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Lynch syndrome and other hereditary cancer predisposition syndromes: insights from establishing an oncogenetic tertiary centre in Romania

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Abstract

Lynch syndrome (LS) is among the most common hereditary cancer predisposition syndromes, caused by germline variants in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*, or by *EPCAM* 3' deletions. Its clinical utility is well established, yet Romanian data remain scarce and germline testing is poorly covered by public healthcare. We retrospectively reviewed 481 consecutive patients referred to a large oncology centre in Timișoara, Romania (2023–2025) for suspected LS or another hereditary cancer syndrome, assessing germline testing yield. Each patient was assessed against the NCCN Colorectal criteria (v1.2023) and their 2026 update (Colorectal, Endometrial and Gastric, v1.2026). A subset of urothelial tract carcinomas was further characterised by whole exome sequencing, MMR immunohistochemistry and *FGFR3* hotspot analysis. The cohort comprised 481 patients (56.3% female), with gastrointestinal (46.2%), gynaecological (23.0%) and reno-urinary (20.3%) malignancies accounting for nearly 90% of cases. Germline testing was performed in 153 patients (31.8%) and identified pathogenic/likely pathogenic variants in 37 (24.2%): 21 in LS genes and 16 in other predisposition genes, predominantly *BRCA1*. Retrospective application of NCCN 2026 core criteria increased eligibility from 183 (38.0%) to 209 patients (43.5%). Category 2B (colorectal and gastric cancer ≥ 50 years) added 109 further eligible patients but no additional Lynch cases, instead capturing three non-Lynch variants (*BRCA1*, *ATM*, biallelic *MUTYH*). Urothelial carcinomas represented 3.9% of the cohort, with two uncertain germline *BRCA2* variants identified, consistent with emerging evidence of a broader *BRCA2*-associated cancer spectrum. Category 2B thus widened eligibility without improving LS detection, though it captured hereditary variants that would otherwise have been missed. Urothelial cancers were strikingly underrepresented despite ranking third among LS-associated malignancies. Germline testing carries clear diagnostic value in this setting, but referral and access remain uneven, an argument for broader reimbursement and for embedding oncogenetic evaluation into routine Romanian oncology care.

1. Introduction

Lynch syndrome (LS) is one of the most common hereditary cancer predisposition syndrome, accounting for approximately 3% of colorectal cancers and a similar proportion of endometrial cancers 1–3, with population prevalence currently estimated at approximately 1 in 279 individuals 4. LS is caused by germline pathogenic variants in one of the DNA mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*, and by 3' deletions of *EPCAM* gene leading to somatic *MSH2* inactivation 5,6.

Cancer risks in LS are substantially increased compared with the general population, although the magnitude and spectrum of risk vary according to the affected MMR gene 7. Pathogenic variants in *MLH1* and *MSH2* are associated with high lifetime risks of colorectal and endometrial cancer, while *MSH2* also confers an increased risk of urinary tract, upper gastrointestinal and other extracolonic cancers 8. *MSH6* variants confer a lower colorectal but comparable endometrial risk, while *PMS2* variants show the lowest penetrance 9.

Upper tract urothelial carcinoma (UTUC) is the third most common LS-associated malignancy, yet its association with LS remains under-recognised, partly owing to the lack of systematic screening. LS has been reported to account for up to 10% of UTUC cases. In a prospective screening study, 13.9% of unselected UTUC tumours screened positive for potential LS, with germline testing confirming LS in 5.2% of cases 10. To address this diagnostic gap, current European guidance recommends germline evaluation for patients with UTUC diagnosed before the age of 60 years or with a suggestive personal or family history, alongside broader integration of urothelial tumours into LS screening pathways 11.

The clinical identification of individuals with suspected LS has traditionally relied on personal and family history criteria, including the Amsterdam II criteria and revised Bethesda guidelines, together with tumour site, histological features, age at diagnosis, and tumour-based screening using MSI and/or MMR immunohistochemistry, followed by confirmatory germline testing 12,13. In recent years, germline testing for LS and other hereditary cancer predisposition syndromes has increasingly shifted towards multigene NGS panels, largely replacing single-gene or small targeted panel testing and improving diagnostic yield 14.

Despite these advances, data on the prevalence and molecular characterisation of LS in the Romanian population remain limited. Access to hereditary cancer genetic testing has historically been constrained by limited reimbursement and fragmented oncogenetic services, and although national reimbursement for selected indications has recently been expanded, comprehensive germline testing for LS is not yet integrated into a publicly funded programme 15–17. As a result, systematically characterised oncogenetic cohorts remain scarce in Romania, limiting reliable estimates of LS prevalence and the broader hereditary cancer burden in routine clinical practice.

In this context, the aim of this study was to characterise the clinical and molecular features of patients evaluated for probable LS at a tertiary oncogenetic centre in Western Romania between 2023 and 2025, and to assess the yield of germline testing in this cohort. Given the recognised underdiagnosis of LS in urothelial malignancies, a secondary aim was to perform molecular characterisation of all urothelial carcinomas evaluated during the study period.

2. Material and methods

Study design and patient selection

This retrospective study included patients evaluated at the cancer genetics service of the OncoHelp Oncology Center in Timișoara, Romania, between January 2023 and December 2025, the first three years since oncogenetic testing was established at the centre.

All consecutive patients referred for genetic evaluation of a suspected inherited cancer predisposition were considered for inclusion. Inclusion criteria centred on LS as the primary syndrome of interest, but were pragmatically broader, given the substantial phenotypic overlap between LS and other hereditary cancer syndromes affecting the same organ systems. Most referrals originated from the institution's oncology service. Referrals fell into two categories: (1) predictive testing in the absence of a personal cancer diagnosis, but with positive family history suggestive of hereditary cancer syndrome (including LS), and

(2) affected patients who had in their personal and/or family history tumours involving one of the three organ systems most frequently implicated in LS: gastrointestinal (colorectal, gastric, intestinal, biliary tree, pancreatic cancers), gynaecological (in female patients, endometrial and ovarian cancers) or reno-urinary tract (urothelial, prostate and renal cancers). Within these organ systems, eligibility was not restricted to classically recognised LS-associated tumour types, in order to reflect real-world clinical referral practice. Breast cancer, although the most common indication for referral at the centre, was included in the study population only when the patient's personal or family history also included cancers involving at least one of the three aforementioned organ systems.

Genetic counselling and testing

Pre-test genetic counselling was provided by the centre's clinical oncogeneticists, who assessed each referral and determined eligibility for germline testing for cancer predisposition according to testing criteria for LS in the NCCN Guidelines applicable at the time of consultation (Genetic/Familial High-Risk Assessment: Colorectal). Testing was performed at external laboratories, mainly Blueprint Genetics (Espoo, Finland) and Fulgent Genetics (El Monte, CA, USA), and consisted of broad NGS multigene panels in the majority of cases. Whole exome sequencing (WES), clinical exome sequencing (TruSight One, Illumina), small NGS panels and targeted variant testing (Sanger sequencing) were used less frequently. Post-test counselling was offered at the centre to all patients who received a genetic result.

NCCN criteria and rationale for germline testing

Each patient's personal and family cancer history was retrospectively assessed against the NCCN clinical criteria for Lynch syndrome genetic testing. Two versions were applied in parallel: Version 1.2023 (Genetic/Familial High-Risk Assessment: Colorectal), in force during the study period, and Version 1.2026 (NCCN Genetic/Familial High-Risk Assessment: Colorectal, Endometrial and Gastric), released after the end of data collection and applied retrospectively for comparison. For the 2026 version, patients were separately classified according to (i) core criteria (category 2A) and (ii) the full criteria set, including category 2B. Clinical indication for testing outside formal NCCN criteria was classified by the documented rationale in the counselling record as: personal cancer history in a Lynch-associated organ system, family history of Lynch-associated cancer, both, unaffected predictive testing based on family history alone, or patient request with no formal criterion identified.

Molecular characterisation of urothelial tumours

Patients from the study cohort referred with urinary tract tumours were offered germline WES from peripheral blood on a research basis. WES was performed on the urothelial sub-cohort to characterise the germline landscape of urothelial carcinomas, given that (i) urothelial cancers are the third most frequent malignancy in Lynch syndrome, (ii) germline testing is not currently reimbursed for urothelial tumours under the Romanian National Oncology Programme, and (iii) while upper tract urothelial carcinoma has long been recognised as part of the Lynch tumour spectrum, bladder urothelial carcinoma has historically been considered a sporadic, environmentally driven malignancy, and its potential hereditary component remains under-characterised 18,19. An exome-based approach was chosen over targeted multigene panels to allow interrogation of genes beyond the currently established spectrum and to enable re-analysis as evidence for novel gene-disease associations accumulates 20,21.

DNA was extracted at the Center for Genomic Medicine, Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania. Library preparation, target enrichment and sequencing were performed at Novogene (Cambridge, UK) using the Twist Human Exome 2.0 (Twist Bioscience, San Francisco, CA, USA) on an Illumina NovaSeq X Plus platform, in short-read, paired-end 2 × 150 bp mode, to a mean target coverage of approximately 50X.

