

Introduction

Background: Use of Next Generation Sequencing (NGS) for gene panels and exomes 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 BRCA testing in patients indicated for hereditary breast/ovarian cancer testing.

Methods: Over 1000 patients indicated for assessment of hereditary breast/ovarian cancer risk under NCCN guidelines were recruited and tested with a 218-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.

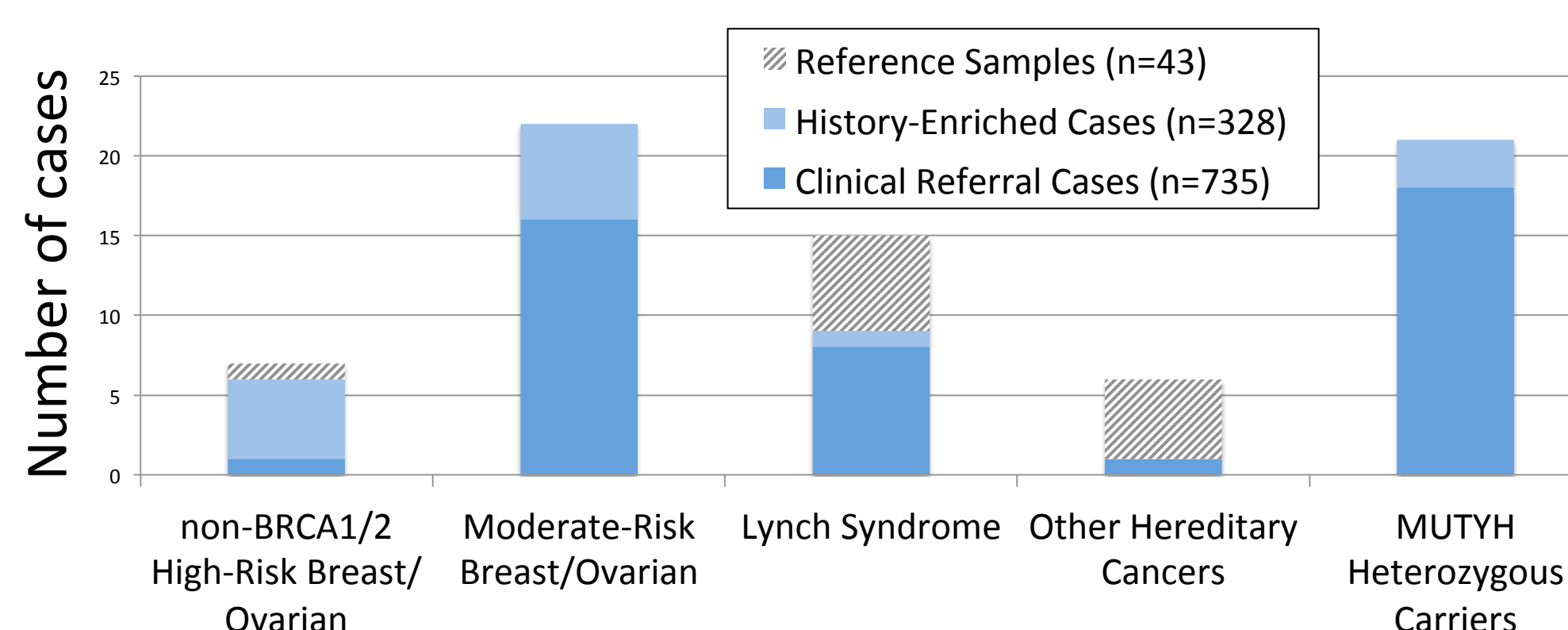
Data Availability: The variants observed in this study and their clinical interpretations have been submitted to ClinVar.

Panel	Gene names
BRCA1/2	BRCA1, BRCA2
Other High-Risk Breast/Ovarian	CDH1, PTEN, STK11, TP53
Moderate-Risk Breast/Ovarian	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

