

Original Article

A Retrospective Investigation of Microsatellite Instability (MSI) Testing in a Tertiary Centre Genetic Laboratory

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ABSTRACT

Aim: Microsatellite instability (MSI) is an important biomarker associated with mismatch repair (MMR) deficiency, hereditary cancer syndromes, and response to immunotherapy. This study aimed to evaluate the distribution of MSI status in a heterogeneous cancer cohort and to investigate the relationship between MSI findings and accompanying germline pathogenic or likely pathogenic variants.

Methods: This retrospective, single-center cohort study included 240 cancer patients who simultaneously underwent MSI analysis (n=240) and germline genetic testing (n=101). Patients were classified as MSI-high (MSI-H), MSI-low (MSI-L), or microsatellite stable (MSS). Demographic features, tumor types, and germline variant profiles were evaluated. Pathogenic and likely pathogenic variants were classified according to American College of Medical Genetics and Genomics criteria. Statistical comparisons between MSI-H and MSS groups were performed where applicable.

Results: The cohort consisted of 126 females (52.5%) and 114 males (47.5%), with a mean age of 55.8±13.5 years. The most common malignancies were colon cancer, pancreatic cancer, endometrial cancer, gastric cancer, and ovarian cancer. MSI-H status was detected in 34 patients (14.2%), MSI-L status in 3 patients (1.2%), and MSS status in 203 patients (84.6%). Pathogenic or likely pathogenic MMR-related germline variants were identified in 7 individuals (31.8% of those tested; n=22), predominantly involving *MSH2*, *MSH3*, and *MSH6* genes. Most of these variants demonstrated clinical concordance with Lynch syndrome-associated tumor types. In contrast, MSS patients more frequently harbored pathogenic variants in non-*MMR* genes, including *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, and *MUTYH*.

Conclusion: MSI-H tumors appeared to have a higher frequency of MMR-associated germline variants, particularly in Lynch syndrome-related cancers. However, clinically actionable germline variants were also identified in MSS tumors, highlighting the importance of combined MSI and germline genetic evaluation in hereditary cancer assessment.

Keywords: Microsatellite instability, Lynch syndrome, mismatch repair, hereditary cancer, germline variant

Introduction

Microsatellite instability (MSI) results from defects in the deoxyribonucleic acid (DNA) mismatch repair (MMR) system and represents one of the most important molecular hallmarks in hereditary and sporadic cancers. Deficiency in *MMR* genes such as *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* deletion leads to the accumulation of insertion-deletion errors within microsatellite regions, ultimately contributing to tumorigenesis [1].

MSI testing has gained increasing clinical importance over the past decades due to its diagnostic, prognostic, and therapeutic

implications. MSI-high (MSI-H) tumors are strongly associated with Lynch syndrome, the most common hereditary colorectal cancer syndrome, but may also occur in sporadic cancers through somatic epigenetic mechanisms, particularly *MLH1* promoter hypermethylation [2].

Beyond colorectal cancer, MSI-H status has been observed in endometrial, gastric, ovarian, pancreatic, small bowel, and several other malignancies [3]. Identification of MSI-H tumors may prompt further germline investigation and guide immunotherapy decisions, particularly with immune checkpoint inhibitors [4].

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Although the association between MSI-H and Lynch syndrome is well established, the overall spectrum of germline variants encountered in routine MSI-tested patient cohorts remains incompletely characterized in many populations. Moreover, the relationship between tumor type, MSI status, and clinically actionable germline variants continues to evolve.

In this study, we aimed to evaluate the demographic and molecular characteristics of a real-world cancer cohort undergoing MSI analysis. We additionally investigated the frequency of pathogenic or likely pathogenic germline variants and assessed whether these variants were concordant with the clinical tumor phenotype.

Methods

This retrospective, single-center study included 240 patients with various malignancies who underwent MSI testing (n=240) and hereditary cancer panel analysis (n=101) at our institution between 2022 and 2026. Clinical and molecular data were collected retrospectively from laboratory records and patient files. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from the patients or their legal guardians. Ethical approval for this study was obtained from the Scientific Research Evaluation and Ethics Committee No. 1 of University of Health Sciences Türkiye, Ankara Etlik City Hospital, (AEŞH-BADEK1-2026-435/date: 06.05.2026).

MSI analysis was performed using fragment analysis methodology with the Seqline Multiplex MSI Analysis Kit (EYS Medikal ve Laboratuvar Projeleri, Ankara, Türkiye) and run on an Applied Biosystems 3500xL Genetic Analyzer platform (Applied Biosystems, Thermo Fisher Scientific, South San Francisco, CA, USA) using somatic tissues obtained from cancer patients.

