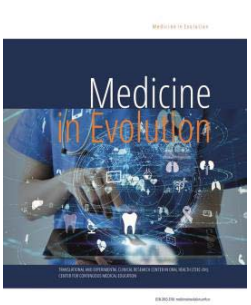
Genetic testing approach in cardiomyopathies comparing NGS panels, WES and WGS



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Abstract

Background: Genetic testing in cardiomyopathies has a great impact on diagnosis and further management. Considering novel technologies developed for DNA sequencing, it is important to understand the indication and limits of genetic testing available, while taking cost-efficiency into account. Aim: The focus of this review is to summarize the current genetic testing approach in cardiomyopathies in order to determine the best patient pathway in reaching a genetic diagnosis. Methods: For this narrative review, we performed a search of several electronic databases, selected and evaluated relevant manuscripts. Results: Each method of genetic testing in cardiomyopathies was assessed in terms of the diagnosis yield, benefits, limitations and turnaround time. Conclusion: Whether the use of whole exome or genome sequencing can improve the performance of genetic diagnosis in cardiomyopathies over standard custom panels is challenging and needs to be determined in future researches.

Keywords: cardiomyopathy, whole genome sequencing, genetic testing, next generation sequencing

INTRODUCTION

Cardiomyopathies are a group of diseases determined by dysfunction of the myocardium leading to heart failure and sudden cardiac death (SCD) [1]. They can be classified into primary (genetic) and secondary (acquired) forms based on etiology. When referring to morpho- functional phenotypes, they can be classified into: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic cardiomyopathy (ACM) [2]. The genetic background should be investigated in order to find a potential inherited type of cardiomyopathy even in presence of a potentially acquired cause or environmental factors [3]. Increasing progress and extensive use of genetic testing has brought more and more evidence of new inherited cardiac gene variants and their impact in disease manifestation and progression [4]. Polygenic risk score (PRS) also named genomic risk score is another variant of genetic testing that may be of importance in the future in the diagnosis work-up of cardiomyopathies. Multiple genetic variants are analyzed in the whole genome, each one being associated to a small risk for the disease. Instead of trying to identify a unique variant responsible the cardiomyopathy, all the identified variants are analyzed and the aggregate risk for the disease is appreciated [2]. Furthermore, genetic investigation for a cardiomyopathy implies not only genetic testing. It ideally involves: documenting a detailed at least 3 generations family pedigree, one on one patient counseling, molecular genetic testing using next generation sequencing (NGS), interpreting the variants according to phenotype and cascade family screening when appropriated for risk stratification in family members. There is a high variability of genes sequences in general population. The probabilistic chance of a genetic result, the yield of testing is higher when testing is realized in an individual with a clear phenotype and the challenge in interpreting the implication of the identified gene variant is easier. However, the yield of genetic diagnosis as well as the difficulty of interpreting the numerous variants of unknown significance in also enhanced by the complexity of the panel used for testing.

Aim and objectives

The aim of this narrative review is to provide a broad comparison between different genetic testing approach in adult patients with primary cardiomyopathies who underwent genetic testing using NGS custom panels, WES or WGS. We analyzed for each method of genetic sequencing: the diagnosis yield, benefits, risks, turnaround time and limitations.

MATERIAL AND METHODS

We performed a search on the following electronic scientific database: PubMed, Google Scholar, Web of Science, and Science Direct. Relevant open access articles employing the association between primary cardiomyopathies and genetic testing were identified. Key words used for the search included: “cardiomyopathy”, “genetic testing”, “next generation sequencing”, “whole genome sequencing”, “whole exome sequencing”. We selected 37 articles, based on a database search published between 2011 and 2024. Manually, we analyzed the reference lists of the selected literature in order to validate the inclusion of genetic cardiomyopathies and the molecular characterization of the cardiac disease based on the genetic testing.

RESULTS AND DISCUSSIONS

The initial literature search yielded 813 records. Of these 273 were excluded based on unavailbility of full content. 512 papers were considered irrelevant and withdrawn, based on the abstract title and/or content. From references review of the 28 articles that remained, 15 additional studies were identified, making the total number of 42 articles. Of these, 34 articles were excluded, based on the criteria detailed in figure 1. After all these exclusions, a total of eight articles remained, from which genetic testing data was extracted.

