Development of a Next Generation Sequencing Panel to Assess Hereditary Cancer Risk that Includes Clinical Diagnostic Analysis of the *BRCA1* and *BRCA2* Genes

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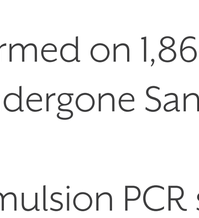
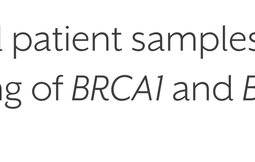
Introduction

- Approximately 7% of breast and 11-15% of ovarian cancers are estimated to be due to germline DNA mutations.
  - The majority of these mutations occur in the *BRCA1* and *BRCA2* genes, but mutations in additional genes have been shown to convey significant cancer risk.
- Sanger DNA sequencing has been the gold standard for detecting genetic variants.
  - Labor-intensive
  - Costly for the analysis of large gene panels
- Next Generation Sequencing (NGS) platforms could be another sequencing alternative.
  - Efficient analysis of larger gene panels
  - Lack of standardization of sample preparation, NGS platforms, and data analysis
- Optimized assay design and validation are critical to maximize the analytical sensitivity and specificity of NGS assays and to ensure high quality interpretation for clinical decision making.

Methods

- Initial assessment of analytical sensitivity and specificity was performed by comparing *BRCA1* and *BRCA2*.
- Testing performed on 1,864 anonymized patient samples which had previously undergone Sanger sequencing of *BRCA1* and *BRCA2*
- RainDance emulsion PCR system used to enrich targets for next generation sequencing
- Barcoded samples for multiple patients were pooled and loaded onto Illumina HiSeq and MiSeq sequencers
- NGS data analysis performed using a combination of commercial and laboratory-developed informatic tools

Results

Sanger Sequencing		Next Generation Sequencing
	=	
↓		↓
Identified 15,878 Variants 681 unique variants 482 disease-associated variants		Identified 15,877 Variants No additional variants found by NGS
↓		↓
Analytical Sensitivity > 99.98% Analytical Specificity > 99.98%		Analytical Sensitivity > 99.96% Analytical Specificity > 99.96% One rare variant missed due to a variant under the primer Sensitivity optimized through process improvements Informatics detection of low coverage regions suggestive of potential deletions or allele dropout

Application - Development of 25 Gene Panel Assay

- Optimization of *BRCA1*/*BRCA2* analysis by NGS facilitated assay validation of 25-gene hereditary cancer panel that includes sequencing and large rearrangement analysis.
- BRCA1/BRCA2 testing is part of the 25 Gene NGS Panel
- We developed and validated a 25-gene NGS hereditary cancer panel (myRisk Hereditary Cancer™) that evaluates a broad number of hereditary cancer syndromes to help define patients' cancer risk and management options with a focus on eight primary cancer sites.

Cancer Genes and Associated Syndromes Targeted by 25 Gene Panel

	Syndrome Name	Breast	Ovarian	Colon	Endometrial	Melanoma	Pancreas	Gastric	Prostate	Other cancer/ Clinical parameters
BRCA1	Hereditary Breast and Ovarian Cancer syndrome (HBOC)	BR	OV				PA		PR	
BRCA2	Hereditary Breast and Ovarian Cancer syndrome (HBOC)	BR	OV			ME	PA		PR	
MLH1	Lynch syndrome		OV	CO	EN		PA	GA		OC
MSH2	Lynch syndrome		OV	CO	EN		PA	GA		OC
MSH6	Lynch syndrome		OV	CO	EN		PA	GA		OC
PMS2	Lynch syndrome		OV	CO	EN		PA	GA		OC
EPCAM	Lynch syndrome		OV	CO	EN		PA	GA		OC
APC	Familial Adenomatous Polyposis (FAP) syndrome/ Attenuated Familial Adenomatous Polyposis (AFAP) syndrome			CO			PA	GA		OC
MUTYH	*MUTYH*-associated Colon Cancer Risk; *MUTYH*-associated Polyposis syndrome (MAP)			CO						OC
CDKN2A	Melanoma-Pancreatic Cancer syndrome					ME	PA			
PALB2	*PALB2*-associated Cancer Risk	BR					PA			
STK11	Peutz-Jegher syndrome	BR	OV	CO	EN		PA	GA		OC
PTEN	PTEN Hamartoma Tumor Syndrome (PHTS)	BR		CO	EN					OC
TP53	Li-Fraumeni Syndrome (LFS)	BR	OV	CO	EN	ME	PA	GA	PR	OC
CDH1	Hereditary Diffuse Gastric Cancer syndrome (HDGC)	BR		CO				GA		
BMPR1A	Juvenile Polyposis Syndrome (JPS)			CO			PA	GA		OC
SMAD4	Juvenile Polyposis Syndrome (JPS)			CO			PA	GA		OC
ATM	*ATM*-associated Cancer Risk	BR					PA			OC
BARD1	*BARD1*-associated Cancer Risk	BR								OC
BRIP1	*BRIP1*-associated Cancer Risk	BR	OV							
CDK4	Melanoma Cancer Syndrome (MCS)					ME	PA			
CHEK2	*CHEK2*-associated Cancer Risk	BR		CO					PR	
NBN	*NBN*-associated Cancer Risk	BR							PR	
RAD51C	*RAD51C*-associated Cancer Risk	BR	OV							
RAD51D	*RAD51D*-associated Cancer Risk		OV							

Technical Components and Validation of 25 Gene Panel

Next Gen Sequencing

- RainDance emPCR prep
- LR-PCR for *PMS2*, *CHEK2*
- Illumina HiSeq, MiSeq NGS
- Sanger confirmation

Large Rearrangements

- Microarray CGH
  - Custom array
  - Confirm LRs
- MLPA for *PMS2*, *CHEK2*
  - Gene vs pseudogene
  - LR vs gene conversion

Review and Reporting

- Informatics, LIMS workflow
- Variant classification
- Unusual case pathway
- Reporting

- **100% concordance** on NGS/Sanger parallel sequencing
  - 100 anonymized DNAs with 3923 variants in hereditary cancer panel genes
- Estimate >99.92% analytical sensitivity, specificity for gene panel
- Large Rearrangements (LR) validated with:
  - Microarray for 23 genes (212 DNAs with 51 genomic positive controls)
  - MLPA for *PMS2* and *CHEK2* (110 DNAs with 5 genomic positive controls)
  - Genomic positive controls supplemented with synthetic controls

Conclusions

- Preliminary analysis of the *BRCA1* and *BRCA2* genes has facilitated assay optimization and led to a comprehensive validation of all 25 genes in the NGS panel.
- Validation studies show that a NGS gene panel designed to meet rigorous quality standards provides clinically actionable results equivalent to those obtained from Sanger DNA sequencing analysis.

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