Fragment analysis data were evaluated according to the manufacturer's recommendations. MSI status was classified as follows: microsatellite stable (MSS): no unstable markers detected; MSI-low (MSI-L): instability detected in less than 30% of analyzed markers (1 marker); MSI-high (MSI-H): instability detected in more than 30% of analyzed markers (2-5 markers).

Hereditary cancer panel analysis was performed using a custom hereditary cancer panel (60 genes), CUSTOM SOLUTION (CHCS_C_v2) v2 by SOPHiA GENETICS (SOPHiA GENETICS SA, Saint-Sulpice, Switzerland) (Supplementary Material 1). Sequencing quality metrics were assessed as part of the routine analytical pipeline. A minimum read depth of 30× was required for reliable variant calling; regions with insufficient coverage were flagged for additional review or confirmatory testing when clinically indicated (excluding copy-number changes). Variants within coding exons and the flanking intronic regions (±20 bp from exon-intron boundaries) were evaluated. Sequence alignment, variant calling, annotation, and quality control were performed using the SOPHiA DDM platform according to the manufacturer's recommendations. Variant interpretation followed the American College of

Medical Genetics and Genomics (ACMG)/ association for molecular pathology guidelines using evidence from population databases (gnomAD), disease-specific databases (ClinVar and Franklin Genoox), published literature, and multiple in silico prediction tools. Clinically relevant variants were confirmed by Sanger sequencing when appropriate as part of routine diagnostic practice. As this was a retrospective study, germline genetic testing was available only to patients who had undergone testing as part of routine clinical care. The decision to perform germline testing was based on individual clinical indications such as personal or family history, tumor characteristics (if available), and suspicion of a hereditary cancer predisposition, rather than on the study protocol. Libraries were sequenced on the NextSeq platform (Illumina, San Diego, CA, USA). Sequence data were analyzed using the SOPHiA DDM platform according to the manufacturer's bioinformatics workflow. Variants were classified according to the ACMG guidelines [5]. The standard workflow of the cohort is displayed in Figure 1.

Statistical Analysis

Descriptive statistics were used to summarize the clinical and demographic characteristics of the cohort. Categorical variables are presented as frequencies and percentages. For continuous variables, such as age at diagnosis, data are expressed as median values.

Results

Patient Characteristics

A total of 240 patients who underwent MSI testing were included in the analysis. The mean age of the cohort was 55.8±13.5 years. Females constituted 52.5% of the cohort (n=126), while males represented 47.5% (n=114).

Clinical classification of the primary malignancies revealed that colorectal cancer was the most frequent diagnosis, occurring in 58 (24.1%) cases; gastric cancer was identified in 19 (7.9%) cases, while rectal cancer, breast cancer, and glioblastoma represented smaller proportions of the cohort.

MSI Status Distribution

Molecular screening for microsatellite status categorized the patients into three groups, identifying 34 cases (14.2%) as MSI-H, 3 cases (1.2%) as MSI-L, and 203 cases (84.6%) as MSS.

Within the MSI-H subgroup, colorectal and endometrial tumors were most common. Colon cancer represented the largest proportion of MSI-H tumors, followed by endometrial and rectal cancers.

The mean age of patients with MSI-H tumors was 59.5±11.3 years, compared with 55.1±13.8 years among MSS patients. The sex distribution in the MSI-H group was relatively balanced (18 males, 16 females).

