The ClinGen-InSiGHT *MUTYH* Variant Curation Expert Panel: Lessons learned and a call to action

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ABSTRACT

The ClinGen-InSiGHT Hereditary Colon Cancer/Polyposis Variant Curation Expert Panel (VCEP) was formed in 2021 to create variant curation specifications for the known genes causing these conditions. The *MUTYH* subcommittee of this VCEP is nearing the release of gene-specific recommendations for ACMG variant classification. *MUTYH*-associated polyposis (MAP) is an autosomal recessive disorder caused by germline biallelic variants in the base excision repair gene *MUTYH*. *MUTYH* encodes a glycosylase that identifies and excises adenines mispaired with the oxidation product 8-oxo-deoxyguanosine (OG), which, if left incorporated, lead to somatic G>T transversions. Through developing ACMG criteria for *MUTYH*, experts from functional, clinical, and computational fields have identified key gaps in understanding transcript expression, functional studies, and clinical features, plus inadequate communication among researchers in these fields. We first identified nomenclature discrepancies because of confusion in application of the Matched Annotation from NCBI and EMBL-EBI (MANE) *MUTYH* transcript. The *MUTYH* MANE Select transcript (NM_001048174) encodes a 521 AA protein. However, transcript NM_001128425, which encodes a 549 AA protein, is the most used by clinical labs. Our VCEP successfully had this second transcript officially recognized as MANE Plus Clinical, harmonizing historical data with current clinical reports. Another issue the VCEP addressed was establishing specific phenotypic descriptions of affected individuals. These are needed since MAP patients present with a variable number of polyps, age of onset, extracolonic features, and association with colorectal carcinoma. Somatic genomic analysis for G>T transversions, mostly done in colorectal cancer, provides strong support for MAP diagnosis, but these tests are rarely done, so data for MAP-specific cohorts are lacking. For ClinGen, *MUTYH* is the first autosomal recessive cancer gene assessed which requires additional ACMG criteria. Per ACMG guidelines, only evidence from "well-established" functional assays can be used for applying criteria PS3 and BS3. Satisfactory functional assays are lacking due to inadequate use of controls and inattention to statistical principles that establish the strength of evidence, despite years of research on *MUTYH*. Improved communication among academics, clinicians, and industry labs is needed. Here, we identify the knowledge gaps in developing *MUTYH*-specific ACMG recommendations in the hopes of galvanizing these communities to generate the needed data for this routinely assessed cancer susceptibility gene.

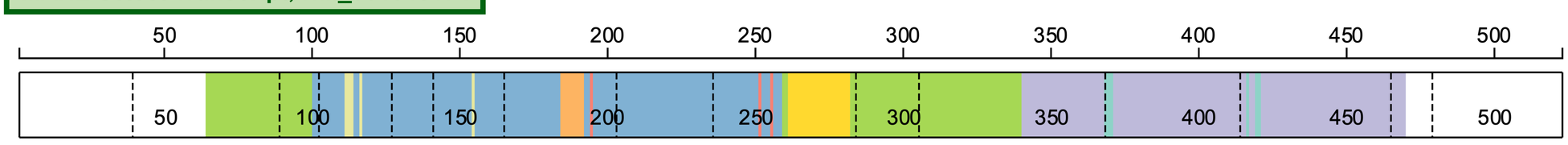
OBJECTIVES

1. Establish ACMG/AMP guidelines for *MUTYH* variant classification
2. Harmonize historical data referencing different *MUTYH* transcripts
3. Specify the *MUTYH*-associated polyposis (MAP) phenotype
4. Set standards for functional assays evaluating *MUTYH* variants

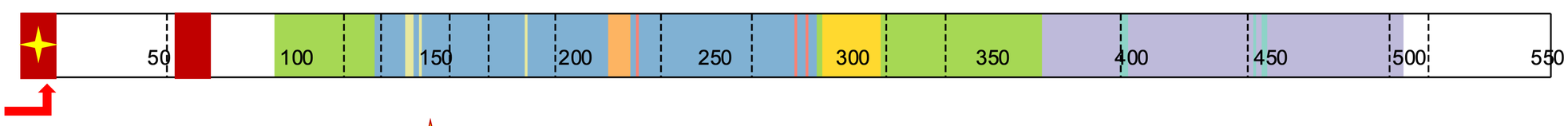
MAP-SPECIFIC PHENOTYPE

Phenotypic Consistency	Phenotype Strong Enough for Moderate	Phenotype Strong Enough for Supporting	Phenotype Less Specific for MUTYH
Phenotype Point per Proband	2	1	0.5
Colorectal Adenomas	≥10 colorectal adenomas at any age **with** a somatic G>T transversion in APC or KRAS genes **OR** Colorectal carcinoma **with** a somatic G>T transversion in APC or KRAS genes	>30 adenomatous polyps **AND** APC excluded by sequencing **AND** Family history of colorectal/duodenal cancer, and/or >10 adenomas, consistent with autosomal recessive inheritance **OR** Any number of colorectal adenomas **with** a somatic G>T transversion in APC or KRAS genes	≥10 colorectal adenomas at any age **OR** Isolated colorectal carcinoma
Duodenal Adenomas		1 or more duodenal adenomas **with** a somatic G>T transversion in APC or KRAS genes **OR** Duodenal carcinoma **with** a somatic G>T transversion in APC or KRAS genes	1 or more duodenal adenomas **OR** Duodenal carcinoma
Extracolonic Manifestations			Extraintestinal neoplasia, irrespective of organ or origin, **with** a somatic G>T transversion in APC or KRAS genes in the tumor samples

