

DEVELOPMENT OF A NOVEL HISTORY WEIGHTING ALGORITHM FOR THE RECLASSIFICATION OF GENETIC VARIANTS IDENTIFIED IN GENES ASSOCIATED WITH LYNCH SYNDROME

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BACKGROUND

- Individuals with clinical and family histories suggestive of Lynch syndrome are appropriate candidates for germline testing for pathogenic mutations in the *MLH1*, *MSH2*, *MSH6* and *PMS2* genes.
- Variants of unknown clinical significance (VUS) make it difficult to determine clinical management. Reclassification of these variants to more clinically definitive categories is crucial for patient management.
- We had previously developed a statistical clinical history weighting algorithm for the reclassification of VUS identified in the *BRCA1* and *BRCA2* genes (Pruss D et al. *Breast Cancer Res Treat.* 2014, 147:119-32).
- We have made significant modifications to this algorithm to allow reclassification of VUS in the *MLH1*, *MSH2*, and *MSH6* genes.

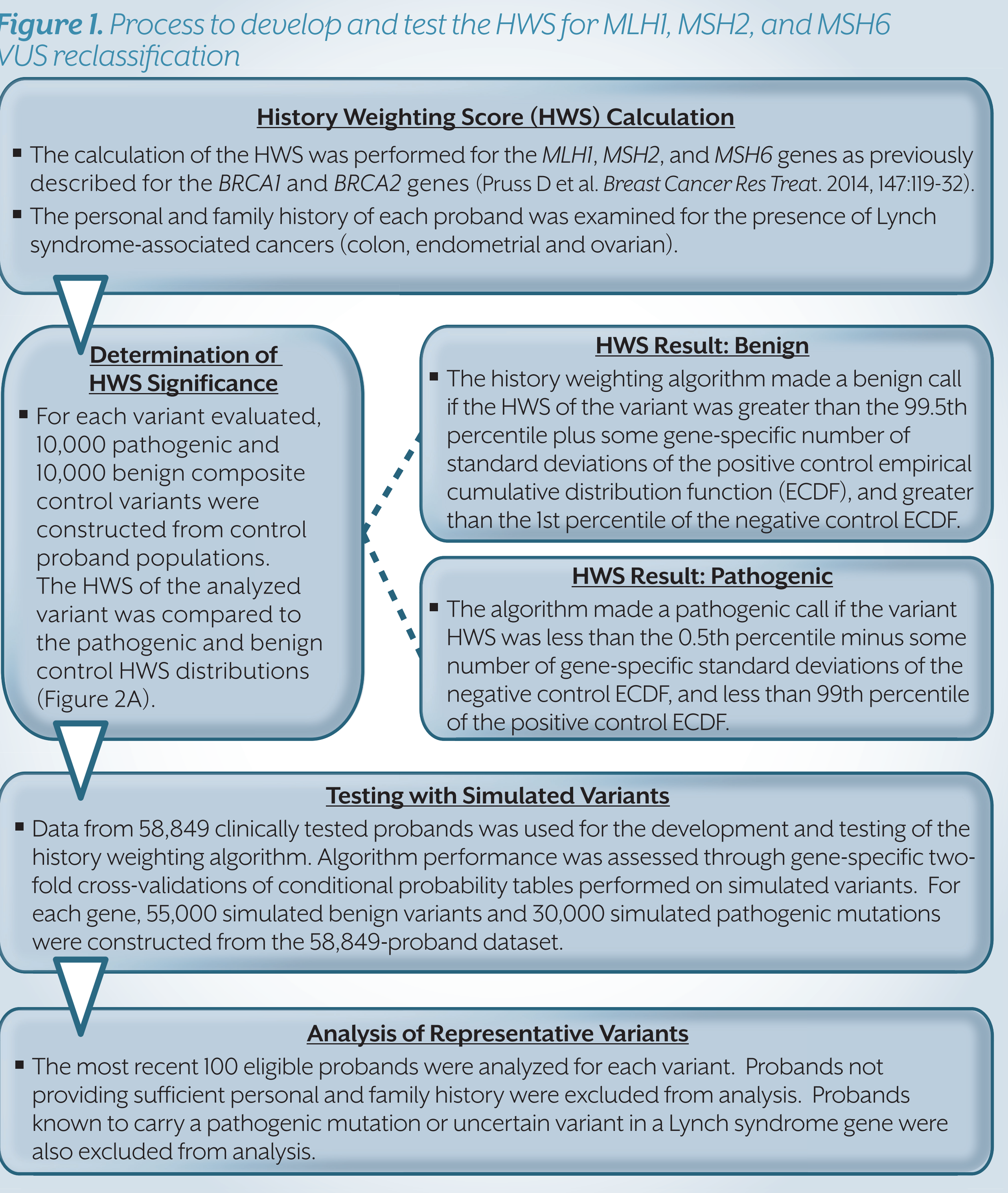
METHODS

Patient Ascertainment

Informed consent for clinical genetic testing, which included sequencing and large rearrangement analysis of the *MLH1*, *MSH2*, and *MSH6* genes, was obtained from all patients. Qualified healthcare providers collected and submitted patient samples along with a test requisition, which requested the following information: proband age, ancestry, personal cancer history and age of diagnosis (if applicable). The proband's family cancer history was also requested and included a list of affected relatives, cancer type(s), and age(s) of diagnosis.

History Weighting Algorithm

The history weighting algorithm was developed and tested for reclassification of VUS identified by sequencing in the *MLH1*, *MSH2*, and *MSH6* genes using clinical and simulated variant data, as described in Figure 1.

