

## Wrestling the Giant New Approaches for Assessing Titin Variant Pathogenicity

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In the recent past, the clinical availability of genetic information has increased exponentially and with it the opportunity to use this information to aid in diagnosis and treatment of cardiovascular disease. One of the greatest challenges we face with this wealth of potentially useful diagnostic information is determining the pathogenicity of the myriad genetic variants identified. Dilated cardiomyopathy (DCM) is a particularly salient example of this challenge. Compared with other inherited cardiovascular diseases, such as long QT syndrome, with a diagnostic yield of genetic testing of  $\approx 80\%$ , only 15% to 40% of DCM genetic tests yield a pathogenic variant.<sup>1</sup> This reduced diagnostic capability is due in part to our lack of precision in defining phenotypes, as well as the sheer size and number of genes thought to be involved, rendering the examination of a large number of variants in an individual cumbersome.

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### Articles, see p 419 and 426

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In the current issue, 2 groups have addressed an exemplar of these challenges: *Titin* (*TTN*).<sup>2,3</sup> *TTN* encodes the largest human protein, consisting of 364 exons that are spliced into transcripts encoding  $\approx 5000$  to 34 000 amino acids. Largely because of its size and the ubiquity of rare variation, many individuals harbor variation in the *TTN* gene (1%–3% differ from the human reference sequence).<sup>4,5</sup> However, significant data support the presence of pathogenic variation in *TTN*, leading to dilated cardiomyopathies, specifically truncating variants in the A-band. Although consistent cosegregation evidence is lacking,<sup>6</sup> such variants clearly have lower prevalence in the healthy population,<sup>5,7</sup> highlighting the importance of critical evaluation of clinically observed variation in *TTN*. In this issue, Hastings et al<sup>3</sup> and Deo<sup>2</sup> each address this challenge with the use of innovative perspective, proposing novel solutions to the problem of variant evaluation and at the same time broadening our perspective on the potential roles of *TTN* in cardiomyopathies.

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Taking a population-level computational approach to this problem, Deo<sup>2</sup> uses 3 key variables to create a predictive model of the pathogenicity of *TTN* truncating variants. To power these analyses, he combines *TTN* truncating variants from 4 previously published cohorts of DCM patients, assembling a case number of over 1700 individuals with DCM and *TTN* truncating variants. Using large exome data sets, including from the Exome Variant Server, the Exome Aggregation Consortium, and the 1000 genomes project,<sup>8-10</sup> he evaluates differences in *TTN* truncating variants between cases and controls. His analysis finds significant differences between variants in these groups based on location relative to the distal C-terminus, likelihood of exon inclusion in cardiac-relevant splice isoforms, and disruption of the expression of Cronos, a shorter isoform embedded in the *TTN* gene. Cronos was recently described and named by Deo and his group. Its promoter lies in the middle of the *TTN* gene, and its expression may explain the increased density of Titin molecules at the M-line versus the Z-line of the sarcomere.<sup>11</sup>

Deo goes on to build a model assigning pathogenic predictive power to each of these characteristics. He then tests the model by classifying variants into risk categories based on these 3 risk factors. He finds that the highest risk category mapped to 100% of variants from kindreds that segregated with disease, as well as 31 of 32 previously described end-stage DCM cases with *TTN* truncation variants. Importantly, however, 28% of control variants still mapped to the high risk category, indicating the importance of the effect of external variables, such as environment and exposure time (age), that could not be quantified. Further, although the drop off in distal C-terminal variants is somewhat inconsistent with prior observations that A-band-contained truncation variants can be pathogenic, other data suggests that distal C-terminal truncation may not prevent sarcomeric integration.<sup>12</sup> This suggests that using a less molecularly predetermined system for detecting risk factors may broaden not only our understanding of protein function, but also allow us to discover important risk factors for pathogenicity. Such a computational approach to variant classification is exciting because it offers a succinct and accessible estimation of variant pathogenicity mapped not only to protein structure and in silico predictions about function but also to patient-level data about disease prevalence.

In contrast, Hastings et al<sup>3</sup> take a more traditional approach to the evaluation of a *TTN* missense variant found in association with a cardiomyopathy with features of left ventricular noncompaction (LVNC). In genetically complex traits with variable expressivity, genetic segregation analysis of families with multiple affected members is a valuable approach. Hastings et al<sup>3</sup> build on this approach by elegantly combining whole genome sequencing with linkage and innovative

biophysical protein product studies to evaluate a *TTN* variant in a 3-generation kindred with 8 affected members.

