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Article

Microsatellite instability in noncolorectal and nonendometrial malignancies in patients with Lynch syndrome

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Abstract

Background: Individuals with Lynch syndrome are at increased hereditary risk of colorectal and endometrial carcinomas with microsatellite instability (MSI-H) and mismatch repair-deficiency (dMMR), which make these tumors vulnerable to therapy with immune checkpoint inhibitors. Our aim is to assess how often other tumor types in these individuals share these characteristics.

Methods: We retrieved the full tumor history of a historical clinic-based cohort of 1745 individuals with Lynch syndrome and calculated the standardized incidence ratio for all tumor types. MSI status, somatic second hit alterations, and immunohistochemistry-based MMR status were analyzed in 236 noncolorectal and nonendometrial malignant tumors.

Results: In individuals with Lynch syndrome MSI-H/dMMR occurred both in Lynch-spectrum and in non–Lynch-spectrum malignancies (85% vs 37%, $P < .01$). MSI-H/dMMR malignancies were found in nearly all non–Lynch-spectrum tumor types. A high percentage (33%) of breast carcinomas with medullary features was observed, and most of them were MSI-H/dMMR. Breast carcinoma with medullary features were shown to be associated with Lynch syndrome (standardized incidence ratio = 38.8, 95% confidence interval = 16.7 to 76.5).

Conclusions: In individuals with Lynch syndrome, MSI-H/dMMR occurs in more than one-half of the malignancies other than colorectal and endometrial carcinomas, including tumor types without increased incidence. The Lynch-spectrum tumors should be expanded to breast carcinomas with medullary features. All malignancies in patients with Lynch syndrome, independent of subtype, should be tested for MSI-H/dMMR in case therapy with immune checkpoint inhibitors is considered. Moreover, Lynch syndrome should be considered an underlying cause of all MSI-H/dMMR malignancies other than colorectal and endometrial carcinomas.

Lynch syndrome is a genetic tumor risk syndrome and is caused by germline pathogenic variants (gPV) in 1 of the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, or *PMS2* or deletion of the 3′-end of *EPCAM* (1,2). Individuals with Lynch syndrome are at high risk to develop colorectal and endometrial carcinomas (CRCs and ECs) (3).

To select individuals at high risk for Lynch syndrome, in many countries CRCs and ECs that develop before the age of 70 years or regardless of age are routinely screened for MMR deficiency (dMMR) and/or presence of microsatellite instability (MSI-H) (4,5). dMMR can be detected in tumor tissues using immunohistochemistry (IHC) and reflects the inactivation of both alleles of 1 of the MMR genes (6). This dMMR causes MSI-H, as base mismatches, and small deletions and insertions within microsatellites, which occur during DNA replication by slippage of the DNA polymerase in repetitive sequences, are not repaired (7,8). Immune checkpoint inhibitor treatments have been proven to be highly effective for MSI-H/dMMR tumors, irrespective of the organ site of origin (9,10). Recently, MSI-H/dMMR was approved as a biomarker for treatment with the PD-1 inhibitor pembrolizumab by the Food and Drug Administration for all solid tumors that have progressed after prior treatments (11) .

Individuals with Lynch syndrome are primarily advised to undergo regular colonoscopies and endometrial screening to identify or prevent these tumors (12). In addition to CRCs and ECs, individuals with Lynch syndrome are also considered at increased risk to develop multiple other primary malignancies in the gastrointestinal tract, pancreas, biliary tract, upper urothelial tract, brain, ovaries, and sebaceous carcinoma (13). These tumors are considered Lynch-spectrum tumors. Data on the fraction of MSI-H/dMMR in malignancies other than CRC and EC are incomplete, which may hamper treatment options. Here, we aimed to

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investigate in patients with Lynch syndrome the fraction of primary malignancies, other than CRC and EC, that have MSI-H/dMMR. We assessed the presence of MSI-H, the absence of the MMR proteins, and the occurrence of second hit alterations in the MMR genes in malignancies other than CRC and EC developed in a historical Lynch syndrome cohort.

Methods

Study cohort

This study included individuals who were identified to have 1 (likely) gPV in an MMR gene (*MLH1*, *MSH2*, *MSH6*, *PMS2*) or in *EPCAM* at the Radboud university medical center, Nijmegen, the Netherlands, between 1997 and January 2020 (N = 1745). Information on tumor development was requested in 2020 from the Dutch nationwide pathology databank. Detailed information on the study cohort and selection is described in the Supplementary Methods (available online). This study was performed in accordance with the standards of the Helsinki Declaration. Ethical approval for this retrospective single-center study was obtained from a local institutional review board of the Radboud university medical center (CMO-2019-6013), Nijmegen, the Netherlands. Informed consent was not necessary because only deidentified pseudonymized data were available and no incidental findings regarding an individual could be made.

