

Introduction

Background: Next Generation Sequencing (NGS) is gaining clinical acceptance, although questions remain about the sensitivity, specificity and clinical implications of these tests. Expanding on our recently published work (Kurian et al., J Clin Oncol, 2014) we considered whether NGS-based panel testing can both replace and supplement traditional BRCA1/2 testing.

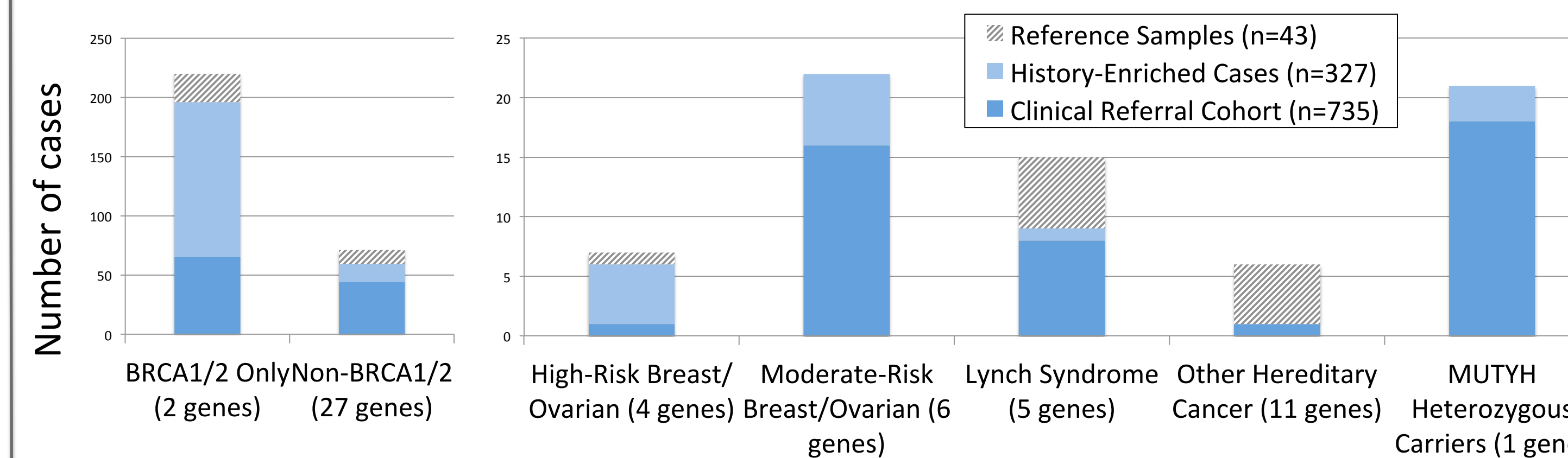
Methods: Over 1000 patients indicated for assessment of hereditary breast/ovarian cancer risk under NCCN guidelines were recruited and tested with a 215-gene NGS panel. In this poster we focus on variants detected in 29 known cancer risk genes. For comparison, most of these patients also previously had traditional clinical genetic testing from an established independent lab. In addition we supplemented the study with 43 reference samples having known (and often technically challenging) variants in these cancer risk genes.

Sub-Panel	Gene names
BRCA1/2	BRCA1, BRCA2
Other High-Risk Breast/Ovarian Cancer	CDH1, PTEN, STK11, TP53
Moderate-Risk Breast/Ovarian Cancer	ATM, BRIP1, CHEK2, NBN, PALB2, RAD51C
Lynch Syndrome	EPCAM, MLH1, MSH2, MSH6, PMS2
Other Hereditary Cancer Syndromes	APC, BMPR1A, SMAD4, CDK4, CDKN2A, PALLD, MET, MEN1, RET, PTCH1, VHL
MUTYH	MUTYH