Bioinformatic analysis and variant interpretation were performed at the Center for Genomic Medicine, using Illumina DRAGEN v4.0 for single-nucleotide variant and indel calling and CNVkit 22 and ExomeDepth 23 for copy-number variant (CNV) analysis, with GRCh38.p14 as the reference genome. Annotation was performed with GeneBe v0.1.0-a.22 (https://genebe.net/) 24 for short variants (under 50 base pairs) and SVAnnotator (https://github.com/ComputationalGenomics/SVAnnotator) for CNV calls. Coding exons and adjacent (+/- 10 bp) intronic regions were analysed. Variants were filtered by minor allele frequency below 1% in gnomAD v4 and prioritised on the basis of genotype and phenotype, with particular focus on genes of the LS spectrum and other genes with definitive, strong or moderate evidence of association with hereditary cancer predisposition. Variants were classified according to the 2015 ACMG/AMP guideline 25 and the 2020 ACMG/ClinGen recommendations for copy number variants 26 as pathogenic, likely pathogenic and variants of uncertain significance.

In addition to germline testing, formalin-fixed paraffin-embedded (FFPE) tissue from the primary tumour was reviewed by the pathologist, who selected tumour-representative regions and confirmed adequate tumour cellularity. Mismatch repair status was assessed by immunohistochemistry for MLH1, MSH2, MSH6 and PMS2. Recurrent hotspot variants at codons 248 and 249 of *FGFR3* (NM_000142.5:c.742C > T, p.(Arg248Cys), R248C; NM_000142.5:c.746C > G, p.(Ser249Cys), S249C) were analysed by Sanger sequencing using BigDye™ Terminator v3.1 chemistry (Thermo Fisher Scientific, Waltham, USA), as previously described 27.

Data collection and database construction

Clinical, family history, and molecular data were retrospectively extracted from all available records within the oncogenetics department. For each patient, the following variables were compiled: age at diagnosis, sex, personal cancer history, family cancer history, and molecular findings including germline test results and, where performed, somatic tests (histological and molecular). Terminology was harmonised across records, and tumour sites were classified into three organ-system categories relevant to LS: gastrointestinal, gynaecological, and reno-urinary. Breast cancer and tumours involving other organ systems were retained as separate categories. Reported cancers in family members were classified by organ system (gastrointestinal, gynaecological, reno-urinary, or other) and by degree of relationship to the index patient, as first-degree relatives (parents, siblings, and children) or other relatives.

Germline variants reported by external laboratories were re-annotated using the standardised HGVS nomenclature and MANE Select transcripts. Each reported variant was reviewed by the molecular geneticist and classified as either a primary finding or an additional / incidental finding on the basis of the gene–phenotype association strength, as curated by ClinGen and the Gene Curation Coalition (GenCC) 28,29. Variants in genes with at least moderate evidence of association with the referral phenotype were considered primary findings, whereas pathogenic, likely pathogenic and uncertain variants in genes with only limited evidence of association were classified as additional / incidental and not considered causal.

Ethics

The study was approved by the Ethics Committees of Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania (approval number 102/03.09.2024) and of the OncoHelp Oncology Center, Timișoara, Romania (approval number 2334/27.09.2024), and was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent for genetic testing at the time of clinical evaluation. Patients included in the urothelial cohort additionally provided written informed consent for participation in research and for the use of their data. Patient data were pseudonymised prior to analysis, with each individual assigned a study identifier.

Statistical analysis

Categorical variables were reported as counts and percentages, continuous variables as median with range. Group comparisons used Fisher's exact test, and pairwise comparisons used the McNemar exact test. All statistical analyses were performed in Python 3.11 using pandas v2.2, NumPy v1.26, SciPy v1.13 and statsmodels v0.14. For all statistical tests applied, significance was defined as a p-value < 0.05. All figures in the manuscript were created with matplotlib v3.9.

3. Results

Study cohort, tumour spectrum and clinical presentation

Between January 2023 and December 2025, the first three years of the streamlined oncogenetic service at the centre, 481 patients referred for genetic counselling met the study criteria. The great majority (462, 96.0%) were affected patients evaluated for a personal cancer diagnosis, and 19 (4.0%) were unaffected individuals referred for predictive testing. Female patients predominated slightly (270, 56.1%). Detailed cohort characteristics are shown in Table 1.

Table 1
Demographic, clinical and family history characteristics of the study cohort. Percentages for primary cancer site are calculated over 552 primary tumours recorded in 462 affected patients.

Characteristic	n (%)	Characteristic	n (%)
Demographics		**Family cancer history (n = 481)**	
Female sex	270 (56.1%)	≥ 1 affected relative (any degree)	367 (76.3%)
Referral indication		≥ 1 first-degree relative	232 (48.2%)
Personal cancer diagnosis	462 (96.0%)	≥ 1 relative of other degree	256 (53.2%)
Predictive testing (unaffected)	19 (4.0%)	Both first-degree and other	121 (25.2%)
Primary cancer site (n = 552 tumours in 462 affected)		No affected relatives	114 (23.7%)
Gastrointestinal	255 (46.2%)	**Family history by organ system**	
Gynaecological	127 (23.0%)	Gastrointestinal	167 (34.7%)
Reno-urinary	112 (20.3%)	First-degree	92 (19.1%)
Other organ system	58 (10.5%)	Other degree	104 (21.6%)
Multiple primary tumours		Gynaecological	90 (18.7%)
Single primary	387 (83.8%)	First-degree	33 (6.9%)
≥ 2 primaries	75 (16.2%)	Other degree	61 (12.7%)
2 primaries	64	Breast	71 (14.8%)
3 primaries	9	Reno-urinary	58 (12.1%)
5 primaries	2	≥ 1 first-degree relative < 50 y	54/232 (23.3%)
Early-onset disease		≥ 1 relative of other degree < 50 y	62/256 (24.2%)
≥ 1 cancer diagnosed before age 50	121 (26.2%)		

Among the 462 affected patients, 552 primary malignancies were recorded. The gastrointestinal tract was the most frequent primary site (46.2% of tumours), followed by gynaecological (23.0%) and reno-urinary (20.3%). Multiple primary tumours occurred in 75 patients (16.2%), most commonly two, and 121

patients (26.2%) had at least one cancer diagnosed before the age of 50. Sex-based differences were modest and non-significant for both multiple primaries (17.3% of women vs 15.0% of men, p = 0.53, Fisher's exact test) and early-onset disease (29.0% vs 22.7%, p = 0.14).

Family cancer history

A positive family cancer history was noted in 367/481 patients (76.3%). First-degree affected relatives were reported in 232 (48.2%) and other distant relatives in 256 (53.2%). Both first-degree and distant affected relatives were reported in 121 (25.2%) families. Gastrointestinal malignancies were the most frequent familial tumours (167 patients, 34.7%), followed by gynaecological (90, 18.7%), breast (71, 14.8%) and reno-urinary cancers (58, 12.1%). Early-onset cancer in an affected relative, diagnosed before the age of 50, was reported at similar frequencies for first-degree relatives (54/232, 23.3%) and for relatives of other degrees (62/256, 24.2%).

Proband–family concordance within the same organ system was significantly stronger for gastrointestinal malignancies (44.0% of affected patients with a gastrointestinal primary had a gastrointestinal family history, vs 25.3% of those with other primaries; OR 2.31, 95% CI 1.56–3.44, p < 0.001) and for reno-urinary malignancies (19.8% vs 8.7%; OR 2.59, 95% CI 1.42–4.73, p = 0.003). For gynaecological malignancies, given that these cancers occur almost exclusively in female relatives, the concordance analysis was restricted to female probands. Among 255 affected women, no significant concordance was observed regarding gynaecological malignancies: 21/124 women (16.9%) with a gynaecological primary tumour had a family history of gynaecological cancer, versus 31/131 (23.7%) with other primary tumours (OR 0.66, 95% CI 0.35–1.22, p = 0.21, Fisher's exact test) (Fig. 1).

Tumour MMR/MSI findings

Tumour MMR/MSI testing was available for 151/462 affected patients (32.7%) and was performed predominantly in colorectal and endometrial cancers, with results available for 98/208 (47.1%) and 27/53 (50.9%) patients, respectively. Testing was considerably less frequent in other tumour types. Among tested cases, 56/151 (37.1%) were classified as dMMR and/or MSI-H and 95/151 (62.9%) as pMMR and/or MSS. dMMR/MSI-H was identified in 39/98 tested colorectal cancers (39.8%) and 14/27 tested endometrial cancers (51.9%), together accounting for 53/56 dMMR/MSI-H cases. Among the 56 dMMR/MSI-H cases, the pattern of protein loss was specified in 46: loss of MLH1/PMS2 in 32, combined loss of MSH2/MSH6 in 5, isolated PMS2 loss in 5, and isolated MSH6 loss in 4. In the remaining 10 cases, mismatch repair deficiency was reported without specification of the protein involved, or was established by microsatellite instability testing alone.