Pathology review

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of 305 malignancies were requested through the Dutch National Tissue Portal. In total, 250 tissue blocks were available. For further analyses, tissue slides of 4 μm were stained with an H&E staining using the Immunologic Autostainer 480 (Immunologic, Duiven, the Netherlands). The H&E stainings were reviewed by a pathologist (RSvdP and/or IDN) to identify neoplastic cell percentage and histological subtype. In total, 14 tissue blocks were excluded due to absence of neoplastic cells in the tissue slide, and the remaining 236 malignancies were used for subsequent analyses (see below and Supplementary Table 1, available online).

Genomic DNA isolation, sequencing, and somatic mutation analysis

To perform sequencing analysis, genomic DNA was isolated from FFPE tumor tissue slides as previously described (14).

To investigate MSI status and second hit alterations in the MMR gene with a gPV, single-molecule molecular inversion probe (smMIP)-based next-generation sequencing (NGS) was performed as previously described (14). Details on the smMIP sequencing and variant selection are described in the Supplementary Methods (available online).

Microsatellite instability analysis

At least 45 out of 57 MSI markers (15) per tumor needed to be assessable to determine the MSI status that was based on instability scores calculated using mSINGS v3.4 (16). A tumor was marked MSI-H if at least 30% of assessed markers were instable, microsatellite stable (MSS) if less than 15% of assessed markers were instable, and MSI-intermediate if 15%-29% of assessed markers were instable (15).

Next to the smMIP panel of 57 MSI markers, the traditional set of 5 mononucleotide markers (NR27, NR21, NR24, BAT25, and BAT26) was analyzed using a pentaplex polymerase chain reaction (PCR) followed by a GeneScan analysis (15).

IHC and MSI-H/dMMR consensus

To investigate dMMR in tumor tissues, IHC of the MMR protein corresponding to the gene with a (likely) gPV was performed. IHC details can be found in the Supplementary Methods (available online).

The MSI status was determined based on MSI analysis using the 57 markers (MSI NGS), MSI analysis based on the 5 makers analyzed by GeneScan (MSI GeneScan), and IHC of the MMR proteins. For a conclusive MSI-H/dMMR consensus, at least 2 techniques needed to be concordant. If only 1 technique was performed because of limitations of the tissue, that result was used as MSI-H/dMMR consensus. In case dMMR and MSI status were discordant, the tumor was reevaluated by a pathologist (RSvdP, IDN). For MLH1-deficient tumors without a second hit, a methylation-specific multiplex ligation-dependent probe amplification was performed to detect *MLH1*-promoter hypermethylation according to the manufacturer's instructions (MRC-Holland, Amsterdam, the Netherlands).

Statistical analysis

Age, sex, country, and birth cohort–adjusted standardized incidence ratios (SIRs) with 95% confidence intervals (CIs) were calculated to compare the cancer-specific incidence in the Lynch syndrome cohort and the general population (17,18). A detailed description of the SIR calculation can be found in the Supplementary Methods (available online).

A 2-sided χ^2 test or Fisher exact test using R v3.6.2 were performed to analyze the association of tumor presence or MSI status with the gene in which individuals with Lynch syndrome have a gPV. *P* values were adjusted for multiple testing using a Bonferroni correction. *P* values less than .05 were considered statistically significant.

Results

Malignancies other than colorectal and endometrial tumors in Lynch syndrome patients

A cohort of 1745 individuals with Lynch syndrome (n = 985 females, 56%) from 457 families (median: 2 individuals per family, range = 1-42, interquartile range = 1-5) was available for this study (Table 1; Figure 1, A), including 394 index patients (23%). Based on the pathology records, 1151 malignancies were diagnosed in 752 patients (43% of the cohort), with a median number of 1 malignancy per patient (range = 1-7) (Table 1; Figure 1, A and B). Males more frequently developed CRC compared with females (284/760 vs 223/985; *P* = 1.3e-10). However, the fraction of CRC and EC combined was not statistically significant different between males and females (284/760 vs 344/985; *P* = 1; Figure 1, B). Malignancies other than CRC and EC were identified in 16% of individuals (n = 287 individuals) and most common were breast (6%; n = 59 females), urinary bladder (3%; n = 44 individuals), and small bowel carcinomas (2%; n = 35 individuals).

