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A Systematic Comparison of Traditional and Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Genes in More Than 1000 Patients

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Gene panels for hereditary breast and ovarian cancer risk assessment are gaining acceptance, even though the clinical utility of these panels is not yet fully defined. Technical questions remain, however, about the performance and clinical interpretation of gene panels in comparison with traditional tests. We tested 1105 individuals using a 29-gene next-generation sequencing panel and observed 100% analytical concordance with traditional and reference data on >750 comparable variants. These 750 variants included technically challenging classes of sequence and copy number variation that together represent a significant fraction (13.4%) of the pathogenic variants observed. For *BRCA1* and *BRCA2*, we also compared variant interpretations in traditional reports to those produced using only non-proprietary resources and following criteria based on recent (2015) guidelines. We observed 99.8% net report concordance, albeit with a slightly higher variant of uncertain significance rate. In 4.5% of BRCA-negative cases, we uncovered pathogenic variants in other genes, which appear clinically relevant. Previously unseen variants requiring interpretation accumulated rapidly, even after 1000 individuals had been tested. We conclude that next-generation sequencing panel testing can provide results highly comparable to traditional testing and can uncover potentially actionable findings that may be otherwise missed. Challenges remain for the broad adoption of panel tests, some of which will be addressed by the accumulation of large public databases of annotated clinical variants. *(J Mol Diagn 2015, 17: 533—544; http://dx.doi.org/10.1016/j.jmoldx.2015.04.009)*

Multigene panel testing has proved useful as a diagnostic tool for disorders where similar phenotypes can be influenced by multiple genes.[1] Recent advances in next-generation DNA sequencing technology (NGS) have enabled these clinical tests and made them increasingly inexpensive to perform.[2,3] For hereditary cancer syndromes, studies have shown that NGS-based panel tests can uncover potentially actionable findings that may be missed by traditional testing paradigms.[4—12] Validation studies of clinical NGS assays for hereditary cancer genes have correspondingly been published,[4,7,11,13,14] and certain guidelines exist for their clinical implementation.[15—18] Patient management experience using these hereditary cancer panels is growing,[4,19,20]

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This paper conforms to the STARD guidelines (*http://www.stard-statement.org*) for reporting of diagnostic cohort studies.

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Table 1 Study Population

Population	Group	No. of patients	Description	Previous testing
Clinical cases (n = 1062)	Clinical referral	735	Patients prospectively accrued following NCCN guidelines for HBOC	Traditional clinical testing for *BRCA1* and/or *BRCA2* in most cases, with occasional testing for other genes
	History enriched (n = 327)	209	Retrospective cases from a clinical biobank containing particularly high-risk patients	
		118	Cases referred because of a known pathogenic variant in family	Clinical single-site testing
Reference samples (n = 43)	Positive reference samples	36	Reference samples selected from public biobanks	Samples carry known pathogenic variants in specific genes
	Genome reference samples	7	Reference samples from public biobanks with high-quality WGS data	Variants in 29 cancer genes extracted from WGS data
Total		1105		

Individuals included in this study, with their selection criteria. Previous test results for *BRCA1* and/or *BRCA2* were available for 92% (n = 975) of the 1062 clinical cases. All of the reference samples had previous test data. The specific type and scope of testing varied (Supplemental Table S2).

HBOC, hereditary breast/ovarian cancer; NCCN, National Comprehensive Cancer Network; WGS, whole genome sequencing.

although the clinical utility of these panels is not yet fully established[21,22] and the appropriate routes for clinical deployment of such tests remain under discussion.[23]

Several technical questions also remain about these new tests. NGS has traditionally had analytical limitations[24] compared with established technologies, such as Sanger sequencing,[25] quantitative PCR,[26] multiplex ligation-dependent probe amplification (MLPA),[27] and copy number microarrays.[28] If panel tests are to replace traditional single-gene tests in appropriate situations, further evidence is required to show that NGS can meet the analytic performance standards of these established methods, particularly on those classes of variants that are known to be most challenging for NGS. In addition, questions have long been raised about the potential for inconsistent variant interpretations between laboratories because of limited access to proprietary data and because of differences in interpretation criteria.[29,30] This is an increasingly relevant area for study, because NGS-based tests from multiple laboratories have emerged in recent years[31] and new guidelines for the interpretation of sequence variants (ISV) have also emerged.[32]

To help address these technical questions, particularly as they apply to hereditary breast/ovarian cancer (HBOC), we tested 1062 patients with an NGS-based 29-gene hereditary cancer panel. These individuals were indicated for HBOC risk assessment under National Comprehensive Cancer Network (NCCN) guidelines,[33] and most had previously received clinical testing for *BRCA1* and/or *BRCA2* from another laboratory. We supplemented these data with additional confirmatory testing and with data from 43 additional reference samples to evaluate NGS performance over more genes and variants. We classified variants following a system based on the recent ISV guidelines, and we used only broadly available, non-proprietary resources to do this. In total, these data allowed us to compare both the analytical and clinical interpretation results from traditional BRCA testing with an NGS-based gene panel.

Materials and Methods

Patients, Samples, and Previous Test Data

Samples were compiled from multiple sources (Table 1 and Supplemental Table S1), and most had previous genetic data available for comparison (Supplemental Table S2).

Seven-hundred thirty-five patients referred for HBOC counseling and/or testing under NCCN guidelines[33] were prospectively recruited at two academic medical centers: the Stanford Clinical Cancer Genetics Program (Stanford, CA; 2002-2012) and the Massachusetts General Hospital Center for Cancer Risk Assessment (Boston, MA; 2013-2014). A further 118 patients referred to either center because of known familial mutations were recruited but considered separately in this analysis. An additional group of 209 patients was recruited at Massachusetts General Hospital Center for Cancer Risk Assessment (2000-2012) on the basis of high-risk personal and family features, but not under uniform criteria, and they are also considered separately. Of the total 1062 patients, 975 (92%) had previously received traditional *BRCA1* and/or *BRCA2* tests from Myriad Genetics (Salt Lake City, UT), and a small subset (4%) had undergone tests for other genes or multigene panels, as had been clinically indicated. A subset of these patients (n = 175) had been analyzed in our prior work using a research panel,[4] although insufficient material remained to retest all 198 patients from that previous study here.

Thirty-six reference samples carrying known pathogenic variants were selected from two public biobanks: the Coriell Institute (Camden, NJ) and the National Institute for Biological Standards and Control (Hertfordshire, UK) (Supplemental Table S3).