Germline testing and diagnostic yield

Of the 481 patients in the study cohort, 153 (31.8%) underwent germline testing during the study period. Testing was performed on broad multigene panels in 127 cases (83.0%), on whole exome or clinical exome sequencing in 14 (9.2%), and on small multigene panels and Sanger sequencing in another 12 (7.8%).

Of 183 patients meeting at least one NCCN 2023 criterion, 61 (33.3%) underwent germline testing, while 122 (66.7%) did not. The proportion tested did not increase with the strength of the clinical indication: 30.9% observed among the patients not meeting any NCCN criterion, 30.6% among those meeting a single criterion and 43.6% among those meeting two or more (p = 0.26, Fisher's exact test). Among the 92 tested patients who did not meet any formal NCCN 2023 criterion, testing was justified by personal cancer history in a Lynch-associated organ system, family history, or both in 60/92 (65.2%) cases, 11/92 (12.0%) were unaffected individuals tested predictively based on family history, and 21/92 (22.8%) were tested at patient request without a formal clinical criterion.

Among the 153 tested patients, 37 (24.2%) carried a P/LP variant considered causal for the referral phenotype, 16 (10.5%) carried variants of uncertain significance as primary findings (after curation), and a further 100 (65.4%) had a negative primary result. Additional or incidental findings were identified in 9 patients (5.9%). Pathogenic and likely pathogenic causal variants were identified across 11 genes (Table 2). Mismatch repair genes associated with LS accounted for the majority of primary findings (21/37, 56.8%): *MLH1* in 10 patients (27.0%), *MSH6* in 6 (16.2%), *MSH2* in 3 (8.1%), and *PMS2* in 2 (5.4%). Homologous recombination repair genes accounted for a further 14/37 primary findings (37.8%), predominantly *BRCA1* (8, 21.6%), together with *ATM* (3, 8.1%), *BRCA2* (1), *BARD1* (1) and *CHEK2* (1). The remaining 2 findings were in *MUTYH* (homozygous) and *SDHB*.

Table 2

Germline pathogenic (P), likely pathogenic (LP) and variants of uncertain significance (VUS) identified in 37 of 153 tested patients (primary findings)

ID	Sex	Age	Gene	Transcript	cDNA	Protein	Zygosity	ACMG	ClinVar ID	Referral phenotype	Family
P1	M	37	*MLH1*	NM_000249.4	c.544A > G	p.(Arg182Gly)	het	P	90258	SBC (x2) (37)	Cousi (27)
P2	M	45	*MLH1*	NM_000249.4	c.306 + 5G > A		het	P	90148	CRC (45)	None
P3	M	45	*MLH1*	NM_000249.4	c.(116+1_117-1)_(884+1_885-1)del	p.?	het	P	661730	GBC (45)	Fathe (35); TCL (5 paterr grandf PC
			MSH2	NM_000251.3	c.557A > G	p.(Asn186Ser)	het	VUS	91133		
P4	M	53	*MLH1*	NM_000249.4	c.143A > C	p.(Gln48Pro)	het	P	89739	CRC (53)	Fathe (48); OM
P5	F	54	*MLH1*	NM_000249.4	c.2041G > A	p.(Ala681Thr)	het	P	17099	CRC (54)	Cousi (49); mater relativ
P6	F	57	*MLH1*	NM_000249.4	c.677G > A	p.(Arg226Gln)	het	P	90318	CRC (32, 40, 44, 57); EC (51)	None
P7	M	58	*MLH1*	NM_000249.4	c.1667G > A	p.(Ser556Asn)	het	P	449776	CRC (58); PrC (?)	None
P8	M	59	*MLH1*	NM_000249.4	c.2059C > T	p.(Arg687Trp)	het	P	90014	CRC (x2) (59)	Mothe
P9	F	69	*MLH1*	NM_000249.4	c.677G > A	p.(Arg226Gln)	het	P	90318	CRC (51, 69); EC (55)	Mothe (55); grandı — GC
P10	M	38	*MLH1*	NM_000249.4	c.545 + 3A > G	p.?	het	P	90260	CRC (37)	Mothe (57) a polyp 40 pol (47); f GC (62
P11	F	42	*MSH2*	NM_000251.3	c.2245G > T	p.(Glu749Ter)	het	P	820955	unaffected	None
P12	F	58	*MSH2*	NM_000251.3	c.1801C > T	p.(Gln601Ter)	het	P	1758	EC (58)	Mothe
P13	F	34	*MSH2*	NM_000251.3	c.596G > A	p.(Cys199Tyr)	het	LP	91147	unaffected	Mothe (53); grandı — BC
P14	F	21	*MSH6*	NM_000179.3	c.3261dup	p.(Phe1088LeufsTer5)	het	P	89364	unaffected	None
P15	F	36	*MSH6*	NM_000179.3	c.2653A > T	p.(Lys885Ter)	het	P	142620	CRC (36)	brothe (39); CUP
P16	F	46	*MSH6*	NM_000179.3	c.2300C > T	p.(Thr767Ile)	het	P	141058	EC (46)	Paterr grandf CRC (5
P17	M	49	*MSH6*	NM_000179.3	c.2653A > T	p.(Lys885Ter)	het	P	142620	CRC (49)	Sister (45); CRC (1 mater uncle (70)
P18	F	53	*MSH6*	NM_000179.3	c.3261dup	p.(Phe1088LeufsTer5)	het	P	89364	EC (53)	Mothe (70); HL (17

Zygosity: het = heterozygous, hom = homozygous. P/LP variants are grouped by gene, in the order: mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*), hom recombination repair genes (*BRCA1, BRCA2, ATM, BARD1, CHEK2*), and other hereditary cancer genes (*MUTYH, SDHB*). **Cancer types**: BC = breast cancer; BD biliary duct cancer; BoC = bone cancer; CRC = colorectal cancer; CUP = cancer of unknown primary; CxC = cervical cancer; EC = endometrial cancer; GBC = gallbladder cancer; GC = gastric cancer; HL = Hodgkin lymphoma; HNC = head-and-neck cancer; LC = lung cancer; OC = ovarian cancer; OeC = oesophageal c OM = ocular melanoma; PC = pancreatic cancer; PrC = prostate cancer; RC = renal cancer; SBC = small bowel cancer; TC = testicular cancer; TCL = T-cell lymp UBC = urothelial bladder cancer.

ID	Sex	Age	Gene	Transcript	cDNA	Protein	Zygosity	ACMG	ClinVar ID	Referral phenotype	Family
P19	M	47	*MSH6*	NM_000179.3	c.2653A > T	p.(Lys885Ter)	het	P	142620	CRC (47)	Sister (50); b CRC (4 mater – skin cousir (25); c CRC, E
P20	F	34	*PMS2*	NM_000535.7	c.65C > G	p.(Ser22Ter)	het	LP	960049	unaffected	Mothe (38); n grandı — BC (
P21	F	36	*PMS2*	NM_000535.7	c.1792C > T	p.(Gln598Ter)	het	LP	957674	GC (36)	None
P22	M	34	*BRCA1*	NM_007294.4	c.2329del	p.(Tyr777MetfsTer15)	het	P	54536	unaffected	Mothe bilater (BRCA multip mater aunts OC/ot
P23	F	39	*BRCA1*	NM_007294.4	c.5266dup	p.(Gln1756ProfsTer74)	het	P	17677	unaffected	Mothe paterr cousir mater — BDC
P24	F	45	*BRCA1*	NM_007294.4	c.2933dup	p.(Tyr978Ter)	het	P	236270	OC (45)	Mothe (65); p cousir (60)
P25	F	48	*BRCA1*	NM_007294.4	c.843_846del	p.(Ser282TyrfsTer15)	het	P	17683	OC (48)	Mater grandı — OC
P26	F	58	*BRCA1*	NM_007294.4	c.3607C > T	p.(Arg1203Ter)	het	P	17671	OC (58)	Mater uncle (83)
P27	F	58	*BRCA1*	NM_007294.4	c.2329del	p.(Tyr777MetfsTer15)	het	P	54536	OC (58)	2 pate nieces (58)
P28	M	66	*BRCA1*	NM_007294.4	c.843_846del	p.(Ser282TyrfsTer15)	het	P	17683	CRC (66)	Mothe (53)
P29	M	69	*BRCA1*	NM_007294.4	c.4986 + 6T > C	p.?	het	LP	37620	PrC (69)	Brothe (67); F grandı — CUP paterr cousir
P30	M	70	*BRCA2*	NM_000059.4	c.3751dup	p.(Thr1251AsnfsTer14)	het	P	51516	PrC (70)	Brothe HNC (smoke
P31	F	42	*ATM*	NM_000051.4	c.6628del	p.(Gln2210SerfsTer25)	het	LP	265548	BC (42)	Mothe (42); n grandı — PC (paterr — LC (
P32	M	55	*ATM*	NM_000051.4	c.7792C > T	p.(Arg2598Ter)	het	P	265383	GC (55)	Sister (50); n BoC (6
P33	M	76	*ATM*	NM_000051.4	c.1564_1565del	p.(Glu522IlefsTer43)	het	P	127340	PrC (76)	Mothe (63); n uncle 70); m grandı — BC/ mater

Zygosity: het = heterozygous, hom = homozygous. P/LP variants are grouped by gene, in the order: mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*), hom recombination repair genes (*BRCA1, BRCA2, ATM, BARD1, CHEK2*), and other hereditary cancer genes (*MUTYH, SDHB*). **Cancer types**: BC = breast cancer; BD biliary duct cancer; BoC = bone cancer; CRC = colorectal cancer; CUP = cancer of unknown primary; CxC = cervical cancer; EC = endometrial cancer; GBC = gallbladder cancer; GC = gastric cancer; HL = Hodgkin lymphoma; HNC = head-and-neck cancer; LC = lung cancer; OC = ovarian cancer; OeC = oesophageal c OM = ocular melanoma; PC = pancreatic cancer; PrC = prostate cancer; RC = renal cancer; SBC = small bowel cancer; TC = testicular cancer; TCL = T-cell lymp UBC = urothelial bladder cancer.

