Comparison of Rare Germline Variants Associated With Cancer Predisposition Syndromes In Taiwan And USA Populations

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BACKGROUND AND PURPOSE

Germline mutations that increase the susceptibility of an individual to certain cancers have been identified in numerous genes, and individuals can be tested for mutations in these genes to understand their inherited lifetime risk for developing cancer [1-5]. Traditional approaches, using Sanger sequencing or qPCR, focus on small groups of genes and therefore present a difficulty to screen for mutations in numerous genes from multiple individuals synchronically. As the landscape of genes that are important to hereditary cancer risk grows, it is becoming increasingly important to develop methods to interrogate multiple genes that may carry mutations. With rapid advances in next-generation sequencing (NGS) technology and bioinformatics, testing of multiple genes from multiple individuals synchonically with panel-based tests is becoming increasingly common.

Expert-Curated, Comprehensive 98-Gene Panel

AIP	*ALK*	*APC*	*ATM*	*BAP1*	*BARD1*	*BLM*	*BMPR1A*	*BRCA1*
BRCA2	*BRIP1*	*BUB1B*	*CDC73*	*CDH1*	*CDK4*	*CDKN1C*	*CDKN2A*	*CEBPA*
CEP57	*CHEK2*	*CYLD*	*DDB2*	*DICER1*	*DIS3L2*	*EGFR*	*EPCAM*	*ERCC2*
ERCC3	*ERCC4*	*ERCC5*	*EXT1*	*EXT2*	*EZH2*	*FANCA*	*FANCB*	*FANCC*
FANCD2	*FANCE*	*FANCF*	*FANCG*	*FANCI*	*FANCL*	*FANCM*	*FH*	*FLCN*
GATA2	*GPC3*	*HNF1A*	*HOXB13*	*HRAS*	*KIT*	*MAX*	*MEN1*	*MET*
MLH1	*MSH2*	*MSH6*	*MUTYH*	*NBN*	*NF1*	*NF2*	*NSD1*	*PALB2*
PHOX2B	*PMS1*	*PMS2*	*PPM1D*	*PRF1*	*PRKAR1A*	*PTCH1*	*PTEN*	*RAD51C*
RAD51D	*RB1*	*RECQL4*	*RET*	*RHBDF2*	*RUNX1*	*SBDS*	*SDHAF2*	*SDHB*
SDHC	*SDHD*	*SLX4*	*SMAD4*	*SMARCA4*	*SMARCB1*	*STK11*	*SUFU*	*TMEM127*
TP53	*TSC1*	*TSC2*	*VHL*	*WT1*	*WRN*	*XPA*	*XPC*	

[Fig.1] The 98 genes included in the CellMax Life DNA Genetic Cancer Risk Test. Genes associated with hereditary cancer syndromes and cancer risk were included when there was sufficient evidence in the literature that mutations in those genes greatly affects cancer risk.