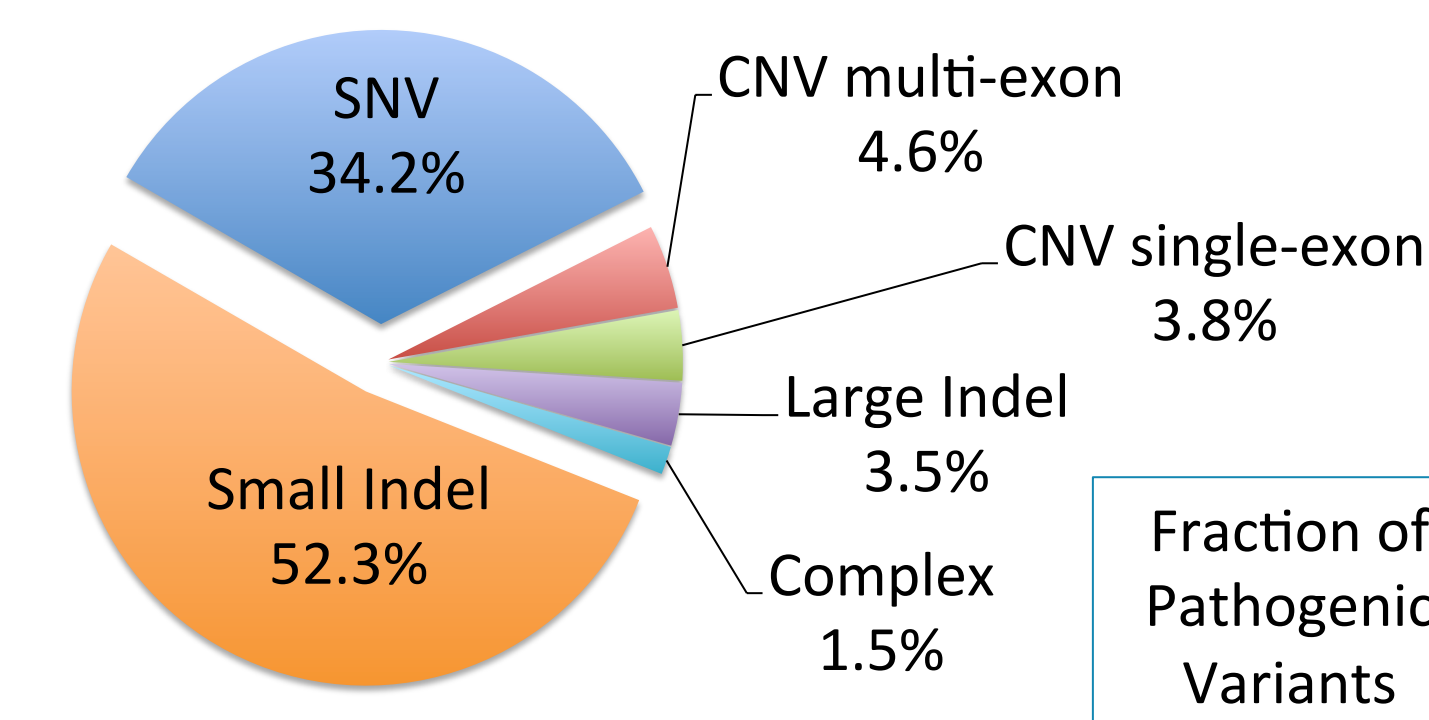
Group	N	Description	Previous Testing
Clinical Referral	735	Patients prospectively accrued following NCCN guidelines	Clinical testing for BRCA1/2 in most cases, with occasional testing for other genes
History Enriched (n=327)	209	Retrospective cases from a clinical biobank containing higher-risk patients	
	118	Cases referred due to known pathogenic variant in family	Clinical single-site testing in all cases
Positive Reference Samples	36	Reference samples selected from public biobanks	Samples carry specific known variants
Genome Reference Samples	7	Reference samples from public biobanks with high-quality whole genome sequencing (WGS) data	Variants in 29 cancer genes extracted from WGS data
Total	1105		

Laboratory Results

Prevalence: As expected, 9% of our Clinical Referral cohort carried a pathogenic variant in BRCA1 or BRCA2. 4% had a pathogenic variant in another cancer risk gene, and most of these were found in moderate penetrance genes or Lynch syndrome genes (even though there was no selection in this study for colon or endometrial cancer). Another 2.5% were heterozygous MUTYH carriers. A higher fraction of our History-Enriched Cohort was positive for high penetrance genes.



