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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 Universty 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-higt (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 Metarial 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).

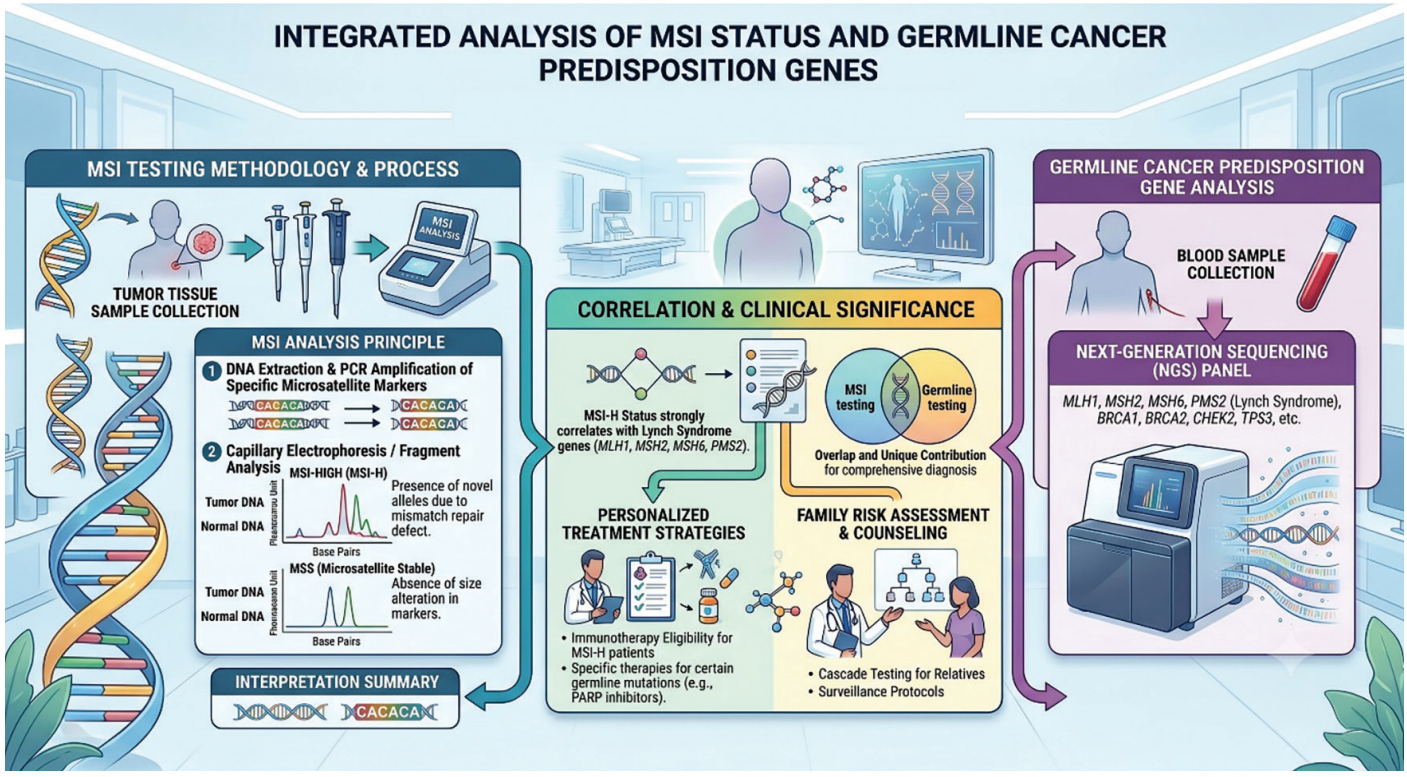

Figure 1. Generalized workflow of the study cohort
MSI: Microsatellite instability, DNA: Deoxyribonucleic acid, PCR: Polymerase chain reaction

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)
P1	66	Male	Stomach	MSS	NA
P2	45	Female	Colon	MSS	NA
P3	67	Male	Colon	MSS	APC (NM_000038.5) c.3920T>A p.(Ile1307Lys)/Het/VUS
P4	50	Female	Pancreatic	MSS	N
P5	76	Female	Ovarian	MSS	N
P6	45	Female	Colon	MSS	NA
P7	71	Female	Sarcoma	MSS	NA
P8	65	Female	Biliary tract	MSS	N
P9	60	Female	Ovarian	MSS	NA
P10	41	Male	Brain (glioblastoma)	MSS	NA
P11	33	Male	Sarcoma	MSS	NA
P12	82	Female	Stomach	MSS	NA
P13	25	Male	Testicular	MSS	NA
P14	71	Male	Colon	MSS	MLH1 (NM_000249) c.1480T>C p.(Cys494Arg), VHL (NM_000551) c.340+657C>G/Het (both)/VUS (both)
P15	59	Female	Ovarian	MSS	BRCA2 (NM_000059.3) c.1587_1590delTAAA p.(Phe529Leufs)/het/ pathogenic
P16	54	Male	Biliary tract	MSS	NA
P17	71	Male	Colon	MSS	RAD51C (NM_058216) c.810C>G p.(Ile270Met)/Het/VUS
P18	55	Female	Ovarian	MSS	RET (NM_020975.6) c.394C>A p.(Leu132Ile)/Het/VUS
P19	45	Male	Pancreatic	MSS	NA
P20	45	Female	Colon	MSI-H	NA
P21	59	Female	Colon	MSI-H	POLE (NM_006231) c.2466G>C p.(Lys822Asn)/Het/VUS
P22	65	Male	Colon	MSS	NA
P23	46	Female	Breast	MSS	NA
P24	60	Female	Biliary tract	MSS	NA
P25	73	Female	Pancreatic	MSS	NA
P26	63	Female	Stomach	MSS	NA
P27	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)
P28	50	Male	Thyroid (papillary)	MSS	NA
P29	34	Female	Sarcoma	MSS	NA
P30	67	Male	Prostate	MSS	NA
P31	51	Female	Endometrial	MSI-H	N
P32	70	Female	Endometrial	MSI-H	STK11 (NM_000455.5) c.238C>G p.Leu80Val/Het/VUS
P33	51	Female	Breast/endometrium (MSI tested in endometrial tissue)	MSI-H	MSH2 (NM_000251.2) c.1222dup p.(Tyr408Leufs*9)/ Het/Pathogenic
P34	59	Male	Pancreatic	MSS	N
P35	43	Male	Rectum	MSS	NA
P36	44	Female	Biliary tract	MSS	NA
P37	62	Male	Colon	MSS	N
