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

Birgitte B. Simen, PhD[¶] and Joseph V. Thakuria, MD[¶§&]

[¶]Veritas Genetics, Danvers, MA 01923, [§]Massachusetts General Hospital, Boston, MA 02114 and [&]Harvard Medical School, Boston, MA 02115

Abstract

The myBRCA HiRisk test is a clinical test detecting mutations in 26 genes associated with increased risk for breast and ovarian cancer. The test is based on next-generation sequencing and multiplex PCR del/dup detection and is 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 variants representing both rare and relatively common pathogenic mutations. Validation results are described.

Introduction

Pathogenic mutations in *BRCA1* and *BRCA2* dramatically increase an individual's lifetime 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.

In addition to these two genes, several others have been identified as contributing to breast and ovarian cancer risk. Veritas Genetics' myBRCA HiRisk test includes 24 genes in addition to *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 next generation sequencer. The assay covers 82,853 base pairs of genomic sequence, including the complete coding regions and splice sites of all 26 genes. Point mutations and small insertions/deletions are detected with >99.9% specificity and sensitivity. For detection of large structural variants (copy number variations), a companion del/dup assay is performed that detects single- and multi-exon del/dups in *BRCA1* and *BRCA2* with very high accuracy (>99.9% specificity and sensitivity). Del/dup analysis of the remaining 24 genes is based on the NGS data and detects multi-exon structural variants. *PMS2* analysis is complicated by the existence of a highly homologous pseudogene. If a pathogenic variant is detected in the last three exons (exons 13-15) of the gene, a long-range PCR

About the authors

Dr. Birgitte Simen, who designed the validation study, co-authored the Nex-StoCT CDC working group Nature Biotechnology publication *Assuring the Quality of Next-Generation Sequencing in Clinical Laboratory Practice* (2). Dr. Simen previously headed NGS assay development at Roche.

Dr. Joseph Thakuria is an acknowledged authority on clinical interpretation of genome data (9). His previous accomplishments include being medical director and co-investigator for the Personal Genome Project at Harvard Medical School.

that excludes the pseudogene is performed and the sequence of the gene verified by Sanger sequencing (1). Del/dup analysis is not performed on this region of the gene.

Validation Study Overview

The validation study was based on CDC, CLIA, CAP, ACMG and CLSI guidelines (2-7). 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 were processed from original patient specimens.

myBRCA HiRisk is a multi-gene HBOC panel identifying germline mutations utilizing full gene next generation sequencing with full coding region coverage. With coverage of over 82,000 base pairs, myBRCA HiRisk is one of the most comprehensive panels currently on the market for screening patients at high risk for breast and ovarian cancer. It is intended for patients with confirmed breast or ovarian cancer and/or for patients with strong family history of breast/ovarian cancer. It is also recommended for patients who previously tested negative for *BRCA1* and *BRCA2* mutations who have personal or family histories suggestive of HBOC. Other indications include a personal or family history of male breast cancer, cancer diagnosed at an early age, different cancers in the same patient or both organs of a pair affected (e.g. cancer in both breasts). As documented in a recently published clinical study performed over 10 years at Massachusetts General, Stanford and Beth Israel Deaconess hospitals, multigene panel testing for HBOC risk assessment may yield "findings likely to change clinical management for substantially more patients than does *BRCA1/2* testing alone". myBRCA HiRisk offers the opportunity to improve clinical outcomes for high-risk individuals and their families and "alter near-term cancer risk assessment and management recommendations for mutation-affected individuals across a broad spectrum of cancer predisposition genes." (8)

Veritas included careful testing of samples from patients of multiple ethnicities, rather than simply assuming that the relevant data were available at the design stage; a point often overlooked in DNA testing. One third of the blood and saliva validation samples with known ethnicity were from non-white donors.