Variant Types: Challenging variants for NGS (e.g. large indels and single-exon CNVs) were rare (0.05% of all variants) but represent a significant fraction (~10%) of the pathogenic variants, underscoring the importance of accurate methods to call these events.



Variants in Analytic Validation	N
Single Nucleotide Variants (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 in 29 Cancer Genes	750

Sensitivity and Specificity: 750 variants were selected for analytic validation from those detected either previously or by the NGS panel. We included all pathogenic variants, all large indels, all CNVs, and a sampling of VUS and benign variants. In spite of the challenging variants included, the NGS panel data had 100% concordance with the traditional (Sanger, qPCR, array) data.

NGS Panel	Variant Present	Variant Not Present	Conclusion
Variant Detected	750 True Positives	0 False Positives	100% Specificity
Variant Not Detected	0 False Negatives	>2500 concordant negative gene tests	100% Sensitivity

Clinical Results

Variant Classification: We used a point-based system consistent with the 2014 draft ACMG/AMP/CAP guidelines for interpretation of sequence variants, and we used only broadly available and not proprietary resources in this classification. Nevertheless, for BRCA1/2 we find that our variant interpretations are substantially concordant for with those from the previous tests where a large, proprietary database was used by the previous testing lab.

Positive vs. not Positive Result for BRCA1/2 (only)	
Agree	99.8%
Disagree	0.2%

% of patients with one or more VUS in BRCA1/2	
Panel Test	5.3%
Previous Test	3.2%

Clinical Impact: We are now completing detailed chart reviews of these patients and, preliminarily, we see the following results for the non-BRCA positives:

- Phenotype:** In most cases (80% to date) the patient's cancer or family history was consistent with known effects of the mutant gene they carry, suggesting that these findings are not incidental. The other 20% could have rare pleiotropic effects, incomplete family histories, or cancers unrelated to their genetics (with the possibility of a linked cancer in their future).
- Actionability:** In most cases (70% to date) the non-BRCA findings would warrant consideration of a change in care under current medical guidelines.
- Counseling and Acceptance:** In patients who consented to return of these results, genetic counseling has proved both feasible and appreciated by the patients in spite of the increased uncertainties (e.g. unclear risk levels and increased prevalence of variants of unknown significance) that result from broad genetic testing vs. BRCA-only testing.