P38	58	Female	Ovarian	MSS	NA
P39	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)
P40	64	Male	Rectum	MSI-H	***MSH6 (NM_000179) c.3556+1G>C/het/pathogenic*** CDH1 (NM_001317184) c.1583A>G p.(Asn528Ser)/Het/VUS
P41	67	Female	Hepatocellular	MSS	PALB2 (NM_024675) c.2882T>C p.(Leu961Pro)/Het/ VUS
P42	64	Male	Colon	MSI-H	NA
P43	75	Female	Rectum	MSS	NA
P44	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)
P45	31	Female	Stomach	MSS	POLE (NM_006231) c.5244G>A p.(Met1748Ile)/Het/VUS
P46	37	Male	Brain (glioblastoma)	MSS	N
P47	57	Female	Endometrial	MSS	NA
P48	78	Female	Colon	MSI-H	N
P49	58	Male	Colon	MSS	NA
P50	72	Female	Biliary tract	MSS	NA
P51	42	Male	Stomach	MSS	NA
P52	61	Male	Colon	MSS	NA
P53	25	Female	Lung	MSS	NA
P54	67	Male	Prostate	MSS	NA
P55	48	Male	Colon	MSS	NA
P56	73	Male	Pancreatic	MSS	NA
P57	60	Male	Colon	MSS	N
P58	56	Male	Colon	MSS	NA
P59	58	Male	Biliary tract	MSI-H	N
P60	53	Female	Urinary tract (bladder)	MSS	NA
P61	61	Male	Colon	MSS	NA
P62	68	Female	Colon	MSI-H	NA
P63	80	Male	Breast	MSS	NA
P64	72	Male	Colon	MSS	NA
P65	72	Female	Rectum	MSS	NA
P66	42	Female	Rectum	MSS	NA
P67	54	Female	Cervix	MSS	NA
P68	47	Female	Stomach	MSS	NA
P69	37	Male	Colon	MSI-H	N
P70	32	Female	Sarcoma	MSS	NA
P71	49	Female	Biliary tract	MSS	RAD51D (NM_001142571) c.540+1G>A/Het/Pathogenic
P72	67	Female	Endometrial	MSS	NA
P73	38	Female	Ovarian	MSI-H	NA
P74	52	Male	Colon	MSS	N
P75	48	Female	Rectum	MSS	N
P76	26	Male	Stomach	MSS	BLM (NM_000057) c.2474C>T p.(Pro825Leu)/Het/VUS
P77	47	Female	Stomach	MSS	NA
P78	66	Male	Melanoma	MSS	NA
P79	70	Female	Endometrial	MSS	N
P80	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)
P81	57	Male	Pancreatic	MSI-H	N
P82	76	Female	Breast	MSS	SMAD4 (NM_005359) c.977T>C p.(Ile326Thr), POLE (NM_006231) c.6124G>A p.(Gly2042Arg)/Het (both)/ VUS (both)
P83	23	Male	Colon	MSS	N
P84	53	Male	Colon	MSI-H	NA
P85	32	Male	Colon	MSI-H	MSH2 (NM_000251) c.1784T>C p.(Leu595Pro)/Het/Likely pathogenic
P86	64	Male	Stomach	MSI-H	NA
P87	26	Female	Brain (glioblastoma)	MSS	NA
P88	57	Female	Pancreatic	MSS	GALNT12 (NM_024642) c.5G>T p.(Trp2Leu)/Het/VUS
P89	71	Female	Breast	MSS	NA
P90	53	Female	Melanoma	MSS	N