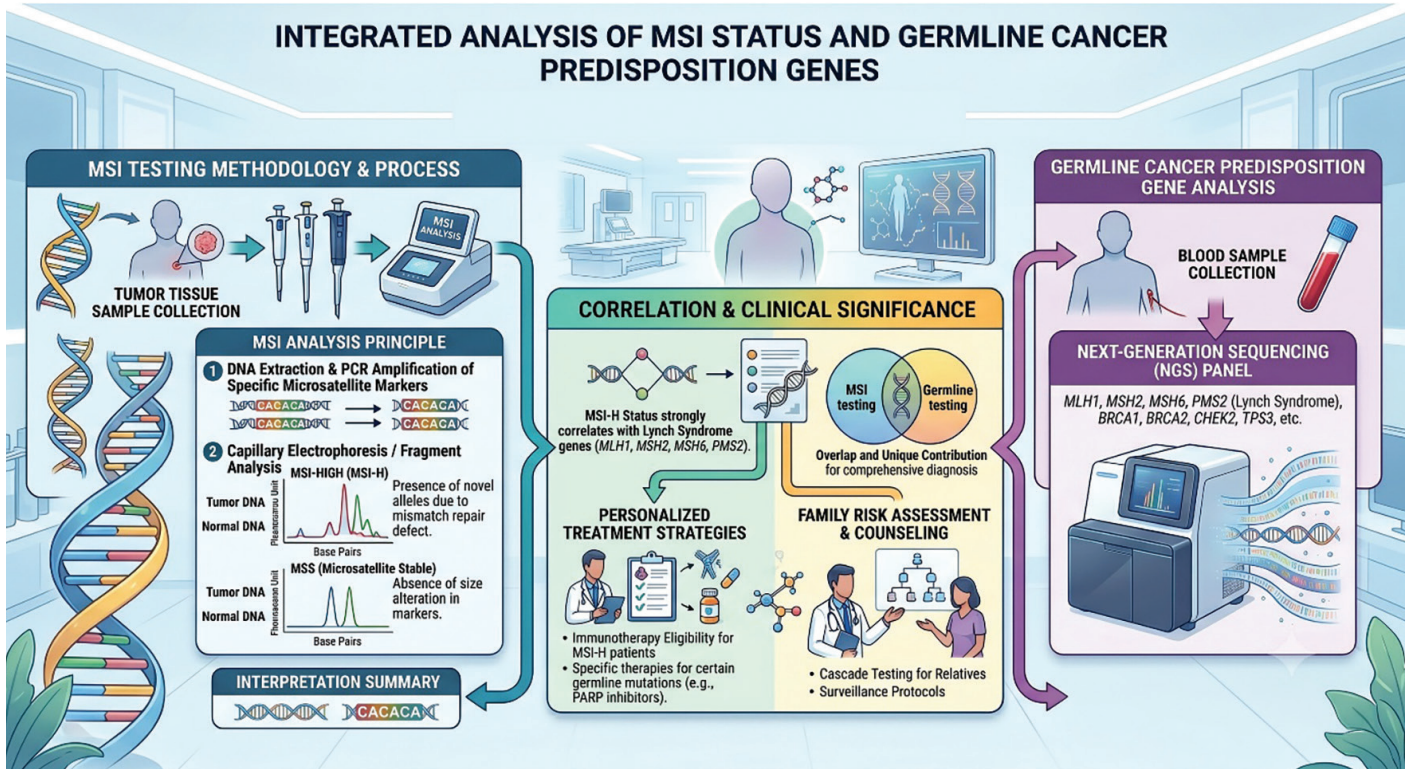


Figure 1. Generalized workflow of the study cohort
 MSI: Microsatellite instability, DNA: Deoxyribonucleic acid, PCR: Polymerase chain reaction

Germline Variant Analysis

A total of 101 patients underwent germline testing simultaneously. Pathogenic or likely pathogenic germline variants were identified in both the MSI-H and MSS groups; however, the molecular spectra differed substantially between the two groups.

Among MSI-H patients, 7 of 34 individuals (31.8% among those tested; $n=22$) harbored pathogenic or likely pathogenic variants involving MMR-associated genes. These included pathogenic or likely pathogenic variants in *MSH2*, *MSH3*, and *MSH6*. Notably, the identified variants were predominantly associated with tumors within the Lynch syndrome spectrum. *MSH2* and *MSH6* variants were identified in patients with colorectal, rectal, and endometrial cancers, supporting the clinical concordance between germline findings and tumor phenotype.

One MSI-H patient with colon cancer carried both a pathogenic *MUTYH* variant and a pathogenic *MSH6* variant, suggesting the possibility of overlapping hereditary cancer susceptibility mechanisms (P#44). In the MSI-L group ($n=3$), no patients were tested for germline variants.

In contrast, MSS patients demonstrated a broader spectrum of non-MMR hereditary cancer-related variants. Pathogenic or

likely pathogenic variants identified in MSS tumors included alterations in *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, *ERCC2*, and *MUTYH*.

Several variants identified in MSS patients were clinically concordant with the presenting tumor type. For example, pathogenic variants in *BRCA1* and *BRCA2* were observed in ovarian and breast cancer patients, respectively, while pathogenic variants in *MUTYH* were identified in patients with gastrointestinal malignancies.

Variant Classification Overview

Across the entire cohort, pathogenic/likely pathogenic variants were identified in 17/101 (16.8%) patients; one patient had dual variants. Variants of uncertain significance (VUS) were observed in 36 patients. All of the detected variants were in a heterozygous state.

Overall, MMR-associated pathogenic or likely pathogenic variants were significantly enriched in the MSI-H subgroup, whereas MSS tumors more commonly demonstrated pathogenic variants involving alternative hereditary cancer predisposition pathways. Detailed characteristics of the cohort are depicted in Table 1.

