Novel high-throughput functional models for *MLH1*, *MSH2*, *PMS2*, and *MSH6* have high accuracy for clinical variant classification

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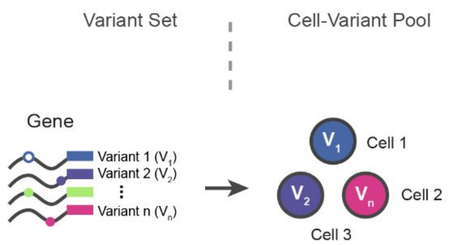
Lynch syndrome, the most common form of hereditary cancer, is caused by pathogenic germline variants in mismatch repair (MMR) genes.

Rationale and Objective

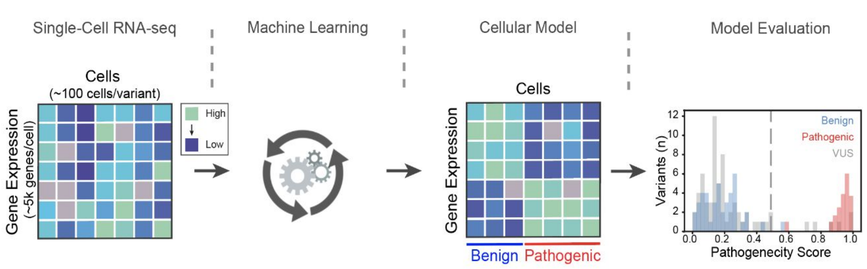
- 1 in 300 individuals have a genetic variant associated with Lynch syndrome (LS).
- Variants of uncertain significance (VUS) in MMR genes hinder diagnoses and clinical management.
- Multiplex assays of variant effects (MAVEs) are high-throughput cellular assays designed to provide functional data on variant activity.
- **Specific Aim: Obtain high-quality functional evidence for variant classification (VC) for select variants from the four LS MMR genes, *MLH1*, *MSH2*, *PMS2*, and *MSH6*.**

Methods

- Pools of variants including pathogenic (P), benign (B), and VUS were generated for the four LS MMR genes. These were introduced into cells such that each cell contained one variant copy.

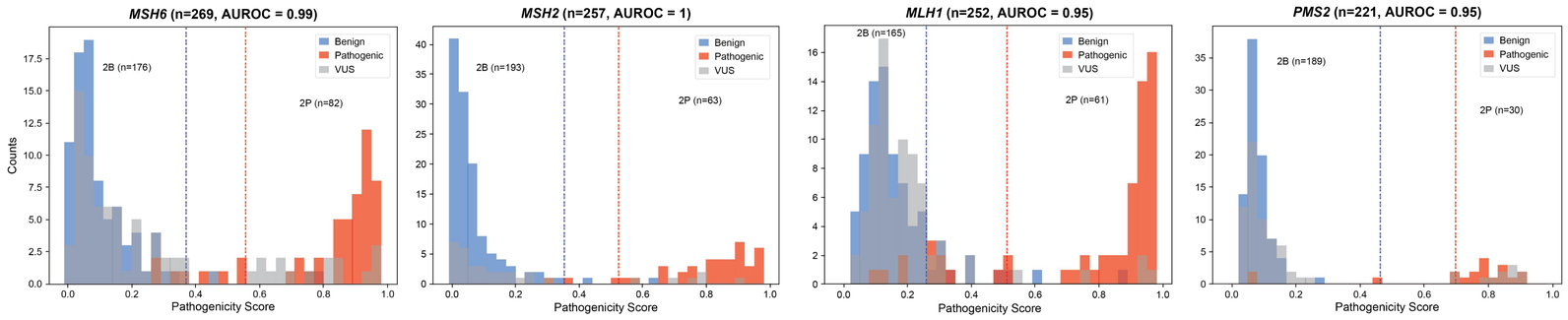


- Engineered cells were captured for single-cell RNA sequencing
- Single-cell RNA expression profiles were normalized and filtered.
- Supervised machine learning was used to find patterns in gene expression that were different between cells harboring established P and B and to assess the accuracy of variant predictions.
- VUS were classified using these trained models.



