

Causes and ways of detection of dilated cardiomyopathy and hypertrophic cardiomyopathy

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Abstract. Inherited cardiomyopathy has a wide variety and complex symptoms, which can cause a severe burden on the patient's family. Researchers have done a lot of research on inherited cardiomyopathy. Among the five inherited cardiomyopathy under the current classification standard, hypertrophic cardiomyopathy and dilated cardiomyopathy are two of the more in-depth studies. Researchers have now identified many of the genes responsible for the two most familiar forms of cardiomyopathy. The pathogenic factors of hypertrophic cardiomyopathy mainly concentrate on the two gene mutations of MYBPC3 and MYH7, and others are fairly rare. The etiology of dilated cardiomyopathy is more complex. LMNA gene variants are relatively common in familial dilated cardiomyopathy. Also, MYH7 and TNNT2 variants are more common causes of this condition. In addition, the researchers discovered part of the pathogenic mechanism of the two diseases and achieved different results of the clinical detection methods and etiological detection methods of the diseases. In clinical testing, myocardial biopsy is still the gold standard, and electrocardiography and echocardiography are widely utilized as clear indicators. In terms of etiology detection, two generations of genetic testing methods with their advantages and disadvantages, but with an accuracy rate higher than 95% have been put into use. This article summarizes information about the two diseases the authors read, including basic information on cardiomyopathy, the gene-level etiology of hypertrophic and dilated cardiomyopathy, and the role of gene sequencing in disease detection, as well as several commonly available methods for the detection of both types of cardiomyopathy.

Keywords: Hypertrophic cardiomyopathy; Dilated cardiomyopathy; Gene.

1. Introduction

Cardiomyopathy is a pathologically abnormal myocardium state which makes drawing blood to the other tissues and organs of the body more difficult for the heart [1]. It is one of the major causes of morbidity and mortality. Among patients who have cardiomyopathy, a significant percentage have genetic-based, inheritable disease, which is called inherited cardiomyopathy.

Inherited cardiomyopathy is a group of myocardium disorders caused by genes that is classified into five types, which are dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic ventricular cardiomyopathy (AVC), and left ventricular noncompaction cardiomyopathy (LVNC). The time of the outbreak of inherited cardiomyopathies varies, mainly at the age of 20 to 50 [2], but some remain asymptomatic until late in life. The diseases have a slow onset and severe progression, and often lead to heart failure in the end-stage of condition, which are difficult to treat. As a result, the disease imposes severe financial and psychological burdens on the patient's family. Because of the severity, people want to research ways to treat these diseases. To achieve this goal, people must first understand the causative factors of the disease. Therefore, many researchers in the field have studied Inherited cardiomyopathies for years.

Standard methods for detecting cardiomyopathy include myocardial biopsy, electrocardiogram, echocardiography, etc. However, a myocardial biopsy can cause damage to the heart, and the potential complications are also more severe. Electrocardiogram and echocardiography can only indicate the patient's physical signs but not the exact cause. So the researchers developed genetic sequencing that genuinely shows the cause of inherited cardiomyopathy.

More than 30 years ago, researchers first discovered the "disease gene" responsible for the pathogenesis of hypertrophic cardiomyopathy, and subsequent studies on the genetic causes of different inherited cardiomyopathy have become increasingly specific. Methods of gene sequencing are also constantly updating. The commonly used first-generation gene sequencing method is the Sanger sequencing technique. Due to the high accuracy and high efficiency of sequencing small-capacity gene fragments, this method is still used by the researchers. However, due to the inefficiency and high cost of sequencing large numbers of genes or genomes utilizing this method, researchers have developed a second-generation gene sequencing technology called next-generation sequencing (NGS). This approach further improves the accuracy of gene sequencing. In addition, since the method can sequence a large number of genes simultaneously, it successfully compensates for the deficiency of Sanger sequencing. However, the cost is relatively high when sequencing a small number of genes. As a result, Sanger sequencing and NGS technology have successfully reached a complementary state. Most of the time, they are simultaneously used for the sequencing of genetic cardiomyopathy pathological genes.