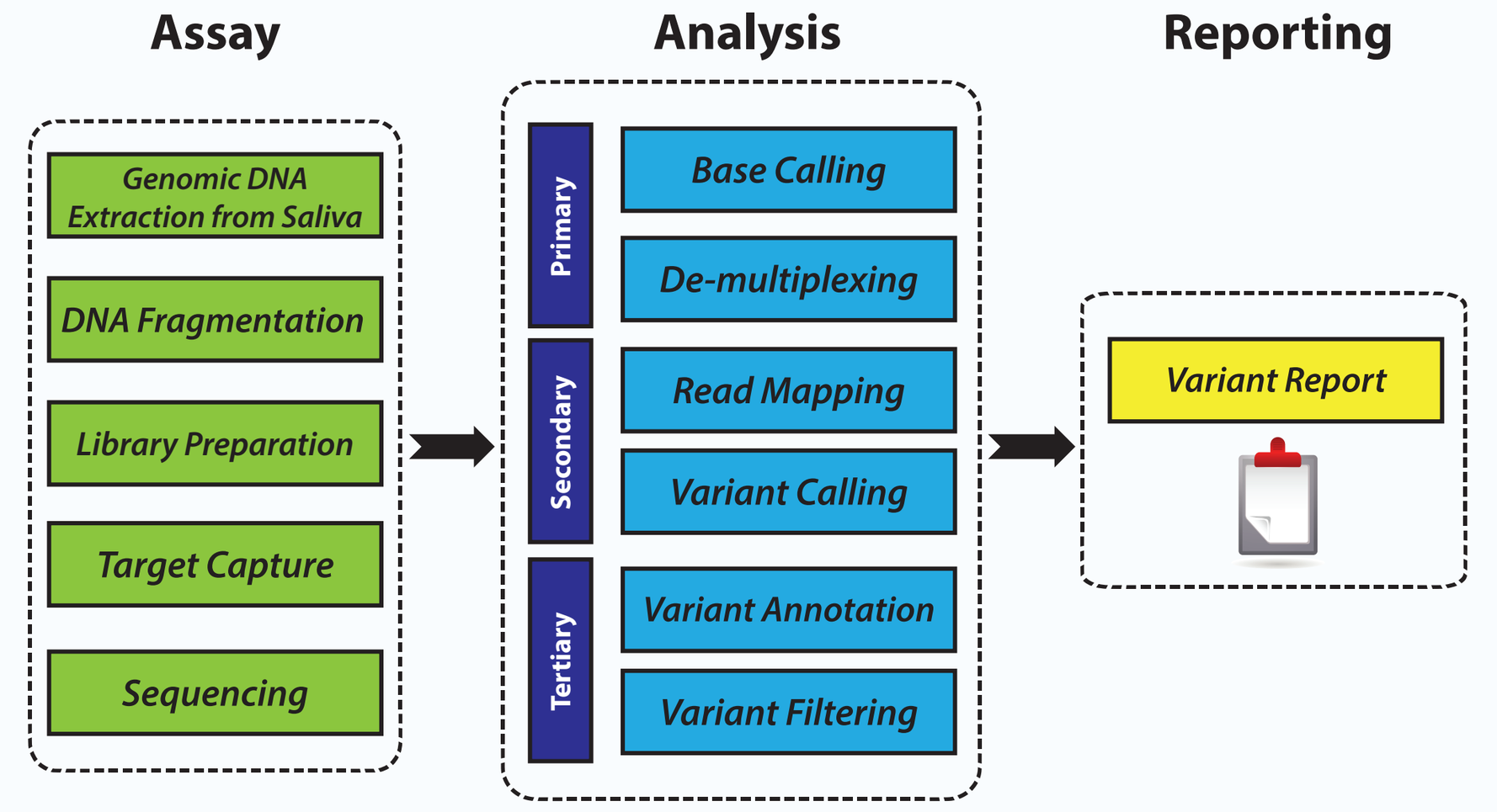
The **CellMax DNA Genetic Cancer Risk Test** was designed to allow the analysis of genes and genetic variants associated with predisposition to cancer. The test targets germline variants detectable in white blood cell DNA. The **98 gene panel [Fig.1]** was selected based on systematic, expert review of the scientific literature to identify genes that predispose to cancer, and focuses on genes for which there is already strong evidence of such predisposition. The focus of this study was to evaluate the analytical and clinical performance of the CellMax DNA Genetic Cancer Risk Test, and to observe the prevalence of highly-penetrant, rare, pathogenic germline variants strongly linked to predisposition of cancers in **patient populations from Taiwan and USA**.

METHODS AND STUDY DESIGN

The CellMax DNA Genetic Cancer Risk Test covers all coding exons, nearby flanking regions (+/- 20bp), and known splice-sites in the 98 gene panel. The test detects single nucleotide substitutions, insertions and deletions in genomic DNA with a mean coverage of >100X for each sample.

As part of this study, we validated the 98 gene panel prior to sequencing the patient samples from the study cohorts. The assay validation design completely took the advantage of the availability of **gold standard reference samples**. The lab performed **end-to-end sample processing and data analysis** with 19 unique gold standard reference samples run over 38 different library preprations throughout the analytical validation. After processing from DNA input through variant calling **[Fig.2]**, the data produced is compared to the high-quality reference calls to assess the assay's analytical performance. A concordance study to compare genotyping performance between blood and saliva-derived DNA was also performed.

CellMax DNA Genetic Cancer Risk Test End-to-End Process


Assay
Analysis
Reporting
Genomic DNA Extraction from Saliva
DNA Fragmentation
Library Preparation
Target Capture
Sequencing
Primary
Secondary
Tertiary
Base Calling
De-multiplexing
Read Mapping
Variant Calling
Variant Annotation
Variant Filtering
Variant Report

[Fig.2] In the CellMax DNA Genetic Cancer Risk Test workflow, genomic DNA is converted to sequence-ready libraries, enriched for target regions, sequenced, and variants are called.

