Clinical Validation of an NGS Assay for Heritable Mutations

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Abstract

Next Generation Sequencing (NGS) technology can help expand clinical use of genetic testing by allowing large numbers of genes to be rapidly tested in patients at low cost. However, broad clinical adoption of NGS requires demonstration of clinical-grade accuracy, in part to demonstrate sensitivity for “difficult” classes of variants, and in part to address clinical concerns about false positive findings from increasingly large gene panels. To address the latter concern, most clinical NGS labs, including ours, confirm certain variants in patients using one or more orthogonal technologies, adding to cost and slowing turn-around times.

We have embarked on a series of studies to measure both the analytic and clinical performance of NGS in our diagnostic laboratory. In one study we apply clinical NGS to individuals which have undergone traditional genetic testing. We also analyze a reference set of well-characterized clinical samples from public sources. We deliberately enriched these cohorts for individuals with known pathogenic variants, in order to help measure diagnostic sensitivity. Among those we further enriched for certain classes of DNA variation known to be challenging for NGS technology (such as indels and CNVs). Analyses focused on identifying high-confidence and invariant positions, and on identifying “difficult” classes for NGS. Sensitivity is assessed by two methods — sequencing depth and base quality — and by clinical validation in the laboratory.

Methods

Our panel covers all coding exons, splice sites, and any other regions with known pathogenic variants in 212 genes. These are targeted using hybridization based enrichment with multiple pull-down chemistries to improve coverage of “difficult” targets (for ex. GC-rich regions). Sequencing is performed on the Illumina platform using 150bp paired-ends to an average depth over 400x. We use a custom-built mapping, variant calling and data QC pipeline. Preliminary interpretations are automatically generated for review, and medical specialists then classify variants according to ACMG guidelines. Confirmation on an orthogonal platform and fill-in is used, when needed, to complete a diagnosis.

Conclusions

Our preliminary results from these studies suggest that careful application of targeted NGS can produce data highly concordant with traditional diagnostic methods.

A. We see very high NGS sensitivity for the genes and variant classes represented in this study.

B. NGS false positive rates appear to be very low. A systematic examination of this in the future may allow us to remove, in some circumstances, the need for orthogonal confirmation in NGS-based genetic diagnostics.

C. By following ACMG guidelines and leveraging widely available tools and information, clinical interpretations consistent with established laboratories can be made. As expected, VUS rates are slightly higher but we expect these to come down rapidly as public clinical data accumulate. Note that family members were not available for these research cohorts, although they would be tested in our diagnostic process when possible to resolve some of the VUS.

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