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Genetic diagnosis in pediatric cardiomyopathy: clinical application and research perspectives

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Abstract

There has been tremendous growth in the application of genetics to the clinical practice of pediatric cardiomyopathy. The identification of the genetic basis for cardiomyopathies is important for establishing a causal diagnosis, providing definitive identification of at risk family members, and providing cost-effective screening and surveillance. Additional research is needed to better understand the genetic heterogeneity of cardiomyopathy in children, the implications of specific genotypes, the best approach to cardiac surveillance and genetic testing, and the utility of genotyping for individual risk stratification. As the technology for evaluation of the human genome continues to improve, there is an increasing need for assessment of clinical relevance and utility. This is coupled with an ongoing need for education and training of professionals to interpret and implement genomics in a clinical setting.

Keywords

cardiomyopathy; mutation; genetic testing; sequencing; pediatric

1. Introduction

The Second International Conference on Cardiomyopathy in Children provided a forum for clinicians and scientists to discuss the current status of clinical care and research relevant to pediatric cardiomyopathy and identify critical questions and areas requiring additional research. There has been tremendous growth in the application of genetics to pediatric cardiomyopathy since the initial conference in 2006. The expanded availability of clinical molecular genetic testing for cardiomyopathy has significantly changed the landscape for clinical evaluation. Additional research is needed to better understand the genetic heterogeneity of cardiomyopathy in children, the implications of specific genotypes, the best approach to cardiac surveillance and genetic testing, and the utility of genotyping for individual risk stratification and, ultimately, more personalized therapy. This report summarizes the current status of genetic testing for pediatric cardiomyopathy and highlights some focus areas for future investigation.

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2. The genetic basis of pediatric cardiomyopathy

Cardiomyopathies with known genetic causes can be broadly classified by pathophysiology into 5 types: hypertrophic (HCM), dilated (DCM), restrictive (RCM), left ventricular noncompaction (LVNC), and arrhythmogenic right ventricular cardiomyopathy (ARVC). The latter is infrequently identified in children. HCM, DCM, RCM, and LVNC all occur in childhood with a bimodal age distribution. One peak occurs in infancy (less than 1 year of age) and is characterized by the worst outcomes and broadest spectrum of causes, while a second peak occurs in adolescence [1, 2]. In children, the underlying causes of cardiomyopathy are more heterogeneous than in adults. Genetic syndromes, neuromuscular disease, inborn errors of metabolism, mitochondrial disorders, and mutations in genes encoding structural components of the cardiomyocyte including the sarcomere and cytoskeleton all contribute to the genetic heterogeneity [3]. As recently as 2007, only one third of children with cardiomyopathy had a well defined etiology based on Pediatric Cardiomyopathy Registry data [1, 2]. For both HCM and DCM, outcomes are demonstrated to be dependent on cause and age of presentation. Therefore, identifying specific etiologies is important for long term management, prognosis, risk stratification, and development of more targeted therapeutics.

In adults, HCM is common, affecting approximately 1 in 500 individuals in the population. It is a highly penetrant genetic disease, most frequently inherited in an autosomal dominant pattern. HCM has largely been considered a disease of the sarcomere, with mutations in beta-myosin heavy chain (*MYH7* gene) and myosin binding protein C3 (*MYBPC3* gene) combining to explain approximately 80% of mutation positive cases. In a recent study of 84 children without extracardiac abnormalities, mutations in sarcomeric genes were identified in 55% (46/84) of subjects [4]. The prevalence of sarcomeric mutations in infants, in whom the assessment or exclusion of extracardiac abnormalities may be problematic, is not yet known. As discussed below, improvement in chromosome based assays such as chromosome microarrays, combined with new availability of molecular testing for a number of genetic syndromes, provides better tools for evaluation of other genetic causes of HCM in children.

