High Accuracy and Expanded Yield from Next-Generation Testing of Multiple Cancer Risk Genes

Stephen Lincoln1, Allison Kurian2, Kristen Shannon3, Michael Anderson1, Yuya Kobayashi1, Geoffrey Nilsen1, Kevin Jacobs1, Leif Ellisen3, James Ford2, and Federico Monzon1

1Invitae, San Francisco, CA 2Stanford University Medical Center, Palo Alto, CA 3Massachusetts General Hospital, Boston, MA

Background

Gene panels assayed using next-generation sequencing (NGS) technologies are moving from research labs into clinical use, with the potential to provide improved diagnostic yield quickly and at low cost. It is important to understand the performance and clinical implications of these emerging tests by comparison with traditional methods and sequential testing.

Methods

Germ-line DNA samples were collected from multiple sources: (a) Research specimens from consented patients referred for HBOC (Hereditary Breast and Ovarian Cancer) testing at two major cancer centers. These patients were independently tested for BRCA1 and/or BRCA2 using traditional methods as part of their care. (b) Reference samples from public biobanks, chosen both to have broad coverage of genes previously assayed and also enriched for pathogenic variants known to be technically challenging for NGS; (c) A small number of reference samples for which high-quality whole-genome sequence data was publicly available.

Samples were blinded and tested on a custom 220 gene NGS panel including 29 hereditary cancer risk genes. Laboratory protocols and custom bioinformatics methods were implemented to detect a spectrum of DNA variation types from the NGS data alone: These include single nucleotide variants (SNVs), small and large sequence insertions and deletions (indels), and small and large deletion and duplication (del/dup) events. NGS results were compared to the previous testing, and any analytical disagreements were sent to a third-party lab to arbitrate, also using traditional methods.

Analytic Results

Of the thousands examined, only 5 discordant variants existed between NGS and the previous testing. In all 5 cases the NGS result was shown to be correct by the third-party lab. Thus, 100% analytical sensitivity and 100% analytical specificity was observed between NGS and all of the confirmed traditional results to date.

Some classes of variation posed specific challenges for NGS and led to the development of new bioinformatic solutions:

- BRCA2: c.9203del126
- Deletion spanning part of but not all of a full exon, into neighboring intron past our pulldown target (or other’s typical PCR primer sites)
- Solution: Split-read algorithm implemented detect these types of event

Clinical Results

Clinical interpretations were also highly concordant, although with a slightly higher rate of VUS (Variants of Unknown Significance) in our BRCA1/2 data (6% vs. 4%). This was expected: We followed ACMG classification guidelines and used only public, and not proprietary reference databases, unlike the traditional BRCA1/2 tests we compared against. We also did not have access to family members in this study to help resolve VUS by segregation analysis.

More HBOC cases saw a reportable finding with the 29 gene panel vs. BRCA1/2 alone. Some, but not all, of these findings were clinically actionable. By virtue of testing more genes, additional VUS were also uncovered, with about 50% of patients having a VUS in one or more of the 29 genes. This too was expected as some genes in the panel have much less data available in the scientific and medical literature than others.

Conclusion

NGS can produce high quality clinical results comparable with traditional testing. Additional useful information can be produced by testing additional genes but with a corresponding increase in VUS. The high NGS specificity observed suggests that validating clinical variants in patients using an orthogonal platform may, over time and in certain circumstances, become less necessary.