Clinical Validation of a Preventative Genetics NGS Assay for CDC Tier 1 & PGx

Moore, T[1], Kloske, D[1], Stoner, M[1], Weber, N[2], Parker, J[1], Vasenkova, I[1]

[1]Kailos Genetics, [2]Keck Graduate Institute

Introduction

Using available evidence-based guidelines and recommendations, CDC Tier 1 genomic applications have the potential for positive impact on public health. Approximately 2 million people in the U.S. are at an increased risk due to genetic changes which predispose them to Hereditary Breast and Ovarian Cancer Syndrome (HBOC); Lynch syndrome (LS) associated with mutations in mismatch-repair genes; or familial hypercholesterolemia (FH) increased risk for heart disease or stroke with mutations leading to very high cholesterol levels from an early age. Many individuals and families affected by these variants are not aware that they are at risk; however, early detection and intervention could significantly reduce morbidity and mortality.

Gene	Category	Disease/Disorder	Coverage
APOB	Hereditary Risks	Familial Hypercholesterolemia	All exons
LDLR	Hereditary Risks	Familial Hypercholesterolemia	All exons
PCSK9	Hereditary Risks	Familial Hypercholesterolemia	All exons
Factor II*	Hereditary Risks	Venous thrombosis	SNPs
Factor V*	Hereditary Risks	Venous thrombosis	SNPs
BRCA1	Hereditary Risks	HBOC/Lynch Syndrome	All exons
BRCA2	Hereditary Risks	HBOC/Lynch Syndrome	All exons
EPCAM	Hereditary Risks	Lynch Syndrome	All exons
MLH1	Hereditary Risks	Lynch Syndrome	All exons
MSH2	Hereditary Risks	Lynch Syndrome	All exons
MSH6	Hereditary Risks	Lynch Syndrome	All exons
PMS2	Hereditary Risks	Lynch Syndrome	All exons
ATM*	Hereditary Risks	Hereditary cancers	All exons
CHEK2*	Hereditary Risks	Hereditary cancers	All exons
NBN*	Hereditary Risks	Hereditary cancers	All exons
PALB2*	Hereditary Risks	Hereditary cancers	All exons
CYP4F2*	PGx	Coagulation medication	SNPs
VKORC1*	PGx	Coagulation medication	SNPs
DPYD*	PGx	Chemotherapy	SNPs
CYP2C19	PGx	SSRI & Opioids	SNPs (34 haplotypes)
CYP2CD6	PGx	SSRI & Opioids	SNPs (129 haplotypes)

Table 1. Prevent Assay gene coverage **Optional*

Reported here are the results of a study to validate a next generation sequencing assay, Kailos Prevent™, designed to detect variants associated with HBOC, LS, and FH; along with the determination of an extensive number of haplotypes, CYP2D6 (129) and CYP2C19 (34), that influence the metabolism of SSRIs and opioids. Included in the study are genetically well characterized samples from Genome-In-A-Bottle and CDC GeT-RM along with dry buccal swabs from patients previously evaluated with hereditary cancer and pharmacogenetic NGS assays.

Methods

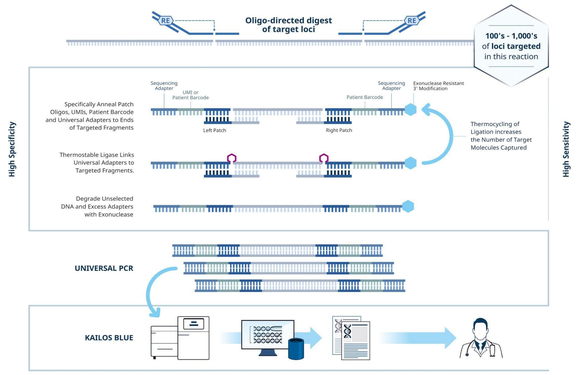


Figure 1. TargetRich capture & enrichment

The Prevent assay utilizes TargetRich™, a proprietary capture and enrichment technique, to remove genomic DNA background and sequencing of over 74,000 targeted bases. This three-step protocol includes the following:

Targeting - A pool of 573 pairs of guide oligos possessing both a type II restriction enzyme recognition site (FokI) and sequences flanking the targeted regions are combined with denatured genomic DNA. Localized double stranded regions are created by the guide oligos at the targeted sites the restriction enzyme cuts at a specific location.

Patching - A pool of 582 pairs of patch oligos comprised of a two-part design: (i) complimentary to the end of the targeted region and (ii) complementary to universal adaptors comprised of a unique molecular identifier (UMI), sample barcodes and P5/P7, are added to the reaction with a thermostable ligase. A high temperature cycled ligation reaction is performed.

Exo/uPCR - The 3' end of the right adapter contains a chemical modification to protect the ligated loci from degradation following the addition of a cocktail of exonucleases; allowing the non-targeted genomic DNA to be digested. The single stranded adapted targets are bead purified prior to universal PCR, sample pooling and loading on the Illumina® flowcell (MiSeq®, NextSeq®).