Table 1. Detailed characteristics of the cohort

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	66	Male	Stomach	MSS	NA
	45	Female	Colon	MSS	NA
	67	Male	Colon	MSS	APC (NM_000038.5) c.3920T>A p.(Ile1307Lys)/Het/VUS
	50	Female	Pancreatic	MSS	N
	76	Female	Ovarian	MSS	N
	45	Female	Colon	MSS	NA
	71	Female	Sarcoma	MSS	NA
	65	Female	Biliary tract	MSS	N
	60	Female	Ovarian	MSS	NA
	41	Male	Brain (glioblastoma)	MSS	NA
	33	Male	Sarcoma	MSS	NA
	82	Female	Stomach	MSS	NA
	25	Male	Testicular	MSS	NA
	71	Male	Colon	MSS	MLH1 (NM_000249) c.1480T>C p.(Cys494Arg), VHL (NM_000551) c.340+657C>G/Het (both)/VUS (both)
	59	Female	Ovarian	MSS	BRCA2 (NM_000059.3) c.1587_1590delTAAA p.(Phe529Leufs)/het/pathogenic
	54	Male	Biliary tract	MSS	NA
	71	Male	Colon	MSS	RAD51C (NM_058216) c.810C>G p.(Ile270Met)/Het/VUS
	55	Female	Ovarian	MSS	RET (NM_020975.6) c.394C>A p.(Leu132Ile)/Het/VUS
	45	Male	Pancreatic	MSS	NA
	45	Female	Colon	MSI-H	NA
	59	Female	Colon	MSI-H	POLE (NM_006231) c.2466G>C p.(Lys822Asn)/Het/VUS
	65	Male	Colon	MSS	NA
	46	Female	Breast	MSS	NA
	60	Female	Biliary tract	MSS	NA
	73	Female	Pancreatic	MSS	NA
	63	Female	Stomach	MSS	NA
	41	Female	Lung	MSS	PALB2 (NM_024675) c.3058_3069dup p.(Gln1020_Gln1023dup), TSC1 (NM_000368) c.3436G>A p.(Asp1146Asn)/Het (both)/VUS (both)
	50	Male	Thyroid (papillary)	MSS	NA
	34	Female	Sarcoma	MSS	NA
	67	Male	Prostate	MSS	NA
	51	Female	Endometrial	MSI-H	N
	70	Female	Endometrial	MSI-H	STK11 (NM_000455.5) c.238C>G p.Leu80Val/Het/VUS
	51	Female	Breast/endometrium (MSI tested in endometrial tissue)	MSI-H	MSH2 (NM_000251.2) c.1222dup p.(Tyr408Leufs*9)/ Het/Pathogenic
	59	Male	Pancreatic	MSS	N
	43	Male	Rectum	MSS	NA
	44	Female	Biliary tract	MSS	NA
	62	Male	Colon	MSS	N
	58	Female	Ovarian	MSS	NA
	62	Male	Colon	MSI-H	MSH6 (NM_000179) c.3740C>T p.(Thr1247Ile)/Het/VUS

Table 1. Continued

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	64	Male	Rectum	MSI-H	<i>MSH6 (NM_000179) c.3556+1G>C/het/pathogenic</i> CDH1 (NM_001317184) c.1583A>G p.(Asn528Ser)/Het/VUS
	67	Female	Hepatocellular	MSS	PALB2 (NM_024675) c.2882T>C p.(Leu961Pro)/Het/ VUS
	64	Male	Colon	MSI-H	NA
	75	Female	Rectum	MSS	NA
	53	Male	Colon	MSI-H	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del), MSH6 (NM_000179) c.691del p.(Val231Tyrfs*15)/ Het (both)/Pathogenic (both)
	31	Female	Stomach	MSS	POLE (NM_006231) c.5244G>A p.(Met1748Ile)/Het/VUS
	37	Male	Brain (glioblastoma)	MSS	N
	57	Female	Endometrial	MSS	NA
	78	Female	Colon	MSI-H	N
	58	Male	Colon	MSS	NA
	72	Female	Biliary tract	MSS	NA
	42	Male	Stomach	MSS	NA
	61	Male	Colon	MSS	NA
	25	Female	Lung	MSS	NA
	67	Male	Prostate	MSS	NA
	48	Male	Colon	MSS	NA
	73	Male	Pancreatic	MSS	NA
	60	Male	Colon	MSS	N
	56	Male	Colon	MSS	NA
	58	Male	Biliary tract	MSI-H	N
	53	Female	Urinary tract (bladder)	MSS	NA
	61	Male	Colon	MSS	NA
	68	Female	Colon	MSI-H	NA
	80	Male	Breast	MSS	NA
	72	Male	Colon	MSS	NA
	72	Female	Rectum	MSS	NA
	42	Female	Rectum	MSS	NA
	54	Female	Cervix	MSS	NA
	47	Female	Stomach	MSS	NA
	37	Male	Colon	MSI-H	N
	32	Female	Sarcoma	MSS	NA
	49	Female	Biliary tract	MSS	RAD51D (NM_001142571) c.540+1G>A/Het/Pathogenic
	67	Female	Endometrial	MSS	NA
	38	Female	Ovarian	MSI-H	NA
	52	Male	Colon	MSS	N
	48	Female	Rectum	MSS	N
	26	Male	Stomach	MSS	BLM (NM_000057) c.2474C>T p.(Pro825Leu)/Het/VUS
	47	Female	Stomach	MSS	NA
	66	Male	Melanoma	MSS	NA
	70	Female	Endometrial	MSS	N
	50	Male	Oesophageal	MSI-H	NA