MANE Select Transcript, NM_001048174



MANE Plus Clinical Transcript, NM_001128425

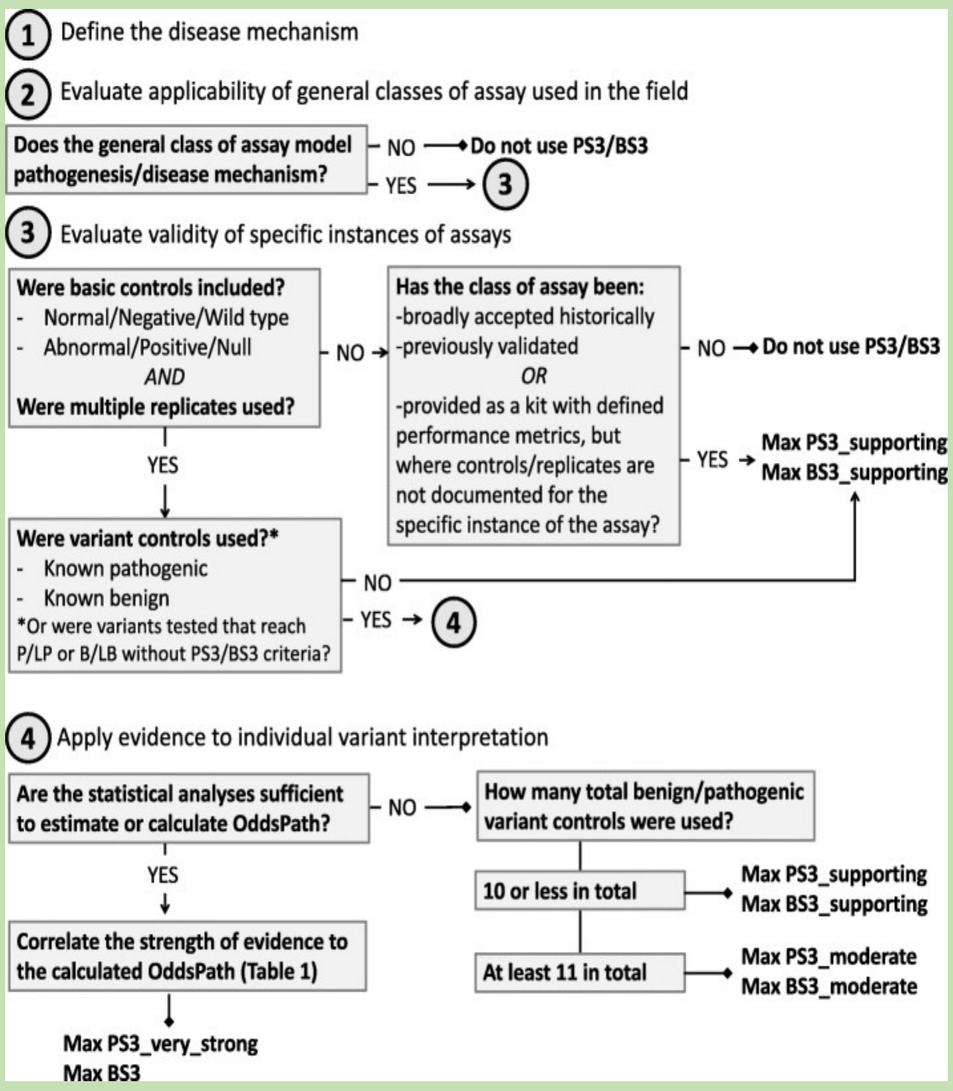


TOE1 gene, which overlaps with *MUTYH*

mitochondrial localization signal

- adenine-specific DNA glycosolase
- endonuclease III
- minor groove reading motif
- helix-hairpin-helix binding domain
- substrate binding pocket
- iron-sulfur binding domain
- DNA glycosolase
- DNA binding and oxoG recognition site
- amino acids not in the MANE Select

FUNCTIONAL ASSAYS FOR PS3 AND BS3



- Guidelines for the use of functional assays as evidence for PS3 and BS3 are outlined in Brnich et al. (2020)
- *MUTYH* assays routinely failed to meet standards for controls, broad historical acceptance, statistical analysis, and multiple replicates
- VCEPs typically provide a list of approved assays for variant assessment
- The *MUTYH* VCEP was limited to outlining required components of future assays

CONCLUSIONS

- *MUTYH* experts have identified large gaps in understanding transcript expression, functional studies, clinical features, and insufficient communication among researchers in these fields
- The *MUTYH* MANE Select transcript (NM_001048174) encodes a 521 AA protein but is not the longest transcript that is used most by clinical labs
- Because of misinterpretation of transcripts (especially when overlapping the TOE1 gene), 8.5% of variants categorized as missense in LOVD and gnomAD are not actually missense in the MANE Select transcript
- Our VCEP successfully lobbied for a second, officially recognized MANE Plus Clinical transcript (NM_001123425, 549 AA, most used by clinical labs) to harmonize historical data with current clinical reports
- Although the MAP-specific phenotype is difficult to define, we hope to encourage more facilities to perform somatic genomic analysis for G>T transversions by setting phenotypic standards using this information
- *MUTYH f*unctional assays validated to classify variants are lacking due to inadequate use of controls, inattention to statistical principles, and lack of controls and replicates that establish the strength of evidence

WORKS CITED

- Brnich et al. (2020). "Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework." *Genome Medicine* 12:3.
- Thet et al. (2023). "Phenotype correlations with pathogenic DNA variants in the *MUTYH* gene." Manuscript submitted.
- Zhou et al. (2016). "Exploring genomic alteration in pediatric cancer using ProteinPaint." *Nature Genetics* 48:4-6.

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