Progress on pathogenic causes made by researchers is varied from different types of inherited cardiomyopathy. Among the five forms, the two types that are studied most deeply are HCM and DCM, the two with the highest incidence. Now, researchers have found more than 1500 related disease-causing gene mutations [3] of inherited cardiomyopathy and have a certain degree of understanding of the pathogenesis of HCM and DCM. The pathogenic genes of the two conditions are highly overlapping, and the same pathogenic gene can cause different kinds of diseases according to individual differences. For example, MYH7 gene mutation can cause both HCM and DCM, as well as TNNT2 gene mutation. However, the causative genes of HCM are fairly narrow. In contrast, the causative genes of DCM are more extensive. Therefore, the related pathogenic genes of HCM are somewhat clear, while the related pathogenic genes of DCM are more private and relatively difficult to determine, which makes it hard to find a disease-causing gene that could be considered a hotspot. But even so, the genetic mutations that cause DCM have certain regularity that can be summarized.

The purpose of writing this review is to build a unique framework by summarizing the relevant knowledge and achievements. In this review, the author will briefly introduce inherited cardiomyopathy, some related genetic mutations and pathogenic mechanisms associated with HCM and DCM, and the importance of gene sequencing technologies in finding them. Also, the authors will introduce the current commonly used ways of detecting cardiomyopathy and briefly touch on the cutting-edge sequencing technologies developed by researchers.

2. Inherited cardiomyopathy (ICs)

2.1. Definition, symptoms and classification

Cardiomyopathy is a pathologically abnormal structural or functional state of the cardiac muscles, excluding ischemic cardiomyopathy and other primary diseases. The disease makes it more difficult for the heart to draw blood to the other tissues and organs in the body as usual.

ICs are cardiomyopathies that are related to genes. Most ICs are single gene disorders and transmit to the child as autosomal dominant at a 50% of chance [4].

Patients may be asymptomatic in the early stage, and as the disease progresses, symptoms include breathlessness, swelling, coughing into bed, trouble falling asleep flatly, fatigue, abdominal bloating due to fluid accumulation, heartbeats which is rapid, pounding or fluttering, thoracic tension or discomfort, lightheadedness, dizziness and loss of consciousness [5].

Severe forms of inherited cardiomyopathy can be fatal, which makes ICs become the commonest reason for sudden cardiac mortality in persons less than 35 years of age and the leading cause of non-traumatic death for young, seemingly healthy athletes [6]. The time of the outbreak of ICs varies, mainly around the age of 20 to 50, but some remain asymptomatic until late in life. The diseases have a slow onset and severe progression, and often lead to heart failure in the end-stage of condition,

which are difficult to treat. The disease imposes severe financial and psychological burdens on the patient's family.

According to the new classification proposed by The European Society of Cardiology, there are five kinds of ICs, which are DCM, HCM, RCM, AVC and LVNC [2].

The two commonest types of inherited cardiomyopathy are DCM and HCM. These two are also primarily studied in the past twenty years.

2.2. HCM

Hypertrophic cardiomyopathy (HCM) is a condition in which the cardiac muscle thickens (hypertrophied) when there is no sufficient degree of hemodynamic stress evidence to explain the appearance of cardiac muscle hypertrophy and systemic diseases. Most researchers agree that amyloidosis-causing increase in ventricular wall thickness should also be considered as HCM [7].

Two-thirds of HCM patients have no apparent complications. Complications such as atrial fibrillation and stroke may occur in the remaining patients. The diagnosis of HCM needs to exclude the influence of related causes that can be secondary to ventricular hypertrophy, such as arterial hypertension, other cardiac problems, poisoning and other pathological and toxicological causes. Similarly, systemic diseases, such as some neurological and metabolic diseases, can also present with symptoms of ventricular hypertrophy, and such reasons should also be considered and excluded [8].

Familial HCM varies significantly between individuals and may present with any disease presentation from asymptomatic to severe hypertrophy, with different degrees of fibrosis and blockage of flow paths. With the aggravation of symptoms, the probability of sudden cardiac death also increases. Factors that may cause sudden death include improper exercise, severe hypertrophy more significant than 3 cm, severe fibrosis, a history of sudden death, and so on [9].