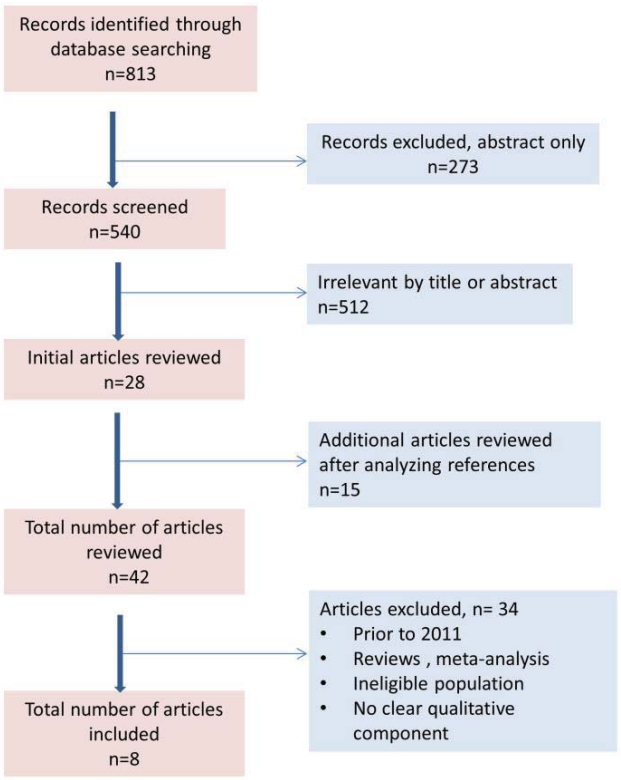

Figure 1. Flowchart describing the systematic literature review

Genetic Testing Strategies

Availability and diversity has increased in the past decades for genetic sequencing. In addition, the turn-around periods are shorter and the costs are lower. Options for genetic analysis vary from sequencing of one gene, a targeted panel of genes related to a specific phenotype, or in some cases, whole exome sequencing (WES) or the whole genome sequencing (WGS). Disease focused panels include genes that are proved to have a moderate to high association to the specific disease [5]. Some examples are: HCM specific panel or DCM specific panel. More complex cardiac panels may include genes with a low gene-disease association. WES sequences the whole Deoxyribonucleic acid (DNA) exons (protein-coding regions) and WGS sequences also the DNA's introns (noncoding regions) and the mitochondrial DNA beside DNA's exons. Testing is usually performed using blood, saliva, or oral swab sample. The DNA is extracted, purified, multiplied and fragmented, then isolated and attached to labeled beads for short-read sequencing. Sequence obtained information are compared with a human genome sequence of reference, and the identified variants in the patient probe are interpreted to determine the correlation with the disease of interest. The process of variant interpretation involves variant type classification. For instance, variants

that imply protein loss-of-function such as frameshift mutations are considered damaging for most genes, yet not all. Genome Aggregation Database, a complex and wide available database of healthy control genome and exome is interrogated for establishing allele prevalence in general population and ethnic groups. Also, the properties of the amino acid are analyzed to determine whether it's change will be tolerated without damaging the protein. The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) have proposed a guideline in order to standardize variant interpretation and facilitate and this complex process [6]. When a causative variant is identified in the index patient, grade one relatives, including those phenotype negative should be tested for identifying presence of that specific variant within the gene using Sanger sequencing. A major challenge in the management of genetic testing in cardiomyopathies is choosing the most cost-efficient method, yet also considering turnaround time of results. Due to the genetically heterogeneous background of cardiomyopathies and the development and availability of Next-generation sequencing (NGS) NGS techniques in the clinical practice, a multi gene panel is desirable. However the disease focused panels may miss important genes with prognostic implication. For instance, TruSightCardio panel (Illumina, San Diego, CA) currently used for genetic testing in cardiomyopathies in the Regional Center of Medical Genetics Timis does not include the FLNC gene, which has clearly proved to determine DCM with high arrhythmogenic risk. Moreover, recently, deep intronic variants have linked to cardiomyopathy phenotypes in previously unexplained or negative genetic cases [7,8]. On the other hand, with higher number of genes analyzed also increases the costs of testing and the probability of identifying variants of uncertain significance (VUS) increasing the difficulty of interpretation. It is therefore evident that the ordering physician should be aware of the benefits and limitations of specific test types in order to select the most appropriate technique [9].

