Optimizing Diagnostic Potential: Impact of Familial Sample Configurations on Exome Sequencing

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Introduction: Accurate variant classification relies on the comprehensive evaluation of multiple lines of evidence, presenting a particular challenge in exome sequencing (ES) due to the assessment of hundreds of potentially clinically relevant variants. Trio-based analysis involving complete sequencing of both parents ("parental trio") increases the diagnostic potential of ES. Specifically, this approach provides real-time inheritance information, leading to clarification of variants of uncertain significance (VUS) and higher diagnostic rates. However, challenges, such as adoption, use of gamete donors, death, availability to take time off work, and family dynamics, limit the collection of parental trios and restrict the available evidence for variant classification. This can be further compounded with a lack of other evidence availability for under-represented populations. Here, we investigate how different familial sample configurations (FSC) impact the clinical utility and reclassification process of ES.

Methods: We retrospectively reviewed 10,921 diagnostic ES cases reported from 2011 to 2021 at one diagnostic laboratory. Cases were categorized based on FSC: parental trio, non-parental trio (3 familial samples submitted but representing <2 parents), duo, or proband-only analysis. We documented the outcomes of the original testing and current reported results, accounting for reclassifications. We recorded the sources of evidence used for reclassification and assessed the extent of reanalysis and reclassification across FSC categories. Statistical analysis included chi-square tests.

Results: In this cohort, 71% (n=7717) of cases underwent parental trio analysis; 13% (n=1451) were proband-only, 11% (n=1158) duo, and 5% (n=595) non-parental trio. Parental trios exhibited significantly higher diagnostic rates and lower VUS rates compared to all other FSC ($p < 0.005$). Notably, negative rates were similar across FSC, suggesting appropriate variant detection and reporting is occurring, but the lack of available evidence for accurate classification of variants causes uncertainty to remain. Over time, diagnostic yield increased across all FSC due to ES reclassification (range 2.1% to 3.7%).

Evidence used for reclassification came from 37 different categories. The most frequent lines of evidence that accounted for reclassifications were: 1) emergence of literature supporting new gene-disease relationships (GDR; 63%; n=524/838), 2) post-ES familial co-segregation testing ("family studies"; 11%; n=95/838), 3) new phenotypic information provided (7%; n=55/838), 4) updated allele frequency data from population databases (i.e. gnomAD; 6% n=50/838), and 5) identification of additional unpublished patients (4%; n=30/838). While the emergence of literature supporting new GDR was the top category for all FSC, parental trios were significantly more likely to benefit from this line of evidence than proband-only cases (66% vs 47%; $p<0.0002$). In contrast, family studies were significantly more likely to impact reclassification for proband-only cases (30% vs. 6%; $p<0.00001$). Non-parental trios and duos were also significantly more likely to benefit from post-ES familial testing than parental trios (21% and 15%, respectively; $p<0.004$). There were no significant differences between FSC for the other three mentioned evidence categories.

Conclusions: Our findings emphasize the diagnostic potential of including samples from both parents in ES analysis. However, parental trios were unavailable for 30% of cases. Alternate FSC in the setting of other inequalities can be compounded, furthering the gap between receiving comprehensive variant

classification. Therefore, it is crucial to maximize the clinical utility of alternative FSC with robust reanalysis and reclassification. In our study, all FSC showed an increase in diagnostic yield following reclassification. The primary driver for reclassification was new evidence supporting GDR, highlighting the importance of ongoing literature curation and proactive reclassification notices. Follow-up familial co-segregation studies and more than 30 other evidence sources also proved impactful, underscoring the importance of holistic variant assessment.