We collected **2,259 patient samples** from two clinical centers for testing on the CellMax DNA Genetic Cancer Risk Test 98 gene panel - **1885 patients from CellMax's CAP laboratory in Taiwan, and 374 patients from CellMax's CAP/CLIA laboratory in California**, many of whom had been referred for hereditary cancer risk counseling or assessment. Blood or saliva specimens were accepted for sample processing. Information on demographics, personal, and family history of cancer was collected at time of sample collection. The mean age of the Taiwan cohort was 43, with 58% males and 42% females, while the US cohort had a mean age of 58 with 40% males and 60% females.

Sequence variant classification as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign and benign was performed according to the American College of Medical Genetics and Genomics (ACMG) sequence variant interpretation guidelines [6]. All classifications were evaluated by a board-certified pathologist and only pathogenic variants were clinically reported.

RESULTS

The analytical sensitivity, specificity and accuracy of the assay panel were **99.95%**, **100%** and **99.99%**, respectively. Similarly, the reportable variants for 30 DNA samples containing clinically important SNVs in *BRCA1/2*, *RET*, *APC*, *MEN1* and *PTEN* were called with no false positives. Paired blood (Reference lab) and saliva (CellMax CLIA lab) samples were run to validate the sample collection protocol. The concordance for the paired blood/saliva sample analysis was found to be **99.99%**.

Analytical Validation Summary

Analytical Validation	Variant Present	Variant Not Present
Variant Detected	1841 true positives	0 false positives
Variant Not Detected	1 false negative	3,898,611 expected reference calls
Results	99.95% Sensitivity	100% Specificity

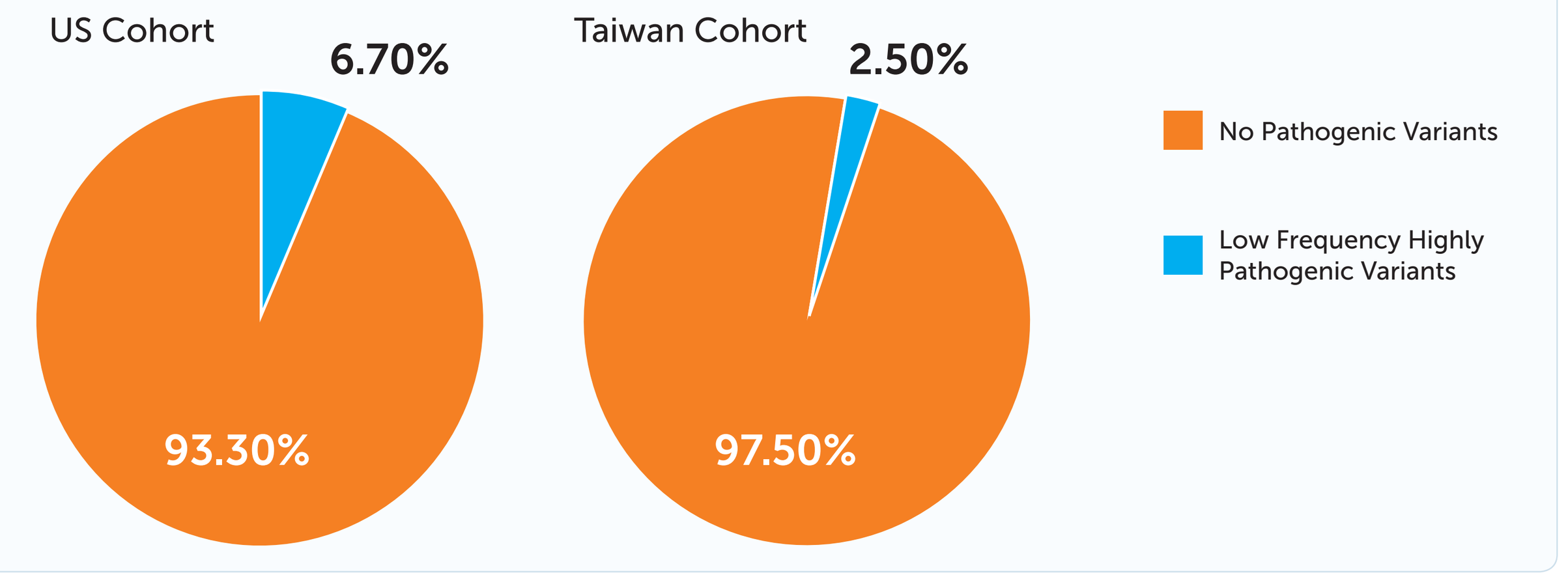
[Fig.3] Three sets of gold standard reference samples and data run over several different library preparations were used to analytically validate the end-to-end sample process and data analysis.