ID	Sex	Age	Gene	Transcript	cDNA	Protein	Zygosity	ACMG	ClinVar ID	Referral phenotype	Family
											cousin (50)
P34	F	48	*BARD1*	NM_000465.4	c.176_177del	p.(Glu59AlafsTer8)	het	P	371931	OC (48)	Mater grandı — OC
P35	M	40	*CHEK2*	NM_007194.4	c.(1008+1_1009-1)_(1095+1_1096-1)del (exon 10 deletion)	p.?	het	LP	4890851	CRC (40); TC (33)	Sister leukae paterr grandı — CRC
			MSH6	NM_000179.3	c.1702T > G	p.(Phe568Val)	het	VUS	1512509		
			MSH6	NM_000179.3	c.1711G > C	p.(Gly571Arg)	het	VUS	2710370		
P36	M	52	*MUTYH*	NM_001128425.2	c.536A > G	p.(Tyr179Cys)	hom	P	5293	CRC (52)	Mater — CUF
P37	M	26	*SDHB*	NM_003000.3	c.286 + 2T > A	p.?	het	P	140773	RC (26)	Multip relativ PC/BC

Zygosity: het = heterozygous, hom = homozygous. P/LP variants are grouped by gene, in the order: mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*), hom recombination repair genes (*BRCA1, BRCA2, ATM, BARD1, CHEK2*), and other hereditary cancer genes (*MUTYH, SDHB*). **Cancer types**: BC = breast cancer; BD biliary duct cancer; BoC = bone cancer; CRC = colorectal cancer; CUP = cancer of unknown primary; CxC = cervical cancer; EC = endometrial cancer; GBC = gallbladder cancer; GC = gastric cancer; HL = Hodgkin lymphoma; HNC = head-and-neck cancer; LC = lung cancer; OC = ovarian cancer; OeC = oesophageal c OM = ocular melanoma; PC = pancreatic cancer; PrC = prostate cancer; RC = renal cancer; SBC = small bowel cancer; TC = testicular cancer; TCL = T-cell lymp UBC = urothelial bladder cancer.

Diagnostic yield increased with family cancer burden. Among tested patients with no affected first-degree relatives, 14/73 (19.2%) carried a pathogenic or likely pathogenic variant, among those with one affected first-degree relative, 7/32 (21.9%), and among those with two or more, 16/48 (33.3%). Testing rates in the same groups were similar, 29.3%, 30.8% and 37.5% respectively, indicating that patients with the highest *a priori* probability of a positive result were only modestly more likely to be tested.

Comparison with NCCN 2026 criteria

The number of patients meeting at least one criterion was 183/481 (38.0%) under NCCN 2023, 209/481 (43.5%) under the 2026 criteria (category 2A), and 318/481 (66.1%) when the category 2B recommendations were included. Compared with NCCN 2023, the 2026 core criteria added 26 patients. Of the 26 newly eligible patients, 23 (88.5%) had a personal diagnosis of endometrial cancer captured by the new universal testing recommendation. The category 2B recommendation added a further 109 patients (135 in total compared with NCCN 2023), of whom 97 (71.9%) had colorectal cancer diagnosed at age 50 or older and 23 (17.0%) had endometrial cancer.

Applied to the 37 tested patients with P/LP variants considered causal for the referral phenotype, sensitivity increased in NCCN 2026 (category 2B included) compared to NCCN 2023 and NCCN 2026 (category 2A), with an increase in detection of patients carrying variants in other hereditary cancer genes (Fig. 2, Fig. 3). The three additional P/LP variants captured only by the category 2B recommendations, in *BRCA1*, *ATM*, and *MUTYH* (biallelic), were identified in patients referred for colorectal or gastric cancer diagnosed at age 50 or older without a family history meeting other criteria. No differences in sensitivity between the three frameworks were significant (McNemar exact test).

Additional findings

Additional variants in cancer genes not considered causal for the referring phenotype were identified in 9/153 tested patients (5.9%). Four (2.6%) involved genes on the ACMG Secondary Findings v3.3 list 30: *BRCA2* (colorectal cancer, 42 y), *PMS2* (breast cancer, 46 y), *TMEM127* (small bowel cancer, 37 y), and *EPCAM* (breast cancer, 56 y). The remaining five (3.3%) involved *SPINK1* (prostate cancer, 75 y), *LZTR1* (colorectal cancer, 70 y; ovarian cancer, 48 y), *ATM* (colorectal cancer, 45 y), *EGFR* (small bowel cancer, 37 y; same patient with the *TMEM127* variant), and a *POLD1* variant (urothelial bladder cancer, 58 y) (Table 3).

Table 3
Additional germline cancer gene variants identified in the study cohort.

ID	Sex	Age	Referral	Primary finding	Gene	Variant, zygosity, classification	ClinVar ID	Family history
P1	M	37	SBC	*MLH1* (see Table 2)	*TMEM127*	NM_017849.4:c.245-1G > T, p.?, het, LP	109	Cousin — CRC (27)
P1	M	37	SBC	*MLH1* (see Table 2)	*EGFR*	NM_005228.5:c.1283G > A, p.(Gly428Asp), het, P	157499	Cousin — CRC (27)
P38	M	42	CRC	Negative	*BRCA2*	NM_000059.4:c.5946del, p.(Ser1982ArgfsTer22), het,P	9325	Paternal aunt — RC/CRC (32); paternal cousin — CRC (43, MSH2+); paternal uncle — brain tumour (74); maternal aunt — CxC (50)
P39	F	56	BC	*MSH6* VUS	*EPCAM*	NM_002354.3:c.604_607del, p.(Lys202LeufsTer7), het, LP	4890852	Mother — PC (65); maternal aunt — OC (42)
P40	F	46	BC	Negative	*PMS2*	NM_000535.7:c.1297A > T, p.(Lys433Ter), het, P	216073	Paternal aunt — LC; paternal cousin — EC
P41	M	75	PrC	Negative	*SPINK1*	NM_001379610.1:c.101A > G, p.(Asn34Ser), het, P	13760	Mother — BC (65)
P42	M	70	CRC	Negative	*LZTR1*	NM_006767.4:c.1373dup, p.(His459ProfsTer210), het, P	967710	Father — tumour (NOS); paternal cousin — CUP (50)
P43	F	48	OC	Negative	*LZTR1*	NM_006767.4:c.890_891del, p.(Tyr297CysfsTer18), het, LP	1073645	Maternal aunt — BC (60); maternal grandfather — UBC; paternal grandmother — BC (60)
P44	F	45	CRC	Negative	*ATM*	NM_000051.4:c.7630-2A > C, p.?, het, P	127447	None
P45	M	58	UBC	Negative	*POLD1*	NM_002691.4:c.835_837del, p.(Glu279del), het, VUS	484336	Father — LC (60); maternal cousin — gynecologic cancer (54); paternal grandfather — PrC (80)

Zygosity: het = heterozygous. **Cancer types**: BC = breast cancer; CRC = colorectal cancer; CUP = cancer of unknown primary; CxC = cervical cancer; EC = endometrial cancer; LC = lung cancer; NOS = not otherwise specified; OC = ovarian cancer; PC = pancreatic cancer; PrC = prostate cancer; RC = renal cancer; SBC = small bowel cancer; UBC = urothelial bladder cancer.

Molecular characterisation of the urothelial sub-cohort

Nineteen patients with urothelial carcinomas were referred for genetic evaluation during the study period, representing 4.0% of the whole cohort. Eleven (57.9%) were female and eight (42.1%) were male. Median age at diagnosis was 56 years (range 36–77). Four patients (21.0%) had upper tract urothelial carcinoma (UTUC), all of the renal pelvis, and 15 (78.9%) had bladder urothelial carcinoma. Positive family cancer history in first- or second-degree relatives was documented in 18/19 patients (94.7%).