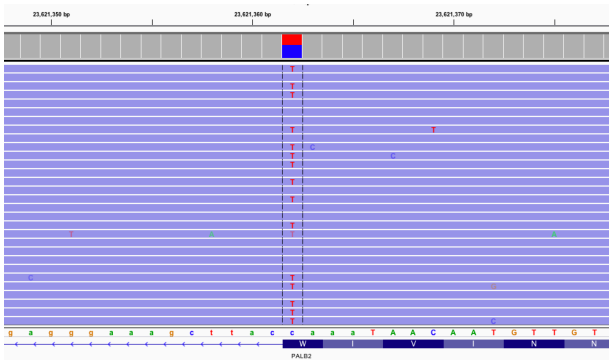
Genes tested in myBRCA HiRisk

ATM	*BARD1*	*BLM*	*BRCA1*
BRCA2	*BRIP1*	*CDH1*	*CHEK2*
*EPCAM**	*FAM175A*	*MEN1*	*MLH1*
MRE11A	*MSH2*	*MSH6*	*MUTYH*
NBN	*PALB2*	*PSM2*	*PTEN*
RAD50	*RAD51C*	*RAD51D*	*STK11*
TP53	*XRCC2*		

*del/dup analysis only

Results

Samples were collected from patients who had previously tested positive for a mutation in the clinic and are currently being monitored clinically for the development of cancer. Blinded clinical samples containing known SNPs, small indels, and large structural variants were included. 87 samples were sequenced, including 69 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 a pathogenic *PALB2* mutation, c.3113G>A (p.Trp1038Ter) with a fraction of the reads visualized in the Integrative Genomics Viewer (10).

Reproducibility and repeatability across multiple runs and different operators were both 100%. All positive samples yielded the expected pathogenic variants. No false negatives or positives were detected (see table).

	Condition Positive (Sanger)	Condition Negative (Sanger)
Test outcome Positive (NGS)	**69**	**0**
Test outcome Negative (NGS)	**0**	**18**

Conclusion

The myBRCA HiRisk test is a clinically validated, highly accurate, laboratory developed test for breast and ovarian cancer risk, covering full coding regions of 26 breast and ovarian cancer associated genes in individuals of varying ethnicities.

References

1. Vaughn CP, Hart KJ, Samowitz WS *et al.* Avoidance of pseudogene interference in the detection of 3' deletions in PMS2. Hum Mutat 32:1063 (2011)
2. Gargis A, Kalman L, Berry M *et al.* Assuring the Quality of Next-Generation Sequencing in Clinical Laboratory Practice. Nature Biotechnology, 30:1033 (2012)
3. Standards and Certification: Laboratory Requirements (42CFR493), *www.ecfr.gov*
4. Aziz N, Zhao Q, Bry L. College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests. Arch Pathol Lab Med (2014)
5. Molecular Pathology Checklist (09.25.2012). College of American Pathologists, *www.cap.org*
6. Rehm HL, Bale SJ, Bayrak-Toydemir P *et al.* ACMG clinical laboratory standards for next-generation sequencing. Gen Med, 15:733 (2013)
7. Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline MM17-A. Vol.28 No.9 (March 2009). Clinical and Laboratory Standards Institute
8. Desmond A, Kurina AW, Gabree M *et al.* Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment. JAMA Oncol 1:943 (2015)
9. Thakuria JV and Murray M. Clinical Interpretation of Genomic Data, chapter in Clinical Genomics: Practical Applications in Adult Patient Care, American College of Physicians (ACP), McGraw Hill, (2014) Columbus, OH
10. Thorvaldsdóttir H1, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform 14:178 (2013)

Acknowledgements

We gratefully acknowledge the patients and volunteers who selflessly donated saliva and blood to this validation study. In addition, we thank the following clinicians who kindly provided reference samples for the validation:

Judy E. Garber, MD, MPH
Director, Center for Cancer Genetics and Prevention, Dana-Farber Cancer Institute
Professor of Medicine, Harvard Medical School

Sapna Syngal, MD, MPH
Director of Research, Center for Cancer Genetics and Prevention, Dana-Farber Cancer Institute
Professor of Medicine, Harvard Medical School

Jan Lubinski, MD, PhD
Head, Division of Genetics and Pathomorphology and Chair of Oncology at the Medical University in Szczecin, Poland
Head, International Hereditary Cancer Center, Pomeranian Medical University, Poland