A retrospective analysis of multigene panel testing for Marfan syndrome, aneurysm, and related disorders: Diagnostic yield and cardiac phenotypic spectrum

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Marfan syndrome (MFS) is caused by mutations in *FBN1* and characterized by cardiovascular, skeletal, and ocular findings. Diagnosis can be challenging due to phenotypic variability and overlap with related disorders. Thoracic aortic aneurysm and dissection (TAAD), in particular, is a life-threatening major component of MFS; however, it also occurs in other syndromes, and genetic causes of isolated TAAD have also been discovered. Identifying a genetic cause of TAAD helps clarify risk and medical management, leading to improved prognosis. Here, we sought to determine the overall diagnostic yield and assess the cardiac phenotypic spectrum of individuals with a mutation identified on a panel of 10 genes associated with predisposition to TAAD.

DNA samples from 722 individuals underwent multigene panel testing via next generation sequencing (NGS) at our clinical diagnostic laboratory from October 2011 through September 2014 for the following genes: *ACTA2, CBS, COL3A1, FBN1, FBN2, MYH11, SLC2A10, SMAD3, TGFBR1,* and *TGFBR2.* In 593 (82.1%) cases, all genes were analyzed concurrently, while the remaining 17.9% of cases took a stepwise approach, beginning with *FBN1* with reflex to the remaining genes. Clinical information submitted by ordering healthcare providers was reviewed.

A total of 98 pathogenic mutations or likely pathogenic variants (mutations) were identified in this cohort in all 8 genes with autosomal dominant (AD) inheritance, including one digenic case with pathogenic mutations in both *FBN1* and *FBN2*. While biallelic mutations in *CBS* and *SLC2A10* (characterized by autosomal recessive inheritance) were not identified, 3.05% (n=22) of individuals were carriers for a mutation in one of these genes. Approximately one third of the mutations detected were novel at the time of testing, supporting a full sequencing approach to testing.

A positive finding was identified in 10.39 % (n=75) of individuals tested. Not surprisingly, the majority of positive findings (65.8%) were detected in *FBN1*, as the prevalence of MFS is the highest, approximately 1:5,000, whereas the prevalence estimates of the other gene conditions are lower or unknown. Mutations in the remaining 7 genes with AD inheritance accounted for an additional 34.2% of positive findings (n=26), thus contributing significantly to the overall diagnostic yield for this panel ($p<0.001$). Mutations in *MYH11*, a gene more recently associated with TAAD, was responsible for 11.5% (n=3) of non-*FBN1* positive findings. Variant(s) of unknown significance were identified in 24.9% (n=180) and no reportable findings (negative) were identified in 64.4% (n=465) of cases.

Reported cardiac phenotypes were compared between *FBN1* positive cases, other positive cases, and negative cases. Clinical information was provided for significantly more of the positive (91%, n=68) than negative (79%, n=367) cases ($p<0.017$). Of these cases, aneurysm or dissection of the aorta or other vessel were reported significantly more in the positive (85.2%; n=58) compared to negative (69.5%; n=255) cases ($p=0.008$). Further, non-aortic involvement was statistically more common in the negative compared to positive cases ($p=0.036$). Cases in which a family history of TAAD was reported were more likely to be in the positive rather than the negative cohort. No significant differences were identified with TAAD involvement in *FBN1* positives compared to other positive cases. Other cardiovascular features reported include mitral valve prolapse and bicuspid aortic valve, which lead to an increased risk for TAAD.

Our data demonstrate that a panel based approach significantly improves diagnostic yield compared to testing for *FBN1* alone. Inclusion of gross deletion/duplication analysis and additional

genes implicated in the expanding TAAD related phenotype are also likely to maximize detection rates and may ultimately improve patient management and outcome.