Tumour MMR immunohistochemistry was performed in 13/19 patients (68.4%) and all tested tumours were MMR-proficient (pMMR). Sanger sequencing of *FGFR3* hotspot variants R248C and S249C was performed on 13/19 tumours (68.4%). *FGFR3* S249C variant (NM_000142.5:c.746C > G) was identified in 4/13 tested tumours (30.8%), affecting three bladder cancers and one UTUC. No R248C variants were identified. Histological grade was high in 6 patients (31.6%) and low in 6 patients (31.6%), while grade could not be assessed in 7 cases (36.8%). Regarding pathological staging, most tumours were classified as pTa (8 patients, 42.1%), followed by pT1 (3 patients, 15.8%), with one case each of pT2 and pT4 (5.3% each); staging data were unavailable for 6 patients (31.6%). Among the 4 patients harbouring an *FGFR3* S249C variant, 2 tumours were high-grade and 2 were low-grade, and staging was predominantly pTa (3/4 cases), with one pT1.

Germline testing was performed in 16/19 patients (84.2%): 13 (68.4%) underwent WES (Material and Methods) and 3 (15.8%) were tested on broad multigene hereditary cancer panels at the patient's request. No P/LP variants considered causal for the referral phenotype were identified. Three variants of uncertain significance were identified (*BRCA2*, p.(Ile2296Met); *BRCA2*, exon 13 duplication, and *TERT*, p.(Arg646Cys)), and one additional finding of uncertain clinical significance was identified in *POLD1* (Table 4).

Table 4
Clinical and molecular characteristics of the urothelial tract carcinoma sub-cohort.

ID	Sex	Age	Referral	MMR IHC	*FGFR3*	Histological grade	Pathological staging	Germline test	Result	ClinVar ID	Family histo
U1	F	59	UTUC-RP	pMMR	S249C	High	pT1	WES	Negative	–	Father — LC
U2	F	69	UTUC-RP	pMMR	negative	High	pT2	WES	Negative	–	Brother — R maternal ur PrC (73)
U3	F	74	UTUC-RP	pMMR	negative	High	pT4	WES	Negative	–	Mother — B
U4	M	74	UTUC-RP, CRC (65), BCC (61)	pMMR	negative	High	pT1	WES	Negative	–	Brother — C
U5	M	36	UBC	—	—	NA	NA	—	—	–	Paternal au CUP (> 65)
U6	F	41	UBC	pMMR	negative	Low	pTa	WES	Negative	–	None
U7	M	43	UBC	pMMR	negative	Low	pT1	WES	Negative	–	Father — CF
U8	M	47	UBC	—	—	NA	NA	Panel	Negative	–	Father — CU paternal un CRC (65); m uncle — UB maternal ur GC (50)
U9	F	48	UBC	pMMR	S249C	Low	pTa	WES	*BRCA2*, NM_000059.4:c.6888A > G, p.(Ile2296Met), het, VUS	252458	Father — pheochrom (30, MEN2, paternal au thyroid + ad cancer (80, RET+); pate grandfather pheochrom (60)
U10	F	48	UBC	—	—	NA	NA	—	—	–	Father — LC
U11	F	51	UBC	pMMR	negative	NA	pTa	WES	Negative	–	Father — CU maternal grandmothe (92); mothe
U12	F	55	UBC	pMMR	—	NA	NA	Panel	Negative	–	Father — CU paternal au CNS tumou
U13	M	56	UBC	—	S249C	High	pTa	WES	*BRCA2*, NM_000059.4:c.(6937+1_6938-1)_(7007+1_7008-1)[2], het, VUS (exon 13 duplication)	3244175	Paternal au (< 50)
U14	M	56	UBC, PrC	—	—	NA	NA	Panel	Negative	–	Nephew — T
U15	F	58	UBC	pMMR	negative	Low	pTa	WES	*TERT*, NM_198253.3:c.1936C > T, p.(Arg646Cys), het, VUS	242220	Maternal au CxC (58); m aunt — head neck cance maternal co EC (43); pat cousin — GI
U16	M	58	UBC	pMMR	negative	High	pTa	WES	*POLD1*, NM_002691.4:c.835_837del, p.(Glu279del), het, VUS (see Table 3, P45)	484336	Father — LC maternal co gynecologic cancer (54) PrC (80)
U17	M	65	UBC	pMMR	S249C	Low	pTa	WES	Negative	–	Sister — EC niece — BC

MMR IHC: mismatch repair protein immunohistochemistry. pMMR = MMR-proficient. **FGFR3**: Sanger sequencing of hotspot codons R248C and S249C on tu DNA. Positive cases carried the S249C variant (NM_000142.5:c.746C > G). **Germline test**: WES = whole-exome sequencing. Panel = broad multigene heredita cancer panel. Result combines the primary germline finding and any additional finding identified through broad testing. **Cancer types**: BC = breast cancer; BC basal cell carcinoma; CRC = colorectal cancer; CUP = cancer of unknown primary; CxC = cervical cancer; EC = endometrial cancer; GC = gastric cancer; LC = l cancer; NA = not available; OC = ovarian cancer; PC = pancreatic cancer; PrC = prostate cancer; RC = renal cancer; TC = testicular cancer; UTUC-RP = upper tra urothelial carcinoma, renal pelvis; UBC = bladder urothelial carcinoma.

ID	Sex	Age	Referral	MMR IHC	*FGFR3*	Histological grade	Pathological staging	Germline test	Result	ClinVar ID	Family histo
											maternal au cancer (?) (maternal co LC (40)
U18	F	69	UBC	—	—	NA	NA	—	—	–	Mother — C
U19	F	74	UBC, CRC	pMMR	negative	Low	pTa	WES	Negative	–	Niece — thy cancer

MMR IHC: mismatch repair protein immunohistochemistry. pMMR = MMR-proficient. **FGFR3**: Sanger sequencing of hotspot codons R248C and S249C on tu DNA. Positive cases carried the S249C variant (NM_000142.5:c.746C > G). **Germline test**: WES = whole-exome sequencing. Panel = broad multigene heredita cancer panel. Result combines the primary germline finding and any additional finding identified through broad testing. **Cancer types**: BC = breast cancer; BC basal cell carcinoma; CRC = colorectal cancer; CUP = cancer of unknown primary; CxC = cervical cancer; EC = endometrial cancer; GC = gastric cancer; LC = l cancer; NA = not available; OC = ovarian cancer; PC = pancreatic cancer; PrC = prostate cancer; RC = renal cancer; TC = testicular cancer; UTUC-RP = upper tra urothelial carcinoma, renal pelvis; UBC = bladder urothelial carcinoma.

4. Discussion

To our knowledge, this represents one of the largest single-centre Romanian cohorts of patients evaluated for LS and other hereditary cancer predisposition syndromes, comprising 481 consecutive referrals to a tertiary oncogenetic service across the first three years of oncogenetics implementation in the centre (2023–2025). The cohort was enriched for tumours arising in organ systems relevant to LS in the personal or family history, with gastrointestinal (46.2%), gynaecological (23.0%) and reno-urinary (20.3%) cancers accounting for nearly 90% of all primary tumours. Germline testing was performed in 31.8% of patients and identified a P/LP variant considered causal for the referral phenotype in 24.2% of tested patients, a diagnostic yield comparable to those reported by other centres 31,32.

However, this diagnostic yield should be interpreted in the context of incomplete access to germline testing, as it reflects only the subset of patients who ultimately underwent molecular analysis. In Romania, public reimbursement for hereditary cancer testing remains limited 15,33. A dedicated genetic testing subprogramme was introduced within the National Oncology Programme in 2023, substantially improving access to molecular diagnostics 34. However, its primary focus has been testing for treatment selection rather than comprehensive assessment of hereditary cancer predisposition. Under the current framework, germline testing in solid tumours is largely restricted to selected patients with advanced breast and ovarian cancers. In colorectal cancer, the programme reimburses tumour-based dMMR testing (from 2023), but does not provide routine confirmatory germline testing for patients with suspected LS. Consequently, patients referred who do not meet these criteria, frequently rely on self-funded testing or research initiatives. Therefore, the observed diagnostic yield should be interpreted with caution and may not be readily generalisable to the entire population of patients meeting clinical criteria for hereditary cancer predisposition in Romania.

Urothelial tract carcinomas represented a distinct group of our cohort (19 patients, 3.9%), a striking under-representation given that urothelial cancers are recognised as the third most frequent malignancy in LS after colorectal and endometrial tumours, particularly among *MSH2* carriers 35–37. Two factors likely contribute to this under-representation. First, referral to genetic evaluation of urothelial patients remains uncommon in Romanian clinical practice. Second, tumour MMR immunohistochemistry, which serves as an important screening step before genetic referral for colorectal and endometrial cancers, is not routinely performed on urothelial specimens. Although no LS–related variants were identified in the urothelial sub-cohort, emerging evidence regarding germline *BRCA2* variants in urothelial cancer susceptibility is relevant to our findings 38. Updated evidence published during the preparation of this manuscript now recognises urothelial carcinoma as part of the *BRCA2* tumour spectrum 38, prompting us to re-analyse the WES data from the urothelial sub-cohort with this broader spectrum in mind. After re-analysis, we identified two heterozygous *BRCA2* variants of uncertain significance in patients with urothelial bladder carcinoma, supporting periodic re-evaluation of variant classification and consideration of additional molecular approaches in these cases 39.