P91	58	Male	Pancreatic	MSS	ERCC3 (NM_000122) c.1606A>T p.(Met536Leu)/Het/VUS
P92	80	Female	Colon	MSS	NA
P93	51	Male	Thyroid (medullary)	MSS	NA
P94	58	Male	Brain (glioblastoma)	MSS	NA
P95	72	Female	Lung	MSS	NA
P96	54	Male	Sarcoma	MSS	NA
P97	39	Female	Stomach	MSS	N
P98	69	Female	Biliary tract	MSS	N
P99	55	Male	Colon	MSS	NA
P100	58	Female	Ovarian	MSS	BRCA1 pathogenic variant (previous external testing)
P101	14	Female	Nerve sheat	MSS	NA
P102	64	Female	Pancreatic	MSS	N
P103	51	Male	Malign melanoma	MSS	NA
P104	54	Male	Biliary tract	MSS	NA
P105	62	Male	Colon	MSS	NA
P106	56	Male	Pancreatic	MSS	N
P107	64	Female	Endometrial	MSS	N
P108	44	Male	Brain (glioblastoma)	MSS	NA
P109	68	Male	Urinary tract	MSS	NA
P110	23	Female	Thyroid (medullary)	MSS	N
P111	28	Female	Biliary tract	MSS	NA
P112	62	Male	Urinary tract (bladder)	MSS	NA
P113	49	Male	Colon	MSS	NA
P114	76	Female	Colon	MSS	NA
P115	76	Female	Colon	MSS	N
P116	44	Male	Wilms tumour	MSS	NA
P117	55	Male	Malignant mesenchymal tumour of the salivary gland	MSI-L	NA
P118	59	Male	Pancreatic/ prostate (MSI performed on prostate tissue)	MSS	***MUTYH (NM_001048171.) c.1395_1397del p.(Glu466del)/het/pathogenic*** ATM (NM_000051.3) c.6497T>C p.(Val2166Ala)/Het/VUS
P119	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)
P120	40	Female	Breast	MSS	BRCA2 (NM_000059.3) c.67+1G>A/Het/Pathogenic
P121	68	Male	Colon	MSI-L	NA
P122	74	Male	Urinary tract (bladder)	MSS	NA
P123	51	Female	Ovarian	MSS	N
P124	43	Female	Biliary tract	MSS	NA
P125	64	Female	Endometrial	MSS	N
P126	77	Male	Colon	MSS	N
P127	49	Female	Biliary tract	MSS	NA
P128	31	Male	Urinary tract (bladder)	MSS	CDH1 (NM_001317184) c.1592C>A p.(Ala531Asp)/ Het/VUS
P129	53	Female	Ovarian	MSS	N
P130	40	Male	Biliary tract	MSS	N
P131	57	Female	Breast	MSS	N
P132	51	Female	Rectum	MSS	NA
P133	60	Male	Lung	MSS	NA
P134	72	Male	Pancreatic	MSS	NA
P135	49	Male	Colon	MSS	NA
P136	45	Female	Pancreatic	MSS	PTEN (NM_001304717) c.176C>A p.(Pro59Gln)/ Het/VUS
P137	46	Female	Pancreatic	MSS	MUTYH (NM_001048171) c.1395_1397del p.(Glu466del)/ Het/Likely pathogenic
P138	54	Male	Stomach	MSS	NA
P139	52	Male	Pancreatic	MSS	N
P140	58	Female	Pancreatic	MSS	NA