Result	Sample	# Reads (M)	% Alignment	% Adapter	Avg Read Depth	On Target, *	Read Bases Over 20% of Mean
33065	NA17265.S1	3.85	99.8%	0.50%	4,250.4	90.6%	82.2%
33150	NA17283.S1	3.15	99.8%	0.31%	3,450.7	90.5%	80.2%
33042	NA17235.S1	5.11	99.8%	1.10%	5,605.9	90.4%	78.2%
33069	NA13707.S1	3.87	99.8%	0.25%	4,249.4	90.4%	82.4%
33071	NA13711.S1	4.00	99.8%	0.34%	4,395.2	90.3%	81.6%
33162	NA17285.S1	3.97	99.6%	0.33%	4,358.6	90.3%	82.4%
33198	NA13707.S1	3.90	99.7%	0.33%	4,289.0	90.3%	81.3%
33168	NA13711.S1	4.02	99.7%	0.30%	4,415.6	90.3%	81.4%
33139	NA17235.S1	4.14	99.8%	1.14%	4,481.7	90.2%	78.9%
33165	NA14788.S1	3.96	99.8%	0.41%	4,368.6	90.2%	82.2%
33047	NA17227.S1	4.24	99.7%	0.72%	4,624.0	90.1%	81.2%
33129	NA17227.S1	4.24	99.7%	0.72%	4,624.0	90.1%	81.2%
33155	NA17298.S1	4.33	99.8%	1.00%	4,742.8	90.1%	81.1%
33148	NA17279.S1	4.23	99.8%	0.62%	4,608.1	90.0%	81.9%

Table 2. Example of sample sequencing metrics

Results

GIAB & previously characterized clinical reference samples were used to determine analytical specificity, sensitivity and accuracy across the reportable range by comparing all variant calls.

Utilizing 5 GIAB samples, testing to evaluate the assay's reproducibility and repeatability were performed in triplicate (i) within a single sequencing run, (ii) across two days and (iii) between technologists. Additionally, these tests included changing sample specific molecular barcodes, thermocyclers and sequencers. The concordance of all variant calls between replicas were calculated utilizing *Kailos Blue*, an internally developed software system.

Assay Performance Characteristics	Assay Performance
Sensitivity	98.96%
Specificity	100%
Accuracy	100%
PPV	99.6%

Table 3. Prevent assay performance

		Variant Concordance Run #			Reproducibility Between Runs
		1	2	3	
GIAB Samples	NA12878	97.0%	96.6%	95.1%	95.9%
	NA23143	97.2%	97.3%	98.0%	96.7%
	NA24149	97.1%	97.2%	97.1%	96.9%
	NA24385	94.2%	95.4%	91.8%	93.9%
	NA24631	95.7%	95.7%	95.7%	95.1%

3 reps/run; Different sequencers; Multiple technicians

Table 4. GIAB sample reproducibility & repeatability

Sensitivity in detecting pathogenic variants (SNPs, small deletions & duplications) in samples with known variants in BRCA1, BRCA2, LDLR, ATM, CHEK2, MLH1, MSH2, MSH6, NBN, PALB2, PMS2 was tested in 7 CDC Cancer Predisposition samples and 21 dry buccal swabs samples characterized by a different cancer predisposition assay.