A recurring challenge in genetic counselling in our cohort was the interpretive burden associated with very broad commercial panels 40,41. Clinical reports frequently included large numbers of variants of uncertain significance and, in some cases, pathogenic variants that are unlikely to be causal for the referral phenotype, for example, monoallelic variants in autosomal recessive genes. This contrasts with recommended reporting practices, which typically apply stricter phenotype-driven curation and consequently report substantially fewer uncertain findings 42.

Our cohort was enriched for the LS-related tumours by design, and the identification of 21 patients with pathogenic variants in mismatch repair genes (56.8% of the 37 causal P/LP findings) reflects appropriate patient selection using the NCCN clinical criteria. The remaining P/LP findings involved genes outside the LS spectrum, most frequently *BRCA1*, but also other significant cancer genes that would have been missed by small NGS panels. The identification of germline pathogenic *BRCA1* variants is not unexpected, as ovarian cancer is a well-established indication for *BRCA1/2* testing independently of LS 43. Current NCCN guidelines recommend universal germline testing with multigene panels for all epithelial ovarian cancer patients, and these panels include both *BRCA1/2* and mismatch repair genes. It is nevertheless worth mentioning that endometrioid ovarian carcinomas are more classically associated with LS 44. Histological subtypes were not systematically captured for the present analysis, but this distinction remains relevant when interpreting P/LP findings in an oncogenetic cohort that includes ovarian cancer referrals.

The retrospective application of the NCCN 2026 criteria to our cohort illustrates the trade-off between higher sensitivity and lower specificity of broader diagnostic criteria. The 2026 core criteria (category 2A) expanded the eligible population by 5.2% relative to NCCN 2023, primarily driven by the new universal testing recommendation for endometrial cancer. The category 2B recommendations expanded the eligible population by a further 22.7% relative to the core criteria (28.2% relative to NCCN 2023), a substantial expansion driven by patients with colorectal or gastric cancer diagnosed at age 50 or older, but did not

identify any additional LS carrier beyond those already captured by the core criteria. Although category 2B did not improve the detection of LS in our cohort, they captured three additional P/LP variants in *BRCA1*, *ATM*, and *MUTYH* (biallelic). This "by-catch" effect further supports the argument that testing eligibility criteria and testing strategy should be considered together: when broader criteria are used to select who to test, the corresponding testing panel should also be broad 45–47.

Another observation from our cohort is that the proportion of patients tested did not increase with the strength of the clinical indication under NCCN 2023 criteria: patients meeting one or more criteria were tested at the same rate as those not meeting any criterion (33.3% vs. 30.9%, respectively). Among the 183 patients who met at least one criterion, 122 (66.7%) did not undergo germline testing during the study period. This pattern suggests that access to testing at our centre is currently determined more by individual circumstances, including patient willingness and ability to fund testing. Addressing this gap will require both broader public reimbursement of germline testing for hereditary cancer syndromes and integration of oncogenetic evaluation into standard oncology care pathways.

Several limitations of our study should be noted. First, the study is retrospective and single-centre, based on the case-mix and referral patterns of a single tertiary service in Western Romania, and the results may not be directly reproducible in other centres. Second, germline testing was not performed systematically according to fixed criteria, it depended mainly on patient self-funding, which introduces bias and precludes reliable estimates of hereditary cancer prevalence in our cohort. Third, tumour MMR immunohistochemistry was not performed uniformly across tumour types, as reflex testing in colorectal cancers was introduced into routine diagnostic practice only progressively during the study period. Fourth, cascade testing of family members was not captured in our cohort. Finally, follow-up of tested patients, including uptake of risk-reducing measures and outcomes was beyond the scope of the present analysis.

5. Conclusion

This study characterises one of the largest single-centre Romanian cohorts of patients evaluated for suspected hereditary cancer predisposition. Germline testing identified P/LP variants in one quarter of tested patients, with mismatch repair genes accounting for about half of all findings. The substantial contribution of non-LS findings across the cohort, together with the additional variants captured by the broader NCCN 2026 criteria, supports the use of broad multigene panels or exome sequencing over small NGS panels. Urothelial tract carcinomas remain markedly under-represented despite being the third most frequent Lynch-associated malignancy in the literature. The limited public reimbursement of germline testing in Romania restricts access and likely underestimates the true burden of hereditary predisposition in the referred population. Broader reimbursement, systematic integration of oncogenetic evaluation into oncology centres, and specialised clinical genetics expertise for report interpretation are essential steps toward equitable access to hereditary cancer risk assessment in Romania.

Abbreviations

ACMG/AMP, American College of Medical Genetics and Genomics / Association for Molecular Pathology; **CI**, confidence interval; **CNV**, copy-number variant; **dMMR**, mismatch repair-deficient; **FFPE**, formalin-fixed paraffin-embedded; **HGVS**, Human Genome Variation Society; **IHC**, immunohistochemistry; **LP**, likely pathogenic; **LS**, Lynch syndrome; **MANE**, Matched Annotation from NCBI and EBI; **MMR**, mismatch repair; **MSI**, microsatellite instability; **MSI-H**, microsatellite instability–high; **MSS**, microsatellite stable; **NCCN**, National Comprehensive Cancer Network; **NGS**, next-generation sequencing; **OR**, odds ratio; **P**, pathogenic; **pMMR**, mismatch repair-proficient; **UBC**, urothelial bladder carcinoma; **UTUC**, upper tract urothelial carcinoma; **VUS**, variant of uncertain significance; **WES**, whole exome sequencing.

Declarations

Author contributions

CVM, APT and ITP conceived and designed the study. ITP, APT, IES and EDN conducted the clinical consultations and collected the data. RB performed the histopathological evaluation of the urothelial tumors. CVM and ACE performed Sanger sequencing (*FGFR3*), bioinformatic analysis and interpretation of WES data for urothelial tumors. CVM, VOG and FN curated the data. CVM performed the statistical analysis and prepared the figures and tables. CVM and IES drafted the manuscript. APT, ACE, ITP and SN revised the manuscript. All authors read and approved the final manuscript.

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Ethics statement

The study was approved by the Ethics Committees of Victor Babeș University of Medicine and Pharmacy, Timișoara (no. 102/03.09.2024), and the OncoHelp Oncology Center, Timișoara (no. 2334/27.09.2024), and was conducted in accordance with the Declaration of Helsinki. Written informed consent was

obtained as applicable, and all data were pseudonymised prior to analysis.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. Sequence data that support new variants in this study have been submitted to the ClinVar database: NM_007194.4(CHEK2):c.(1008+1_1009-1)_(1095+1_1096-1)del, ClinVar ID: SUB16470686; NM_002354.3(EPCAM):c.604_607del, p.(Lys202LeufsTer7), ClinVar ID: SUB16470715.

Publicly available:

NM_007194.4(CHEK2):c.(1008+1_1009-1)_(1095+1_1096-1)del — Variation ID 4890851, accession VCV004890851.1, https://www.ncbi.nlm.nih.gov/clinvar/variation/4890851/

NM_002354.3(EPCAM):c.604_607del, p.(Lys202LeufsTer7) — Variation ID 4890852, accession VCV004890852.1, https://www.ncbi.nlm.nih.gov/clinvar/variation/4890852/

