future directions are summarized in **Table 4**.

Conclusions

The efficiency of next generation sequencing promises to elucidate the genetic contributions to many diseases. Next generation sequencing has facilitated the development of various different sequencing options to choose from when testing patients with inherited arrhythmia

syndromes. Yet the implementation of next generation sequencing into clinical practice carries unique challenges. The data generated from this tool requires thoughtful analysis in order to be of practical use. With improvements in cost, further advances in sequencing technology, larger reference genome data banks for comparison, and improved functional assessment of identified variants in coming years, next generation sequencing is likely to become a familiar instrument in the routine management of patients with suspected inherited arrhythmia syndromes.

Acknowledgments

Funding: This work was supported by grants from the National Institutes of Health (1RO1HL092577, 1RO1HL104156, 1K24HL105780, HL065962). Dr. Lubitz is also supported by NIH/NHLBI K23HL114724 and a Doris Duke Charitable Foundation Clinical Scientist Development Award 2014105. Dr. Ellinor is also supported by an Established Investigator Award from the American Heart Association (13EIA14220013) and by support from the Fondation Leducq (14CVD01).

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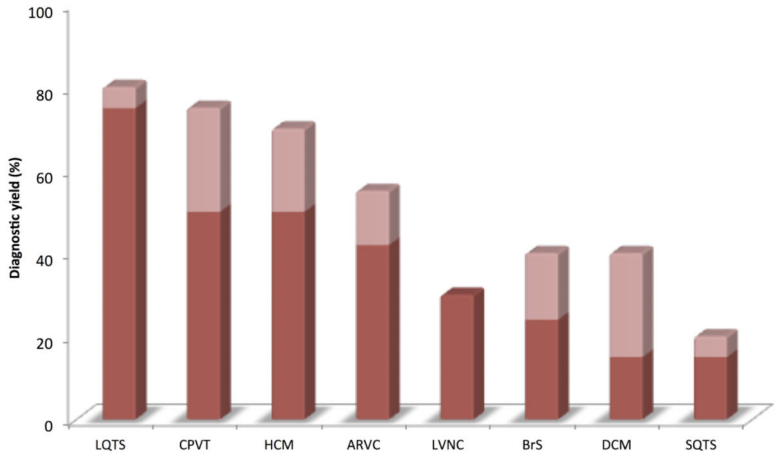


Figure 1. The diagnostic yield of genetic testing varies by condition

Percentage yields are drawn from Sturm and Hershberger.[9] Dark red represents the lower bounds of diagnostic yield estimates, and the light red represents upper bounds of estimates. The potential yield of genetic testing is expected to rise with genetic testing approaches that encompass more genes.

LQTS = long QT syndrome; CPVT = catecholaminergic polymorphic ventricular tachycardia; HCM = hypertrophic cardiomyopathy; ARVC = arrhythmogenic ventricular cardiomyopathy; LVNC = left ventricular noncompaction cardiomyopathy; BrS = Brugada Syndrome; DCM = dilated cardiomyopathy; SQTS = short QT syndrome