2.3. DCM

DCM is defined as left ventricular dilation and left ventricular systolic dysfunction, excluding the influence of abnormal stress conditions or coronary artery disease enough to lead to systemic systolic dysfunction.

DCM is highly insidious and progresses slowly, with only mild expansion and no apparent symptoms in the early stages of the disease. Statistics demonstrate that familial DCM is more frequent among males, primarily in persons between 26 and 45 years of age and very few in elderly patients.

There are many causes of DCM. Myocardial ischemia, valvular disease, hypertension and other reasons can cause left ventricular dysfunction and lead to DCM. DCM with no other identifiable cause is classified as idiopathic DCM. Nonischemic DCM is often familial, and when an apparently affected case in a family is identified, another affected relative may be present in up to 50% of the family if relevant noninvasive testing is performed on other first-degree related members of the family [9]. Clinical statistics show that in several families with patients who fully meet the diagnostic criteria for DCM, nearly 40% of the patient's relatives are affected [10]. The main clinical manifestations of DCM are ECG changes, atrial or ventricular arrhythmias, stroke and even sudden death.

3. Genetic causes of inherited cardiomyopathies

3.1. Gene mutation of HCM

HCM is an autosomal dominant inheritance. Research conducted over the past 30 years has identified ten related genes and more than 1500 related mutations [3] associated with HCM.

A study of 197 HCM cases involving nine causative genes identified 97 different mutations in 124 of them. Among the nine genes (MYBPC3, MYH7, TNNT2, etc.), the two most common pathogenic gene mutations were: MYBPC3 accounted for 42%, and MYH7 accounted for 40%. There are also TNNT2 mutations (6.5%), and TNNI3 mutations (6.5%) that are more common than other rare gene mutations [11].

In another statistic, MYBPC3 mutations accounted for 25% of the total sample, while MYH7 mutations accounted for 12% [12].

Thus, these two gene mutations are the most familiar reasons of HCM.

The prognosis of HCM caused by different causative genes varies. More than 90% of MYBPC3 gene variants have an excellent or moderate prognosis in HCM, but MYH7 gene mutations are more associated with malignant prognosis. TNNT2 and TNNT3 are related to different prognoses, but their proportion is small, and their indication is relatively less clear [11]. Also, statistics show that patients with MYBPC3 mutations have a later onset of disease than patients with MYH7 mutations [12].

However, the genotype-phenotype link in HCM has not been fully established because the causative genetic variants in HCM are highly private, and the phenotypes of genetic variants in first-degree relatives are often different [2].

3.2. Pathogenic mechanism of HCM

A major characteristic of HCM is the enhanced sensitivity of myofilament cells to Ca^{2+} , as this will result in compromised muscle strength development and increased rate of ATP consumption, affecting cardiomyocyte diastolic function.

According to Frank-Starling's law, the heart adjusts stroke volume according to the degree of ventricular filling. This is an ability called length-dependent myofilament activation (LDA). When left ventricular hypertrophy occurs, the degree of ventricular filling reduce, resulting in blunting LDA capacity. The specific mechanism is that left ventricular hypertrophy leads to a decrease in filling degree, and thus the stroke volume of the ventricle will be adjusted and reduced, resulting in a reduction of the force of myocardial cells and a shortening of the length of the sarcomere. The increased Ca^{2+} sensitivity with shortened sarcomere length is smaller than that of regular sarcomere length, so the operating range of LDA is reduced. LDA blunting is responsible for ventricular diastolic dysfunction.

The efficiency of myocardial contraction relates to the tension cost of the muscle. When MYBPC3 and MYH7 mutations occur, the homeostasis of myocardial contraction and relaxation are disrupted, myocardial contractility will become stronger and relaxation tension will become weaker, and the consumption of ATP will exceed the regular consumption [3]. Therefore, under the exact tension cost, the myocardial contraction efficiency of HCM patients will be lower than that of healthy people, which is also why HCM patients may experience diastolic ventricular disorder and cardiac insufficiency.

