

Enhancement of History Weighting Analysis to Accurately Classify Variants in High and Moderate Risk Cancer Panel Genes

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BACKGROUND

- Historically, sequencing analysis to detect Hereditary Breast and Ovarian Cancer syndrome (HBOC) associated pathogenic mutations was performed for *BRCA1* and *BRCA2* alone.
- Previously, we have developed a statistical family History Weighting Algorithm (HWA), which accurately reclassifies variants of uncertain significance (VUS) in *BRCA1* and *BRCA2* as pathogenic or benign based on the severities of personal and family cancer histories associated with each specific variant.¹
- We have enhanced this algorithm to include analysis of genes associated with an increased risk of breast cancer that are included in pan-cancer panel testing.

METHODS

PATIENT ASCERTAINMENT

- Informed consent for was obtained for clinical genetic testing using small or pan-cancer panels (Table 1).
- Qualified healthcare providers completed a test requisition form, providing proband age, ancestry, personal cancer history and age of diagnosis (if applicable). A list of affected relatives including cancer type(s) and age(s) of diagnosis was also requested.

Table 1. Clinical Genetic Testing

Test	Genes Included
Small Panel*	<i>BRCA1, BRCA2</i>
Pan-Cancer Panel**	<i>BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, EPCAM, ATM, CHEK2, PALB2, MUTYH, APC, PTEN, TP53, STK11, SMAD4, CDH1, BARD1, BRIP1, CDKN2A, CDK4, BMPR1A, RAD51C, RAD51D</i>

*Sequencing performed for all genes; Large rearrangement (LR) may have been performed
**Sequencing and LR analysis for all genes, except for *EPCAM* (LR analysis only)

HISTORY WEIGHTING ANALYSIS

- HWA data obtained from small panel testing and pan-cancer panel testing, including *BRCA1* and *BRCA2*, were determined to have no significant cohort differences (data not shown). As such, the cohorts were combined.
- The HWA was based on the previously described methodology¹ and updated to utilize data from the combined cohort for analysis of *BRCA1* and *BRCA2*, as described below. Additional modifications were made to the HWA for analysis of *ATM*, *CHEK2*, and *PALB2*.
- HWA performance was assessed through analysis of simulated variants for each gene, and positive (PPV) and negative predictive values (NPV) were calculated on a per gene basis, as appropriate.

HISTORY WEIGHTING SCORE (HWS) CALCULATION

- The personal and family history (P/FHx) of each proband carrying the variant of interest was scored for the presence of gene-associated cancer(s).
- Based on empirical analysis of >1 million patients, a statistical weight was assigned to the P/FHx of each proband carrying the specific variant. These weights were combined to determine the final HWS for the variant of interest.

COMPARISON OF VARIANT-SPECIFIC HWS TO CONTROLS

- Variant-specific HWSs were compared to pathogenic and benign control HWS distributions composed of HWS scores from 10,000 pathogenic and 10,000 benign composite control variants (Figure 1A).

HWS RESULT: BENIGN

The variant-specific HWS was >99.5th percentile plus a gene-specific number of standard deviations of the positive control HWS distribution, and >1st percentile of the negative control HWS distribution.

HWS RESULT: PATHOGENIC

The variant-specific HWS was <0.5th percentile minus a gene-specific number of standard deviations of the negative control HWS distribution, and <99th percentile of the positive control HWS distribution.

HWA TESTING

- Algorithm performance was assessed through gene-specific two-fold cross-validations of conditional probability tables performed on simulated variants for *BRCA1* and *BRCA2*.
- Testing utilizing data from all available probands was performed on simulated variants in *ATM*, *CHEK2* and *PALB2*.

RESULTS

Table 2. Simulated variant testing results for *BRCA1* and *BRCA2*. PPV and NPV are adjusted for prevalence.

		HWA Classification - Pathogenic				HWA Classification - Benign			
		Fold 1		Fold 2		Fold 1		Fold 2	
Gene	True Classification	# Pathogenic Calls	PPV	# Pathogenic Calls	PPV	# Benign Calls	NPV	# Benign Calls	NPV
<i>BRCA1</i>	Pathogenic 25,500 trials	24,523	0.9978	24,870	0.9960	282	0.9983	224	0.9987
	Benign 50,500 trials	16		29		50,032		49,735	
<i>BRCA2</i>	Pathogenic 25,125 trials	22,898	0.9988	21,570	0.9980	493	0.9982	852	0.9969
	Benign 50,125 trials	5		8		49,629		49,670	

Table 3. Simulated variant testing results for *ATM*, *CHEK2* and *PALB2*. NPV is adjusted for prevalence.

		HWA Classification		
		Pathogenic	Benign	NPV
<i>ATM</i>	Pathogenic 25,031 trials	23,527	440	0.9980
	Benign 5,031 trials	11	5,013	
<i>CHEK2</i>	Pathogenic 25,031 trials	23,756	171	0.9983
	Benign 5,031 trials	10	4,985	
<i>PALB2</i>	Pathogenic 25,125 trials	24,737	218	0.9990
	Benign 5,125 trials	16	5,098	

- The HWA was developed and tested on a clinical dataset consisting of >1 million probands tested for hereditary cancer risk using panel testing.
- Two-fold cross validations performed on >75,000 pathogenic or benign simulated variants resulted in PPV and NPV of ≥0.9960 for *BRCA1* and *BRCA2* (Table 2).
- Analysis of additional variants simulated from our pan-cancer panel-tested patient dataset yielded NPV of ≥0.9980 for the *ATM*, *CHEK2* and *PALB2* genes (Table 3).
- PPV were not calculated for *ATM*, *CHEK2*, and *PALB2* as the HWA is not currently designed to upgrade variants within these genes.

CONCLUSIONS

- We have modified our HWA to allow for combined use of genetic and clinical data obtained from both *BRCA1/BRCA2* testing alone and larger pan-cancer panel testing.
- Extensive testing of the HWA indicates that it is highly accurate for upgrading and downgrading VUSs in *BRCA1* and *BRCA2* to more definitive clinical classifications.
- Additional HWA modifications demonstrate that this technique can be used to accurately reclassify variants in *ATM*, *CHEK2*, and *PALB2*, for which use of other reclassification techniques is severely limited.
- As additional data is obtained through ongoing patient testing, it may be possible to extend the use of the HWA to more genes within the current or a future pan-cancer gene panel.

REFERENCES

1. Pruss D et al. *Breast Cancer Res Treat.* 2014; 147:119-32