Table 1. Continued					
Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	57	Male	Pancreatic	MSI-H	N
	76	Female	Breast	MSS	SMAD4 (NM_005359) c.977T>C p.(Ile326Thr), POLE (NM_006231) c.6124G>A p.(Gly2042Arg)/Het (both)/ VUS (both)
	23	Male	Colon	MSS	N
	53	Male	Colon	MSI-H	NA
	32	Male	Colon	MSI-H	MSH2 (NM_000251) c.1784T>C p.(Leu595Pro)/Het/Likely pathogenic
	64	Male	Stomach	MSI-H	NA
	26	Female	Brain (glioblastoma)	MSS	NA
	57	Female	Pancreatic	MSS	GALNT12 (NM_024642) c.5G>T p.(Trp2Leu)/Het/VUS
	71	Female	Breast	MSS	NA
	53	Female	Melanoma	MSS	N
	58	Male	Pancreatic	MSS	ERCC3 (NM_000122) c.1606A>T p.(Met536Leu)/Het/VUS
	80	Female	Colon	MSS	NA
	51	Male	Thyroid (medullary)	MSS	NA
	58	Male	Brain (glioblastoma)	MSS	NA
	72	Female	Lung	MSS	NA
	54	Male	Sarcoma	MSS	NA
	39	Female	Stomach	MSS	N
	69	Female	Biliary tract	MSS	N
	55	Male	Colon	MSS	NA
	58	Female	Ovarian	MSS	BRCA1 pathogenic variant (previous external testing)
	14	Female	Nerve sheat	MSS	NA
	64	Female	Pancreatic	MSS	N
	51	Male	Malign melanoma	MSS	NA
	54	Male	Biliary tract	MSS	NA
	62	Male	Colon	MSS	NA
	56	Male	Pancreatic	MSS	N
	64	Female	Endometrial	MSS	N
	44	Male	Brain (glioblastoma)	MSS	NA
	68	Male	Urinary tract	MSS	NA
	23	Female	Thyroid (medullary)	MSS	N
	28	Female	Biliary tract	MSS	NA
	62	Male	Urinary tract (bladder)	MSS	NA
	49	Male	Colon	MSS	NA
	76	Female	Colon	MSS	NA
	76	Female	Colon	MSS	N
	44	Male	Wilms tumour	MSS	NA
	55	Male	Malignant mesenchymal tumour of the salivary gland	MSI-L	NA
	59	Male	Pancreatic/ prostate (MSI performed on prostate tissue)	MSS	<i>MUTYH</i> (NM_001048171.) c.1395_1397del p.(Glu466del)/het/pathogenic ATM (NM_000051.3) c.6497T>C p.(Val2166Ala)/Het/VUS
	48	Male	Colon	MSS	NA