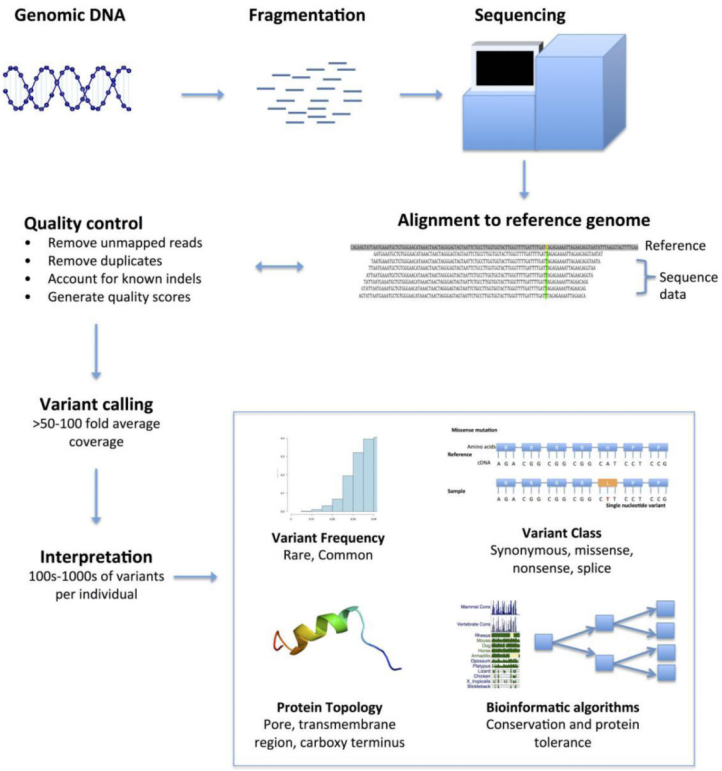
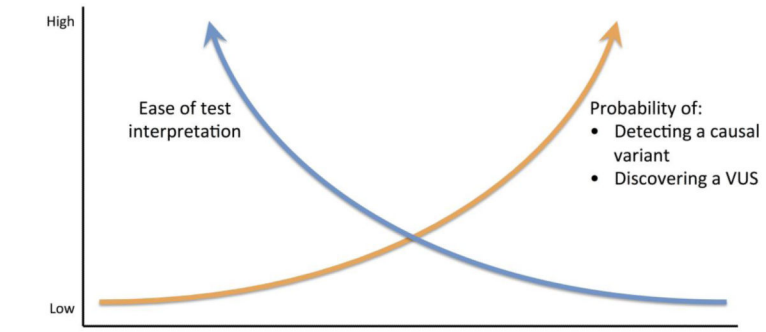


Figure 2. Illustration depicting the flow of next generation sequencing data

The figure displays the basic processing steps involved in the performance and interpretation of next generation sequencing for an isolated individual undergoing exome sequencing. In addition to using bioinformatic annotations, pathogenicity of identified variants can be inferred by using information on functional characterization information and family segregation, if available.



Type of testing	Targeted panel	Comprehensive panel	Exome	Genome
Genes included	5-20	30-80	20,000	All + noncoding
Bases sequenced	20K-75K	250K	60 million	3 billion

Figure 3. Schematic displaying the tradeoffs between increasingly comprehensive sequencing panels available with next generation sequencing

The numbers of bases sequenced using each sequencing approach are estimates. VUS = variant of unknown significance

Table 1

Inherited arrhythmia syndromes and their predominant genetic causes.

Condition	Predominant gene(s)*
Long QT syndrome	*KCNQ1, KCNH2, SCN5A*
Catecholaminergic polymorphic ventricular tachycardia	*RYR2, CASQ*
Brugada syndrome	*SCN5A*
Cardiac conduction system disease	*SCN5A*
Short QT syndrome	*KCNH2, KCNQ1, KCNJ2*
Hypertrophic cardiomyopathy	*MYBPC3, MYH7, TNNI3, TNNT2, TPM1*
Arrhythmogenic ventricular cardiomyopathy	*DSC2, DSG2, DSP, JUP, PKP2, TMEM43*
Dilated cardiomyopathy (with conduction system disease)	*TTN, (LMNA, SCN5A)*
Left ventricular noncompaction cardiomyopathy	*LBD3*
Restrictive cardiomyopathy	*MYH7, TNNI3*
Sudden unexplained death & sudden infant death syndrome	*KCNQ1, KCNH2, SCN5A, RYR2*
Atrial fibrillation	Predominant forms are polygenic

*The reader is referred to other sources for comprehensive reviews on the genetic bases of these conditions. [1-8]

Table 2

Aggregated consensus guideline indications for genetic testing in inherited arrhythmia syndromes.

Condition	Diagnosis / Strong suspicion	Relative is index case with known mutation	Reference
Long QT syndrome	I[1] / IIb[2]	I	*1*
Catecholaminergic polymorphic ventricular tachycardia	I	I	*1*
Brugada syndrome	IIa	I	*1*
Cardiac conduction system disease	IIb[3]	I	*1*
Short QT syndrome	IIb	I	*1*
Hypertrophic cardiomyopathy	I	I	*1,2*
Arrhythmogenic ventricular cardiomyopathy	IIa[4] / IIb[5]	I	*1*
Dilated cardiomyopathy	I[6] / IIa[7]	I	*1*
Left ventricular noncompaction cardiomyopathy	IIa	I	*1*
Restrictive cardiomyopathy	IIb	I	*1*
Out of hospital cardiac arrest	I[8]	–	*1*
Sudden unexplained death & sudden infant death syndrome	IIb[9]	I	*1*
Atrial fibrillation	–	–	*1*

