A Comparison of Traditional and 29-Gene Panel Testing for Hereditary Breast and Ovarian Cancer in Over 1000 Patients

Stephen Lincoln[1], Allison Kurian[2], Andrea Desmond[3], Meredith Mills[2], Michele Gabree[3], Yuya Kobayashi[1], Michael Anderson[1], Shan Yang[1], Geoffrey Nilsen[1], Marina Rabideau[1], James Ford[2] and Leif Ellisen[3]

[1]Invitae Corporation, San Francisco, CA [2]Stanford University, Palo Alto, CA [3]Massachusetts General Hospital, Boston, MA

Overview

Multi-gene panels for hereditary cancer are now entering clinical use. To help develop the most appropriate genetic counseling practices for these panels it is important to have careful measurements of their yield and performance by comparison with traditional genetic tests. Recently we described clinical results from panel testing of 198 patients (Kurian et al., J Clin Oncol 2014) which we expand on here in a larger patient population.

We collected over 1000 patients from two clinical centers, all of whom had been referred for hereditary breast/ovarian cancer risk counseling or assessment. 735 of these patients were prospectively recruited following NCCN criteria (family history and/or personal history) in order to best represent clinical experience. A separate 327 particularly high-risk cases were also collected either from a clinical biobank or from patients referred because of a known pathogenic variant in their family. We also supplemented this study with non-clinical reference samples. Previous test results on these individuals were available that we used in comparison to the 29-gene panel test results.

Group	N	Description	Previous Testing
Prospective Clinical	735	Prospective clinical cases	Clinical testing for BRCA1/2 and/or other genes (depending on case)
High-Risk Clinical (Total 327)	209	Retrospective cases from a clinical biobank generally containing higher-risk individuals	
	118	Cases referred due to known pathogenic variant in family	Clinical single-site testing
Reference Samples	36	Reference samples from public biobanks	Samples carry known pathogenic variants
Well-Characterized Genomes (WCGs)	7	Reference samples from public biobanks with high-quality whole genome sequencing (WGS) data	Variants in 29 cancer genes extracted from WGS data; most of these are benign
Total	**1105**		

Because of both the large number of individuals in this study and the specifics of how these cases were selected, a diverse set of DNA alterations was present:

	Type	N	Details
Sequence	Single Nucleotide Variants (SNVs)	548	
Sequence	Deletions <10bp	125	
Sequence	Deletions ≥10bp	9	126, 40, 19, 15, 11 bp
Sequence	Insertions <5bp	31	
Sequence	Insertions ≥5bp	4	24, 5 bp
Sequence	Delins/Haplotype	6	
Sequence	Homopolymer-Associated SNV	1	MSH2
Copy Number	Single Exon Deletions	9	BRCA1, BRCA2, MSH2, PMS2
Copy Number	Deletions 2 Exons to Whole Gene	10	BRCA1, MSH2, RAD51C
Copy Number	Single Exon Duplications	4	BRCA1, MLH1
Copy Number	Duplications 2 Exons to Whole Gene	6	BRCA1, BRCA2, NBN, SMAD4
	Total	**753**	

Laboratory Results

Analytic Performance: We see 100% concordance between the 29-gene next-generation sequencing (NGS) panel and the previous test results whenever corresponding tests were performed. Overall the panel had 100% sensitivity and 100% specificity compared to traditional methods. While certain classes of variation are known to be challenging for NGS (e.g. large sequence indels and small copy-number del/dups) all such variants in this study were correctly reported by the NGS methods and bioinformatics pipeline we used.

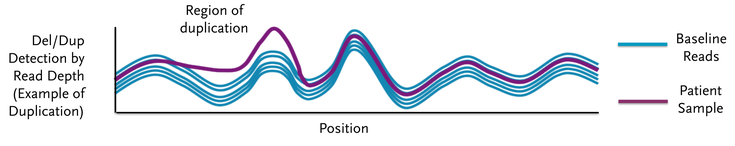
Result		Detail	Conclusion
False Negatives	**0**	All 607 previously reported variants were also detected by the NGS panel	**100% Sensitivity**
False Positives	**0**	607 variants detected by the NGS panel were also previously reported All 146 other variants detected by the NGS panel were independently confirmed All 1911 previously negative gene tests were also negative by the NGS panel	**100% Specificity**

Variant Classification: We see 99.8% concordance for BRCA1/2 pathogenicity assessments between the panel test and the previous test results.