Conclusions

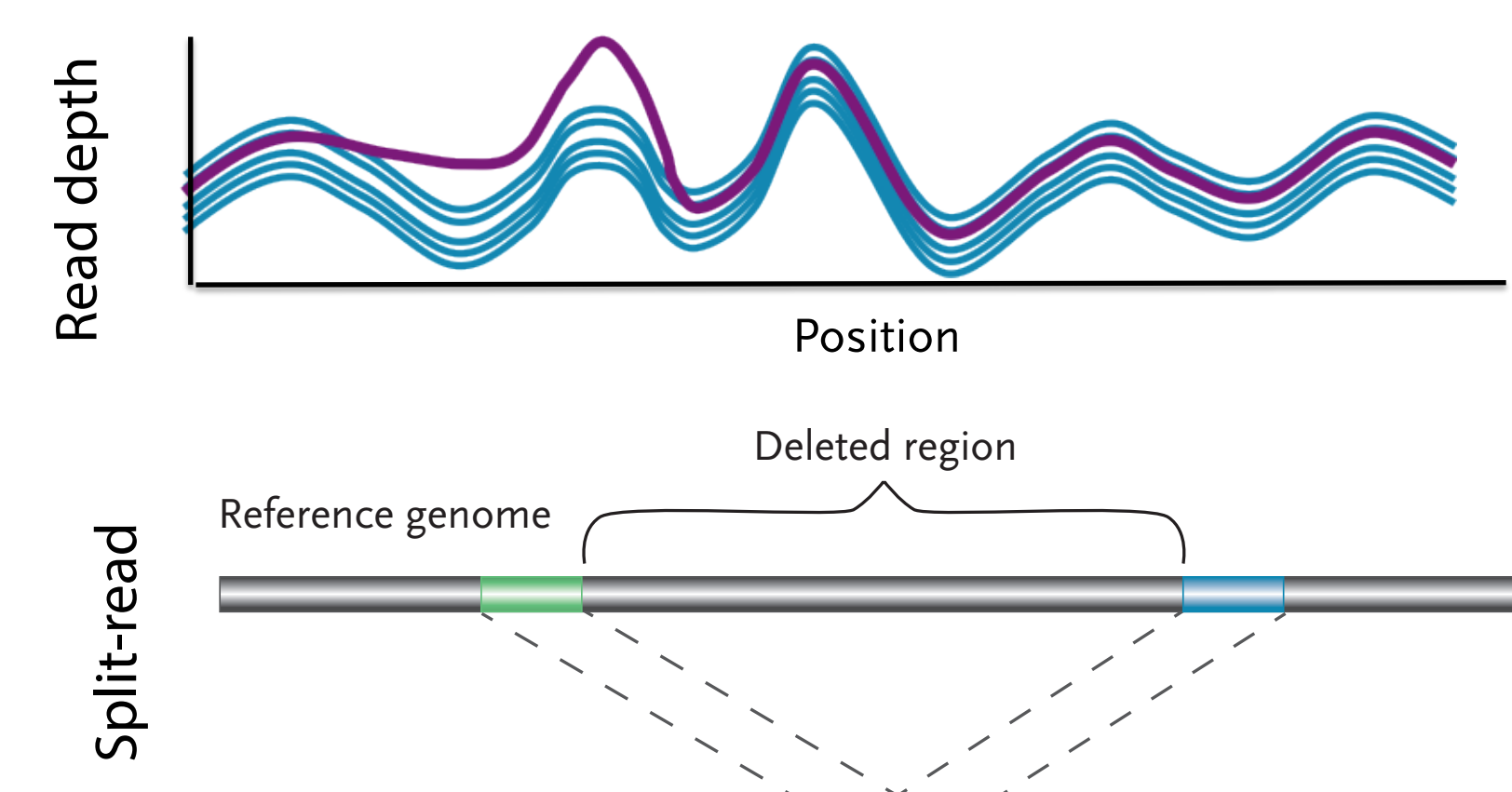
NGS can be a viable replacement for traditional genetic testing for hereditary cancers and other syndromes, with the additional benefit of cost-effectively increasing diagnostic yield in a clinically actionable manner.

Orthogonal confirmation of clinical NGS results remains a strongly recommended practice, although the high concordance of traditional and NGS data that we observe suggests that the benefit of confirmation merits careful consideration over time.

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Technical Methods: Detection of CNVs from Clinical NGS Data

We used a combination of read-depth and split-read analysis to detect CNVs and found that these approaches tend to complement each other. Events detected by split-read analysis are often those that are hard for read-depth approaches (they also can be similarly hard for traditional methods). Examples include CNVs that start or end in the middle of an exon. Laboratory techniques to minimize coverage variability and to quantify remaining variability have proven critical to achieving high sensitivity and specificity for small (1-2 exon) deletions and duplications.



Data Release

The variants observed in this study and their clinical interpretations have been submitted to ClinVar.

