Family Matters: How Segregation Studies Reclassify Variants in Thoracic Aortic Aneurysm and Dissection Multigene Panels

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Multigene panels for thoracic aortic aneurysm and dissection (TAAD) have been available for clinical diagnostic testing at Ambry Genetics since 2011, with panels ranging in size from 3-20 genes. The current panel involves analysis of 20 genes (*ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MED12, MYH11, MYLK, NOTCH1, PLOD1, PRKG1, SKI, SLC2A10, SMAD3, TGFB2, TGFBR1*, and *TGFBR2*). While multigene testing increases diagnostic yield, detection of inconclusive variants of unknown significance (VUS) is a potential outcome of any gene sequencing analysis, with larger panels often increasing the VUS rate. At least 1 in 4 patients had a VUS identified on our 20 gene panel. Inconclusive results can be challenging for clinicians to explain and for patients to comprehend. In an effort to reclassify VUS into clinically meaningful results (i.e. benign or pathogenic), Ambry engages in ongoing segregation analysis through the Family Studies Program. Through retrospective review of TAAD cases referred to the Family Studies Program from October 2011 through June 2015, we analyzed the uptake of VUS segregation studies and its effectiveness in the reclassification of VUS for TAAD-related genes. Of the 328 cases reported with one or more VUS, 101 cases (30.8%) were referred to our Family Studies Program by the ordering clinician. After pedigree and clinical history review, 83 cases (82.2%) were approved for segregation analysis and 18 cases (7.8%) deemed uninformative were excluded from family study. Reasons for exclusion included: 1) inconsistency of the patient's clinical and/or family history with the gene of interest, 2) uncertain phenotype of potentially informative relative(s), 3) recent reclassification of the VUS, or 4) lack of informative relatives for testing. Of the 83 cases approved for segregation analysis, 73.4% successfully submitted specimens, with 19 cases submitting specimens on 1 relative and 46 cases submitting specimens on 2 or more relatives. The 61 cases entering segregation analysis mostly involve alterations in *FBN1* (n=33), *FBN2* (n=23), *MYH11* (n=20) and *TGFBR2* (n=7). Over half of approved cases yielded informative data, with 17 cases (27.9%) contributing to VUS reclassification, including 6 upgrades to pathogenic mutation or likely pathogenic and 11 downgrades to likely benign or benign. Reclassifications were based on *de novo* status (n=4) or co-segregation of VUS genotype with disease phenotype in conjunction with additional lines of evidence, such as *in silico* data, functional studies and allele frequencies from published cohorts (n=13). Another 17 cases (27.9%) were informative; however, additional lines of evidence are still needed to attain reclassification status. Cases yielding uninformative segregation results (43.4%) had one or more of the following factors: 1) approved relative(s) were unavailable for testing, 2) uncertain phenotype of the family study participant, and 3) insufficient number of informative meioses to reach a conclusion. Our experience with TAAD family studies illustrates the power and clinical utility of segregation analysis, with over half of referred cases contributing to reclassification of inconclusive results, ultimately benefiting patients and their families. Thus, while cases may not be referred due to lack of available family members for testing, lack of proband interest, or the clinician deeming the case uninformative, clinicians and patients should be encouraged to pursue family studies. Given published high *de novo* rates in *FBN1* and *TRFGB2* (25 and 75%, respectively), parental testing can be particularly informative in individuals with VUS in these genes. Not surprisingly, cases with 2 or more relatives undergoing segregation analysis were more likely to result in reclassified variants. In summary, the success of segregation analysis through a laboratory family studies program for variant reclassification purposes can result in a high yield of informative data when an adequate number of informative meioses are attained, accurate genotype-phenotype correlations are established and clinicians and families actively engage in the family study process.