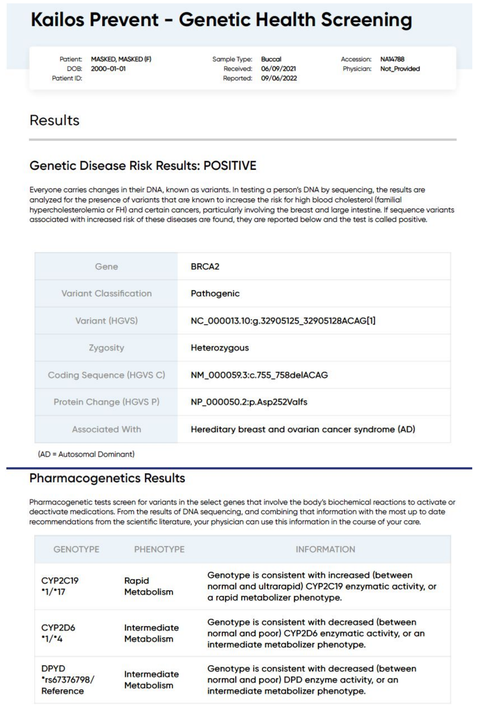
Sample ID	CYP2D6	CYP2C19	Germline Variants
LKG-100595	*1/*1	*2/*9	ATM, rs1290350674, NC_000011.9:g.108198393dup HET, NM_000051.3 c.6997dupA, p.Thr2333Asnfs
LKG-100607	*1/*1	*1/*2xN	ATM, rs587779834 INC_000011.9:g.108155009del
LKG-100609	*1/*1	*4/*35	ATM, rs587779817, NC_000011.9:g.108121754_108121755GA[1]
LKG-100603	*1/*2	*1/*9	CHEK2, rs555607708, INC_000022.10:g.29091857del
LKG-100604	*1/*2	*35/*41	CHEK2, rs121908698, NC_000022.10:g.29121230C>T
LKG-100611	*17/*17	*1/*2	CHEK4, rs17879961, NC_000022.10:g.29121087A>G
LKG-100593	*1/*1	*11/*5	MLH1, rs587779035, NC_000003.11:g.37055921A>G HET, NM_000249.3:c.678-2A>G
LKG-100594	*1/*17	Not Called	MLH1, Novel HET, NM_000249.3 c.2002_2006delGAAGA p.Glu668fs
LKG-100598	*17/*17	*1/*2	MLH1, rs267607760, NC_000003.11:g.37050399A>G
LKG-100591	*1/*1	*1/*2	MSH2, Novel, NM_000251.2 c.328A>T p.Lys110*
LKG-100600	*1/*2	*2/*2	MSH2, NC_000002.11:g.47635682T>G
LKG-100599	*1/*17	*1/*4	MSH6, rs1553333421, NC_000002.11:g.48033639dup
LKG-100601	*1/*2	*1/*2	MSH6, rs267608064, NC_000002.11:g.48026756_48026757del
LKG-100605	*2/*17	*1/*35	NBN, rs587776650, NC_000008.10:g.90983445_90983449del
LKG-100596	*1/*2	*17/*43	NBN, rs764884516, NC_000008.10:g.90965833_90965834delinsT
LKG-100608	*1/*17	*1/*2	PALB2, rs180177110, NC_000016.9:g.23641218G>A
LKG-100610	*1/*17	*2/*4	PALB2, rs515726126, NC_000016.9:g.23647109dup
LKG-100592	*1/*17	*3/*35	PMS2, rs121434629, NC_000007.13:g.6045549C>A HET, NM_000535.7: c.137G>T , p.Ser46Ile
LKG-100597	*1/*1	*1/*2	PMS2, rs587779340, NC_000007.13:g.6043425T>A
LKG-100602	*1/*1	*2/*4	BRCA1, rs80357906, NC_000017.10:g.41209082dup
LKG-100606	*1/*1	*1/*2	BRCA1, rs45553935, NC_000017.10:g.41209139A>G

Table 5. Dry buccal swabs with pathogenic variants

Conclusions

The Preventative Genetics assay was analytically validated with key performance characteristics surpassing the laboratories performance requirements including: sensitivity (>98%), specificity (100%), accuracy (100%) and PPV (99.6%). Repeatability and reproducibility assessments were determined within a sequencing run (96.2%) and between multiple sequencing processes (95.8%). The determination of genotypes for the cytochrome P-450 and all other genes was 100% for SNPs, small deletions and duplications.

The Preventative Genetics Assay is suitable for use as a preventative screening to identify the small but significant group of individuals that are at an elevated risk of serious disease or reactions, enabling appropriate care to mitigate risk and potentially improve outcomes.



Kailos Prevent – Genetic Health Screening

Patient: MASKED, MASKED (F)
DOB: 2000-01-01
Patient ID:
Sample Type: Buccal
Received: 06/09/2021
Reported: 09/06/2022
Accession: NA14788
Physician: Not_Provided

Results

Genetic Disease Risk Results: POSITIVE

Everyone carries changes in their DNA, known as variants. In testing a person's DNA by sequencing, the results are analyzed for the presence of variants that are known to increase the risk for high blood cholesterol (familial hypercholesterolemia or FH) and certain cancers, particularly involving the breast and large intestine. If sequence variants associated with increased risk of these diseases are found, they are reported below and the test is called positive.

Gene	BRCA2
Variant Classification	Pathogenic
Variant (HGVS)	NC_000013.10:g.32905125_32905128ACAG[1]
Zygosity	Heterozygous
Coding Sequence (HGVS C)	NM_000059.3:c.755_758delACAG
Protein Change (HGVS P)	NP_000050.2:p.Asp252Valfs
Associated With	Hereditary breast and ovarian cancer syndrome (AD)

(AD = Autosomal Dominant)

Pharmacogenetics Results

Pharmacogenetic tests screen for variants in the select genes that involve the body's biochemical reactions to activate or deactivate medications. From the results of DNA sequencing, and combining that information with the most up to date recommendations from the scientific literature, your physician can use this information in the course of your care.

GENOTYPE	PHENOTYPE	INFORMATION
CYP2C19 *1/*17	Rapid Metabolism	Genotype is consistent with increased (between normal and ultrarapid) CYP2C19 enzymatic activity, or a rapid metabolizer phenotype.
CYP2D6 *1/*4	Intermediate Metabolism	Genotype is consistent with decreased (between normal and poor) CYP2D6 enzymatic activity, or an intermediate metabolizer phenotype.
DPYD *rs67376798/ Reference	Intermediate Metabolism	Genotype is consistent with decreased (between normal and poor) DPD enzyme activity, or an intermediate metabolizer phenotype.

Figure 2. Extracts of the Prevent Assay clinical report

Bibliography

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