The degree of cardiac insufficiency may be related to the content of intracellular variant proteins. Also, there is evidence of excess cellular reactive oxygen species (ROS) in HCM patients and animal models of HCM [13]. Uncontrolled ROS components can negatively affect cells. Therefore, the excessive intracellular content of ROS has also become one of the pathological mechanisms aggravating the HCM phenotype.

Compared to thick-filament gene (MYBPC3, MYH7) mutations, thin-filament gene (TNNT2, TNNT3) mutations will lead to relatively mild and less typical left ventricular dilatation in HCM patients over 18. But thin filament gene mutations are more likely to lead to cardiac insufficiency and heart failure [14]. Furthermore, TNNT2 mutations are associated with mild or no left ventricular hypertrophy (which may account for the low detection rate of this genetic variant in HCM patients), but increased risk for sudden death [11].

3.3. Utility of genetic testing in HCM

Taking the presence of non-familial cases, about 30% of HCM cases can be detected through genetic testing. And since the causative genes of HCM are relatively concentrated in two gene variants, MYBPC3 and MYH7, gene detection is highly directed.

The results of genetic testing can also guide treatment and prognosis. For example, if a mutation of the MYH7 gene occurs, due to its relatively poor prognosis [11], preparation for the consequences can be made in advance. Similarly, if TNNT2 mutation is showed in the test results, family members

should strengthen the monitoring of the patient to increase the timeliness of rescue in the event of sudden death.

3.4. Gene mutation of DCM

The gene spectrum associated with DCM is extensive, and studies have shown that there are more than 40 causative genes that can cause DCM, and more than 100 related genes considering human and animal genes. These include relatively concentrated genetic variation as well as very private genetic variation. Various genetic mutations can be divided into several categories, namely nuclear envelope protein mutation, sarcomere protein mutation, and some other mutations [15].

The nuclear envelope protein mutations most associated and concentrated in familial DCM are LMNA mutations, which account for 5% to 8% of familial DCM [15]. LMNA gene variants may manifest as extracardiac features and DCM with or without conduction system condition. Conduction system disease caused by this mutation is highly associated with sudden cardiac death, with a frequency of up to 46% [16].

The related genes of sarcomere protein mutation are very similar to HCM. The most common ones are MYH7 and TNNT2. This high overlap prompted researchers to investigate whether the two diseases were related.

3.5. Pathological manifestations of DCM

The pathological mechanism of DCM is highly complex, and different gene mutations can lead to different pathogenic mechanisms. Electrophysiological findings usually precede the onset of DCM and may be the only manifestations that can be characterized as cardiac. Recent evidence suggests that de-isomerized genes used to known as ARVC causative may also play a roll in the etiology of DCM. DCM may also have muscle damage and may be a predominant manifestation or characteristic of several multisystemic disorders.

3.6. Utility of genetic testing in DCM

Compared with the sarcomere disease HCM that has relatively concentrated pathogenic genes, DCM shows a significantly greater degree of locus heterogeneity with a constantly growing number of genes involved.

Due to the genetic heterogeneity in DCM, most mutations show a very low prevalence [9]. Given this factor, the number of gene testing samples typically required to detect DCM can be significant. However, due to the high detection rate of familial DCM among first-degree relatives, genetic testing can still be used as an effective means of screening for hidden dangers of the disease.

4. Analysis of inherited cardiomyopathy

4.1. Endomyocardial biopsy

Endomyocardial biopsy (EMB) is a way of detection that of obtaining cardiac tissue from the ventricle for pathological diagnosis and research, to monitor rejection after heart transplantation. EMB is also used to diagnose cardiomyopathy, infectious and neoplastic diseases.

Typically, the femoral vein or right internal jugular vein are the target positions that an EMB device will be inserted into. Then the device will be advanced into the right ventricle (RV) to collect a sample from the interventricular septum. Commonly used EMB devices are 1.66 mm or larger. Severe trauma at the biopsy site is caused and limited maneuverability is provided due to the large device compared to the small space within the ventricle.