P141	58	Male	Colon	MSI-H	MSH3 (NM_002439) c.2179C>T p.(Arg727*)/ Het/Pathogenic
P142	59	Male	Pancreatic	MSS	APC (NM_000038.5) c.4364A>G p.(Asn1455Ser)/Het/VUS
P143	50	Male	Colon	MSS	APC (NM_000038)c.8120G>A p.(Gly2707Asp)/Het/VUS
P144	46	Male	Brain (glioblastoma)	MSS	NA
P145	34	Female	Brain (glioblastoma)	MSS	NA
P146	52	Female	Breast	MSS	RB1 (NM_000321) c.74C>T p.(Pro25Leu)/Het/VUS
P147	55	Female	Biliary tract	MSS	MSH3 (NM_002439.4) c.1307C>T p.(Ile945Met)/Het/VUS
P148	77	Female	Hepatocellular	MSS	NA
P149	61	Male	Biliary tract	MSS	NA
P150	47	Female	Sarcoma	MSS	MUTYH (NM_001048171) c.892-2A>G, POLE (NM_006231) c.5794C>T p.(Arg1932Cys)/Het (both)/VUS (both)
P151	53	Male	Colon	MSS	N
P152	44	Female	Sarcoma	MSS	N
P153	85	Female	Unknown primary	MSS	NA
P154	64	Female	Sarcoma	MSS	NA
P155	61	Male	Biliary tract	MSS	NA
P156	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
P157	65	Male	Pancreatic	MSS	NA
P158	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)
P159	63	Female	Ovarian	MSS	NA
P160	59	Female	Endometrial	MSS	NA
P161	70	Male	Stomach	MSS	NA
P162	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)
P163	72	Female	Brain (glioblastoma)	MSS	NA
P164	59	Male	Unknown primary	MSS	NA
P165	43	Female	Pancreatic	MSS	NA
P166	59	Male	Oesophageal	MSI-L	NA
P167	63	Female	Colon	MSS	N
P168	50	Female	Endometrial	MSS	N
P169	80	Female	Biliary tract	MSS	NA
P170	51	Male	Pancreatic	MSS	NA
P171	49	Female	Ovarian	MSS	RAD51D (NM_001142571) c.862T>C p.(Trp288Arg)/Het/VUS
P172	33	Female	Ovarian	MSS	N
P173	60	Female	Endometrial	MSI-H	NA
P174	38	Male	Biliary tract	MSS	NA
P175	72	Male	Colon	MSI-H	N
P176	67	Male	Stomach	MSS	N
P177	70	Female	Ovarian	MSS	NA
P178	43	Male	Stomach	MSS	N
P179	49	Female	Colon	MSS	EPCAM (NM_002354.2) c.491+5G>A/Het/VUS
P180	72	Female	Colon	MSI-H	N
P181	41	Female	Stomach	MSS	N
P182	70	Female	Colon	MSS	NA
P183	73	Female	Biliary tract	MSS	N
P184	67	Male	Lung	MSS	NA
P185	57	Male	Pancreatic	MSS	ATM (NM_000051) c.3576G>A p.(Lys1192=)/Het/Pathogenic
P186	55	Male	Gastrointestinal stromal tumour	MSS	NA
P187	61	Female	Pancreatic	MSS	NA
P188	32	Female	Ovarian	MSS	NA
P189	57	Female	Colon	MSS	NA
P190	41	Male	Lung	MSS	NA
P191	53	Female	Colon	MSS	N