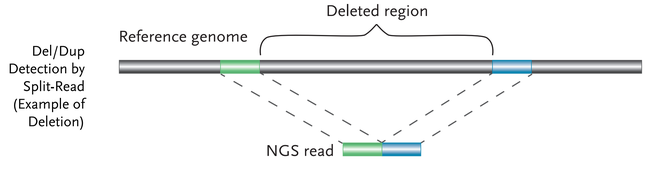
Technical Aside: Del/Dup Calling by NGS

NGS was used to detect not only sequence alterations but also copy-number changes by two methods in this study:

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 demonstrated clinical levels of performance for events as small as an 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 an event that starts or ends in the middle of an exon.



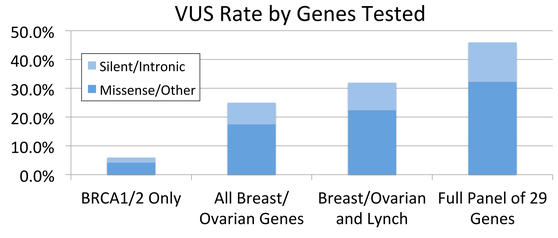
Clinical Results

Panel testing has the potential to provide more comprehensive assessment of risk than traditional testing. Of the 866 BRCA1/2 negative clinical cases, 39 (4.5%) carried pathogenic variants in other dominantly inherited cancer risk genes. Most (2/3) of these pathogenic variants were in genes with known associations with breast and ovarian cancer, while most others (1/3) were in Lynch syndrome genes which confer risk of ovarian cancer and which have been suggested to play a role in breast cancer. In addition, 23 individuals (2.7%) were heterozygous carriers of pathogenic variants in MUTYH.

As expected, pathogenic variants in high-penetrance genes other than BRCA1/2 are not common in our prospective cohort (our high-risk group accounts for all but one of the six TP53 and CDH1 positives, for example). However, the rate of moderate risk and Lynch gene positives is roughly comparable between the prospective and high-risk groups (3.7% vs. 4.1%) suggesting that the risk-based procedures traditionally used to select patients for BRCA testing may not perform similarly well for broader panel tests.

New types of incidental findings are possible with panel tests. Among the patients who previously had received single-site testing for familial BRCA mutations, two were found to be negative for those mutations but were found by the panel test to carry pathogenic variants in different genes (ATM and MSH2, respectively).

Of course, as more genes are tested, rates of VUS (Variants of Uncertain Significance) increase beyond those typically seen in more limited tests.



Positive Findings By Gene in Clinical Cases

Gene	N	
BRCA1	119	Breast/Ovarian
BRCA2	79	Breast/Ovarian
PTEN		Breast/Ovarian
TP53	2	Breast/Ovarian
STK11		Breast/Ovarian
CDH1	4	Breast/Ovarian
PALB2	5	Breast/Ovarian
CHEK2	5	Breast/Ovarian
ATM	9	Breast/Ovarian
BRIP1	1	Breast/Ovarian
RAD51C	3	Breast/Ovarian
NBN		Breast/Ovarian
MLH1	1	Lynch
MSH2	2	Lynch
MSH6	2	Lynch
EPCAM		Lynch
PMS2	4	Lynch
APC		Other Cancers
BMPR1A		Other Cancers
SMAD4		Other Cancers
CDK4		Other Cancers
CDKN2A	1	Other Cancers
PALLD		Other Cancers
MET		Other Cancers
MEN1		Other Cancers
RET		Other Cancers
PTCH1		Other Cancers
VHL		Other Cancers
MUTYH	23	Other Cancers

Discussion

Counseling breast/ovarian cancer patients and their families for panel test results can include a level of uncertainty and complexity: Some genes reported by panels confer only a modest increased risk; the spectrum of cancers in a family may or may not fit the spectrum commonly associated with a reported gene; VUS rates increase with panel testing; and finally counselors must address the reproductive and family implications of multiple genes in panels which have both dominant and recessive modes of inheritance. Despite these challenges, the use of panels is increasing. As shown here, panels can generate reliable data and panels can uncover findings relevant to patient management that are otherwise missed. Data such as those reported here may help guide the ongoing discussion of ways to meet these genetic counseling challenges.

Results from this study have been submitted to ClinVar and are also available from the laboratory's web site at http://clinvitae.invitae.com