As a result, the use of EMB is decreasing, although this is the reference method for many diagnostics and is supported by cardiology organizations. [17].

4.2. Electrocardiogram and echocardiography

Electrocardiogram (ECG) is a technique that record changes in electrical activity of the heart during each cardiac cycle from the body surface using an electrocardiograph.

Echocardiography is a technique that uses ultrasound to detect the periodic activities of various structures, such as the cardiac wall, ventricle and valves. It displays the features on the monitor as a curve to show the relationship between the related actions and time of each structure.

The use of an ECG and echocardiography can help doctors detect possible problems with a patient's heart. ECG abnormalities can also reflect disease severity and provide helpful indications for risk stratification and management [18]. However, these two approaches are more used clinically and can only give valid results if the patient's heart is electrically or structurally abnormal. And in more cases, people will take the initiative to conduct both tests after they have obvious symptoms. Also, these two methods do not explain the pathological causes for cardiomyopathy, and there is no way to suggest the risk of ordinary people without abnormality.

4.3. Sanger sequencing and NGS sequencing

Sanger sequencing is a technique of determining the nucleotide sequence of DNA. The method is also called the “chain termination method” [19]. The advantage of Sanger sequencing is that this method is fast and cost-effective when detecting a low number of genes. However, it has lower sensitivity, lower discovery power, and lower scalability. When trying to sequence long DNA fragments with massive genes, it will be very time- and money-consuming [20]. Next-generation sequencing (NGS) is a massively parallel sequencing technology that delivering extremely high throughput, scale, and speed. It is used to identify the nucleotides order in the targeted genomes or regions of DNA or RNA [21]. NGS enables rapid and in-depth DNA or RNA sequencing and whole-genome sequencing (WGS) of target segments. It allows researchers to complete large-scale genetic sequencing in a relatively short time, enabling them to identify hereditary diseases, to characterize mutations which stimulate the progression of cancer and to follow disease outbreaks.

The advantages of NGS are clear that it has higher sensitivity, faster turnaround time, broader coverage and lower limit. It is really cost-effective when sequencing massive target genes. However, it is less cost-effective and more time-consuming when detecting a low volume of samples. The principle of NGS and Sanger sequencing are somewhat similar. DNA polymerase adds fluorescent nucleotides in order onto the DNA template strand. The specific fluorescent tags will indicate different particular nucleotides. The most significant difference between the two ways is the volume [20]. Also, NGS is more accurate than Sanger. Experiments show that NGS achieves a modest increase in sensitivity without compromising sequence accuracy [22]. In a test done in 2013, NGS is used and achieved 100% sensitivity (95% confidence: 97.76%–100%) and nearly 100% specificity. And variants not covered by Sanger sequencing are included in the results obtained by the NGS test, indicating that the accuracy of NGS might be even better than that of Sanger sequencing [23]. The two approaches can complement each other perfectly if used properly: Sanger for small-scale measurements and NGS for large-scale sequencing. This is why Sanger is still a commonly used sequencing method after the advent of NGS.

5. Conclusion

For people, preventing a disease is more important than treating a disease. Therefore, probing the causative factors of ICs remains an important topic since no way to prevent this disease has been found so far. In general, a large number of pathogenic genes related to ICs, more specifically HCM and DCM, have been detected, but the pathogenic mechanisms of some genes are still unclear. The same causative gene has been identified between HCM and DCM, but the relationship between the two is still inconclusive. Researchers are still working on more details in this area. The two generations of gene sequencing techniques can complement each other, and new methods have been developed. However, the shortcomings of the new methods still need to be filled, and some

advantages still have room for further development. Research in this area is continuing, and more perfect sequencing methods may appear in the future.