Advantages and disadvantages of NGS targeted gene panels

Approaches to genetic testing based on Next-generation sequencing (NGS) enables the diagnostic of a causative genetic variant including more than two hundred genes involved in the etiology of cardiomyopathies or channelopathies. Custom panels provide this precision without driving up sequencing costs to achieve the required depth of target regions. Multiple reports have published the outcome of genetic testing using different panels with genes ranged from 19 to 173, in a single test. [10,11] Usually, in clinical practice, it is feasible to choose a specific focused gene panel according to the suspected cardiomyopathy subtype. The correlation between genotype and phenotype in cardiomyopathies vary a lot as variants in certain gene can determine different phenotypes. [12,13] Variants in lamin A *LMNA* gene can determine DCM or arrhythmogenic cardiomyopathy (ACM), while variants in the MYH7 gene can linked to DCM, HCM and left ventricular non-compaction cardiomyopathy (LVNC). In this context, genetic testing using 2 or more disease targeted panels may become useful and provide better diagnosis yield, especially when phenotype in not well defined. The responsible variant detection rate is usually higher in familial vs. sporadic cases. Multiple studies have reported different diagnostic yield, slightly depending on the type of cardiomyopathy tested. For instance, a research performed by Gómez et al. [14] published a diagnostic yield of 25% using a panel of 9 genes in 76 patients with hypertrophic cardiomyopathy. In a study ruled by Cuenca et al. [15] that used an NGS panel with 126 genes, identified a DCM-causing variant in 73% of cases with familial DCM who were ongoing heart transplant. On the other hand, a study conducted in our Genomic Center of Timisoara, which included patients with familial and sporadic non ischemic DCM from 5 universitary centers of Romania, resulted in a diagnosis yield of 50.8%. [16] The difference in the diagnostic rate can be explained by the complexity of panel design, the number of genes but also the specific selected genes and, of course, by the selection of patients. Although

panel-based NGS techniques are appealing based of the fact they are cost-effective, easy fast, NGS based on ampliseq has some weaknesses. Only 94% from the targeted regions is covered at the panel design stage. Furthermore, recently identified variants may not be included in the panels used currently in clinical practice. For example, mutations in *FLNC* gene has been reported to be involved in the etiology of HCM and DCM cases but it is not included in the TruSight Cardio Illumina Panel that we use in our Genomic Center. Ouellette et al. stated that larger panels for cardiomyopathies had a higher rate of detecting variants of uncertain significance (VUS) in comparison to a disease specific focused panel (87% vs. 30%). In the study we performed on 122 patients with DCM, we have identified a rate of VUS of 30% [17]. Detecting VUS may produce confusion for the physician, the patient and his family, an unwanted consequence of extensive gene panels testing. Another limitation would be the amplification accuracy at the level of polymerase chain reaction (PCR) stage due to content high in GC (guanine-cytosine). As a consequence, a part of the coding regions will remain unsequenced. Despite the fact that targeted custom panels maximize sequencing economy, they are not suitable for broad discovery research.

Advantages and Disadvantages of WES

Whole-exome sequencing focuses on the genomic protein coding regions (exons) that represent around 2% of the genome. This is where most of the genetic variants related to the disease are found. WES requires supplementary reagents (probes) and an extra step: hybridization but it is a cost-effective method compared to WGS. WES has proved to achieve a comprehensive coverage of coding variants such as single nucleotide variants (SNVs) and insertions-deletions. Retter et al. analyzed an extensive cohort of 3040 clinical cases and resulted that WES provided an overall diagnostic rate of 28.8%. More specifically, for proband only cohorts, the diagnostic yield was 23.6%, increased to 31% when 3 family members were investigated [18]. Other papers have reported a diagnostic yield ranging between 22% - 57%, depending on the patient's phenotype and design of study. A study conducted by et al. shows that WES was able to detect likely pathogenic or pathogenic variants for almost half of HCM patients. However, according to a study published by Mak TSH et al, the use of WES did not increase the diagnostic yield versus the 4 commercial panels [19]. One of the limitation of WES is that it can miss valuable information by not detection variants that are localized outside the exome. Particularly variants located in regulatory regions are important for gene regulation and expression. Moreover, WES cannot identify structural variants, large insertions or deletions.

Advantages and Disadvantages of WGS

One of the major advantages of WGS is that it provides a more comprehensive view of an individual's genetic makeup. It can determine the order of the nucleotides in a patient's DNA and can uncover variation in any part of the human genome, including coding-noncoding and mitochondrial DNA (mtDNA) regions. In some instances, WGS is the better option because DNA variations outside protein-coding regions can affect gene activity and protein production, potentially leading to genetic disorders. This can be useful especially for determining rare or new variants that have been missed by WES. WGS provides detection of deep intronic gene variants that have demonstrated to have pathogenic significance [20]. Findings about the good results and improved diagnosis yield of WGS-based testing in hypertrophic cardiomyopathy [21] and DCM have emerged [22]. A powerful criteria that favors WGS as a testing method is the potential for identifying the genetic background of unclear or negative cases but phenotype-positive. Bagnall et al. found that WGS was able to identify deep intronic splice variants in the MYBPC3 gene in 4 out of 46 patients who had previously a negative HCM genetic testing on NGS custom panel [23]. However, one of the biggest limitations of WGS is that it is most expensive, due to the larger amount of data analyzed. WGS also requires more computational resources and sophisticated bioinformatics

expertise to decipher, increasing the time required for analysis. Moreover, WGS can determine false positive results, especially in less frequent genetic variants and make the interpretation of results more challenging.