DCM is also frequently inherited as an autosomal dominant condition. In adults, approximately 30% of patients with non-ischemic DCM have an identifiable mutation when genetic testing is performed[5]. Greater than 30 genes, many of which encode sarcomeric or cytoskeletal proteins, have been shown to cause DCM. No data are currently available for the prevalence of mutations in these genes in children with DCM, although clearly there are shared genetic causes. However, equally clear is the fact that inborn errors of metabolism and genetic syndromic causes are responsible for a significant number of DCM cases in infancy. In adolescence, neuromuscular disorders including Duchenne muscular dystrophy, account for a large number of cases. More precise delineation of causation in the large number of children assumed to have idiopathic disease is a necessary prerequisite to developing directed therapy.

Recently, sarcomeric mutations have also been identified as causative in subjects with LVNC or RCM, although genetic testing results for large cohorts are lacking for both adult and pediatric populations [6-10]. LVNC is also commonly identified in patients with genetic syndromic conditions or inborn errors of metabolism. Examples include Barth syndrome, an X-linked disorder with mitochondrial dysfunction and abnormal cardiolipin metabolism, and 1p36 deletion syndrome. RCM is the least common cardiomyopathy and cause has only rarely been identified in the pediatric population, although case reports of association with neuromuscular disorders exist.

As more patients undergo genetic evaluation or testing, there is increasing recognition of the phenotypic variability resulting from mutations in genes encoding sarcomeric or cytoskeletal proteins. For example, mutations in the *MYH7* gene are well recognized as causing HCM. More recently, mutations in this contractile protein have been identified in patients with DCM, RCM, and LVNC as well. This pleiotropy is attributed, in part, to the fact that mutations in discrete protein domains can disrupt specific protein-protein interactions or functional properties of the protein, with the cardiac phenotype being dependent on the precise site of the mutation within the protein. However, phenotypes can be divergent even within a family with the same mutation, indicating that modifying genetic or environmental factors also contribute to the phenotypic presentation. As genetic testing has become more comprehensive, patients with multiple pathogenic mutations have been identified. In addition, there is some preliminary evidence that multiple rare variants, or even common polymorphisms, may act in concert in sporadic cardiomyopathy cases, explaining a more complex inheritance model. Understanding genetic and phenotypic heterogeneity in cardiomyopathy requires a more complete base of knowledge of disease associated variants and modifying genetic influences and will require additional research.

3. Recommendations for clinical genetic evaluation and testing

A Heart Failure Society of America Practice Guideline was published by Hershberger et al. in 2009 to provide evidence based guidelines for cardiac surveillance and genetic testing in patients with possible familial or genetic causes of cardiomyopathy [11]. The guidelines address the level of evidence for clinical validity and clinical utility in making recommendations. Key components of the genetic recommendations include: 1) generate a 3 generation pedigree for all patients with cardiomyopathy 2) perform ongoing clinical screening for cardiomyopathy in asymptomatic first degree relatives 3) consider referral to centers with specific expertise in genetic evaluation and family-based management 4) consider genetic testing in the most clearly affected family member to facilitate family screening and management 5) provide genetic and family counseling and incorporate genetic testing results into the clinical and family data to develop a care plan.

Evaluation by a medical geneticist is important for cardiomyopathy associated with genetic syndromic conditions, neuromuscular disorders, and inborn errors of metabolism for directing management of extra-cardiac symptoms and for facilitating the diagnostic evaluation necessary to establish the diagnosis.

4. Availability of genetic testing

Clinical genetic testing should be performed in a laboratory that is accredited by Clinical Laboratory Improvement Amendment (CLIA) standards. Genetic testing for cardiomyopathy is changing rapidly and comprehensive molecular panels are currently available. Because of the heterogeneity of causes in pediatric cardiomyopathy, it is difficult to create an algorithm for application of genetic testing that is widely applicable, since testing should be guided by the differential for a specific patient. Clinical genetic testing is available for a large number of sarcomeric and cytoskeletal genes in commercial laboratories. Currently, GeneTests (<http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests>) lists 13 laboratories performing molecular testing for familial cardiomyopathy, of which 6 are in the United States. Each laboratory differs with regard to the number of genes tested on their individual cardiomyopathy panels, cost, and technology platform. Medical geneticists and genetic counselors are trained to facilitate testing – including identification of the most appropriate family member for testing, pre-test counseling regarding utility and possible outcomes, discussion of insurance coverage and the Genetic Information Nondiscrimination Act

(GINA), and interpretation of results after completion of testing – and their expertise should be considered as part of the diagnostic evaluation [11].

