Complexities in Hereditary Cancer Variant Classification: Three Case Examples

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BACKGROUND

- Variant classification is a complex and essential component of genetic testing.
- The ACMG guidelines provide useful guidance, but they are written broadly for all genes and genetic conditions.[1]
- The broad ACMG guidelines must be reconciled with gene-specific rules for classification.
- This can lead to discrepancies in variant classification between laboratories.
- Here we describe three variants with discrepant classifications, detailing our laboratory's approach to classification.

METHODS

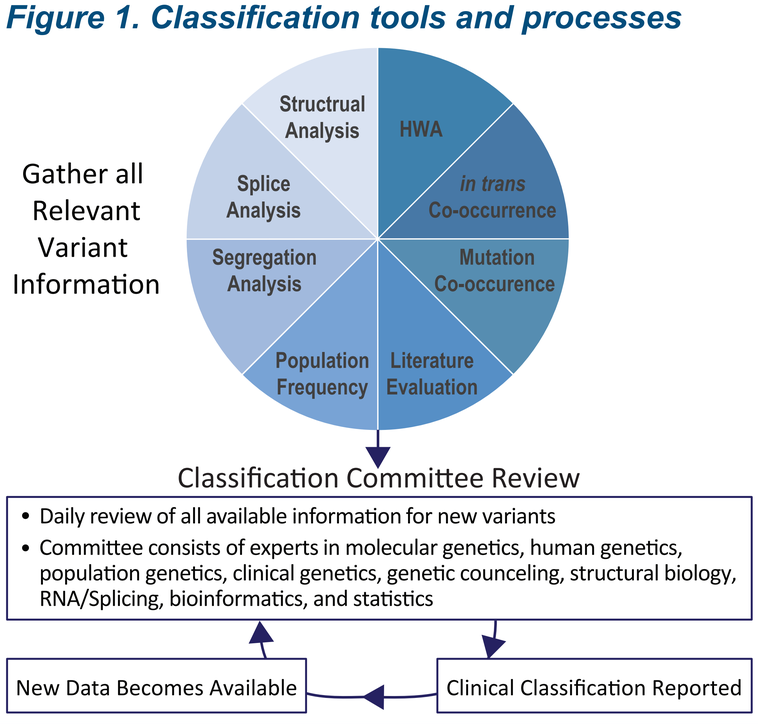
VARIANT CLASSIFICATION

- Our variant classification includes thorough analysis of all classification tools by a committee of experts based on ACMG guidelines and laboratory protocols.[1,2]
- New variants are classified based on evidence available at the time of initial observation, and data is continually reviewed to determine if there is sufficient evidence for the reclassification of any variants (Figure 1).[2]

HISTORY WEIGHTING ALGORITHM (HWA)

- One of the tools used by our laboratory for reclassification is the HWA: a powerful statistical tool that compares the phenotypes of individuals with a given variant to matched controls with known pathogenic or benign variants in the same gene.
- The HWA has been validated with >99.5% positive and negative predictive values.[3]

Figure 1. Classification tools and processes



RESULTS

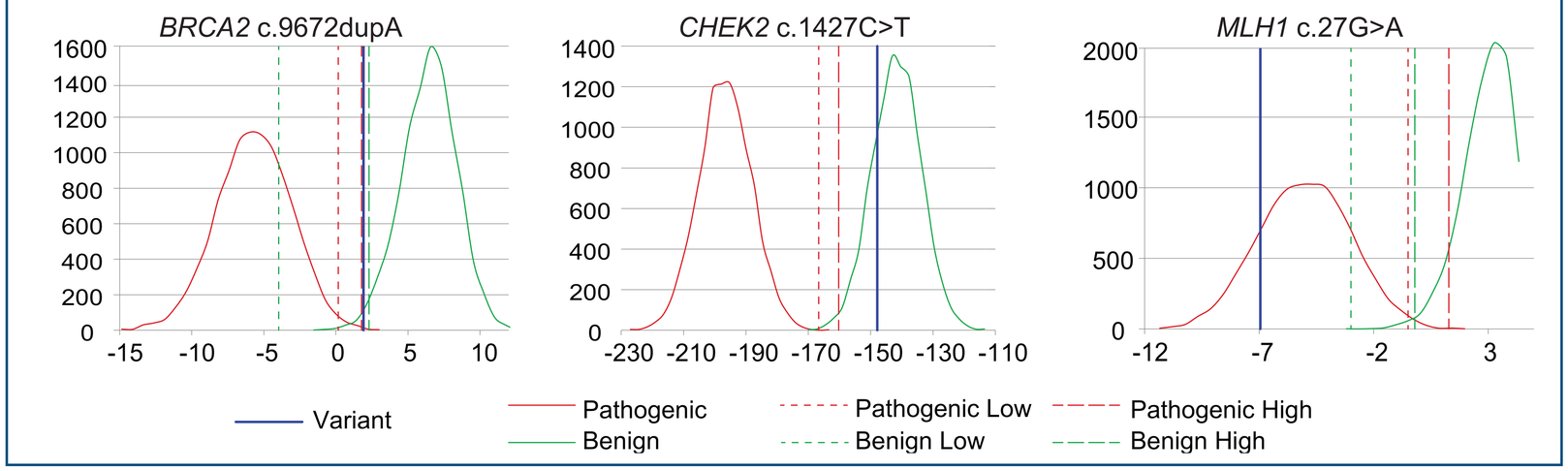
Table 1. Case studies of variants in BRCA1, CHEK2, and MLH1 that resulted in variant reclassification

	BRCA2 c.9672dupA (p.Tyr3225Ilefs*30)	*CHEK2* c.1427C>T (p.Thr476Met)	*MLH1* c.27G>A (p.Arg9Arg)
Initial classification and # of observations	Pathogenic 84 observations	Uncertain 443 observations	Benign 12 observations
Reasons for initial classification	• Expected to result in premature protein truncation. • Observed *in trans* with PV in individual with FA features.	• Missense variant. • Studies suggest reduced kinase activity but data insufficient for definitive classification.	• Not predicted to affect splicing.
Reasons for re-classification	• HWA suggests associated cancer histories are inconsistent with known *BRCA2* PVs (Figure 2). • A similar variant appears benign using HWA and has been seen *in trans* with other PVs in individuals with and without FA features.[4]	• HWA shows no association with BC risks expected for known *CHEK2* PVs (Figure 2).	• HWA shows association with strong personal and family cancer history and is consistent with other *MLH1* PVs (Figure 2). • Only found via Lynch-specific testing. • No splice defect was identified.
Current classification	**Special Interpretation**	**Likely Benign**	**Uncertain**

PV, Pathogenic Variant; FA, Fanconi Anemia; BC, Breast Cancer

Figure 2. HWA graphs illustrating classification calls for three case examples.

The variant-specific history weighing score (HWS) is compared to those of 10,000 benign and 10,000 pathogenic composite control variants. Variant classification categories are defined by thresholds based on composite control HWS distributions



CONCLUSIONS

- Variant classification is a complex and continuous process.
- The examples presented here stress the importance of re-evaluating previously classified variants using standard and novel classification tools.
- Although the ACMG guidelines provide useful guidance for variant classification, classification discrepancies will continue to exist even with proper interpretation and use of these guidelines given differences in thresholds, experience, and expertise.

REFERENCES

1. Richards S, et al. *Genet Med.* 2015; 17(5):405-424.
2. Eggington JM, et al. *Clin Genet.* 2014; 86(3):229-237.
3. Morris B, et al. *BMC Genet.* 2016; 17(1):99.
4. Rosenthal ET, et al. *Clin Genet*. 2015; 88(6): 533-541.

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