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.

Figure 1. Process to develop and test the HWS for MLH1, MSH2, and MSH6 VUS reclassification

History Weighting Score (HWS) Calculation

- The calculation of the HWS was performed for the MLH1, MSH2, and MSH6 genes as previously described for the BRCA1 and BRCA2 genes (Pruss D et al. Breast Cancer Res Treat. 2014, 147:119-32).
- The personal and family history of each proband was examined for the presence of Lynch syndrome-associated cancers (colon, endometrial and ovarian).

Determination of HWS Significance

 For each variant evaluated, 10,000 pathogenic and 10,000 benign composite control variants were constructed from control proband populations. The HWS of the analyzed variant was compared to the pathogenic and benign control HWS distributions

(Figure 2A).

HWS Result: Benign

The history weighting algorithm made a benign call if the HWS of the variant was greater than the 99.5th percentile plus some gene-specific number of standard deviations of the positive control empirical cumulative distribution function (ECDF), and greater than the 1st percentile of the negative control ECDF.

HWS Result: Pathogenic

The algorithm made a pathogenic call if the variant HWS was less than the 0.5th percentile minus some number of gene-specific standard deviations of the negative control ECDF, and less than 99th percentile of the positive control ECDF.

Testing with Simulated Variants

■ Data from 58,849 clinically tested probands was used for the development and testing of the history weighting algorithm. Algorithm performance was assessed through gene-specific twofold cross-validations of conditional probability tables performed on simulated variants. For each gene, 55,000 simulated benign variants and 30,000 simulated pathogenic mutations were constructed from the 58,849-proband dataset.

Analysis of Representative Variants

■ The most recent 100 eligible probands were analyzed for each variant. Probands not providing sufficient personal and family history were excluded from analysis. Probands known to carry a pathogenic mutation or uncertain variant in a Lynch syndrome gene were also excluded from analysis.

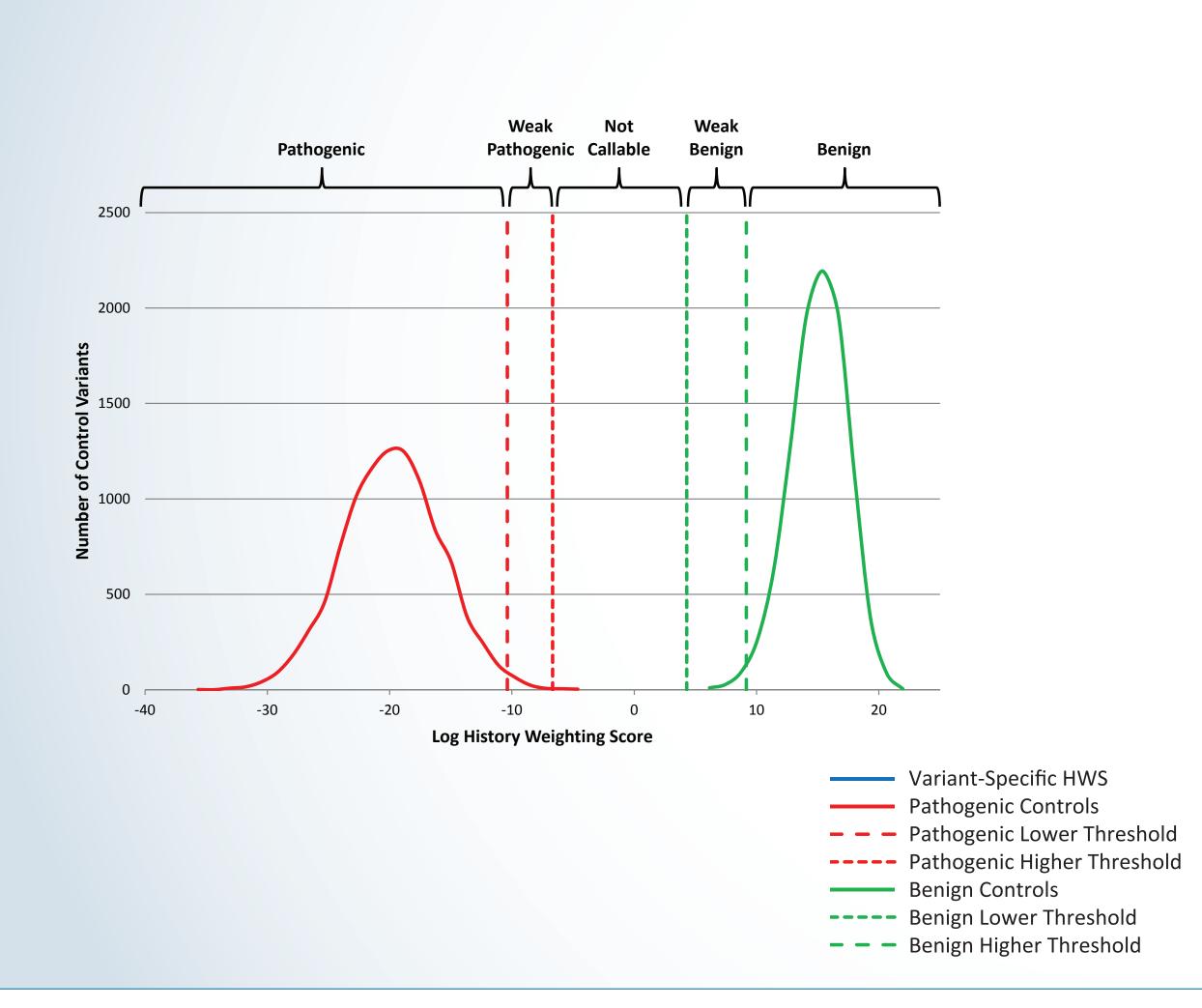
RESULTS

The history weighting algorithm was developed and tested on a clinical dataset consisting of 58,849 probands tested for germline mutations in the MLH1, MSH2, and MSH6 genes.

