Commercial Clinical Genetic Sequencing Panels for Evaluating Patients with Familial Disease—Are They Ready for Prime Time?

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I recently saw a patient with idiopathic dilated cardiomyopathy (DCM) who I had been following for 10 years. Her sister had recently been diagnosed as having a DCM. Because of the positive family history, the physicians caring for her sister had ordered a commercially available heart failure "clinical genetic testing panel." The panel consisted of full exon sequence data on 27 genes that had been associated with the development of a DCM or with the development of left ventricular non-compaction (LVNC).

The genetic panel revealed an informative nucleotide substitution in a region of the gene encoding desmin that had not been reported previously. The report noted that "no definitive disease-causing mutations were detected by sequence analysis of the 27 genes"; however, it went on to remark that while the clinical significance of the variant was not clear "a pathogenic role [for the variant] would be supported if it occurred *de novo* in this individual, or if it co-segregates with a cardiomyopathy phenotype in this family." The report also noted that the variant occurred in a "hot spot" in the desmin gene. Subsequently, my patient, her parents, and an unaffected sibling were genotyped for the single nucleotide polymorphism in the desmin gene: none of the family members harbored the variant. Thus, the desmin mutation did not segregate in the sib pair making it doubtful that it was pathogenetic. However, we could not exclude the far less likely possibility that the mutation in the desmin gene arose *de novo* in one sister and that heart failure occurred in the second sister due to an entirely different cause. Nonetheless, this case pointed out important concerns about the growing availability of commercial gene panels for human disease: the need to follow established guidelines when pursuing genetic analysis and the importance of the early involvement of clinical teams having expertise in genetic counseling and the ability to interpret molecular genetic test results in patients presumed to have a familial etiology of their disease.

It was reassuring to see that the residents and fellows who cared for the proband were intrigued by the family's history of familial DCM; however, they were not cognizant of the guidelines for performing genetic evaluation. The academic medical center did not have a cardiovascular genetics expert, a geneticist or a genetic counselor available, limiting the team's ability to effectively counsel the family and evaluate the results of the testing. This is of particular importance in the case of rare mutations where the results from genetic panels may provide the clinician with the false sense that genetic testing in patients with heart failure is straightforward or simple. Genetic testing can be informative for predicting either prognosis or outcomes for certain diseases where a defined and well-established mutation has been conclusively associated with the development of disease; however, when pursuing complex diseases such as idiopathic DCM, the need for geneticists with particular training in the specific area of interest is of paramount importance. For example, selected mutations in the cytoskeleton (dystrophin, actin, desmin, vinculin, lamin A/C) and the contractile or calcium handling proteins of the sarcolemma (beta-myosin heavy chain, troponin T, phospholamban) have been associated with the development of idiopathic DCM. The pathophysiologic relevance of some of these mutations have been demonstrated by the fact that transgenic mice with specific mutations in these genes develop a heart failure phenotype while *in vitro* studies in cell systems or zebrafish can demonstrate significant alterations in protein expression or function. However, different mutations in the same gene can result in a very different cardiomyopathy phenotype and a different outcome and even for a specific single mutation, there is often genetic heterogeneity. Therefore, a different phenotype can be seen in different families harboring the same variant and varying phenotypes can be found even within members of the same family having the same genotype. Genetic testing in patients with DCM is also confounded by the fact that the etiology of sporadic DCM is generally unknown and there is a relatively low frequency of involvement of any one genetic mutation in patients with either sporadic or familial DCM. Genetic testing can also be confounded by reduced penetrance in individuals—and thus, patients harboring a mutation who have no clinical evidence of the phenotype require assiduous follow-up for disease surveillance.[1]

Genetic heterogeneity is also seen in familial hypertrophic cardiomyopathy—the most thoroughly studied of all genetic cardiomyopathies. Mutations have been identified that disrupt proteins of the thick filament (β-myosin heavy chain, myosin-binding protein-C, essential and regulatory myosin light chains), the thin filament (actin, troponin T and I, tropomyosin), or the supporting architecture of the contractile proteins (titin). While each of these mutations is associated with a common phenotype—cardiac hypertrophy—each can be associated with significantly different outcomes and can be variously modified by pharmacologic therapy. Bick et al. demonstrated the extensive heterogeneity in hypertrophic cardiomyopathy mutations.[2] They screened 3,600 subjects for mutations in 8 sarcomeric genes associated with cardiac hypertrophy. Only 4 of the 22 individuals who were found to have pathogenic variants actually had cardiac

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hypertrophy; however, the presence of a rare missense mutation in a sarcomeric gene was associated with an increased risk of a cardiovascular event suggesting that some of the rare variants might have significance despite lacking a hypertrophic phenotype.

Despite the inherent difficulties in identifying disease-causing mutations in even large families with DCM, family screening has become a critically important part of the clinical approach to this disease. Indeed, Moretti et al. found that family screening—even in the absence of an identified mutation—can identify patients with DCM at an early stage of the disease and in so doing, improve their survival.[3] However, any clinicians who undertake genetic testing using commercial panels should be thoroughly conversant with practice guidelines in clinical genetics provided by professional societies and authored by experts in clinical and molecular genetics. Recent guidelines established by the Heart Failure Society of America for evaluating families with heritable forms of DCM provide a guidepost for both clinicians and clinical investigators and can be applicable to other familial diseases for which guidelines have not yet been established.