Comparison between advantages and disadvantages of targeted gene panels, WES and short read WGS are presented in table I.

Table I. Comparison between different genetic testing techniques

Method	**NGS targeted panels**	**WES**	**Short read WGS**
Diagnosis yield	20-61% [14,15,16]	22- 73% [21,22,23]	50-57% [25,26]
Advantages	-High coverage -First step method when specific cardiomyopathy is suspected -Custom design of panel content -Detection of copy number variations (cnv) -Focused analysis -Cost efficient -Low storage and computational burden -Short turnaround time	-Convenient prescreening method -Quick sequencing and data analysis - Detection of copy number variations (CNV) -Exome wide analysis, allows virtual (dynamic) panel analysis -Medium cost -Medium storage and computational burden	-Uniform coverage -Less sequencing bias, stable, no PCR -Extensive method -Detection of non-coding variants -Accurate detection of copy number variants -Useful in difficult to target regions -could identify repeat expansions (low accuracy)
Limitations	-Limited detection based on gene panel content -Not suitable for broad discovery research -Does not identify repeat expansions	-Does not identify repeat expansions	- Lower coverage - More expensive - High computational and storage burden -Longer turnaround time compared to panels and WES

WESs and WGSs techniques are still mostly used in the research field over clinical practice when evaluating patients for inherited cardiomyopathies. WES and WGS have proved a potential of providing incidental gene variants related to cardiac disease, when testing patients without positive familial history [25]. In a study that involved 2628 individuals who underwent testing using WES, 11 were determined to have pathogenic variants linked to cardiomyopathies. However, on 25 years of follow-up, only 2 of these 11 people developed cardiac dysfunction [26]. The literature and database of genetic variants are constantly improved, thus variants are likely to be reclassified over time and VUS may become likely pathogenic or pathogenic variants. However, due to increased amount of uncertainty regarding cardiovascular genetics, there is still debate within about how to order and interpret these tests. Cardiologists should work in a cardio-genetics team together with the genetician in order to provide best management for the patient and his family. New technologies and tools are developing in order to provide continuous update and knowledge about variants, such as the CardioClassifier tool [27,28] or ClinGen [29]. Long-read sequencing, or third-generation sequencing, offers a number of advantages over short-read sequencing, yet it is currently more expensive compared to short read [30]. Thus, long read DNA sequencing approach will not be the focus of this future research.

Genetic variant's impact on clinical management

Genetic testing is not only providing the definite etiological diagnosing but also influences treatment and further management. In HCM patients, genetic testing provides

definite differentiation between genetic cardiomyopathies and phenocopies, for example, transthyretin (TTR)-cardiac amyloidosis, Fabry disease or other glycogen storage disorders [31]. By detecting the etiology of the disease earlier, targeted therapies are started sooner with impact on preventing disease progression and cascade family screening for the variant identified in the proband is initiated. The importance of determining the genetic basis of a cardiomyopathy arises also, from the impact of specific variant, among other risk factors, in stratifying the risk of life-threatening ventricular arrhythmias. Among patients with LMNA pathogenic variants, Wahbi et al. generated a risk score (https://lmna-risk-vta.fr/) that estimates the 5-year risk of malignant ventricular arrhythmia in these patients [32]. Other gene that has proved to induce a high risk for life threatening ventricular tachyarrhythmias is Phospholamban (PLN). PLN p. Arg14del variant along with other risk factors: left ventricular (LV) ejection fraction, inverted T waves, low voltaged ECG, the amount of PVC's over 24 hours are variables used in the 5-year risk SCD calculator in these patients [33]. These risk stratification tools are of great importance since they are capable of rising the indication for ICD implantation in primary SCD prevention. All these emphasize the important impact of genetic testing in personalized disease specific management.

CONCLUSIONS

Determining the genetic background of a cardiomyopathy is indispensable in the era of precise, personalized medicine and arises from the impact on patient's clinical management and family screening. Finding the right balance between the diagnostic yield, costs and the rate of incidental findings remains one of the major challenges in the field of genetic testing. Whether the use of whole exome or genome sequencing can improve the performance of genetic diagnosis in cardiomyopathies over standard custom panels is yet to be determined in future studies.

The authors declare that they have no competing interests.

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