References

1. Ryan, N. A. J. et al. The proportion of endometrial cancers associated with Lynch syndrome: a systematic review of the literature and meta-analysis. *Genet. Med.* **21**, 2167–2180 (2019).
2. Hampel, H. et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J. Clin. Oncol.* **26**, 5783–5788 (2008).
3. Abu-Ghazaleh, N., Kaushik, V., Gorelik, A., Jenkins, M. & Macrae, F. Worldwide prevalence of Lynch syndrome in patients with colorectal cancer: systematic review and meta-analysis. *Genet. Med.* **24**, 971–985 (2022).
4. Win, A. K. et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.* **26**, 404–412 (2017).
5. Maratt, J. K. & Stoffel, E. Identification of Lynch syndrome. *Gastrointest. Endosc Clin. N Am.* **32**, 45–58 (2022).
6. Niessen, R. C. et al. Germline hypermethylation of MLH1 and EPCAM deletions are a frequent cause of Lynch syndrome. *Genes Chromosomes Cancer*. **48**, 737–744 (2009).
7. Campoy, S., Drouet, Y., Rochefort, P., Bonadona, V. & Lasset, C. Cancer risks in Lynch syndrome carriers: a systematic review and meta-analysis. *J. Natl. Cancer Inst.* **118**, 589–602 (2026).
8. Dominguez-Valentin, M. et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet. Med.* **22**, 15–25 (2020).
9. Ten Broeke, S. W. et al. Cancer risks for PMS2-associated Lynch syndrome. *J. Clin. Oncol.* **36**, 2961–2968 (2018).
10. Metcalfe, M. J. et al. Universal point of care testing for Lynch syndrome in patients with upper tract urothelial carcinoma. *J. Urol.* **199**, 60–65 (2018).
11. Masson-Lecomte, A. et al. European Association of Urology guidelines on upper urinary tract urothelial carcinoma: summary of the 2025 update. *Eur. Urol.* **87**, 697–716 (2025).
12. Vasen, H. F., Watson, P., Mecklin, J. P. & Lynch, H. T. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. *Gastroenterology* **116**, 1453–1456 (1999).
13. Umar, A. et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J. Natl. Cancer Inst.* **96**, 261–268 (2004).
14. Hodan, R. et al. Genetic/familial high-risk assessment: colorectal, endometrial, and gastric, version 3.2024, NCCN Clinical Practice Guidelines in Oncology. *J. Natl. Compr. Canc Netw.* **22**, 695–711 (2024).
15. Cătană, A. et al. Hereditary breast cancer in Romania—molecular particularities and genetic counseling challenges in an Eastern European country. *Biomedicines* **11**, 1386 (2023).
16. Iordache, P. D. et al. Identification of Lynch syndrome risk variants in the Romanian population. *J. Cell. Mol. Med.* **22**, 6068–6076 (2018).
17. Ciucă, A., Moldovan, R. & Băban, A. Developing genetic counselling services in an underdeveloped healthcare setting. *J. Community Genet.* **12**, 539–548 (2021).
18. Huang, D., Matin, S. F., Lawrentschuk, N. & Roupret, M. Systematic review: an update on the spectrum of urological malignancies in Lynch syndrome. *Bladder Cancer*. **4**, 261–268 (2018).
19. Nassour, A. J. et al. Relative risk of bladder and kidney cancer in Lynch syndrome: systematic review and meta-analysis. *Cancers (Basel)*. **15**, 506 (2023).
20. Hinić, S. et al. Exome-based cancer predisposition gene testing can provide a genetic diagnosis for individuals with heterogeneous tumor phenotypes. *Eur. J. Hum. Genet.* **33**, 803–809 (2025).
21. Deignan, J. L. et al. Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **21**, 1267–1270 (2019).
22. Talevich, E., Shain, A. H., Botton, T. & Bastian, B. C. CNVkit: genome-wide copy number detection and visualization from targeted DNA sequencing. *PLoS Comput. Biol.* **12**, e1004873 (2016).
23. Plagnol, V. et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics* **28**, 2747–2754 (2012).

24. Stawiński, P. & Płoski, R. Genebe.net: implementation and validation of an automatic ACMG variant pathogenicity criteria assignment. *Clin. Genet.* **106**, 119–126 (2024).
25. Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
26. Riggs, E. R. et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet. Med.* **22**, 245–257 (2020).
27. Billerey, C. et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. *Am. J. Pathol.* **158**, 1955–1959 (2001).
28. Thaxton, C. et al. Utilizing ClinGen gene-disease validity and dosage sensitivity curations to inform variant classification. *Hum. Mutat.* **43**, 1031–1040 (2022).
29. DiStefano, M. T. et al. The Gene Curation Coalition: a global effort to harmonize gene-disease evidence resources. *Genet. Med.* **24**, 1732–1742 (2022).
30. Lee, K. et al. ACMG SF v3.3 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **27**, 101454 (2025).
31. Pearlman, R. et al. Prospective statewide study of universal screening for hereditary colorectal cancer: the Ohio Colorectal Cancer Prevention Initiative. *JCO Precis Oncol* **5**, (2021). PO.20.00525.
32. Dixon, K. et al. Integrating tumor sequencing into clinical practice for patients with mismatch repair-deficient Lynch syndrome spectrum cancers. *Clin. Transl Gastroenterol.* **12**, e00397 (2021).
33. Vlad, I. C. et al. Preoperative germline genetic testing and surgical timing in breast cancer: implementation of national reimbursement programs: a retrospective cohort study. *Med. (Kaunas)*. **62**, 1397 (2026).
34. McCrary, J. M. et al. Genetic counselling legislation and practice in cancer in EU Member States. *Eur. J. Public. Health*. **34**, 666–675 (2024).
35. Lonati, C. et al. Upper tract urothelial carcinoma in the Lynch syndrome tumour spectrum: a comprehensive overview from the European Association of Urology—Young Academic Urologists and the Global Society of Rare Genitourinary Tumors. *Eur. Urol. Oncol.* **5**, 30–41 (2022).
36. Joost, P., Therkildsen, C., Dominguez-Valentin, M., Jönsson, M. & Nilbert, M. Urinary tract cancer in Lynch syndrome; increased risk in carriers of MSH2 mutations. *Urology* **86**, 1212–1217 (2015).
37. van der Post, R. S. et al. Risk of urothelial bladder cancer in Lynch syndrome is increased, in particular among MSH2 mutation carriers. *J. Med. Genet.* **47**, 464–470 (2010).
38. Sasagawa, H. et al. BRCA1 and BRCA2 pathogenic variants increase the risk of four less common cancer types. *ESMO Open.* **11**, 106900 (2026).
39. Börcsök, J. et al. Detection of molecular signatures of homologous recombination deficiency in bladder cancer. *Clin. Cancer Res.* **27**, 3734–3743 (2021).
40. Rehm, H. L. et al. The landscape of reported VUS in multi-gene panel and genomic testing: time for a change. *Genet. Med.* **25**, 100947 (2023).
41. Slavin, T. P. et al. Clinical application of multigene panels: challenges of next-generation counseling and cancer risk management. *Front. Oncol.* **5**, 208 (2015).
42. Mighton, C. et al. Points to consider for the reporting of variants of uncertain significance in germline genetic and genomic testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **28**, 102583 (2026).
43. Konstantinopoulos, P. A. et al. Germline and somatic tumor testing in epithelial ovarian cancer: ASCO guideline. *J. Clin. Oncol.* **38**, 1222–1245 (2020).
44. Mitric, C. et al. Mismatch-repair deficiency, microsatellite instability, and Lynch syndrome in ovarian cancer: a systematic review and meta-analysis. *Gynecol. Oncol.* **170**, 133–142 (2023).
45. Whitworth, J. et al. Interpretation of constitutional cancer predisposition gene variants in 14 765 individuals in the 100 000 Genomes Project cancer arm: a retrospective cohort analysis. *Lancet Oncol.* **27**, 502–511 (2026).
46. Tung, N. et al. Selection of germline genetic testing panels in patients with cancer: ASCO guideline. *J. Clin. Oncol.* **42**, 2599–2615 (2024).
47. Samadder, N. J. et al. Comparison of universal genetic testing vs guideline-directed targeted testing for patients with hereditary cancer syndrome. *JAMA Oncol.* **7**, 230–237 (2021).

Figures

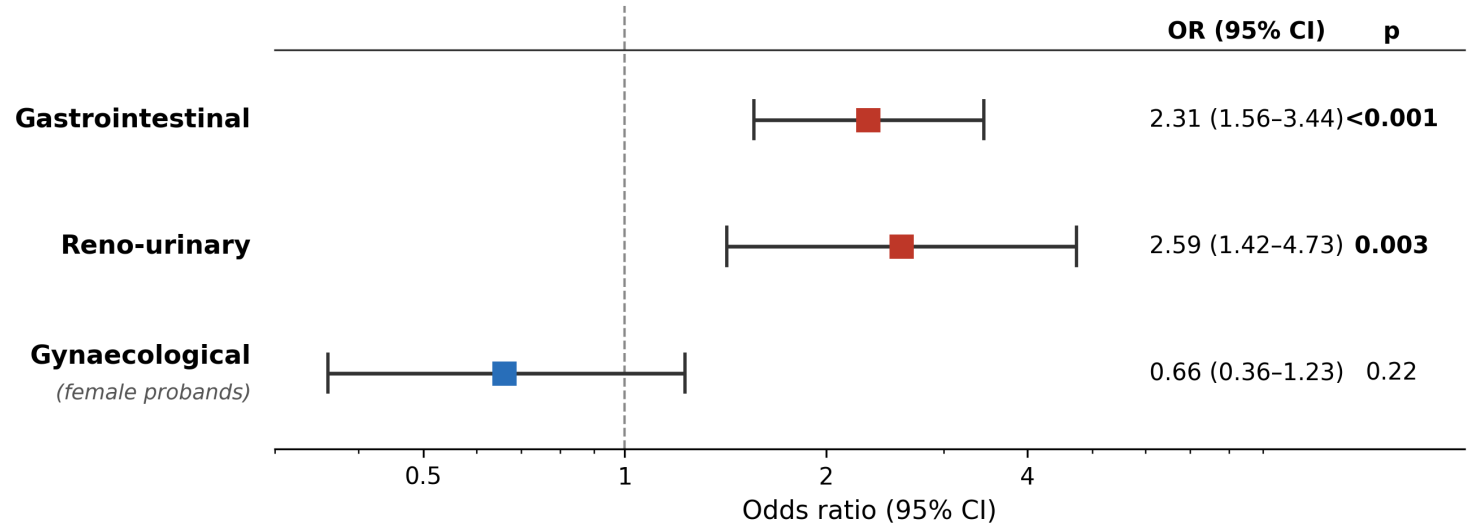


Figure 1

Association between the proband's tumour type and family history of cancer in the same organ system among 462 affected patients. Odds ratios (ORs) with 95% confidence intervals (CIs) compare patients with a tumour in a given organ system with those without, according to whether at least one relative had cancer in the same organ system. P values were calculated using Fisher's exact test.