Targeted MMR MAVE models provide high-quality functional evidence for VC

- Ninety-nine percent (n= 642/649) of targeted variants across the three models were able to reach strong predictions for either pathogenicity (positive predictive value ≥95%) or benign (negative predictive value ≥95%).



- 25% of MMR VUS can be reclassified by these targeted models, impacting 2144 reports.

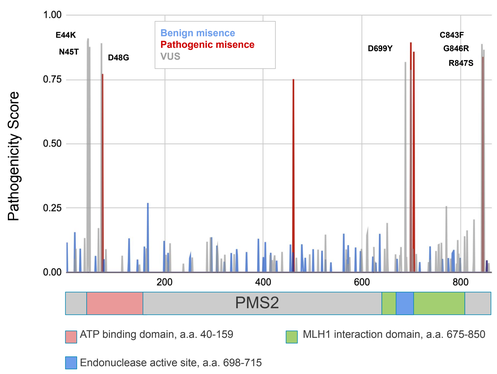
Classifications	VUS>B	VUS>LB	VUS>VUS	VUS>LP	VUS>P	LB>B	LP>P
TAR-MLH1	0	10	92	3	0	2	0
TAR-MSH2	2	15	32	2	0	23	0
TAR-MSH6	0	58	30	4	0	0	2
TAR-PMS2	1	4	100	1	0	3	3
Total	3	87	254	10	0	28	5

Reports	VUS>B	VUS>LB	VUS>VUS	VUS>LP	VUS>P	LB>B	LP>P
TAR-MLH1	0	557	3562	6	0	65	0
TAR-MSH2	44	337	328	3	0	2527	0
TAR-MSH6	0	838	379	10	0	0	56
TAR-PMS2	93	256	7744	0	0	3817	126
Total	* 137	*1988	12013	* 19	0	6409	182

These models will accelerate discovery

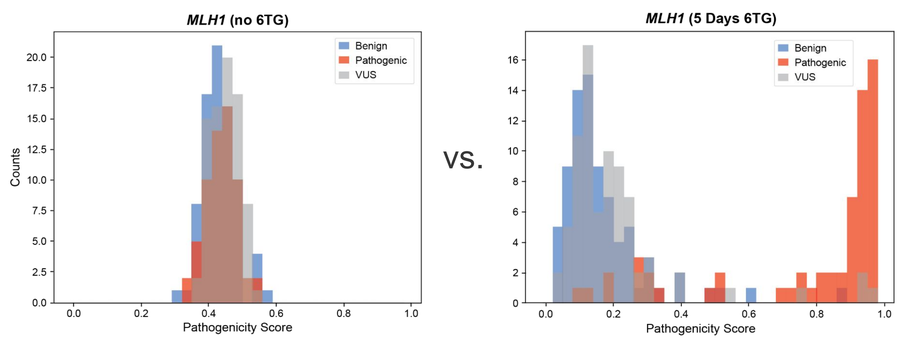
- Mapping variants by gene structure identifies critical domains.

  Example: PMS2 pathogenic **VUS** cluster with known **pathogenic** variants.



- An established cellular system is a powerful tool to functionally test compounds with variant-specific resolution.

  Example: Our MMR MAVE models required cells to be cultured with a chemical that causes mismatches. *This illustrates the feasibility of chemical administration leading to variant effect.*



Conclusions

- The *MSH2*, *MLH1*, *PMS2*, and *MSH6* MAVE models provide a high-quality source of functional evidence for variant classification and will impact many patients.
- These models hold potential for revealing mechanistic insight into MMR protein function and are promising systems with which to characterize potential therapeutics.

Disclosures: DS and coauthors are stockholders and employees of Invitae.