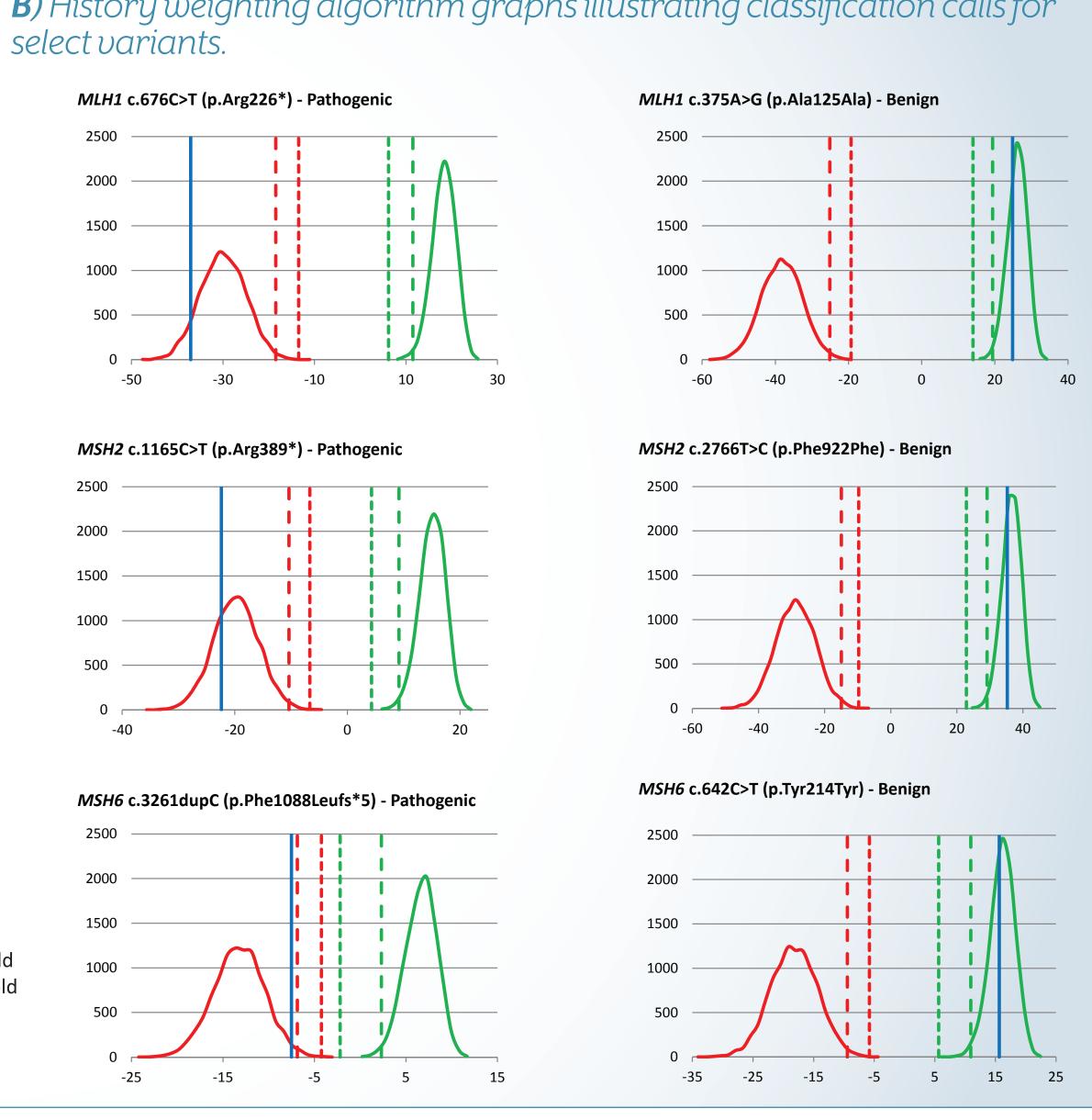
- Two-fold cross-validations of conditional probability tables performed on 85,000 simulated pathogenic or benign variants resulted in positive predictive values (PPV) and negative predictive values (NPV) of >0.995 for each gene, indicating that the algorithm is highly robust and accurate for the reclassification of variants identified within these genes (Table 1).
- Analysis of representative true variants that were previously classified, using other methodologies, as either pathogenic or benign illustrates the accuracy and clinical utility of the history weighting algorithm (Figure 2).

Table 1. Results of testing with MLH1, MSH2, and MSH6 simulated variants. PPV and NPV were adjusted for prevalence. History # of Pathogenic PPV # of Pathogenic True Weighting Classification Calls (Fold 1) (Fold 1) Calls (Fold 2) (Fold 2) Pathogenic 29,016 29,533 30,000 trials 0.9986 0.9972 Pathogenic 55,000 trials Pathogenic 28,511 29,371 30,000 trials MSH2 Pathogenic 0.9956 0.9991 Benign 55,000 trials Pathogenic 26,527 27,041 30,000 trials MSH6 Pathogenic 0.9960 0.9986 Benign 25 55,000 trials History PPV # of Benign NPV True # of Benign Weighting Gene Classification Calls (Fold 1) Calls (Fold 2) (Fold 2) Pathogenic 224 83 30,000 trials MLH1 0.9972 0.9990 Benign Benign 54,798 54,199 55,000 trials Pathogenic 379 176 30,000 trials MSH2 0.9971 0.9987 Benign Benign 54,659 54,862 55,000 trials Pathogenic 446 743 30,000 trials 0.9981 0.9968 MSH6 Benign Benign 54,607 54,673 55,000 trials

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.







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.

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