References

- [1] Elizabeth M. McNally, et al. Genetic mutations and mechanisms in dilated cardiomyopathy [J]. *J Clin Invest.* 2013;123(1):19-26.
- [2] Jeffrey A. Towbin. Inherited Cardiomyopathies [J]. *Circulation Journal*, 2014;78(10):2347-2356.
- [3] Paul J.M. Wijnker, Jolanda van der Velden. Mutation-specific pathology and treatment of hypertrophic cardiomyopathy in patients, mouse models and human engineered heart tissue [J]. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 2020;1866(8):165774.
- [4] Chris Miles, et al. Inherited cardiomyopathies [J]. *Cardiology Clinical Academic Group, Molecular and Clinical Sciences Institute, St George's University of London, London, UK. BMJ* 2019; 365: 11570.
- [5] <https://www.mayoclinic.org/diseases-conditions/cardiomyopathy/symptoms-causes/syc-20370709>
- [6] Polakit Teekakirikul, et al. Inherited Cardiomyopathies. 2013;15(2):158-170.
- [7] Perry Elliott, et al. Classification of the cardiomyopathies: a position statement from the european society of cardiology working group on myocardial and pericardial diseases [J]. *European Heart Journal*, 2008;29(2):270–276.
- [8] Wolf CM. Hypertrophic cardiomyopathy: genetics and clinical perspectives [J]. *Cardiovasc Diagn Ther.* 2019;9(Suppl 2): S388-S415.
- [9] Daniel Jacoby, William J. McKenna, Genetics of inherited cardiomyopathy [J]. *European Heart Journal*, 2012;33(3):296–304.
- [10] Luisa Mestroni, et al. Familial dilated cardiomyopathy: Evidence for genetic and phenotypic heterogeneity [J]. *Journal of the American College of Cardiology.* 1999;34(1):181-190.
- [11] Pascale Richard, et al. and for the EUROGENE Heart Failure Project [J]. *Hypertrophic Cardiomyopathy. Circulation.* 2003; 107: 2227–2232
- [12] Gilles Millat, et al. Prevalence and spectrum of mutations in a cohort of 192 unrelated patients with hypertrophic cardiomyopathy [J]. *European Journal of Medical Genetics.* 2010;53(5):261-267.
- [13] Paul J.M. Wijnker, et al. Hypertrophic Cardiomyopathy: A Vicious Cycle Triggered by Sarcomere Mutations and Secondary Disease Hits [J]. *Antioxidants & Redox Signaling.* 2019;31(4):318-358.
- [14] Raffaele Coppini, et al. Clinical Phenotype and Outcome of Hypertrophic Cardiomyopathy Associated with Thin-Filament Gene Mutations [J]. *Journal of the American College of Cardiology.* 2014; 64(24): 2589-2600.
- [15] Dellefave L, McNally EM. The genetics of dilated cardiomyopathy [J]. *Curr Opin Cardiol.* 2010; 25(3): 198-204.
- [16] van Berlo, J.H., et al. Meta-analysis of clinical characteristics of 299 carriers of LMNA gene mutations: do lamin A/C mutations portend a high risk of sudden death [J]. *Journal of Molecular Medicine.* 2005; 83: 79–83
- [17] Rikard Grankvist, Arvin Chireh, Mikael Sandell, Abdul Kadir Mukarram et al. Myocardial micro-biopsy procedure for molecular characterization with increased precision and reduced trauma [J]. *Scientific Reports.* 2020;10, Article number: 8029
- [18] Finocchiaro, G., et al. The electrocardiogram in the diagnosis and management of patients with dilated cardiomyopathy [J]. *Eur J Heart Fail.* 2020; 22: 1097-1107.
- [19] <https://www.sigmaaldrich.com/JP/ja/technical-documents/protocol/genomics/sequencing/sanger-sequencing>
- [20] <https://www.illumina.com/science/technology/next-generation-sequencing/ngs-vs-sanger-sequencing.html>
- [21] <https://www.illumina.com/science/technology/next-generation-sequencing.html>
- [22] Philip L. Tzou, et al. Comparison of an In Vitro Diagnostic Next-Generation Sequencing Assay with Sanger Sequencing for HIV-1 Genotypic Resistance Testing [J]. *Journal of Clinical Microbiology.* 2018; 56(6)

- [23] Sikkema-Raddatz, B. et al. Targeted Next-Generation Sequencing can Replace Sanger Sequencing in Clinical Diagnostics [J]. Human Mutation, 2013; 34: 1035-1042.