Summary of Results

- Germline pathogenic variants were detected in **47/1885 (2.5%)** and **25/374 (6.7%)** of individuals in the Taiwan and US cohort respectively **[Fig.4]**.
- Pathogenic *BRCA1* and *BRCA2* mutations comprised **8.5% and 17%** of total positive cases in Taiwan, and **4% and 0%** of total positive cases in the US.
- Outside of *BRCA1* and *BRCA2*, additional cases of rare pathogenic mutations in Taiwan **35/47 (75%)** and the US **24/25 (96%)** associated with increased cancer risk were identified with our panel **[Fig.5]**, **which would not have been identified if only *BRCA1/2* testing was used**. These genes include *MUTYH*, *MLH1*, *RET*, *ATM*, *APC*, *CHEK2*, *ERCC2*, *PALB2*, *PTCH1*, *WRN*, *BARD1*, *BRIP1*, *PMS2*, *PTEN*, *RAD51D*, *SDHD*, *RUNX1*, *BLM*, *HOXB13*, and *MSH6* **[Fig.6]**.
- **76% of non-*BRCA1/2* findings in the US** and **40.5% of non-*BRCA1/2* findings in Taiwan** are in genes which have well-defined national guidelines outlining care and would warrant consideration of a change in patient care.
- The pathogenic variants within non-*BRCA1/2* genes are associated with breast/ovarian cancer genes (*ATM*, *CHEK2*, *PALB2*), MUTYH-associated Polyposis syndrome gene (*MUTYH*), and Lynch syndrome genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*).

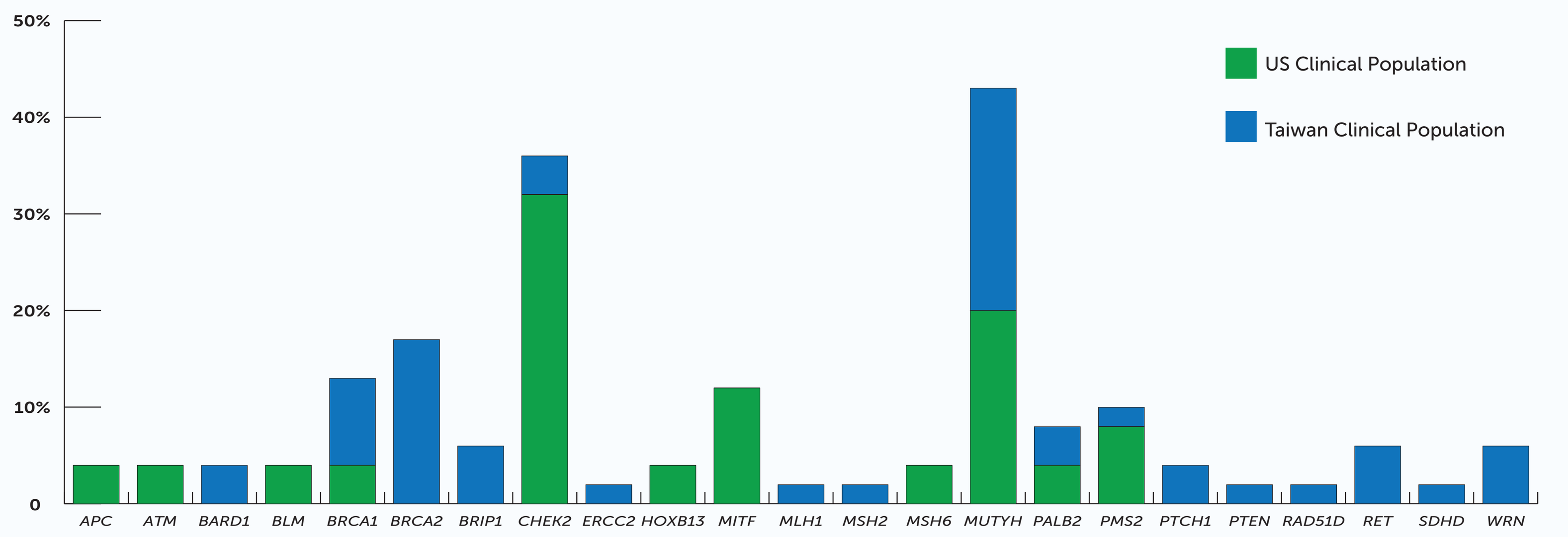
Percentage of Pathogenic Variants in Two Diverse Clinical Populations

