Exome Sequencing Covers >99% of Mutations Identified on Targeted Next Generation Sequencing Panels

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Description:

BACKGROUND: With the expanded availability of next generation sequencing (NGS)-based clinical genetic tests, clinicians must weigh the superior coverage of targeted gene panels with the greater number of genes included in diagnostic exome sequencing (DES) when considering their first-tier testing approach. This decision may be particularly challenging for diseases with significant genetic and phenotypic heterogeneity. To date, few studies have examined the analytic sensitivity of DES using position-specific basepair coverage. Here, we aim to predict the analytic sensitivity of DES using mutations identified on targeted NGS panels as a reference.
METHODS: Our internal database was queried for all pathogenic and likely pathogenic variants ("mutations") detected on targeted NGS multi-gene panel testing at our clinical diagnostic laboratory. Multi-gene panels targeted a range of disorders including hereditary cancer, X-linked intellectual disability (XLID), primary ciliary dyskinesia (PCD), Marfan syndrome, thoracic aortic aneurysms and dissections, and related disorders (Marfan/TAAD), and other cardiovascular diseases such as cardiomyopathies and arrhythmias. All mutations included in this analysis were identified by NGS and confirmed by Sanger sequencing. Corresponding nucleotide positions for these mutations were interrogated in data from 100 randomly-selected clinical DES samples to quantify the sequence coverage at each position. DES samples were prepared as previously described using the SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI), and the enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 or 2500 (Illumina, San Diego, CA). Mutations were interpreted as 'detected' if exome coverage at the respective nucleotide positon was >10x. Coverage at the flanking nucleotides was averaged for insertions, and for indels and deletions, coverage was recorded as the minimum of the first and last nucleotides.
RESULTS: A total of 1563 different mutations identified on targeted NGS multi-gene panel testing were included in this analysis, representing 96 genes implicated in 5 disease categories. Mutations in cancer susceptibility genes accounted for 87.1% of mutations analyzed (n=1362), with each of the other disease categories each accounting for < 4% of mutations studied. Single nucleotide subsitutions were the most common type of mutation included in this analysis (n=691, 44.2%), followed by small deletions (n=486, 31.1%), intronic mutations (n=185, 11.8%), small duplications (n=137, 8.8%), and insertions and indels (each 2%). The lengthiest variants assessed were a 40-nucleotide deletion in BRCA1, and a 20-nucleotide duplication in BARD1.
Considering that coverage was assessed among 100 individual DES samples for each mutation (156,300 individual assessments), a total of 99.7% (n=155,772) of mutations would likely have been detected on DES. For 97.1% of mutations (n=1517), coverage at the respective nucleotide positions was >10x across all 100 DES samples. For the

remaining 46 mutations, the number of DES samples with adequate coverage ranged from 36 to 99. The mutation detection rate varied by disease, with the highest detection observed for PCD (54/55 mutations detected across all 100 DES samples) and lowest for XLID (17/23 mutations detected across all 100 DES samples); however, the lower detection for XLID may be a result of a small sample size. When assessed on the individual level, 98.6% of mutations in XLID genes would have been detected by exome sequencing since all mutations not detected across all 100 DES samples were still detected in the majority of DES samples.
CONCLUSIONS: Despite current estimates that 90-95% exome-wide coverage is achieved with exome sequencing, results from this position-specific comparative analysis limited to disease-causing mutations demonstrate that exome sequencing is expected to perform well (>99.5%) for a range of inherited diseases. This data suggest the use of exome sequencing may achieve similar results/diagnostic yield when compared to panel based tests and may be an appropriate option to consider when indicated.

Learning Objectives:

Determine the analytic sensitivity of exome sequencing using mutations identified on targeted next generation sequencing panels as a reference

Review previous literature comparing targeted panels to exome sequencing

Review benefits and limitations of a targeted panel vs. exome sequencing first-tier testing approach

Define the implications of these results on clinical genetic testing strategies

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