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EDITORIAL

Pediatric Primary Dilated Cardiomyopathy Gene Testing and Variant Reclassification: Does It Matter?

Jeffrey A. Towbin, MD

In the late 1990s and early in the 21st century, the interest in discovering the genetic basis of dilated cardiomyopathy (DCM) started in earnest. These studies, performed in individual research laboratories, identified increasing numbers of potential disease-causing genes as technology improved, especially genes encoding the sarcomere and cytoskeleton.[1–3] This led to the development of commercial genetic testing for DCM, with individual genes or small gene panels of ≈5 genes being offered. Over the years, the panels increased in the number of genes analyzed because of improved technology, such as next-generation sequencing, and now has gotten to the point where current panels exist that have nearly 100 genes offered.[4] As clinical genetic testing started to become commonplace and results were dispatched in a more timely manner, a red flag was raised by clinicians receiving the results of testing for their patients. It was widely assumed by clinicians and families that the genetic testing results would be able to answer the question of cause of the patient's disease and be a definitive diagnostic tool for family members, as well as being a blueprint to clinical management and prognosis. However, the clinical laboratory reports that followed were not as expected. Although some clinical reports from commercial laboratories provided reports for individual patients that were able to classify the identified variants somewhat definitively as either pathogenic or benign, many results were reported with the term that became the bane of the clinician and family, a variant of uncertain significance (VUS), demonstrating that genetic testing results were not binary. Many clinicians view a VUS as being the worst-case scenario because this information does not enable clinicians to help the patients and their families better understand their disease causation, the likelihood of others in the family who might be at-risk carriers, or prognosis, and left the clinician wondering how to proceed. In some instances, clinicians unfamiliar with genetics misinterpreted the meaning of VUSs and thought they were actionable variants. In the arena of caring for children with primary DCM, lack of clarity and lack of understanding of these results can lead to poor decisions and potentially serious harm. This disconnect on the utility of genetic testing has also been amplified by the fact that only ≈30% of individuals with DCM who are tested are identified as having a pathogenic variant. There is significant genetic heterogeneity, and, in children, there

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See Article by Quiat et al.

Correspondence to: Jeffrey A. Towbin, MD, Le Bonheur Children's Hospital, FOB Room 344, 49 N. Dunlap Street, Memphis, TN 38103.
E-mail: jtowbin1@uthdc.edu

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appears to be an age-related association. An improvement in the ability to more definitively determine the pathogenicity or the lack thereof of genetic variants would go a long way in planning the care of these patients and their family members. Variability in the interpretation of variants between laboratories has always been an issue, but in the current setting of massively increased data generation because of next-generation sequencing, these issues are amplified and therefore necessitated more thorough guidance and ultimately led to the development of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines, published in 2015, a more structured approach for variant interpretation.[5] These guidelines were intended for evaluating variants in genes with established disease causality and not for novel variants in "candidate" genes. Application of the Association for Molecular Pathology guidelines, which established a 5-tier classification system (pathogenic, likely pathogenic, uncertain significance, likely benign, or benign) and specified lines of evidence necessary for clinical interpretation, has facilitated more uniformity of variant interpretation. The ClinGen framework was also created to determine the strength of evidence supporting gene-disease associations.[6]

