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EDITORIAL COMMENT

Transcriptomics and Beyond in Dilated Cardiomyopathy*

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Over the last 2 decades our understanding of the genetic basis of dilated cardiomyopathy (DCM) has evolved from a strict focus on rare, single gene variants with Mendelian inheritance to a more nuanced view of DCM as a complex disease caused by interaction of disease-relevant genetic, environmental, and modifying factors. This evolution has been driven in large part by continuing advancements in genome-wide testing technologies and a concomitant shift toward omics-driven clinical care and research.

Because a definitive genetic cause can only be identified in less than one-half of all cases of idiopathic DCM, there remains considerable potential for novel discovery. Integration of -omics data alongside genetic testing results promises to address many of our existing knowledge gaps and to facilitate ongoing precision medicine efforts in DCM. RNA-sequencing, in particular, has emerged as an invaluable tool for detecting expression imbalances caused by genetic affects in noncoding regulatory and enhancer regions, which are typically excluded from exome-sequencing efforts.

In this issue of *JACC: Basic to Translational Science*, Verdonschot et al[1] present RNA-sequencing data from cardiac biopsies derived from 82 patients with DCM enrolled in the Maastricht Cardiomyopathy Registry. Uniform manifold approximation and projection clustering methods were used to cluster transcripts from 708 unique genes into 4 distinct transcriptomic clusters (clusters 1-4) based on differential gene expression. Expression clusters were then tested for gene ontology and pathway enrichment and cross-referenced to clinical and gene-sequencing data to factor in cluster-specific enrichment of disease-relevant genetic variation. These data are presented alongside publicly available RNA-sequencing and clinical data from an additional 89 White patients with late-stage DCM from the Myocardial Applied Genomics Network (MAGNet) Consortium. MAGNet Consortium data were used for validation of clustering methods and for comparison to the Maastricht cohort.

A genetic variant was detected in 1 of 47 DCM-associated genes in one-half of all tested patients from the Maastricht cohort (41 of 82, 50%). Pathogenic variants were most frequently detected in *TTN* (31%) and *LMNA* (~8.5%), reflecting previous prevalence estimates of 13%-20% (*TTN*) and 4%-6% (*LMNA*) in other DCM cohorts.[2] Although no genotype completely mapped to a specific cluster, cluster 1—defined by up-regulation of glycolytic and down-regulation of β-oxidation pathway genes—was notably enriched for truncating *TTN* variants. Likewise, patients with no identifiable genetic cause were enriched in cluster 4, which was defined by up-regulation of genes involved in extracellular matrix remodeling and cardiomyocyte function and morphology. Whereas the study design is limited in its ability to identify the root cause of any of the reported gene expression changes, observed metabolic affects may ultimately reflect adaptation of the

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cardiac muscle to other causative factors, such as the pathogenic truncating *TTN* variants enriched in cluster 1. Identifying which gene expression changes represent primary causes of DCM and which changes reflect secondary affects from other causative factors will be an important avenue for future research.

Despite such limitations, the breadth of the reported data speaks to the pathophysiological complexity of DCM and the importance of including changes in the cardiac transcriptome alongside traditional assessments of genotype and clinical phenotype. Differences in gene expression between the Maastricht and MAGNet DCM cohorts also highlight the need for high fidelity clinical staging of patients included in transcriptome analysis. RNA-sequencing data capture samples in a particular cellular state and the resulting differential gene expression is reflective of a specific pathophysiological moment in disease progression. As such, careful phenotyping of included patient cohorts is vital for accurate and robust clustering analyses. Although there was no significant difference observed in combined clinical endpoint across the 4 expression clusters in the Maastricht cohort (defined as cardiovascular death, heart transplantation or left ventricular assist device implantation, heart failure requiring non-elective hospitalization, or life-threatening arrhythmia), a trend toward increased clinical severity was observed for clusters 1 and 4 relative to clusters 2 and 3. These trends may partially explain the difference in metabolic enrichments reported for clusters 1 and 2, with differences in metabolic processes between the 2 clusters representing different stages in remodeling of the dilated heart. How individual genotypes influence disease progression alongside other primary or secondary gene expression changes will be a guiding question in development of future precision medicine-based treatment plans.

Because timing is likely to play a significant role in reconciling gene expression profiles with observed clinical outcomes, meta-analyses using biological samples collected from patients at similar stages of disease progression may be more successful than individual studies at identifying disease-relevant genes and pathways. Meta-analyses are also likely to clarify discrepancies in differential gene expression across smaller data sets and to provide additional statistical power for clustering analyses. For example, a recent meta-analysis completed using publicly available data from 3 RNA-sequencing studies identified 39 genes that were not reported as differentially expressed in any of the individual studies.[3] These identifications were attributed to the increase in statistical power afforded by the larger combined sample size of the meta-analysis. Whereas some overlap in differentially expressed genes was observed across the 3 studies, a greater proportion of genes were found to be unique to each individual analysis. Whether these between-study differences in gene expression are related to intrinsic differences in cohort genotypes, disease progression, or other unidentified factors remains an unanswered question.

Given the well-known phenotypic and genetic heterogeneity of DCM, some discrepant findings across studies should not be surprising. Researchers and clinicians have long struggled to explain clinical variability in DCM, both within and between families. It is likely that some inter- and intrafamilial phenotypic variability will be explained as we start to unravel how individual genetic and nongenetic influences interact to affect progression of disease. Combined use of transcriptomic, epigenomic, and proteomic data, as in a recent study by Liu et al,[4] promises to provide more comprehensive insight into global gene expression changes in DCM, while simultaneously interrogating the resulting affects on downstream signaling pathways or protein interactions. Allele-specific expression analyses should also help to assess the role of cis-regulatory events such as differential messenger RNA splicing, nonsense-mediated decay, X-inactivation, locus imprinting, or RNA interference in DCM.[5]

Future research must continue to account for the possibility of multiple DCM-causative genetic insults in individual families, including the potential for noncoding variants to influence the pathophysiological and metabolic progression of DCM. That common coding variants, occurring in more than 1% of the general population, may also be disease-relevant, remains an underexamined, but equally important consideration. Interpretation of results across individual studies will need to continue to account for differences in sample sizes and sample collection methods, including timing of sample collection, for any gene expression analyses. Additionally, the extent and potential affects of previously initiated therapeutic interventions will need to be examined because drug treatments are likely to have both intended and unintended effects on gene expression and cardiac metabolomics. Animal models for DCM should continue to be leveraged, and RNA-sequencing of these models are likely to provide additional insights into gene expression differences observed in human cohorts. Continued collection of large and carefully phenotyped DCM data sets will be paramount and should lead to the identification of novel DCM genes and pathways, as well as disease-relevant circulating biomarkers that may prove viable as screening or

therapeutic targets. Although these investigations remain enormously challenging, the potential for making clinically relevant discoveries and developing meaningful therapeutic interventions is significant.

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