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Wrestling the Giant: Experience with *TTN* Testing for Cardiomyopathies

Authors: Jill S. Dolinsky, Christian Antolik, Tami Johnston, Lily Hoang, Brittany Dougall, Stephanie Gutierrez, Catherine Potente, Brigette Tippin Davis, Barbara A. Boggs

Intro: The *TTN* gene is expressed in striated muscle and encodes the protein titin, which spans half the distance of the sarcomere (from Z-disc to M-band) and is the largest known protein. Mutations in *TTN* constitute a substantial proportion of genetic risk for DCM, with mutation rates up to 25% in probands with familial dilated cardiomyopathy (DCM) and 18% in sporadic DCM cases. However, there is also a high burden of missense variants of unknown significance (VUS) detected in *TTN*. This makes the decision to choose a multigene panel (MGP) that includes *TTN* challenging when patients do not have a clear diagnosis of DCM. We sought to determine the characteristics and phenotypic spectrum associated with *TTN* pathogenic alterations, and to explore the decision to choose a panel including *TTN* for probands with cardiomyopathies.

Methods: A retrospective analysis was performed using clinical history and next generation sequencing (NGS) data from patients who underwent MGP testing that included *TTN* (n=234), or NGS with a targeted chromosomal microarray to detect gross deletions/duplications (del/dups) (n=266). Panels ranged in size from 31-85 genes. Clinical history was extracted from test requisitions, clinic notes and pedigrees. Phenotypic comparisons were performed using a Fisher's exact test.

Results: Among 500 individuals tested for *TTN* by MGP testing, the positive rate was 5.8% overall, with all pathogenic alterations classified as variant, likely pathogenic (VLP). When stratified by phenotype, VLPs were detected in 11.4% of probands with a reported history of DCM (n=19/166), 9.1% of probands with left ventricular noncompaction (LVNC) (n=2/22), and in 0.7% of those with a reported history of HCM only (n=1/142). Among the remaining probands with *TTN* VLPs, one had a personal and family history of sudden cardiac arrest only while the others had a family history of DCM or complex histories including multiple forms of cardiomyopathy including DCM. Significantly more probands with DCM had a VLP in *TTN* than those with HCM ($p<0.001$). Pathogenic alterations were detected in 57 probands with DCM who underwent MGP testing, 33.3% of which were *TTN* VLPs. The vast majority of VLPs were detected in the A-band of *TTN*, including 23 nonsense or frameshift alterations, 1 splice site alteration and 3 gross deletions. Two VLPs were identified in the I-band (1 splice site, 1 nonsense) , and one nonsense alteration was detected in the M-band. Among cases in which testing included gross del/dup analysis, 10% of all *TTN* pathogenic alterations were gross deletions within the M-band. Among HCM-only probands who underwent MGP testing when the inclusion of *TTN* was optional, a panel including *TTN* was selected in 10.8% (22/203) of cases. Variants of unknown significance (VUS) were seen in 48.4% of probands, with the majority (61.6%) having only 1 *TTN* VUS, 28.5% having 2 *TTN* VUS, and 9.9% having 3-5 *TTN* VUS, with missense alterations accounting for 95.1% of VUS in *TTN.*

Conclusions: Our data supports *TTN* as a major contributor to genetic predisposition for DCM, accounting for 33.3% of pathogenic alterations identified in DCM probands. *TTN* does not appear to be a significant contributor to increased risk for HCM. However, when given a choice, clinicians selected a

panel including *TTN* in nearly 10% of HCM-only cases. While our data demonstrates a 9.1% positive rate among patients with LVNC, there could be bias in clinician interpretation of the patient's phenotype and thus should be interpreted with caution. VLPs occur primarily in the A-band of *TTN*, which is included in the both the major (shorter) isoform and minor (longer) isoform expressed in cardiac tissues and are predominantly loss of function alterations. Despite the high diagnostic yield for *TTN* among DCM probands, the missense VUS rate is a substantial burden, with nearly half of all probands with at least 1 VUS, including nearly 10% with 3 or more VUS in *TTN*. Therefore, the option to exclude *TTN* from MGP testing for probands with no clinical indication of DCM is reasonable.