In this issue of the *Journal of the American Heart Association* (*JAHA*), Quiat et al report a retrospective genetic analysis of all patients with childhood primary DCM who presented to their institution between 2008 and 2018, a 10-year period in which technology and DCM gene panel sizes changed significantly.[7] A cohort of 63 pediatric probands with primary DCM was evaluated. Only 18% of those who underwent cardiomyopathy-specific gene testing had a family history of cardiomyopathy or sudden death. A disease-causing variant was identified in 19 of 63 probands (30%), with 47% occurring de novo. No new disease-causing variants were identified. The authors identified 116 variants that were classified at the initial gene testing, with 8 being interpreted as being pathogenic, 11 being likely pathogenic, 90 being reported as VUSs, 3 being likely benign, and 2 reported as benign variants, with 2 others unclassified. The detection rate was 30% for disease-causing variants in these probands, a similar result as seen in other studies on DCM in children.[8–10] Twelve affected genes were identified hosting pathogenic variants, likely pathogenic variants, and VUS favoring pathogenic, including genes that encode proteins critical to sarcomere function (*MYH7*, *TTN*, *TNNT2*, *TNNI3*, *MYBPC3*, *TPM1*, and *BAG3*) and proteins important for cellular structure (*LMNA* and *DES*), cell junctions/desmosomes (*PKP2* and *DSP*), and lysosomal function (*LAMP2*), which is similar to that seen in adults with DCM.[11] Variant classification was based on prior methods. Reclassification of variants was performed by the authors using the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines and ClinGen, and this resulted in the downgrading of 29% of VUSs (26/90) to either likely benign or benign, whereas four of the likely pathogenic variants were reclassified as a VUS-favor pathogenic and one was reclassified as a pathogenic variant; therefore, a decrease in the number of VUSs from 60% to 52% was found, and the proportion of likely benign or benign variants increased from 14% to 24%. In probands with a positive family history and pathogenic or likely pathogenic variants (n=9), 3 variants were inherited and 6 were de novo mutations. In 6 probands with a potentially disease-causing VUS, familial testing helped clarify inheritance, with 3 occurring de novo and 3 being inherited from individuals with a normal cardiac phenotype at the time of testing. The most frequently identified pathogenic variants were found in the sarcomere protein-encoding genes *TNNT2* (n=5) and *MYH7* (n=3), with all 3 *MYH7*-positive patients presenting in infancy, and those with *TNNT2* variants presenting in either early infancy (n=2) or adolescence (n=3). The percentage of genetic tests identifying pathogenicity was mildly increased when the gene testing panels were expanded over the time of the study, with panels having >50 genes finding 27% with pathogenic variants versus 20% for panels having <50 genes. This was not significantly affected by variant reclassification.

So how does this help us? The authors report that 46% of the 63 affected children either underwent heart transplantation or died during the 37-month follow-up period, with patients hosting a disease-causing variant and presenting after 1 year of age having decreased transplant-free survival, with a composite outcome of death or transplant of 91% (10/11 probands) compared with probands presenting after 1 year of age and not hosting a pathogenic variant (13/29; 45%). Familial cascade testing of the healthy parents of 9 probands with pathogenic or likely pathogenic variants identified 3 variants as inherited and 6 variants as de novo. Six probands hosted a potentially disease-causing VUS; familial testing identified 3 occurring de novo and 3 being inherited from individuals with a normal cardiac phenotype at the time of testing, thereby helping to clarify the inheritance or lack thereof of the variant. The authors conclude that their results highlight the importance of genetic and phenotypic evaluation in parents and first-degree relatives of probands for the interpretation of genetic testing results in DCM probands.

This study, albeit being performed in a small cohort, demonstrates that reclassification of genetic variants using the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines and ClinGen may facilitate more definitive "calls" on causality or no causality in clinical

genetic testing of children, in this case reducing the uncertainties (VUSs) by ≈30% and increasing the percentage of children having a benign genotype. This does allow for the family members of those children identified as having benign variants to require follow-up evaluation less frequently or potentially not at all by a cardiologist. Unfortunately, in this study, the percentage of VUSs continued to be high, demonstrating a continued need to not only continue to recommend evaluation of first-degree relatives clinically and genetically but also continue to expect that clinical genetic testing be reevaluated by the commercial facility on a routine basis to ensure that the interpretation remains the correct result. It is worth noting that, in the best-case scenario, using the expertise of genetic counselors is important to evaluate the data received as well as presenting the data to the families and providing counseling. Whether larger and larger panels and other advances, such as use of whole genome sequencing, will be helpful in defining the cause of pediatric DCM in the future is controversial at this time and will remain so until other breakthroughs in technology and computing ability occur. The use of genetic testing for genotype-phenotype correlation and risk stratification of children with primary DCM is still imperfect, and the small patient numbers herein do not provide the data necessary to alter our ability to improve outcomes but could help to improve our ability to provide more definitive answers to families about the level of risk or lack of risk that they have on the basis of the genetic test result. The red flags, however, will continue until that time.

ARTICLE INFORMATION

Affiliations

From the Division of Pediatric Cardiology, Heart Institute, Le Bonheur Children's Hospital, Memphis, TN (J.A.T.); Division of Pediatric Cardiology, Department of Pediatrics, Heart Institute, University of Tennessee Health Science Center, Memphis, TN (J.A.T.); Pediatric Cardio-Hemato-Oncology, St. Jude Children's Research Hospital, Memphis, TN (J.A.T.).

Disclosures

None.

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