Because randomized clinical trials that demonstrate a survival benefit of genetic testing (the *sine quo non* of traditional guideline recommendations) are largely absent, the expert panel that created the Heart Failure Society guidelines developed a novel hierarchy to grade the types of evidence that support the various processes involved in genetic testing.[4] They defined Level A recommendations as based on a specific genetic test or clinical test that has a high correlation with the specific cardiomyopathic phenotype in reasonably large studies from multiple centers; Level B—a specific genetic test or clinical test that has a high correlation with the cardiomyopathic disease of interest in small or single center studies; and Level C—a specific genetic test or clinical test that correlates with the cardiomyopathic disease of interest in case reports. The second criterion that is a staple of practice guidelines—clinical utility strength of evidence—has also been modified by the Heart Failure Society committee: Level A—represents a single randomized trial; Level B—utilizes data from cohort and case control studies, *post hoc* analysis, subgroup analysis, meta-analysis, prospective observational studies or registries; and Level C—rests on expert opinion based on observational studies, epidemiologic findings, or safety reporting from large-scale use in practice.

The Heart Failure Society guidelines recommend with a high level of evidence (A or B) that: (1) All patients with cardiomyopathy should have a careful family history and creation of a pedigree that includes at least three generations in order to identify individuals who may be at risk of developing heart failure; (2) Asymptomatic first-degree relatives should undergo clinical screening for cardiomyopathy (echocardiography); (3) Clinical screening should be performed at intervals in asymptomatic at-risk relatives who are known to carry the disease-causing mutation; (4) Clinical screening for cardiomyopathy should be performed on asymptomatic at-risk first-degree relatives who have not undergone genetic testing or who have not been identified as having a disease-causing mutation; (5) Affected individuals as well as unaffected and affected family members should undergo genetic counseling before actual testing is performed; (6) Genetic testing should begin with the family member who is most clearly affected; (7) Definitive genetic testing should be carried out in a fully accredited molecular genetic testing laboratory that has met Clinical Laboratory Improvement Amendment (CLIA) standards although initial studies may be carried out as part of a research protocol; and, (8) Patients and family members should understand the clear distinctions between testing for research purposes and clinical purposes.

The Heart Failure Society guidelines also recommend that: practitioners caring for patients and families with genetic cardiomyopathy "are encouraged" to "consider" referral to centers with expertise in genetic cardiomyopathy who can facilitate both expert genetic counseling as well as participation in research studies. I would argue that the guidelines should state definitively that all patients should be referred to a center where they and their families can receive formal genetic counseling and have their genotype reviewed by cardiac geneticists who have a thorough understanding of the strengths and pitfalls of genetic testing as well as an up-to-date knowledge of new technology and the identification of new disease-causing genetic variants. Only through experienced cardiac geneticists will patients and family members be provided with a well thought out plan for all family members, both affected and unaffected, that includes recommendations regarding the frequency and stringency of screening for signs of disease, interventions to educate family members regarding risks and symptoms, and the threshold for instituting preventive measures such as an implantable cardiac defibrillator in family members at risk for sudden cardiac death or the potential benefits of the early inception of medical therapy in at risk but still asymptomatic individuals. Interestingly, the only specific genetic test that is recommended by the guidelines for patients with idiopathic DCM is for the LMNA gene because of its high frequency in those with familial (7%) or apparent sporadic disease (3%). However, this information is now out of date as a number of additional genetic tests are now recommended for patients with DCM.

Finally, it is important that payers recognize the importance of genetic testing in patients with heritable diseases. The charge for the commercial "dilated cardiomyopathy genetics panel" was $5,460 if an insurance company was billed (the insurance company declined in this case), $2,500 if the individual patient was billed, and $3,375 if an academic institution was billed. Targeted costs for genotyping the members of the proband's family were substantially less. The charges for the original panel appeared to be quite high in view of the fact that commercial research laboratories now charge less than $1,000 for whole genome sequencing and these high charges may make genetic testing using a commercial panel problematic for some families. Therefore, it is important to point out to payers that the decrease in overall health care costs that can accrue from early surveillance and early treatment in family members that carry a genotype that segregates with an inherited disease far outweighs the costs of even commercial tests. In addition, we found that university-associated CLIA certified laboratories had significantly lower costs than did commercial companies.

In summary, genetic testing for patients with DCM is evolving rapidly and is now commercially available. Genetics testing for even relatively rare diseases is becoming increasingly available and most geneticists would suggest that genetic testing should be carried out even when there is between 5% and 10% sensitivity. For DCM, the sensitivity is at least 25% and may be higher and therefore appears useful and indicated, particularly for familial diseases where cascade testing of at-risk relatives can be undertaken to help assess their genetic risk and guide intensity of surveillance echocardiography. For now, however, this remains an area of medicine best left to centers of excellence that house multidisciplinary teams of molecular geneticists, genetic

counselors, heart failure specialists, and clinical geneticists with a specialized interest in heart muscle diseases. Together, the team can provide accurate interpretation of genetic data, patient education and counseling, short- and long-term surveillance, and disease-specific clinical recommendations: a level of patient support that is not available in a commercial "genetic panel." Our recent experience also points to the need to better train our students, residents, and fellows in the didactics of the rapidly evolving field of genomics/genetics so that they will be able to translate the discovery of new disease-causing genes in the future to better care for patients at risk for both common and rare familial and sporadic genetic diseases. CTS

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