In addition to advances in the molecular genetic testing panels for isolated (familial) cardiomyopathy, there have been advances in tests for syndromic conditions associated with cardiomyopathy. The development of chromosome microarray analysis allows the identification of submicroscopic chromosomal abnormalities that were previously undetectable. Use of this cytogenetic test should be considered in children with a suspected genetic syndrome of unknown etiology. Molecular genetic testing for Noonan syndrome, a common syndrome associated with HCM, is now available as a gene panel. Advances in newborn screening allow for the identification of a number of inborn errors of metabolism associated with cardiomyopathy, including fatty acid oxidation disorders, before metabolic decompensation. Newborn screening tests differ by state and practitioners need to be aware of the specifics for their practice region. The availability of molecular tests for neuromuscular and mitochondrial disorders also continues to increase, although the diagnostic evaluation for a suspected mitochondrial disorder remains difficult for patients with cardiomyopathy.

5. The utility of genetic testing

There is reason for optimism that genetic testing will improve the care of patients with cardiomyopathy [12]. Because testing has been clinically available for a relatively short time period, there are no published studies to compare outcomes in individuals that received testing versus those that did not. Furthermore, studies designed to estimate the benefit to at risk family members are difficult to design and implement. The utility of genetic testing is an important topic that has received recent attention [11-15].

Benefits of genetic testing include establishing a causal diagnosis, providing definitive identification of at risk family members, and providing cost-effective screening and surveillance. Particularly in the pediatric setting, where cardiomyopathy is so heterogeneous, the benefit of establishing a causal diagnosis should not be underestimated. It is, in fact, critical for early and proper management of extra-cardiac symptoms for syndromic, metabolic, or neuromuscular cases.

In order to use genetic testing most effectively, it is important to understand its limitations in addition to the benefits. Limitations will be discussed in more detail below, and include the facts that 1) genotype-phenotype correlations are still emerging 2) testing does not always yield unambiguous results and 3) some causative genes remain unidentified. Each of these limitations represents a unique area for future research.

Currently, there are insufficient data regarding the prognostic information provided by specific genotypes. After initial (over)enthusiasm about genotype-phenotype correlations and the ability to risk stratify based on mutation type, testing larger cohorts has demonstrated a more complex reality. This was, in fact, a predicted outcome of more globally applied genetic testing and should not be viewed as a cause for negativity regarding the importance of identifying the molecular etiology. Research-based identification of causative genes has historically relied on an ascertainment bias in which the most severely affected individuals, with highly penetrant mutations, are the first identified. Thus, for the majority of genetic disease, discovery of the genetic basis and description of the phenotype is followed by an expansion of the phenotypic spectrum and modification and re-interpretation of initial molecular results. Genetic testing provides important benefits to clinical care as described above. However, clinical research is required to determine the full implication of genetic results in aggregate. Re-classification of mutations with incorporation of clinical information, refinement of genotype-phenotype predictions, and identification of

susceptibility alleles or modifier genes are all research needs that can only be addressed with information derived from large cohorts. There are important opportunities for further development of our understanding of the genetic basis of pediatric cardiomyopathy and the development of algorithms for clinical management.

Genetic testing in the cardiomyopathy population, particularly in HCM, results in some of the highest diagnostic yields of any type of genetic test. Nevertheless, negative and indeterminate tests (variants of uncertain significance) are inevitable and are intrinsic to the limitations of our current technical and clinical knowledge base. Interpretation of genetic testing is strengthened by evaluating results in large populations derived from clinical genetic testing programs that avoid the biases that occur when a small number of subjects are studied in a research setting. There are specific criteria for determining the pathogenicity of a variant, but novel rare variants pose problems for interpretation. Ultimately, each commercial laboratory decides the final interpretation based on the published literature, bioinformatic prediction programs, clinical information, and their internal testing and reporting practices. An understanding of the possible outcomes of the test, and how each possible outcome will be utilized clinically, is important for both the care provider and the patient. Future research also is needed in development of strategies to assess functional consequences of rare variants.