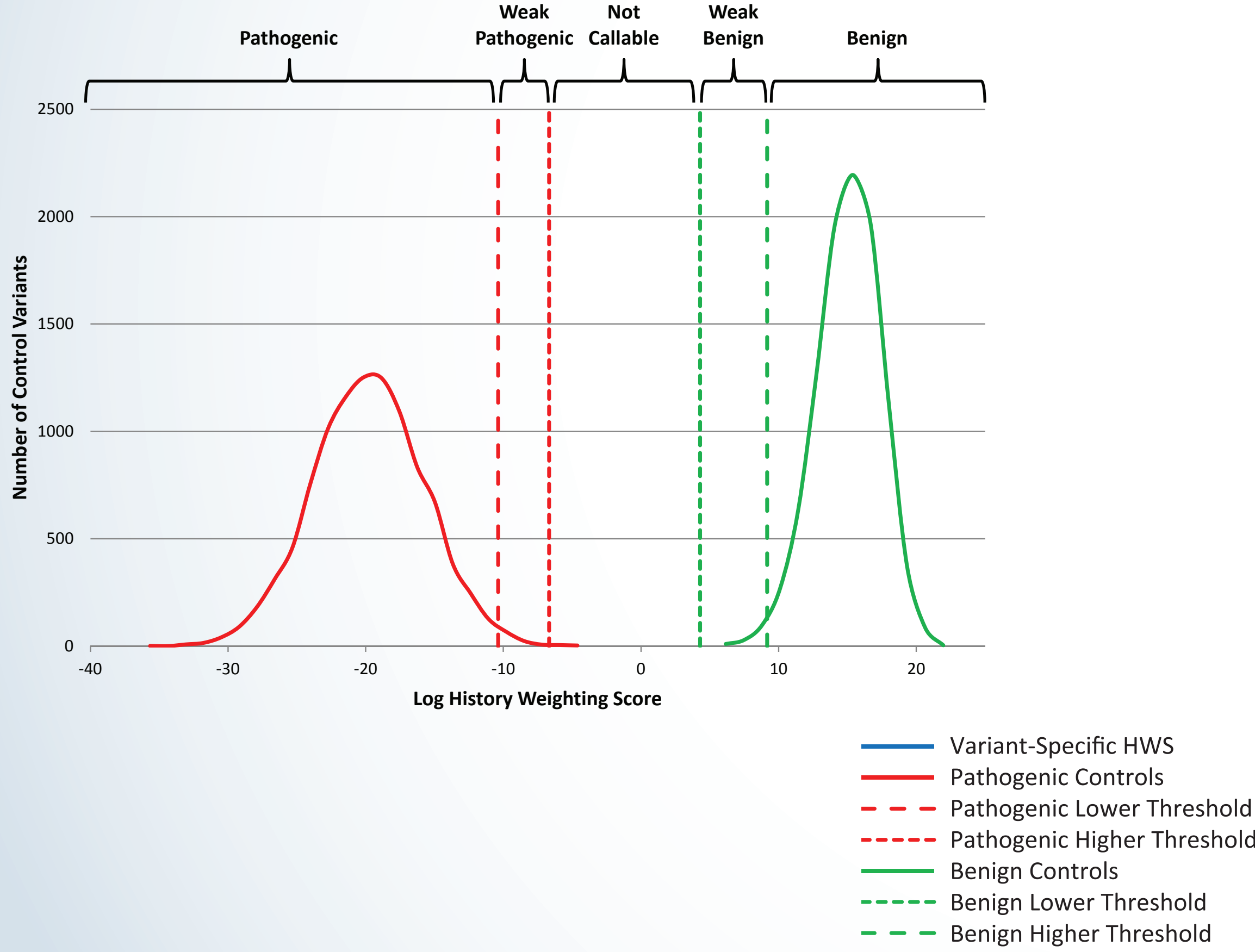


RESULTS

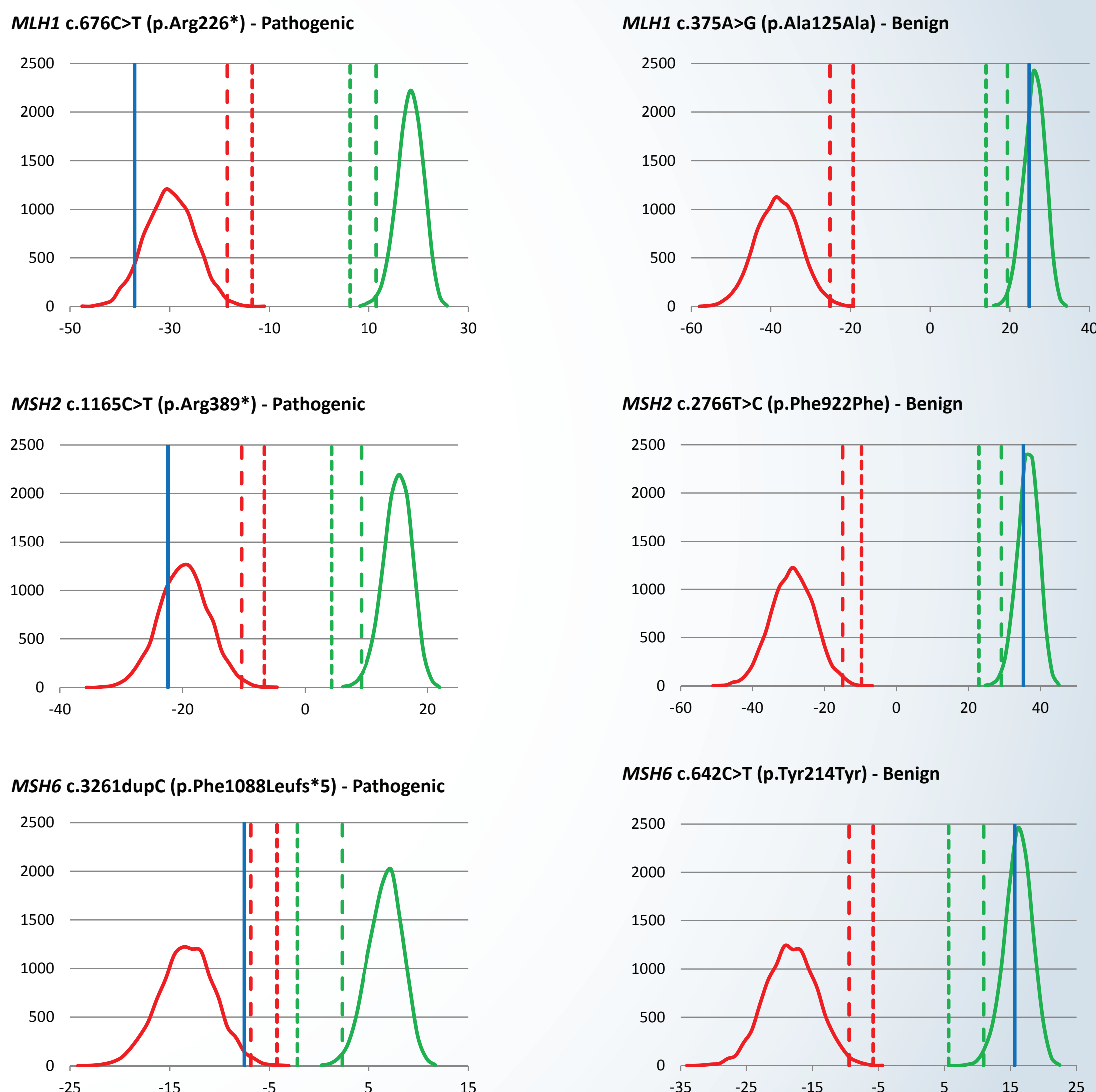
Table 1. Results of testing with *MLH1*, *MSH2*, and *MSH6* simulated variants. PPV and NPV were adjusted for prevalence.