Table 1. Continued

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	40	Female	Breast	MSS	BRCA2 (NM_000059.3) c.67+1G>A/Het/Pathogenic
	68	Male	Colon	MSI-L	NA
	74	Male	Urinary tract (bladder)	MSS	NA
	51	Female	Ovarian	MSS	N
	43	Female	Biliary tract	MSS	NA
	64	Female	Endometrial	MSS	N
	77	Male	Colon	MSS	N
	49	Female	Biliary tract	MSS	NA
	31	Male	Urinary tract (bladder)	MSS	CDH1 (NM_001317184) c.1592C>A p.(Ala531Asp)/ Het/VUS
	53	Female	Ovarian	MSS	N
	40	Male	Biliary tract	MSS	N
	57	Female	Breast	MSS	N
	51	Female	Rectum	MSS	NA
	60	Male	Lung	MSS	NA
	72	Male	Pancreatic	MSS	NA
	49	Male	Colon	MSS	NA
	45	Female	Pancreatic	MSS	PTEN (NM_001304717) c.176C>A p.(Pro59Gln)/ Het/VUS
	46	Female	Pancreatic	MSS	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del)/ Het/Likely pathogenic
	54	Male	Stomach	MSS	NA
	52	Male	Pancreatic	MSS	N
	58	Female	Pancreatic	MSS	NA
	58	Male	Colon	MSI-H	MSH3 (NM_002439) c.2179C>T p.(Arg727*)/ Het/Pathogenic
	59	Male	Pancreatic	MSS	APC (NM_000038.5) c.4364A>G p.(Asn1455Ser)/Het/VUS
	50	Male	Colon	MSS	APC (NM_000038)c.8120G>A p.(Gly2707Asp)/Het/VUS
	46	Male	Brain (glioblastoma)	MSS	NA
	34	Female	Brain (glioblastoma)	MSS	NA
	52	Female	Breast	MSS	RB1 (NM_000321) c.74C>T p.(Pro25Leu)/Het/VUS
	55	Female	Biliary tract	MSS	MSH3 (NM_002439.4) c.1307C>T p.(Ile945Met)/Het/VUS
	77	Female	Hepatocellular	MSS	NA
	61	Male	Biliary tract	MSS	NA
	47	Female	Sarcoma	MSS	MUTYH (NM_001048171) c.892-2A>G, POLE (NM_006231) c.5794C>T p.(Arg1932Cys)/Het (both)/VUS (both)
	53	Male	Colon	MSS	N
	44	Female	Sarcoma	MSS	N
	85	Female	Unknown primary	MSS	NA
	64	Female	Sarcoma	MSS	NA
	61	Male	Biliary tract	MSS	NA
	46	Female	Breast	MSS	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del)/het/likely pathogenic NF1 (NM_000267) c.2629A>G p.(Met877Val)/Het/VUS
	65	Male	Pancreatic	MSS	NA
	51	Female	Sarcoma/ breast (MSI tested in sarcoma tissue)	MSS	NA

Table 1. Continued					
Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	63	Female	Ovarian	MSS	NA
	59	Female	Endometrial	MSS	NA
	70	Male	Stomach	MSS	NA
	63	Female	Endometrial	MSS	BARD1 (NM_000465.3) c.827C>G p.(Thr276Ser), MSH6 (NM_000179) c.3763G>A p.(Asp1255Asn)/Het (both)/VUS (both)
	72	Female	Brain (glioblastoma)	MSS	NA
	59	Male	Unknown primary	MSS	NA
	43	Female	Pancreatic	MSS	NA
	59	Male	Oesophageal	MSI-L	NA
	63	Female	Colon	MSS	N
	50	Female	Endometrial	MSS	N
	80	Female	Biliary tract	MSS	NA
	51	Male	Pancreatic	MSS	NA
	49	Female	Ovarian	MSS	RAD51D (NM_001142571) c.862T>C p.(Trp288Arg)/Het/VUS
	33	Female	Ovarian	MSS	N
	60	Female	Endometrial	MSI-H	NA
	38	Male	Biliary tract	MSS	NA
	72	Male	Colon	MSI-H	N
	67	Male	Stomach	MSS	N
	70	Female	Ovarian	MSS	NA
	43	Male	Stomach	MSS	N
	49	Female	Colon	MSS	EPCAM (NM_002354.2) c.491+5G>A/Het/VUS
	72	Female	Colon	MSI-H	N
	41	Female	Stomach	MSS	N
	70	Female	Colon	MSS	NA
	73	Female	Biliary tract	MSS	N
	67	Male	Lung	MSS	NA
	57	Male	Pancreatic	MSS	ATM (NM_000051) c.3576G>A p.(Lys1192=)/Het/Pathogenic
	55	Male	Gastrointestinal stromal tumour	MSS	NA
	61	Female	Pancreatic	MSS	NA
	32	Female	Ovarian	MSS	NA
	57	Female	Colon	MSS	NA
	41	Male	Lung	MSS	NA
	53	Female	Colon	MSS	N
	62	Female	Endometrial	MSI-H	N
	51	Male	Pancreatic	MSS	N
	79	Female	Stomach	MSS	NA
	33	Male	Rectum	MSS	NA
	56	Male	Pancreatic	MSS	MUTYH (NM_001048171) c.692G>A p.(Arg231His)/Het/Likely Pathogenic
	62	Female	Cervix	MSS	NA
	69	Female	Thyroid (papillary)	MSS	NA

