Landscape of Variants of Unknown Significance in *FBN1* Identified in Patients with TAAD and Marfan Syndrome

Lan Qin, Robert Huether, Sara Calicchia, Tami Johnston, Jade Tinker, Arthur Dean, Tina Pesaran, Hsiao-Mei Lu, Brigette Tippin Davis, Jill S. Dolinsky

Ambry Genetics Corporation, Aliso Viejo, CA 92656

Background: Thoracic aortic aneurysm and dissection (TAAD) is characterized by dilatation and/or dissection of thoracic aorta. Without surgical repair, enlargement of the aorta typically progresses and leads to sudden rupture of the aorta wall, a life threatening condition. Genetic etiologies for TAAD are highly heterogeneous, some of which are associated with additional syndromic features, such as *FBN1* mutations associated with Marfan syndrome, while other etiologies cause isolated TAAD. Therefore, the accurate molecular diagnosis and variant classification is of great importance in the management of patients' cardiovascular complications and potential comorbidities.

Methods: To evaluate the burden of *FBN1* VUS in TAAD-related tests a retrospective review was performed for 1062 patients who underwent a TAAD or TAAD-related next generation sequencing (NGS) panel at a genetic testing laboratory. The review included ethnic variation, significance of population statistics, conservation, and *in silico* prediction (PolyPhen/SIFT) in evaluating pathogenicity of missense variants, and contribution of gross deletion/duplication (del/dup) analysis to the overall VUS rates. All patients underwent *FBN1* sequencing, 227 of which also underwent *FBN1* del/dup analysis. 982 further underwent NGS with or without del/dup analysis for additional 2-19 TAAD-associated genes. In line with American College of Medical Genetics and Genomics standards and guidelines, detected alterations were classified to five categories by the laboratory as pathogenic, likely pathogenic (VLP), variant of unknown significance (VUS), likely benign (VLB) and benign. Ethnic backgrounds were self-reported and classified as Caucasian, African American, Ashkenazi Jewish, Asian, Hispanic or Middle Eastern.

Results: The overall diagnosis rate was 10.08% (107/1062) for all TAAD-related panels, with the majority of pathogenic mutations or VLP identified in *FBN1* (73/107, 68.2%). In addition, VUS were identified in almost 1 in 4 cases (263/1062, 24.8%), with *FBN1* as the second most frequent gene in which VUS were identified in this cohort (65), next to *FBN2* (71). Cases were predominantly of non-Jewish Caucasian descent (638/798, 79.9%), however, VUS rates were highest in individuals of Ashkenazi Jewish descent (8/17, 47.1%) followed by Middle Eastern descent and the lowest in non-Jewish Caucasians (145/638, 22.7%). In addition to single nucleotide variants or small insertions/deletions, del/dups in *FBN1* were detected, including 6 mutations and 2 VUS, thus contributing 3-fold to diagnostic yield compared to VUS burden and accounting for 17% (6/36) of *FBN1* positive results when del/dup analysis was included. To obtain insight into the properties of *FBN1* VUS and facilitate variant classification and interpretation, we analyzed VUS compared to missense pathogenic mutations or VLP in the *FBN1* gene, including variants identified by our standalone *FBN1* testing. VUS in *FBN1* do not display a distinct distribution in the protein sequence compared with pathogenic variants and VLP. However, they show different patterns in terms of Grantham, Polyphen-2 and SIFT scores. The scores of VUS from these *in silico* tools derived from computational prediction algorithms are similar to those of pathogenic rather than the benign alterations, although the statistical significance varies. Conversely, allele frequencies of the VUS are significantly higher than pathogenic/VLP variants (p value <0.01) according to the ExAC database.

Conclusion: Multigene panel testing is efficient in identifying disease-causing mutations in genetically heterogeneous life-threatening cardiovascular condition TAAD, the majority of which are identified in *FBN1* in this clinical cohort. Interpretation of *FBN1* VUS is a challenging common reality and the frequencies of *FBN1* VUS vary depending on ethnicity. Continually emerging population frequency data, specialized bioinformatics tools, and functional assays are important in interpreting the pathogenicity of *FBN1* VUS, and need to be considered in the context of each other and the patients' clinical phenotype.