Tumor Characteristics Provide Evidence for Mismatch Repair (MMR) Variant Pathogenicity

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BACKGROUND

- Pathogenic/likely pathogenic(VLP) variants in mismatch repair (MMR) genes (*MLH1, MSH2, MSH6,* and *PMS2*) increase risk for Lynch syndrome and other cancers[1].
- MMR deficiency results in microsatellite instability (MSI)[2]. Tumor MSI testing and immunohistochemical (IHC) analysis for MMR protein expression are often used in clinical screening for Lynch syndrome (Espenschied *et al.,* 2017 CGA meeting, poster #126).
- These quantifiable tumor characteristics may be useful in assessing germline MMR variant pathogenicity[3].

METHODS

- Clinical information was obtained for patients who underwent germline multigene panel testing that included MMR genes in 2012-2016 and consented to research.
- Among 3,627 patients with available MSI or IHC information, 69 with conflicting MSI/IHC status (unstable/normal or stable/abnormal) and 238 carriers of non-MMR pathogenic/VLP variants in major cancer genes were excluded.
- Of the remaining 3,320 with MSI (*n*=1,055) or IHC (*n*=2,954) results (*n*=689 with both), we tested associations between MMR carrier status and MSI/IHC phenotype using Fisher's exact test, and estimated MSI/IHC likelihood ratios (LR = %pathogenic variant carriers/%non-carriers). Variant pathogenicity was assessed according to ACMG guidelines[4,5].
- For each variant, we computed the tumor characteristic LR (TCLR) as the product of the carriers' MSI/IHC LRs. While TCLR>1 and TCLR<1 thresholds may suggest pathogenic and benign, respectively, we considered TCLR>10 and TCLR<0.1 as stronger evidence for predicting pathogenic or benign.

RESULTS

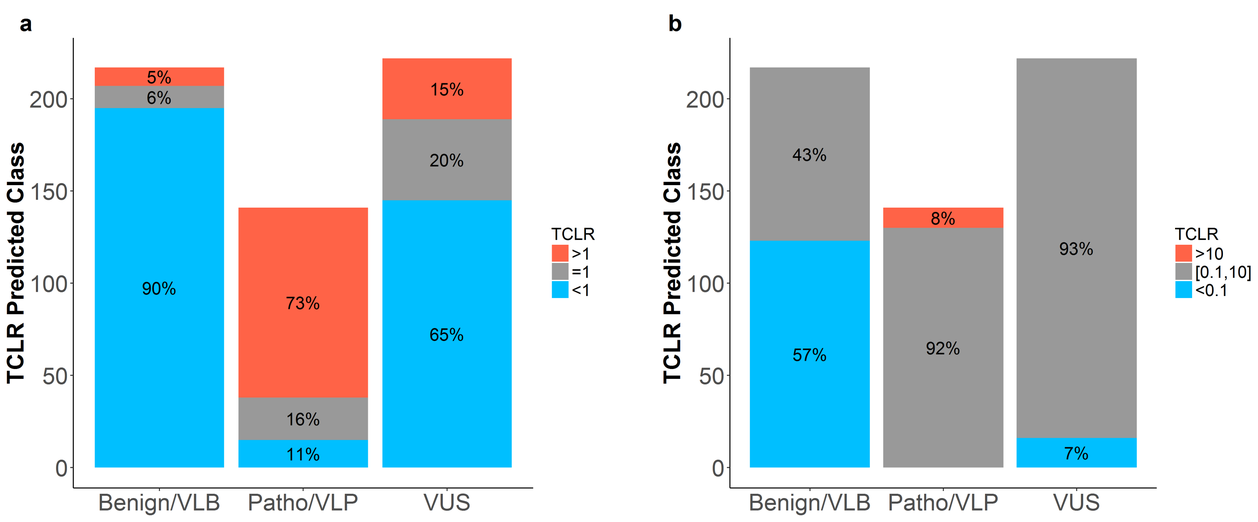
- Carriers of MMR pathogenic/VLP variants were significantly more likely to have abnormal MSI/IHC (Table 1).
- Overall, we identified 141 MMR pathogenic/VLP variants, 217 benign/likely benign(VLB) variants, and 221 variants of uncertain significance (VUS).
- Among pathogenic/VLP variants, 73.0% have TCLR>1 and none have TCLR<0.1; 89.9% of benign/VLB variants have TCLR<1 and none have TCLR>10 (Figure 1).
- Moreover, 15% of VUSs have TCLR>1 and 65% have TCLR<1, suggesting some may be reclassified. While no VUS have TCLR>10, 15 (7%) have TCLR<0.1, providing evidence for predicted classification as benign/VLB (Table 2).

Table 1. Estimation of tumor characteristic LR

	PC[a]	NC[a]	%PC/%NC	OR (95% CI)	P-value
MSI/IHC	n (%)	n (%)	LR		
Unstable/abnormal	181 (91.9%)	915 (29.3%)	3.136	27.3 (16.2-49.0)	<0.0001
Stable/normal	16 (8.1%)	2208 (70.7%)	0.115		
MSI/IHC[b]	n (%)	n (%)	LR		
Unstable/abnormal	181 (91.9%)	1019 (30.3%)	3.030	26.0 (15.5-46.7)	<0.0001
Stable/normal	16 (8.1%)	2342 (69.7%)	0.117		

[a]PC: Pathogenic/VLP variant carrier; NC: non-Pathogenic/VLP variant carrier
[b]includes the non-MMR pathogenic/VLP variant carriers as a sensitivity analysis

Figure 1. Using TCLR to inform variant classification



Among 217 benign/likely benign (Benign/VLB) variants, 141 pathogenic/likely pathogenic (Patho/VLP) variants and 221 VUS, the TCLR-informed classification is based on the following thresholds:

a. TCLR >1 for Patho/VLP and TCLR <1 for Benign/VLB
b. TCLR >10 for Patho/VLP and TCLR < 0.1 for Benign/VLB

Table 2. VUSs with TCLR <0.1

Gene	RefseqID	Variant	TCLR	#Carrier	ClinVar[6]
PMS2	NM_000535	c.2007-6C>G	2.31×10^{-6}	7	VLB
MSH6	NM_000179	c.628-7C>A	0.002	3	VLB
PMS2	NM_000535	c.497T>C	0.005	4	VLB, VUS
PMS2	NM_000535	c.620G>A	0.005	4	VLB, VUS
MSH2	NM_000251	c.-116G>T	0.013	2	VUS
MSH2	NM_000251	c.-140G>T	0.013	3	VUS
MSH2	NM_000251	c.-189T>C	0.013	2	VUS
MSH2	NM_000251	c.-225G>C	0.013	2	VLB, VUS
MSH2	NM_000251	c.835C>G	0.013	2	VUS
MSH6	NM_000179	c.-8C>T	0.013	2	VUS
MSH6	NM_000179	c.866_867delGCinsAA	0.013	2	VUS
MSH6	NM_000179	c.2006T>C	0.013	2	VUS
MSH6	NM_000179	c.3478G>A	0.013	3	VUS
PMS2	NM_000535	c.2350G>A	0.013	2	VUS
PMS2	NM_000535	c.2395C>T	0.013	2	VUS

SUMMARY

- MSI/IHC-based TCLRs provide evidence for or against pathogenicity of MMR variants.
- Used independently or in conjunction with other evidence, the TCLR has the potential to inform variant classification, and may have important implications for future genetic testing and clinical management

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