Next-Generation Panel vs. Traditional Testing of Hereditary Breast and Ovarian Cancer Genes in a Large Clinical Population

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Introduction

Next-generation sequencing (NGS) of gene panels has gained clinical acceptance, although important questions remain about these tests. Expanding on our recently published work (Kurian et al., *J Clinical Oncol.* 2014), we considered whether NGS panel testing can both replace and supplement traditional genetic tests for hereditary breast/ovarian cancer (HBOC). Specifically, we evaluated whether the spectrum of pathogenic variants detectable by traditional laboratory methods (e.g., Sanger sequencing, qPCR, MLPA, and arrays) can also be detected by NGS. We also compared BRCA1/2 variant interpretations produced using only publicly available resources and following the 2014 draft ACMG ISV guidelines to those interpretations produced previously by an independent laboratory using a large proprietary database. Finally, we examined the clinical relevance and potential actionability of the non-BRCA1/2 results uncovered by panel testing but generally not by the traditional testing paradigm for HBOC.

Study Design

1062 patients and 43 reference samples were tested for sequence and del/dup (copy-number) variants using a 29-gene NGS panel.

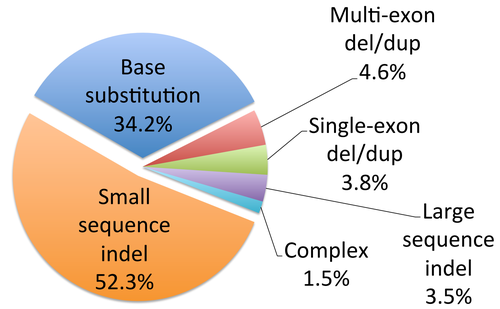
Group	N	Description	Previous Testing
Clinical Referral	735	Patients prospectively accrued following NCCN guidelines	Clinical testing for BRCA1/2 (92% cases) and/or other genes (4%)
History Enriched (n=327)	209	High-risk retrospective cases from a clinical biobank	
	118	Cases referred due to a known mutation in the family	Clinical single-site testing in all cases
Positive Reference Samples	36	Reference samples from public biobanks	Samples carry specific known mutations
Genome Reference Samples	7	Reference samples from public biobanks with high-quality whole-genome sequences (WGS)	Variants in 29 cancer genes extracted from WGS data
Total	**1105**		

	Clinical Referral	History-Enriched Retrospective	Familial Mutation
Total Patients	735	209	118
Gender			
Male	9 (1.2%)	8 (3.8%)	14 (11.9%)
Female	726 (98.8%)	201 (96.2%)	104 (88.1%)
Age at testing (years)			
35 and under	84 (11.4%)	23 (11.0%)	41 (34.8%)
<50 (49-36)	260 (35.4%)	92 (44.0%)	34 (28.8%)
>=50	391 (53.2%)	94 (45.0%)	43 (36.4%)
Self-Reported Ethnicity			
African	6 (0.8%)	1 (0.5%)	1 (0.8%)
Asian	48 (6.5%)	4 (1.9%)	3 (2.5%)
Asian Indian	17 (2.3%)	0 (0.0%)	1 (0.8%)
Caucasian	543 (73.9%)	130 (62.2%)	92 (78.0%)
Hispanic	29 (3.9%)	1 (0.5%)	4 (3.4%)
Ashkenazi Jewish	59 (8.0%)	42 (20.1%)	6 (5.1%)
Multiple	18 (2.5%)	6 (2.9%)	5 (4.2%)
Unknown/other	15 (2.0%)	25 (12.0%)	6 (5.1%)
Personal Cancer History			
Breast Ca	503 (68.4%)	138 (66.0%)	16 (13.6%)
Ovarian Ca	42 (5.7%)	17 (8.1%)	0 (0.0%)
Colorectal Ca	9 (1.2%)	3 (1.4%)	1 (0.8%)
Endometrial Ca	12 (1.6%)	2 (1.0%)	0 (0.0%)
Pancreatic Ca	2 (0.3%)	0 (0.0%)	1 (0.8%)
No personal Hx Ca	167 (22.7%)	47 (22.5%)	95 (80.5%)

Analytical Results

In these 1105 individuals, 58,708 variants were observed by the 29-gene NGS panel, with the vast majority being benign polymorphisms. 607 previously reported variants could be directly compared to the NGS panel data. Another 143 NGS-only variants were selected for orthogonal confirmation, for a total of 750.

Variants Used in Analytic Validation	N
Base substitutions (SNVs)	549
Sequence deletions <10 base pairs	125
Sequence insertions <5 base pairs	31
Sequence insertions ≥5 base pairs	4
Sequence deletions ≥10 base pairs	9
Complex variants	6
Single-exon deletions	9
Single exon duplications	4
Deletions of multiple exons or whole gene	10
Duplications of multiple exons or whole gene	6
Total	**750**



Of 260 pathogenic variants observed in the 1062 patients, 13.4% were of types known to be challenging for NGS. Nevertheless, we observed 100% analytical concordance.