Gene	History Weighting Classification	True Classification	# of Pathogenic Calls (Fold 1)	PPV (Fold 1)	# of Pathogenic Calls (Fold 2)	PPV (Fold 2)
<i>MLH1</i>	Pathogenic	Pathogenic 30,000 trials	29,016	0.9986	29,533	0.9972
		Benign 55,000 trials	28		57	
<i>MSH2</i>	Pathogenic	Pathogenic 30,000 trials	28,511	0.9956	29,371	0.9991
		Benign 55,000 trials	52		11	
<i>MSH6</i>	Pathogenic	Pathogenic 30,000 trials	26,527	0.9960	27,041	0.9986
		Benign 55,000 trials	25		9	
Gene	History Weighting Classification	True Classification	# of Benign Calls (Fold 1)	NPV (Fold 1)	# of Benign Calls (Fold 2)	PPV (Fold 2)
<i>MLH1</i>	Benign	Pathogenic 30,000 trials	224	0.9972	83	0.9990
		Benign 55,000 trials	54,798		54,199	
<i>MSH2</i>	Benign	Pathogenic 30,000 trials	379	0.9971	176	0.9987
		Benign 55,000 trials	54,659		54,862	
<i>MSH6</i>	Benign	Pathogenic 30,000 trials	446	0.9981	743	0.9968
		Benign 55,000 trials	54,607		54,673	

Figure 2. A) Illustration of a history weighting algorithm graph. The variant-specific HWS is compared to those of 10,000 pathogenic and 10,000 benign composite control variants. Variant classification categories (top) are defined threshold lines based on composite control HWS distributions.



B) History weighting algorithm graphs illustrating classification calls for select variants.



CONCLUSIONS

- The history weighting algorithm is a powerful and highly accurate tool for the reclassification of VUS identified by sequencing in the *MLH1*, *MSH2*, and *MSH6* genes, allowing for better identification and clinical management of high risk patients.
- The history weighting algorithm in combination with other reclassification tools has reduced our laboratory's VUS rate to <2% for the *MLH1* and *MSH2* genes, and to ~3% for the *MSH6* gene, while maintaining our classification accuracy of >99%.
- There is currently insufficient data to use this algorithm for the reclassification of *PMS2* variants. With additional data and modifications, the history weighting algorithm may be utilized for the reclassification of variants identified in *PMS2* and other cancer-associated genes and also in other autosomal dominant disorders.