Finally, it is clear that we haven't yet identified all genetic causes of cardiomyopathy. Diagnostic yield varies depending on the type of cardiomyopathy. Patients should be counseled carefully about the interpretation of negative testing and its implications with regard to at risk family members. In addition to identification of novel causative genes, future research will also be directed at delineating the importance of regulatory regions, enhancers, microRNA, and copy number variation in the development of disease. Recent technical developments have led to a number of general discoveries about genetic disease and genomic architecture, but these have not yet been applied in a rigorous way to the field of pediatric cardiomyopathy. Studies directed toward understanding complex inheritance patterns and gene x environment interactions will provide additional information about disease susceptibility.

6. Genomics and medicine

Isolated cardiomyopathy is most frequently the result of point mutations or small insertions or deletions in genes encoding sarcomeric or cytoskeletal proteins. Although recurrent mutations have been identified in multiple families, many disease causing mutations are private, occurring in a single family. This lack of a mutational "hot spot", combined with the marked locus heterogeneity in cardiomyopathy, makes genetic testing challenging. Using traditional Sanger sequencing, there is significant cost and labor involved in testing the large number of implicated disease causing genes. Recent developments in higher throughput sequencing – massively parallel or next generation sequencing – allows simultaneous interrogation of a much larger number of genes and is beginning to impact both the cost and comprehensive nature of clinically available genetic testing [16, 17]. Full exome (all gene coding regions) sequencing is now well developed for research use, and the push for development of low cost whole genome sequencing persists. Third generation sequencers are under rapid development. Taken together, all evidence points to the fact that the historical limitations to sequence based testing are obsolete.

The challenge no longer lies with the technical ability to generate the molecular data, but rather with the ability to reproducibly analyze and interpret it. These are not small challenges, and they will require similar levels of research effort to match the successes of technical platform development. Importantly, development of methods for analysis of

genetic data is dependent on the research participation of the medical community since accurate genetic interpretation requires phenotype information. As the technology for evaluation of the human genome continues to improve, there is an increasing need for professionals to apply and interpret genetic testing in a thoughtful and clinically meaningful way. Cardiologists, geneticists, and genetic counselors need cross discipline training in order to provide the best care to patients with cardiomyopathy and their at risk family members. Similar models exist in the field of cancer genetics, and such partnerships are well established for adult cardiomyopathy in some European countries. A number of opportunities exist to further promote interdisciplinary collaboration, of which development of subspecialty training programs or less formalized certification programs in cardiovascular genetics are two options.

The increased availability of genetic testing for pediatric cardiomyopathy provides an opportunity to be more precise in the categorization of our patients with regard to underlying etiology. The first challenge is to utilize available clinical testing to improve diagnosis based on cause. Subsequently, there is a clear need for longitudinal clinical studies in which careful phenotyping and outcome measures are evaluated in the context of the known genetic diagnosis. A long term goal should be to determine the utility of genetic testing for genotype-phenotype prediction and risk stratification. *A priori* knowledge of a patient's genotype should be used for the development of more sensitive measures of disease and to improve the ability to monitor disease progression. Studies of large cohorts are required in order to begin to identify genetic and environmental modifiers of disease.

7. Conclusions

An understanding of the genetic basis of cardiomyopathy has come a long way from the initial description of *MYH7* mutations in HCM. Genetic testing has transitioned from a research-only tool to an important component of the diagnostic evaluation of patients with cardiomyopathy and extension of care to at risk family members. The incorporation of genetic testing into clinical care is cost effective and allows for earlier detection and improved prevention of adverse events. Pediatric cardiomyopathy has unique challenges because it is rare and its causes are more heterogeneous than its counterpart in adults. Cause-based diagnostic precision is important for the best patient care. As technological advances continue to increase, there is an ongoing need for research that directly addresses the relevance, utility, and implementation of genomics in a clinical setting. This is coupled with an ongoing need for education and training of professionals to interpret and utilize this new array of tools and develop additional clinical applications.

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