Country	# of Patients	Mean Age	% Males	% Females	Cases with pathogenic variant identified	Percentage of pathogenic variant cases
USA	374	58	40%	60%	25	**6.70%**
Taiwan	1885	43	58%	42%	47	**2.50%**



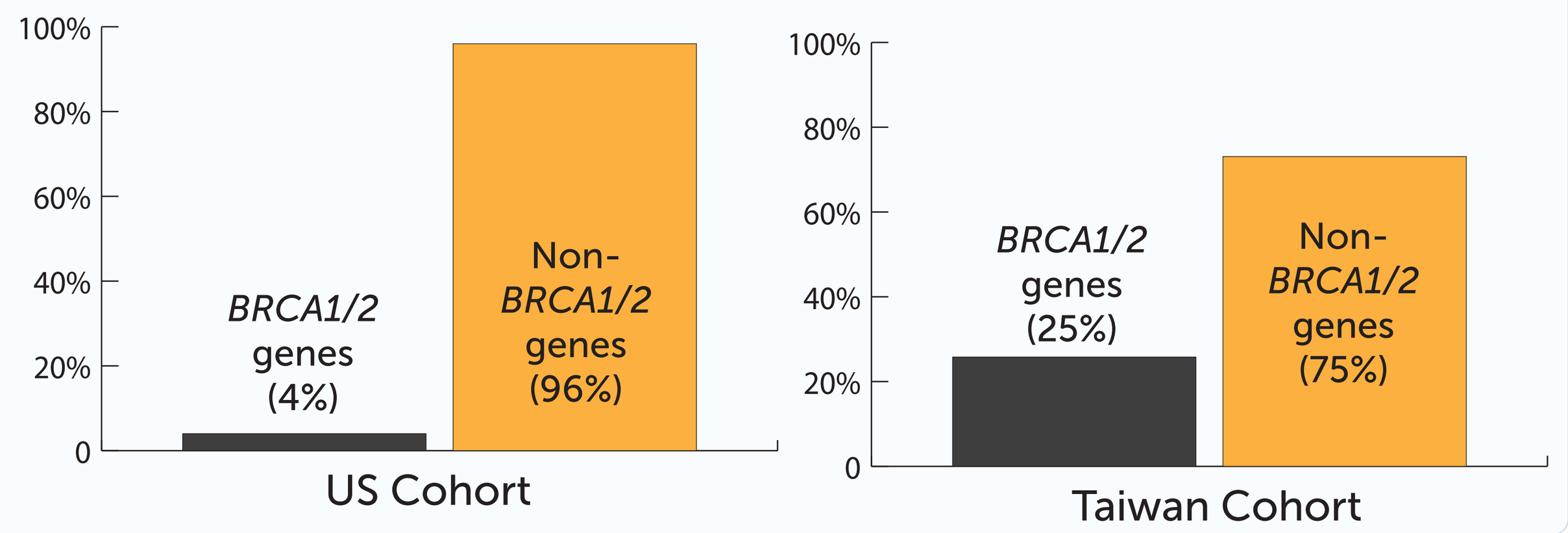
[Fig.4] The study cohorts consisted of 374 patients from the US and 1885 patients from Taiwan. These patients were referred for hereditary cancer risk counseling or assessment. Prevalence of pathogenic mutations was observed to be higher in the US cohort.

Distribution of Identified Pathogenic Variants By Gene In Two Clinical Populations



[Fig.6] Mutation prevalence among patients from two ethnically diverse populations. *BRCA1/2* was more common in the Taiwan cohort, whereas *CHEK2*, *PMS2*, and *MITF* were more common in the US cohort.

Percentage of *Brca1/2* vs Other Pathogenic Mutations in Two Clinical Populations



[Fig.5] *BRCA1/2* testing alone would only have identified 12 individuals from the Taiwan cohort and 1 individual in the US cohort. CellMax Life multi-gene panel testing identified other important pathogenic mutations in 35 additional patients from Taiwan and 24 additional patients from the US.

CONCLUSIONS

We have demonstrated in 2 clinical cohorts the analytical and clinical validity of a 98 gene panel for the detection of rare pathogenic germline variants associated with cancer predisposition. High sensitivity and specificity of this panel was demonstrated irrespective of the use of blood or saliva as samples. This study provides useful data of the prevalence for many cancer predisposition genes in two diverse ethnic groups. The frequencies of pathogenic mutations within these cohorts revealed a disparity between racial/ethnic groups with similar risks. Multi-gene panel testing using next-generation sequencing provides more comprehensive, cost effective, and clinically actionable assessment compared to traditional single gene hotspot testing for hereditary cancer risk.

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