Single-nucleotide polymorphism array genotyping with 300 000 genetic markers was performed for all 13 family members of which 5 were unaffected, and whole genome sequencing was performed on 2 affected individuals who were first cousins. Genomic regions identical by descent were identified through linkage analysis, and potentially pathogenic variants were selected based on an autosomal dominant inheritance model. After filtering variants that did not segregate with disease, or were unlikely to be functionally associated with cardiac disease, a missense variant in *TTN* was identified. The variant was further investigated by several biophysical experiments, suggesting partially disrupted secondary structure and increased degradation of the protein. In addition, the authors' experiments showed that the altered folding impaired the binding of Titin to Telethonin in mammalian cells. Telethonin is part of the stretch receptor in the sarcomere, and variants in this protein have previously been described that cause DCM by reducing its binding affinity for Titin.<sup>13</sup> Thus, although the study does not provide a precise mechanism of underlying disease pathways, this biophysical evaluation of variant protein structure indicates its potential pathogenicity. It also supports data suggesting that disruption in Titin's association with Telethonin alters myocardial contractility.<sup>13</sup> This represents an innovative *in vitro* and *in silico* method for evaluation of variant pathogenicity.

Historically, LVNC is a condition defined by prominent left ventricular trabeculae, a thin compacted layer and deep intertrabecular recesses of the left ventricle.<sup>14</sup> This results from failure of the spongiform myocardium to compact during development.<sup>15</sup> However, the radiographic diagnosis of LVNC is based solely on the ratio of trabecular to compacted myocardium on echocardiography or magnetic resonance imaging.<sup>16</sup> This radiographic phenotype has been associated with many different cardiomyopathies and some noninherited exposures (eg, hemodynamic changes in sickle cell anemia, pregnancy, and chronic renal failure), suggesting that its underlying cause may be more variable than its original definition.<sup>17</sup> In addition, advances in imaging resolution may have contributed to increased frequency of diagnosis of LVNC both alone and in combination with other conditions.

In several kindreds, LVNC-associated cardiomyopathy has been observed to have an autosomal dominant inheritance pattern with variable expressivity. In these families, it has been associated with variants in a variety of functionally related genes such as those encoding sarcomeric, cytoskeletal, nuclear-envelope proteins, z-line components, and developmental signaling pathways.<sup>18</sup> These variable functional categories suggest convergence of multiple different factors (genetic and perhaps also nongenetic) on this phenotype. Further, traditionally, truncating variants in *TTN* have segregated with DCM.<sup>7</sup> That a missense variant could confer such well-segregated and well-characterized LVNC cardiomyopathy is a surprising and novel finding.

Taken together, Deo<sup>2</sup> and Hastings et al<sup>3</sup> describe innovative techniques for examining the causality of candidate variants in the setting of one of the most complicated genes in the human genome. This work builds on prior reports in other cardiomyopathy-related genes, including the cardiac myosin heavy chain,

which combine population genomics data with sequence-to-function translation of protein alterations to predict variant pathogenicity.<sup>19</sup> Such methods look toward an era of variant evaluation informed by more sophisticated computational models of complex genes. The pursuit of these computational and biophysical assays of variant pathogenicity will require the continued expansion of large disease-specific cohorts. Further, the phenotypic categorization of complex diseases and traits, such as DCM and LVNC, requires increased precision. A sophisticated gene-level understanding of pathobiologic mechanisms will provide a scaffold on which to build a new disease ontology. Such a diagnostic framework may, in future, supplant the blunt instrument of imaging-based phenotyping to more precisely target therapy to specific disease mechanisms.

Still, much remains to be done before we can bring this elegant work into the realm of the everyday practice of precision cardiovascular medicine.<sup>20</sup> The hypothesis that missense variants in *TTN* can cause significant disease should be supported by model-system level evidence. Additionally, identification of other kindreds with similar *TTN* missense genotype–phenotype segregation will be necessary to convince many investigators of this mechanism of disease. On a population level, the investigation of *TTN* missense variants in cohorts with detailed phenotype and genotype data may also shed light on their pathogenicity. Models such as Deo's could be immediately applied (the data and code are publicly available), but the large percentage of unaffected individuals carrying high-risk variants remains to be explained. Incorporation of demographic features and environmental factors, such as pregnancy and other risk factors for DCM, may shed light on the variable expressivity and penetrance noted here. Finally, as coverage of high throughput sequencing improves and the size, detail, and number of relevant data sets expand, our power to predict causality will increase. As our understanding of complex inheritance grows, methods and models like those presented by Hastings et al<sup>3</sup> and Deo<sup>2</sup> open the door to the inclusion of environmental and polygenic modulators of expressivity in the precise diagnosis and treatment of disease.

## Disclosures

None.

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