These indications represent a summary of class I and II indications based on consensus guideline statements. [1,2]

[1] Strong clinical suspicion, or asymptomatic with QTc > 500 (adult) or > 480 (prepuberty) on repeated occasions

[2] Asymptomatic with QTc > 480 (adult) or >460 (prepuberty)

[3] Isolated CCD or with concomitant congenital heart disease, especially with family history of cardiac conduction system disease

[4] Task force clinical diagnosis of arrhythmogenic ventricular cardiomyopathy

[5] Task force possible arrhythmogenic ventricular cardiomyopathy

[6] With conduction disease or family history of sudden death

[7] To confirm diagnosis with familial dilated cardiomyopathy

[8] If exam suggests channelopathy or cardiomyopathy

[9] If evaluation suggests long QT syndrome or catecholaminergic polymorphic ventricular tachcyardia

Table 3

Comparison of genetic testing panels between select companies for several different phenotypes, excluding whole exomes and genomes.

	Transgenomic www.transgenomic.com	GeneDx www.genedx.com	Correlagan www.correlagen.com	Partners Laboratory for Molecular Medicine www.personalizedmedicine.partners.org/Laboratory-For-Molecular-Medicine/Default.aspx	Genes in common
	Number of genes tested in panel				
Long QT syndrome	13	12	–	–	12
Brugada syndrome	9	7	–	–	7
Hypertrophic cardiomyopathy	12	18	–	18	12
Dilated cardiomyopathy	13	38	6	27	6
Arrhythmogenic ventricular cardiomyopathy	5	7	5	8	5
Pan-cardiomyopathy panel	–	76	–	51	45
Pan-arrhythmia panel	25	30	–	–	23

Table 4

Challenges and future directions in the application of next generation and other sequencing technologies in practice.

Challenge	Problem	Potential solutions / future directions
Costs	• Overall sequencing panel costs typically range from $2500-$5000, though out of pocket expenses may be as low as ≤10% of this for patients in whom laboratories are contracted with their insurers • Costs are prohibitive for many patients	• Increased contracting between genetic testing laboratories and payers • Reduction in test costs with improved technology
Genome coverage	• Next generation sequencing panels do not have uniform coverage across the genome	• Improved capture probe chemistry and sequencing technology
Determining variant pathogenicity	• Bioinformatic methods are imperfect • Published literature is heterogeneous and does not always address variant transmission with disease in families or functional characterization of variants • Most discovered variants are private to families and not represented in the published literature • Different laboratories use different algorithms for determining pathogenicity, which may not be consistent with one another	• Increase in large scale population and disease-specific sequencing databases for reference • Improved bioinformatic models to predict pathogenicity • Development of high-throughput disease-specific functional characterization assays to introduce and test variants • Agreement of standardized elements of variant pathogenicity between laboratories
Legal and ethical considerations	• Some assurances are not guaranteed by the Genetic Information Nondiscrimination Act, including discrimination from disability, life, and long-term care insurance on the basis of genetic testing results • Increasingly comprehensive genetic sequencing panels may incidentally identify actionable disease causing or pharmacogenetic variants; the obligations of providers and testing laboratories to report such incidental discoveries is unclear • Annotations of variants as pathogenic, VUS, or non-disease causing may change over time as a result of increasing information; the burden on providers and laboratories to update and report annotations is unclear	• Advocacy and legal reform to ensure protections to patients undergoing genetic testing • Agreement of standard reporting practices for both incidentally discovered potentially actionable variants • Agreement of standards for updating genetic variant annotations reported to patients
Role of genetic variation in complex (polygenic) diseases	• The role of testing for genetic variation underlying complex diseases is unclear • It is unknown whether polygenic variation contributes to or modifies presumed Mendelian disease risk	• Population based and clinical trial data to determine the clinical effectiveness of utilizing genetic testing information in practice • Genome-wide association studies and large-scale sequencing to better understand the genetic architecture of inherited arrhythmia syndromes