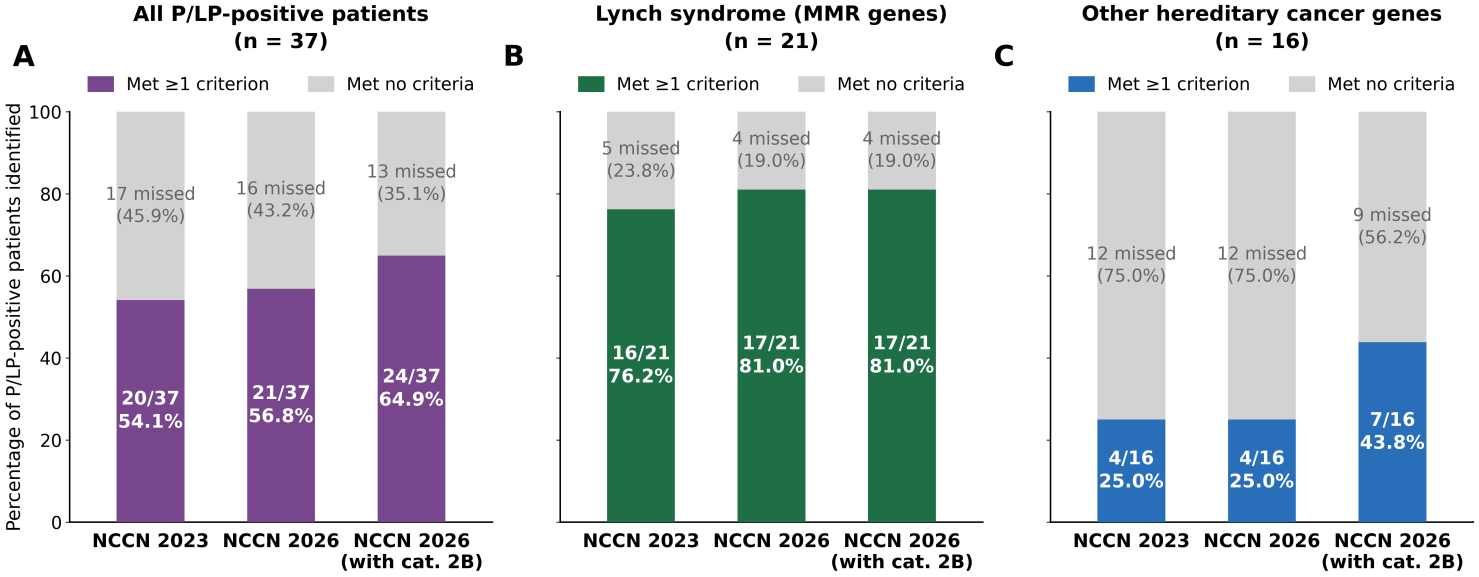


Figure 2

Sensitivity of the NCCN eligibility criteria for LS for identifying patients with germline pathogenic (P) and likely pathogenic (LP) variants. (A) All P/LP-positive patients considered to carry a variant causal for the referral phenotype (n = 37). (B) Patients with P/LP variants in LS mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*; n = 21). (C) Patients with P/LP variants in other hereditary cancer predisposition genes (*BRCA1*, *BRCA2*, *ATM*, *BARD1*, *CHEK2*, *MUTYH*, *SDHB*; n = 16). Three versions of the guideline are compared: version 1.2023, version 1.2026 core (category 2A), and version 2026 with category 2B recommendations included. Absolute counts and percentages of patients captured and missed are indicated on each bar.

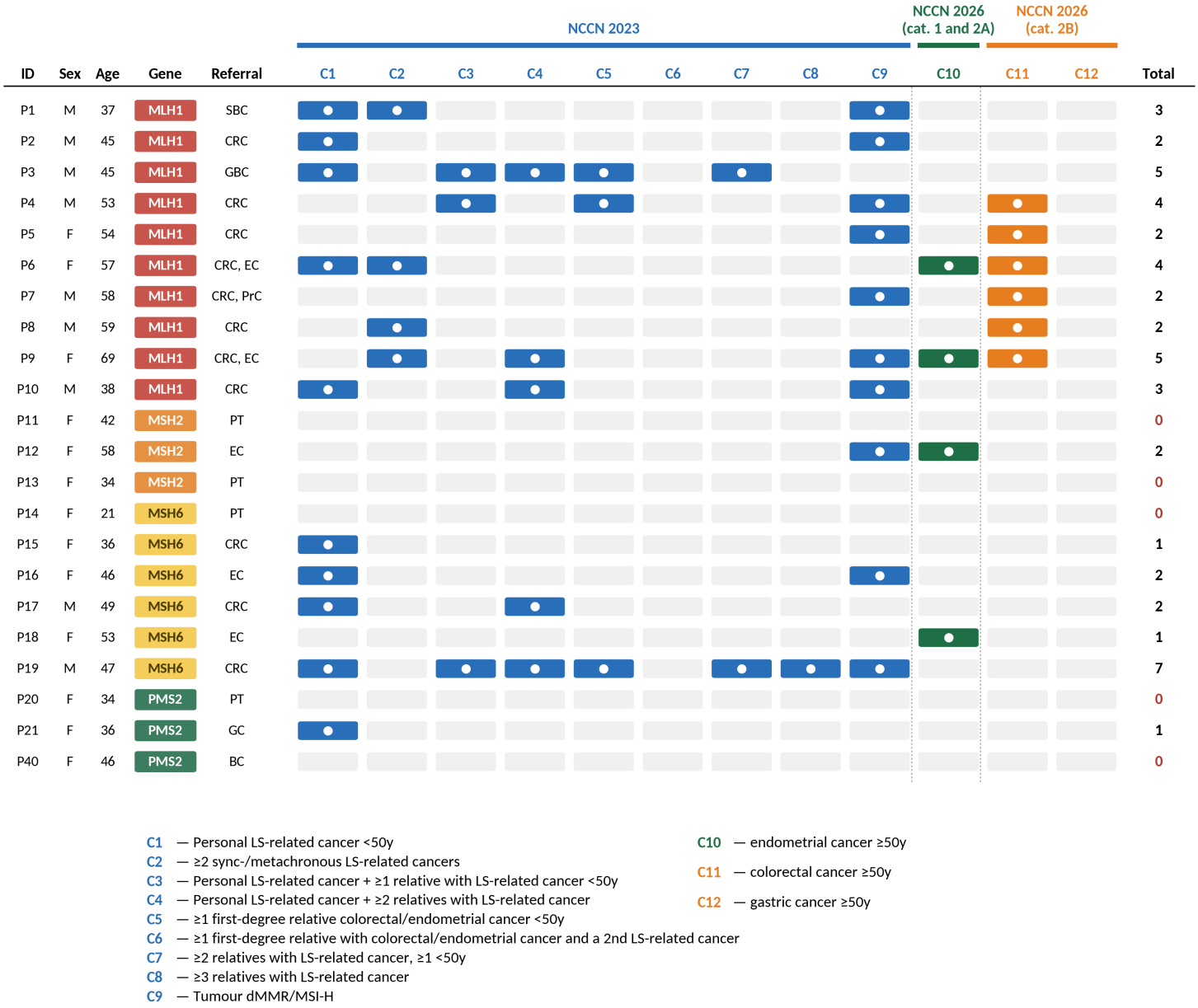


Figure 3

NCCN LS testing criteria in the 22 patients identified as carriers of a P/LP variant in a LS mismatch repair gene (*MLH1*, *MSH2*, *MSH6*, *PMS2*), including one *PMS2* variant identified as an additional finding in a female patient with breast cancer (P40). Each row represents a patient. Coloured cells indicate criteria met, depending on the guideline version. Patients are ordered by total number of criteria met. C1–C12, NCCN criteria; LS, Lynch syndrome; CRC, colorectal cancer; EC, endometrial cancer; GC, gastric cancer; GBC, gallbladder cancer; SBC, small bowel cancer; BC, breast cancer; PT, predictive testing (unaffected proband); dMMR, mismatch repair-deficient; MSI-H, microsatellite instability–high.