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Title:
High-throughput characterization of *BRCA1* and *BRCA2* splicing defects

Abstract:
Statement of purpose: Advances in DNA Next Generation Sequencing (NGS) techniques resulted in the detection of many thousands of germline unclassified variants with unknown functional impact. Variants of unknown significance (VUS), especially in clinically actionable genes such as the hereditary breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* (OMIM 113705 and 600185 respectively), pose significant challenges to the medical community and patients. Among the variants that are frequently classified as VUS are those with unclear effects on splicing. Currently, there are no cost effective and high-throughput assays to quantify and characterize germline splicing defects in a time-frame necessary for clinical testing. Here we discuss the validation and implementation of a novel high-throughput RNA-NGS assay designed to perform *quantitative* and *qualitative* characterization of splicing VUS.
Methods: We compared the gold-standard mRNA splicing assays recommended by members of the ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium, including Capillary Electrophoresis and Sanger sequencing of subcloned transcripts, to high-throughput RNA-NGS assays. Following the ENIGMA protocol we performed cDNA analysis of lymphoblastoid cell lines (LCLs) generated by the kConFab consortium from carriers of *BRCA1* or *BRCA2* variants known to be associated with splicing defects, and of control LCLs, blood samples, and breast tissue. In parallel, we performed RNA-NGS assays and compared the results derived from these analyses to evaluate sensitivity and specificity of our assays.
Summary of Results: Using these techniques described above, we were able to detect and characterize the splicing aberrations described in the literature by the ENIGMA consortium. Differences in protocols allowed us to determine whether RNA-NGS assays are a reliable alternative for clinical characterization of *BRCA1* and *BRCA2* splicing alterations. RNA-NGS provides a cost effective and high-throughput alternative to the gold-standard with a reduced turnaround time, thereby improving the interpretation of splicing variants detected on clinical genomic tests.

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