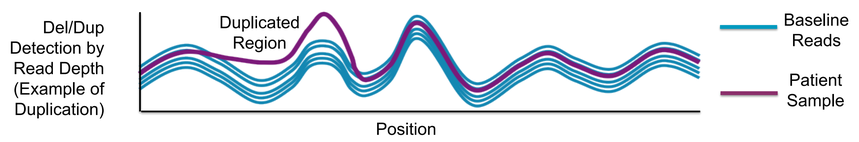
Previous Testing and/or Orthogonal Confirmation

NGS Panel	Present	Not Present	Result
Detected	750 True Positives	0 False Positives	**100% Sensitivity** CI Seq: 100%–99.7% CI Del/dup: 100%–92.8%
Not Detected	0 False Negatives	15.0M True Negative bps (Seq) 22.2K True Negative Exons (Del/dup)	**100% Specificity** CI Seq: 100%–99.99998% CI Del/dup: 100%–99.989%

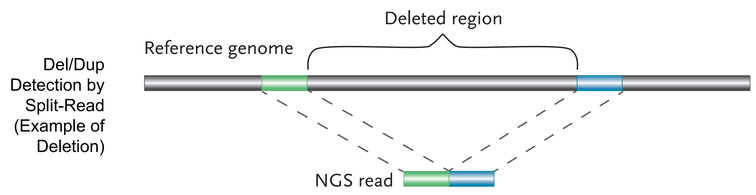
Technical Methods

NGS was used to detect not only sequence alterations (via GATK, Freebayes, and Coalgen) but also copy-number changes using two methods:

Read-depth: In NGS data, read depth varies across genomic regions, although the pattern of relative depths is reproducible. Deviations from this pattern indicate copy-number changes. Using a coordinated set of laboratory protocols and data-analysis methods (much as has been successfully done with microarrays), we validated performance for events as small as one exon in most genes.



Split-read: NGS reads that span the breakpoint of a deletion or duplication show distinct patterns when mapped to the reference genome. Events detected by split-read analysis can be exactly those that are harder for both read-depth approaches and traditional probe-based methods (such as microarrays, qPCR, or MLPA)—for example, events that start or end in the middle of an exon.



Variant Classification

Net clinical results for BRCA1/2 were 99.8% concordant with prior results. The prevalence of variants of unknown significance (VUS) was 6% of cases, which compares with the 4% VUS rate for the previous laboratory in this patient cohort.

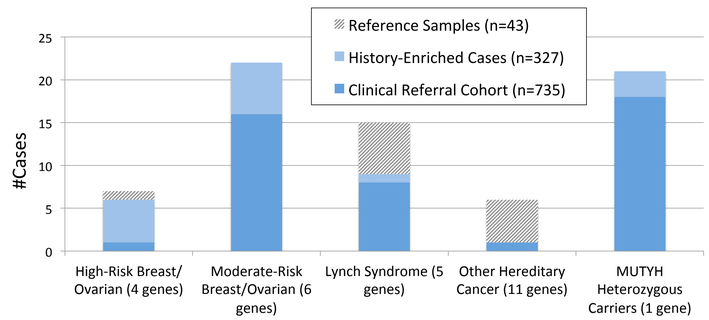
Previous BRCA1/2 Testing

BRCA1/2 Results from 29-Gene NGS Panel	Positive	Uncertain	Negative	Total
Positive	188			188 (19.3%)
Uncertain	2	30	8	787 (80.7%)
Negative		1	746	
Total	190 (19.5%)	785 (80.5%)		975 (100%)

Net result concordance: 99.8% (973/975 patients)
Uncertain (VUS-only) reports: 4.1% (40/975) vs. 3.2% (31/975)

Clinical Implications

Approximately 4% of all BRCA-negative cases in the prospective cohort (n=735) were positive for another dominant-acting cancer-risk gene. This compares to 9% of patients who were BRCA1/2 positive, for a >40% increase in yield.



We separately examined the detailed personal and family histories of these patients who carried pathogenic variants in non-BRCA1/2 genes. In summary:

- In the vast majority of cases (>90%), the spectrum of cancers in the patient and/or family fit the known effect(s) of the gene they carry, suggesting that these results are clinically relevant.
- In the majority of cases, these non-BRCA1/2 findings would warrant consideration of a change in management for the patient and/or positive family members over and above care recommendations based on personal and family history alone.

Conclusions

With appropriate bioinformatics and laboratory methods, NGS can deliver comparable analytic performance to traditional genetic tests. Variant interpretations using new ACMG guidelines and public resources are similar to those from a lab using proprietary resources. Panels can both be a viable replacement for traditional tests and may provide additional clinically actionable findings.