Table 1. Continued

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	39	Female	Breast	MSS	N
	48	Female	Breast	MSS	MSH2 (NM_000251.2) c.70C>T p.(Gln24*)/ Het/Pathogenic
	65	Male	Colon	MSS	NA
	73	Male	Prostate	MSS	N
	60	Female	Endometrial	MSI-H	MSH2 (NM_000251.2) c.70C>T p.(Gln24*)/Het/Pathogenic
	65	Female	Endometrial	MSS	RAD51C (NM_058216) c.1039A>G p.(Arg347Gly)/ Het/VUS
	59	Female	Breast	MSS	EPCAM (NM_002354.2) c.-9_7dup (p.Pro3Argfs*33), TSC2 (NM_000548.4) c.4678G>A (p.Ala1560Thr)/Het (both)/VUS (both)
	65	Male	Stomach	MSS	NA
	61	Female	Pancreatic	MSS	N
	47	Male	Pancreatic	MSS	NA
	67	Male	Lung	MSS	NA
	81	Male	Colon	MSS	NA
	37	Female	Lung	MSS	NA
	37	Male	Brain (glioblastoma)	MSS	NA
	50	Male	Colon	MSS	TSC1 (NM_000368.4) c.106+16T>C , AXIN2 (NM_004655.3) c.203G>A p p.(Arg68Gln)/Het (both)/ VUS (both)
	35	Female	Thyroid	MSS	NA
	61	Female	Endometrial	MSS	N
	62	Male	Pancreatic	MSS	N
	48	Male	Colon	MSS	NA
	77	Male	Stomach	MSI-H	BRIP1 (NM_032043.2) c.2284C>T p.(Arg762Cys), FH (NM_000143.3) c.730C>T p.(Leu244Phe), MSH3 (NM_002439.4) c.3382A>G p.(Met1128Val), TSC2 (NM_000548.4) c.4664G>A p.(Ser1555Asn) /Het (all)/VUS (all)
	70	Female	Rectum	MSS	NA
	65	Male	Renal cell cancer/ rectum (MSI tested in both tissues)	MSS	NA
	33	Female	Colon	MSS	APC (NM_000038) c.5528C>T p.(Pro1843Leu)/Het/VUS
	46	Male	Unknown primary	MSS	NA
	63	Male	Small bowel	MSI-H	N
	45	Female	Small bowel	MSI-H	MLH1 (NM_000249) c.1832T>C p.Ile611Thr/Het/VUS
	35	Male	Brain (astrocytoma)	MSS	NA
	56	Female	Colon	MSS	NA
	73	Male	Colon	MSS	NA
	70	Female	Endometrial	MSI-H	NA
	58	Female	Brain (glioblastoma)	MSS	NA
	47	Male	Lung	MSS	NA
	52	Male	Colon	MSS	NA
	53	Female	Colon	MSS	NA
	62	Female	Endometrial	MSI-H	NA

Table 1. Continued

Patient ID	Age	Sex	Cancer type	MSI status	Hereditary cancer panel (variant-if detected/zygosity/ACMG classification)
	32	Female	Colon	MSS	EPCAM (NM_002354.2) c.28G>C p.(Gly10Arg), CHEK2 (NM_001005735.1) c.551A>C p.(Lys184Thr)/Het (both)/VUS (both)
	42	Male	Colon	MSS	NA
	65	Female	Ovarian	MSS	FLCN (NM_144997.7) c.203G>A p.Ser68Asn, POLD1 (NM_002691.4) c.2048G>A p.Arg683His/Het (both)/VUS (both)
	67	Male	Colon	MSI-H	N
	62	Male	Stomach	MSI-H	NA
	61	Male	Rectum	MSI-H	MSH2 (NM_000251.2) c.942+3A>T/Het/Pathogenic
	77	Female	Endometrial	MSI-H	NA

N: hereditary cancer panel performed but no causative variant was found, MSI-H results were written in bold, pathogenic alterations were written in bold and italic
ACMG: American College of Medical Genetics and Genomics, MSI: Microsatellite instability, MSS: Microsatellite stabil, MSI-L: MSI-low, MSI-H: MSI-high NA: Not available, Het: Heterozygous, VUS: Variant of unknown significance

Discussion

In this retrospective study, we evaluated MSI status and accompanying germline variant profiles in a heterogeneous cancer population. Our findings demonstrate that MSI-H tumors were strongly enriched for pathogenic or likely pathogenic variants affecting MMR-associated genes, particularly among tumors classically associated with Lynch syndrome.

The observed MSI-H rate of 14.2% in our cohort is generally consistent with previously reported frequencies in mixed gastrointestinal and endometrial cancer populations [3,6]. MSI-H tumors are known to occur most frequently in colorectal and endometrial cancers, a finding that was similarly reflected in our dataset. Although previous studies in the Turkish population have investigated MSI in relatively modest patient cohorts and predominantly within specific tumor groups, the present study represents, to our knowledge, the first analysis conducted in such a large cohort encompassing a broad tumor spectrum in which MSI could be observed with correlation to germline variants [7-9].

