Design and enhanced validation of a 36-gene guideline-compliant inherited cancer panel

Genevieve Gould, PhD; Greg Hogan, PhD; Xin Wang, PhD; Alex Robertson, PhD; Mark Theilmann; Lindsay Spurka; Peter Grauman; Valentina Vysotskaia, PhD; Matthew Leggett; Jared Maguire; Peter Kang, MD; Imran S. Haque, PhD; Kaylene Ready, MS CGC; Eric A. Evans, PhD

South San Francisco, California

Introduction

The past two decades have brought many important advances in our understanding of the hereditary susceptibility to cancer. Multiple studies have provided convincing evidence that identification of germline mutations associated with hereditary cancer syndromes can lead to reductions in morbidity and mortality through targeted risk management options. Here, we describe the Counsyl *Inherited Cancer Screen* for detecting single-nucleotide variants (SNVs), short insertions and deletions (indels), and copy number variants (CNVs) in 36 genes, listed below.

	Cancers with Increased Risk*										
Gene	Br	Ov	En	Co	Pa	Ga	Pr	Th	Me	Ne	Guidelines**
APC				●	●	●		●			●
ATM	●				●						●
BARD1	●	●									
BMPR1A				●		●					●
BRCA1	●	●			●		●				●
BRCA2	●	●			●		●		●		●
BRIP1		●									●
CDH1	●			●		●					●
CDK4									●		
CDKN2A					●				●		
CHEK2	●			●							●
EPCAM		●	●	●	●	●					●
GREM1				●							●
MEN1										●	
MLH1		●	●	●	●	●					●
MRE11A	●	●									
MSH2		●	●	●	●	●					●
MSH6		●	●	●	●	●					●
MUTYH				●							●
NBN	●										
PALB2	●				●						●
PMS2		●	●	●	●	●					●
POLD1			●	●							●
POLE				●							●
PTEN	●		●	●				●			●
RAD50	●	●									
RAD51C		●									●
RAD51D		●									●
RET								●		●	●
SDHA										●	
SDHB										●	
SDHC										●	
SMAD4				●		●					●
STK11	●	●	●	●	●	●					●
TP53	●	●	●	●	●	●	●	●	●		●
VHL										●	

Breast **En**dometrial **Pa**ncreatic **Pr**ostate **Me**lanoma
Ovarian **Co**lorectal **Ga**stric **Th**yroid **Ne**uroendocrine

* Table not representative of all possible cancer risks.
** Gene with clinical management guidelines from NCCN[1–5].

Study design

The validation study included reference cell lines and previously tested patient specimens encompassing technically challenging variants such as CNVs and large indels. We also performed in-silico CNV simulations.

Measure(s)	Variant Type(s)	Source	n
Accuracy, Sensitivity, Specificity	SNPs and INDELs	1000 Genomes and Hap-Map Projects	85
Intra-assay Reproducibility	SNPs and INDELs	NIST Standards	8
		Patient Samples	13
Inter-assay Reproducibility	SNPs and INDELs	NIST Standards	8
		Patient Samples	84
Sensitivity	Single- and multi-exon CNVs	Patient Samples	50
Sensitivity	Challenging INDELs (≥ 5 bp)	Patient Samples	83
Sensitivity	Alu Insertions	Patient Samples	5

Study results

Accuracy—DNA from reference cell lines

SNPs & INDELs		
True Positives	4146	
False Positives	0	
True Negatives	48383	
False Negatives	0	
Accuracy	100.00%	[99.993, 100.00%]
Sensitivity	100.00%	[99.91, 100.00%]
Specificity	100.00%	[99.992, 100.00%]

True Negatives defined as number of homozygous reference calls made at sites for which an alternative variant was observed in at least one sample in the cohort.

Reproducibility

Source	n	Reproducibility Measure	SNPs	INDELs
NIST Standards	8	Intra-assay	3649 / 3649 **100%** [99.90, 100%]	62 / 62 **100%** [94, 100%]
	8	Intra-assay	2439 / 2439 **100%** [99.85, 100%]	34 / 34 **100%** [90, 100%]
Patient Samples (blood or saliva)	13	Intra-assay	1774 / 1774 **100%** [99.79, 100%]	35 / 35 **100%** [90, 100%]
	84	Intra-assay	9609 / 9609 **100%** [99.96, 100%]	172 / 173* **99.4%** [96.8, 100%]

*Single discordance at a homopolymer site in an intron of the *ATM* gene that is difficult to sequence. The called alleles are classified as benign and were not subject to manual review.

Technically challenging variants

We tested samples with challenging INDELs (those ≥ 5 bp), CNVs of various sizes (single-exon to full gene, deletions and duplications in 15 genes). To test the sensitivity of our assay and bioinformatics pipeline for known Alu insertion detection, we included 5 positives in two genes.

	INDELs	CNVs	Alus
True Positives	83	50	5
False Positives	0	0	0
True Negatives	3569	1000	5
False Negatives	0	0	0
SENSITIVITY	100% [96, 100%]	100% [93, 100%]	100% [48, 100%]
SPECIFICITY	100% [99.9, 100%]	100% [99.6, 100%]	100% [28, 100%]

INDELs by size:
5-10 bp (n=63), 11-20 bp (n=9), >20 bp (n=11)

Alu Insertions:
BRCA2 c.156_157insAlu (Portuguese Founder Mutation, n=2)
ATM c.8010+13_8010+14insAlu (n=3)

In-silico simulated CNVs

Testing patient and reference samples with known CNVs is the existing gold standard to assess CNV detection performance. However, many regions go untested by this method as there are no controls available with known CNVs of all sizes in all genes. Thus, to more comprehensively assess our ability to call CNVs ranging in size from single-exons to whole genes, we generated synthetic deletions and duplications for all 36 genes on our panel.

Deletion Sensitivity (by Simulation):
99.60% [99.50%, 99.72%]

Duplication Sensitivity (by Simulation):
99.00% [98.82%, 99.19%]

Conclusion

We assessed test performance across a broad range of genomic alteration and clinical specimen types to support clinical use related to risk of developing hereditary cancers. The test is now offered by Counsyl's laboratory, which is CLIA certified (05D1102604), CAP accredited (7519776), and NYS permitted (8535).

REFERENCES: 1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer Risk Reduction Version 2.2015. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed August 1st, 2015. **2.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer Screening and Diagnosis Version 1.2015. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed August 1st, 2015. **3.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)for Gastric Cancer Version 3.2015. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed August 1st, 2015. **4.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High Risk Assessment: Breast and Ovarian Version 01.2016. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed February 20th, 2016. **5.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High Risk Assessment: Colorectal Version V.2.2015. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed November 2nd, 2015. for Neuroendocrine Version 1.2015. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed August 1st, 2015. To view the most recent and complete version of the guidelines, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc.

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