Validation of the myBRCA Hereditary Breast and Ovarian Cancer Test for Population Screening

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Abstract

The myBRCA test is a targeted next generation sequencing assay for detecting mutations in *BRCA1* and *BRCA2*, performed in Veritas Genetics' CLIA approved clinical laboratory. The test utilizes saliva or whole blood and was carefully validated on a diverse set of samples with *BRCA1* and *BRCA2* variants representing both rare and relatively common pathogenic mutations. Validation studies showed that the sensitivity and specificity of the assay are both greater than 99.9%.

Introduction

Pathogenic mutations in BRCA1 and BRCA2 dramatically increase an individual's risk of developing breast and ovarian cancer. In many cases, pathogenic mutations in these two well-known tumor suppressor genes result in frameshift leading to premature truncation and decreased or non-functional tumor suppressor protein products. Pathogenic BRCA1 or BRCA2 mutations cause hereditary breast and ovarian cancer syndrome (HBOC), and, to a lesser extent, also increase risk for other cancers such as prostate cancer, pancreatic cancer, and melanoma.

Veritas Genetics has developed a next generation sequencing-based clinical test for BRCA1 and BRCA2. Extracted genomic DNA is processed by multiplex amplification with gene specific primers, and the resulting PCR products are sequenced on an Illumina NextSeq 500 next generation sequencer. The assay covers 16426 base pairs of genomic sequence, including the complete coding regions and splice sites in *BRCA1* and *BRCA2*. Point mutations and small insertions/deletions are detected.

The validation study was based on CDC, CLIA, CAP, ACMG and CLSI guidelines (1-6). Both types of sample material, blood and saliva, were processed from start to finish. Cell line DNAs with additional mutations were also included to ensure a full spectrum of validation variants, but the majority of samples was processed from original patient specimens.

myBRCA is intended for anyone who wants to know their BRCA1 and 2 mutational status. To this end, Veritas built in careful testing of samples from multiple ethnicities, rather than simply assuming that the relevant data was available at the design stage; a point often overlooked in DNA testing. More than a quarter of the blood and saliva validation samples (27.7%) were from non-white donors,

About the authors

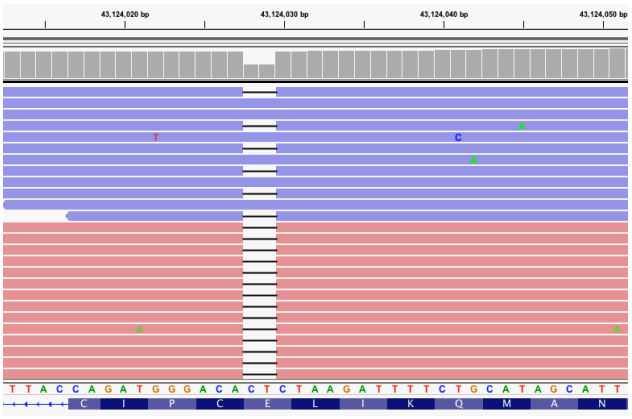
Dr. Birgitte Simen, who designed the validation study, co-authored the CDC working group Nature Biotechnology publication *Assuring the Quality of Next-Generation Sequencing in Clinical Laboratory Practice* (1). Dr. Simen previously headed NGS assay development at Roche.

Dr. Joseph Thakuria is an acknowledged authority on clinical interpretation of genome data (7). His previous accomplishments include being medical director and co-investigator for the Personal Genome Project at Harvard Medical School.

including persons of African-American, Hispanic, East Asian, South Asian and Middle Eastern ancestry.

Results

BRCA1 and *BRCA2* positive samples were collected from women who had previously tested positive in the clinic and are currently being monitored clinically for the development of cancer. 188 samples were sequenced, including 108 positive saliva and blood samples. The analysis was performed blinded to exclude interpretation bias. All positives counted were pathogenic, i.e., this analysis was not "padded" by including benign polymorphisms as true positives. An example of a validation sample mutation is shown below.



Result for a sample with BRCA1 frameshift mutation, p.Glu23ValfsTer17, a known pathogenic mutation, with a fraction of the reads visualized in the Integrative Genomics Viewer (8). Red and blue colors indicate forward and reverse sequencing reads, respectively.

Reproducibility and repeatability across multiple runs and different operators were both 100%. All positive samples yielded the expected pathogenic variants, which were additionally confirmed by Sanger sequencing. More than 2,000 individual positions in regions flanking these variants were compared to ensure that expected negative positions were true negatives. No false negatives or positives were detected (see table).

	Condition Positive (Sanger)	Condition Negative (Sanger)
Test outcome Positive (NGS)	108	0
Test outcome Negative (NGS)	0	2305

Data tracking and analysis

The integrated proprietary laboratory management system (LIMS) and in-house bioinformatics pipeline are very important components in the myBRCA process. To ensure that these function in a safe and secure manner, they were validated as recommended by CAP (3,4). In addition to the assay validation described above, Veritas created synthetic reads with realistic error rates, molecular barcodes, and pathogenic variants for separate pipeline validation. Downsampling was also performed to simulate low coverage. 100% of the tested variants were detected under all conditions. Finally, all expected National Institute of Standards and Technology reference polymorphisms were detected in a Hapmap sample (NA12878) that was sequenced as part of the validation.

Veritas Genetics' comprehensive database contains over 8,000 known variants in BRCA1 and 2. Pathogenic variants are extensively curated. In our validation studies, the incidence of variants of unknown significance (VUS rate) was only 2.3%, maximizing the information reported to guide genetic counseling and spare patients unnecessary anxiety.

Conclusion

The myBRCA test is a clinically validated, highly accurate, laboratory developed test for breast and ovarian cancer risk, covering full coding regions of BRCA1 and BRCA2 in people with a wide range of ethnicities.

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