P192	62	Female	Endometrial	MSI-H	N
P193	51	Male	Pancreatic	MSS	N
P194	79	Female	Stomach	MSS	NA
P195	33	Male	Rectum	MSS	NA
P196	56	Male	Pancreatic	MSS	MUTYH (NM_001048171) c.692G>A p.(Arg231His)/Het/Likely Pathogenic
P197	62	Female	Cervix	MSS	NA
P198	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)
P199	39	Female	Breast	MSS	N
P200	48	Female	Breast	MSS	MSH2 (NM_000251.2) c.70C>T p.(Gln24*)/ Het/Pathogenic
P201	65	Male	Colon	MSS	NA
P202	73	Male	Prostate	MSS	N
P203	60	Female	Endometrial	MSI-H	MSH2 (NM_000251.2) c.70C>T p.(Gln24*)/Het/Pathogenic
P204	65	Female	Endometrial	MSS	RAD51C (NM_058216) c.1039A>G p.(Arg347Gly)/ Het/VUS
P205	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)
P206	65	Male	Stomach	MSS	NA
P207	61	Female	Pancreatic	MSS	N
P208	47	Male	Pancreatic	MSS	NA
P209	67	Male	Lung	MSS	NA
P210	81	Male	Colon	MSS	NA
P211	37	Female	Lung	MSS	NA
P212	37	Male	Brain (glioblastoma)	MSS	NA
P213	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)
P214	35	Female	Thyroid	MSS	NA
P215	61	Female	Endometrial	MSS	N
P216	62	Male	Pancreatic	MSS	N
P217	48	Male	Colon	MSS	NA
P218	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)
P219	70	Female	Rectum	MSS	NA
P220	65	Male	Renal cell cancer/ rectum (MSI tested in both tissues)	MSS	NA
P221	33	Female	Colon	MSS	APC (NM_000038) c.5528C>T p.(Pro1843Leu)/Het/VUS
P222	46	Male	Unknown primary	MSS	NA
P223	63	Male	Small bowel	MSI-H	N
P224	45	Female	Small bowel	MSI-H	MLH1 (NM_000249) c.1832T>C p.Ile611Thr/Het/VUS
P225	35	Male	Brain (astrocytoma)	MSS	NA
P226	56	Female	Colon	MSS	NA
P227	73	Male	Colon	MSS	NA
P228	70	Female	Endometrial	MSI-H	NA
P229	58	Female	Brain (glioblastoma)	MSS	NA
P230	47	Male	Lung	MSS	NA
P231	52	Male	Colon	MSS	NA
P232	53	Female	Colon	MSS	NA
P233	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)
P234	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)
P235	42	Male	Colon	MSS	NA
P236	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)
P237	67	Male	Colon	MSI-H	N
P238	62	Male	Stomach	MSI-H	NA
P239	61	Male	Rectum	MSI-H	MSH2 (NM_000251.2) c.942+3A>T/Het/Pathogenic
P240	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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