Reclassification of Uncertain Variants Identified in High and Moderate Cancer Risk Genes Using History Weighting Analysis

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BACKGROUND

- We have previously developed and implemented a statistical family History Weighting Algorithm (HWA), which accurately reclassifies variants of uncertain significance (VUS) as pathogenic or benign based on the severities of personal and family cancer histories associated with each specific variant.[1]
- This algorithm was specific to small gene panels composed of *BRCA1*/*BRCA2* or Lynch syndrome genes (*MLH1*, *MSH2*, *MSH6*).
- We have expanded the use of this algorithm to incorporate data obtained from pan-cancer panel testing and reclassify VUSs in additional genes.

METHODS

PATIENT ASCERTAINMENT

- Informed consent for clinical genetic testing (Table 1) was obtained. Qualified healthcare providers completed a test requisition form, which requested: 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 Panels*	HBOC: *BRCA1, BRCA2*	LS: *MLH1, MSH2, MSH6, PMS2**, EPCAM***
Pan-Cancer Panel†	*BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, EPCAM, ATM, CHEK2, PALB2, MUTYH, APC, PTEN, TP53, STK11, SMAD4, CDH1, BARD1, BRIP1, CDKN2A, CDK4, BMPR1A, RAD51C, RAD51D*	

HBOC: Hereditary Breast and Ovarian Cancer; LS: Lynch Syndrome
*Sequencing performed for all genes; Large rearrangement (LR) may have been performed
**Not included in all LS testing
†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 was analyzed and significant cohort differences were not present (data not shown). As such, the cohorts were combined.
- HWA was based on the previously described methodology[1] and updated to utilize data from the combined cohort for analysis of *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, and *MSH6*, as described below. Additional modifications to the HWA were made to allow 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*, *BRCA2*, *MLH1*, *MSH2* and *MSH6*.
- Testing utilizing data from all available probands was performed on *ATM*, *CHEK2* and *PALB2* simulated variants.

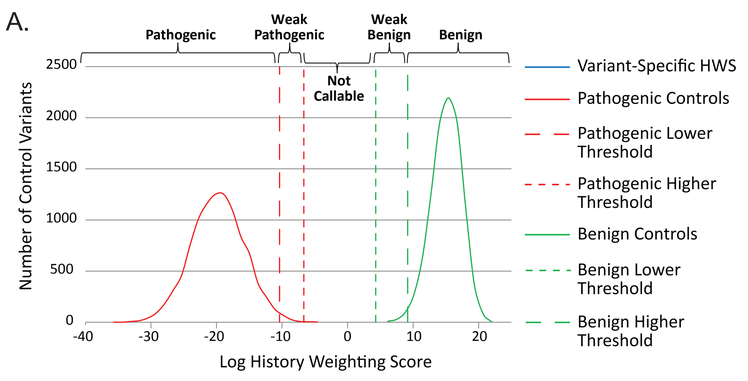
RESULTS

- 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 >0.996 for *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, and *MSH6* (Table 2).
- Analysis of additional variants simulated from our full 25-gene panel-tested patient dataset yielded NPV of > 0.998 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.

Table 2. *Simulated variant testing results for BRCA1, BRCA2, MLH1, MSH2 and MSH6. 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
BRCA1	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	
BRCA2	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	
MLH1	Pathogenic 25,500 trials	24,880	0.9962	24,836	0.9978	176	0.9978	166	0.9979
	Benign 50,500 trials	60		35		49,748		49,765	
MSH2	Pathogenic 25,500 trials	24,775	0.9988	24,262	0.9986	111	0.9991	164	0.9987
	Benign 50,500 trials	12		14		50,329		50,243	
MSH6	Pathogenic 25,500 trials	23,787	0.9987	24,014	0.9965	180	0.9990	88	0.9995
	Benign 50,500 trials	9		25		50,262		49,754	

Figure 1. *A) Illustration of a HWA graph. The variant-specific HWS is compared to those of 10,000 deleterious and 10,000 benign composite control variants. Variant classification categories (top) are defined by thresholds based on composite control HWS distributions. B) HWA graphs illustrating classification calls for select variants.*



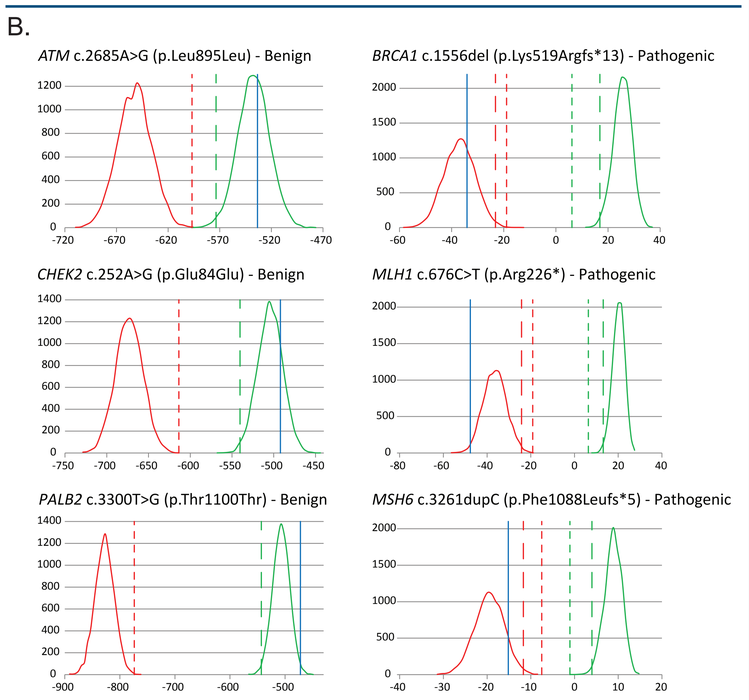


Table 3. *Simulated variant testing results for ATM, CHEK2 and PALB2. NPV is adjusted for prevalence.*

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

CONCLUSIONS

- We have modified our HWA to allow for combined use of genetic and clinical data obtained from both small gene panel and larger pan-cancer panel testing.
- Extensive testing of the HWA indicates that it is highly accurate for upgrading and downgrading VUSs to more definitive clinical classifications, depending on the gene.
- Preliminary analysis of data obtained from the combined HWA indicates that variants affecting approximately 9,000 patients may be able to be given a more definitive classification using the updated algorithm.
- As additional data is obtained through ongoing patient testing it may be possible to extend the use of the HWA to more variants within *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *ATM*, *CHEK2*, and *PALB2* and potentially 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

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