A Retrospective Review of Family Studies in Reclassifying Variants of Unknown Significance Detected in Cardiomyopathy Multigene Panels

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Multigene panel tests (MGPT) for cardiomyopathy (CM) have been available for clinical diagnostic testing at Ambry Genetics since 2012, with panels ranging from 2-85 genes. While MGPT increases diagnostic yield, detection of variants of unknown significance (VUS) is a potential outcome of any testing. At least 50% of patients had ≥ 1 VUS identified on our CM panels. In an effort to reclassify VUS into clinically meaningful results, Ambry utilizes segregation analysis through the Family Studies Program (FSP). A retrospective review of CM cases referred to the FSP from 2012 through 2015 analyzed the uptake of segregation studies and its effectiveness in reclassification. Of the 421 cases reported with ≥ 1 VUS, 48 cases (11.4%) were referred to our FSP by the ordering clinician. After pedigree and clinical history review, 44 cases (91.7%) were approved and 4 cases (8.3%) were excluded due to inconsistency of the patient's clinical and/or family history with the gene(s) of interest or lack of/uncertain phenotype of potentially informative relatives. Of 28 cases submitted for family study, over half yielded informative data, with 8 cases (28.6%) contributing to VUS reclassification. Reclassifications were based on *de novo* status (3 alterations in *RYR2, MYH7* and *TNNI13)* or co-segregation of VUS genotype with disease phenotype in conjunction with additional lines of evidence, such as functional studies or allele frequencies from published cohorts (5 alterations in *TTN*, 2 in *MYH7*, 1 in *TNNI3*, 1 in *RYR2*, 1 in *MYOM1,* 1 in *KCNH2* and 1 in *BAG3*). Another 11 cases (39.3%) were informative but require additional data for reclassification. Our experience with CM family studies illustrates the power and clinical utility of segregation analysis in VUS reclassification, given 28.6% of participating families obtained a reclassification. In summary, family study results in a high yield of informative data when an adequate number of informative meioses (>2-3) are attained, accurate genotype-phenotype correlations are established and clinicians/families actively engage in the process.