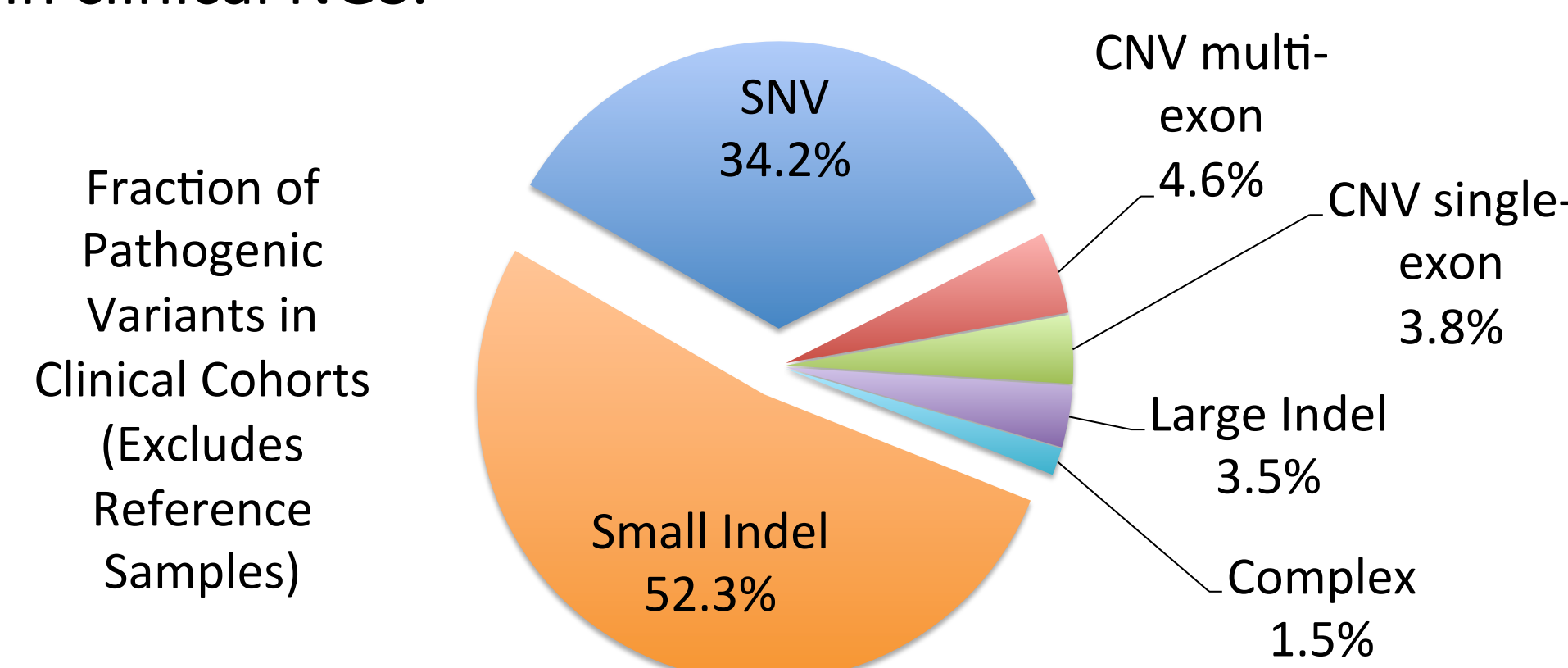
Group	N	Description	Previous Testing
Clinical Referral	735	Patients prospectively accrued following NCCN guidelines	Clinical testing for BRCA1/2 in most cases, with occasional testing for other genes
History Enriched (n=327)	209	Retrospective cases from a clinical biobank containing higher-risk patients	Clinical single-site testing in all cases
	118	Cases referred due to known pathogenic variant in family	
Clinical 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 given our selection criteria, 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 in moderate penetrance breast/ovarian genes or Lynch syndrome genes (even though there was no specific 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 BRCA1/2 and the other high penetrance genes, although the prevalence of moderate risk and Lynch genes was similar to that in the Clinical Referral Cohort.



Variant Types: Challenging variants for NGS (e.g. indels >10bp and single-exon CNVs) were rare (0.05% of the total) but represented a significant fraction (10%) of the pathogenic variants, underscoring the importance of accurate methods to call these events in clinical NGS.



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, our NGS data had 100% concordance with the traditional (Sanger, qPCR, microarray) data.

Traditional Data (Previous Testing and Confirmations)			
	Variant Present	Variant Not Present	Conclusion
NGS Panel	Variant Detected	750 True Positives	0 False Positives 100% Specificity
	Variant Not Detected	0 False Negatives	>30,000 individual gene tests with concordant negative results 100% Sensitivity

Clinical Results

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

- **Clinical Relevance:** 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 patients (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).
- **Clinical 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.
- **Unexpected Findings:** In spite of the apparent relevance of these positive findings to the clinical situations, unexpected results did occur. Among the 118 patients who previously had received single-site testing for familial BRCA variants, two were found to be negative for those events but were found by the panel to carry pathogenic variants in different genes (ATM and MSH2, respectively). Two other patients were positive for both BRCA1 and BRCA2. These results were confirmed and the patients indeed have multiple risk genes segregating in their families.

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.

Acknowledgements

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Technical Aside: 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.