Importantly, pathogenic or likely pathogenic MMR-related variants were identified in approximately a third of MSI-H cases. Variants involving *MSH2*, *MSH3* and *MSH6* demonstrated particularly strong clinicopathological concordance with Lynch syndrome-associated tumor types. These findings support the clinical utility of integrating germline testing with MSI analysis, especially in patients with colorectal, rectal, and endometrial malignancies. However, it should be treated carefully since this does not prove that MSI-H is a highly sensitive standalone marker for germline MMR deficiency [10].

One patient harbored concurrent pathogenic alterations in both *MUTYH* and *MSH6*. Although the clinical implications of multiple inherited cancer susceptibility variants remain incompletely understood, recent studies suggest that multi-locus inherited neoplasia syndromes may contribute to variable phenotypic presentations and modified cancer risk profiles.

Another notable observation was the detection of clinically actionable germline variants in MSS tumors. Although MSS status generally lowers the suspicion for Lynch syndrome, hereditary cancer predisposition cannot be excluded solely on the basis of MSI negativity [11]. Pathogenic variants involving *BRCA1*, *BRCA2*, *ATM*, *RAD51D*, and *MUTYH* identified in MSS tumors highlight the genetic heterogeneity of hereditary cancer syndromes and emphasize the importance of phenotype-driven germline evaluation.

The identification of *BRCA*-associated variants in breast and ovarian cancer patients further supports the internal clinical consistency of the dataset. Similarly, the detection of *MUTYH* pathogenic variants in gastrointestinal malignancies aligns with the established association between *MUTYH*-associated polyposis and colorectal tumorigenesis.

Our study has several strengths. First, it reflects real-world clinical practice by including multiple tumor types rather than a single disease-focused cohort. Second, the simultaneous evaluation of MSI status and germline findings allowed assessment of genotype-phenotype concordance in routine diagnostic settings.

Study Limitations

Several limitations must be acknowledged in the interpretation of these findings. First, approximately 20 samples from the initial cohort were excluded due to insufficient DNA quality, preventing reliable MSI analysis and possibly affecting the overall sample size. In addition, detailed family history, immunohistochemistry data, somatic testing results, treatment response information, and comprehensive histopathological findings were not uniformly available for all patients, limiting detailed genotype-phenotype correlation analyses. Furthermore, genetic testing of hereditary cancer genes was not systematically validated for copy-number variations using gold-standard methods, which may have resulted in an underestimation of large genomic rearrangements.

Segregation studies and functional validation assays could not be performed for several variants, particularly those classified as VUS. Because the study was conducted at a tertiary referral center, sampling bias is likely; such centers often receive more complex or high-risk cases. Additionally, the relatively limited number of MSI-H cases may have reduced the statistical power for subgroup analyses. Another limitation is the differential availability of germline testing across study groups, which may have introduced verification bias and affected comparisons of germline variant frequencies among MSI categories. The absence of advanced epigenetic investigations, such as DNA methylation profiling of *MMR* genes, limited our ability to fully characterize the mechanisms underlying deficient MMR in cases without detectable germline mutations. Despite these limitations, our cohort reflects real-world clinical practice and provides valuable insight into the relationship between MSI status and hereditary cancer predisposition across a broad and heterogeneous cancer population.

Conclusion

This study supports the clinical utility of MSI-H as an indicator of germline MMR deficiency, while emphasizing the importance of comprehensive germline sequencing for a complete diagnostic evaluation. The discovery of discordant cases and a significant number of non-MMR hereditary mutations underscores the necessity of a multifaceted testing strategy in oncology. Future clinical guidelines should continue to promote a combination of phenotypic and genotypic testing to optimize the detection of hereditary syndromes and enhance personalized therapeutic interventions.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Scientific Research Evaluation and Ethics Committee No. 1 of Ankara Etlik City Hospital, University of Health Sciences Türkiye (AEŞH-BADEK1-2026-435/date: 06.05.2026).

Informed Consent: Written informed consent was obtained from the patients or their legal guardians.

Footnotes

Authorship Contributions

Concept: A.K., Design: A.K., Data Collection or Processing: Ö.B.Ç.Ö., Analysis or Interpretation: A.K., Ö.B.Ç.Ö., Literature Search: A.K., Writing: A.K., Ö.B.Ç.Ö.

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Supplementary Material 1 Link: <https://d2v96fxpocvxx.cloudfront.net/f6118de3-f656-440d-95f2-50c620149951/content-images/292dc4b9-04a8-41c0-ae1e-79fe411ee295.pdf>

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