SIRs for Lynch-spectrum malignancies

To determine the incidence of malignancies other than CRC and EC in our study cohort compared with the general population, we calculated the SIRs. Increased SIRs were observed for malignancies within the Lynch-spectrum (ie, small bowel [97.0, 95% CI = 63.9 to 141.1], ureter [53.6, 95% CI = 32.3 to 83.7], renal pelvis [31.7, 95% CI = 18.1 to 51.4], ampulla of Vater [13.1, 95% CI = 2.6 to 38.2], stomach [7.0, 95% CI = 4.4 to 10.6], and ovarian carcinomas [5.9, 95% CI = 3.7 to 8.9]), but we also observed an increased

Table 1. Number of tumors, median age, gene distribution, and MSI-H/dMMR count per sex and tumor tissue type

	Total tumors, No.[a]	Tumors in males, No.[a]	Tumors in females, No.[a]	Median age at diagnosis (range), y[b]	*MLH1*[c]	*MSH2/EPCAM*[c]	*MSH6*[c]	*PMS2*[c]	MSI-H/dMMR tumors/all analyzed tumors
Cohort									
Males	528	NA	NA	53 (18-87)	133 (25%)	170 (32%)	146 (28%)	79 (15%)	NA
Females	623	NA	NA	54 (16-91)	137 (22%)	163 (26%)	201 (32%)	122 (20%)	NA
Lynch-spectrum tumor types									
Colorectum	591	337	254	50 (16-90)	169 (29%)	166 (28%)	142 (24%)	114 (19%)	NA
Endometrium	172	0	172	54 (32-78)	25 (15%)	40 (23%)	77 (45%)	30 (17%)	NA
Small bowel	37	28	9	54 (31-86)	7 (19%)	18 (49%)	7 (19%)	5 (13%)	27/28 (96%)
Ureter	27	19	8	61 (45-84)	2 (7%)	13 (48%)	11 (41%)	1 (4%)	13/18 (72%)
Renal pelvis	20	9	11	61 (50-79)	5 (25%)	12 (60%)	3 (15%)	0 (0%)	12/13 (92%)
Ampulla of water	6	5	1	69 (31-78)	1 (16.6%)	1 (16.6%)	3 (50%)	1 (16.6%)	4/5 (80%)
Stomach	28	15	13	63 (38-89)	9 (32%)	6 (22%)	9 (32%)	4 (14%)	13/16 (81%)
Ovary	25	0	25	47 (37-76)	5 (20%)	9 (36%)	8 (32%)	3 (12%)	10/13 (77%)
Pancreas	5	0	5	67 (53-73)	2 (40%)	2 (40%)	1 (20%)	0 (33%)	4/4 (100%)
Brain	3	1	2	66 (36-70)	1 (33%)	2 (67%)	0 (0%)	0 (0%)	2/3 (67%)
Non–Lynch-spectrum tumor types									
Bladder	54	43	11	62 (39-84)	11 (20%)	23 (43%)	18 (33%)	2 (4%)	18/34 (53%)
Kidney	14	6	8	52 (32-73)	6 (43%)	3 (21%)	4 (29%)	1 (7%)	1/11 (9%)
Prostate	29	29	0	64 (47-75)	3 (10%)	8 (28%)	13 (45%)	5 (17%)	5/18 (28%)
Breast	65	0	65	60 (28-91)	14 (22%)	12 (18%)	20 (31%)	19 (29%)	13/36 (36%)
Esophagus	7	2	5	69 (53-77)	2 (28.6%)	1 (14%)	2 (28.6%)	2 (28.6%)	4/7 (57%)
Melanoma	18	7	11	51 (27-87)	2 (11%)	2 (11%)	9 (50%)	5 (28%)	0/7 (0%)
Lung	9	5	4	58 (37-69)	1 (11.1%)	2 (22.2%)	3 (33.3%)	3 (33.3%)	0/3 (0%)
Other	41	22	19	53 (23-80)	5 (12%)	13 (32%)	17 (41%)	6 (15%)	9/20 (45%)

[a] Number of tumors per category. NA = not applicable.
[b] Median and range of age at diagnosis in years per category.
[c] Number of tumors in individuals with a germline pathogenic variant in 1 of the mismatch repair genes: *MLH1*, *MSH2/EPCAM*, *MSH6*, or *PMS2*.

SIR for urinary bladder (4.0, 95% CI = 2.8 to 5.6), kidney (2.8, 95% CI = 1.5 to 4.9), and prostate carcinomas (1.9, 95% CI = 1.2 to 2.8) (Figure 1, C left). Furthermore, we observed less tumors for lung carcinomas than expected for the general Dutch population. When excluding index individuals, the SIRs of Lynch-spectrum tumors and urinary bladder carcinomas remained statistically significant (Supplementary Figure 1, A, available online).

MSI-H/dMMR in malignancies other than colorectal and endometrial tumors in patients with Lynch syndrome

Overall, 236 malignancies other than CRC and EC were available for MSI, IHC, and/or somatic second hit analysis, of which 39% (92/236) were the first tumor that developed in a patient. Based on the consensus of 3 techniques, 57% of investigated tumors (n = 135) were MSI-H/dMMR, 6% of tumors (n = 15) were MSI-intermediate, 35% (n = 82) were MSS, and 2% (n = 4) had inconclusive MSI results (Figure 1, C right; Supplementary Figure 1, B and C, available online). The unambiguous IHC result of 93%-95% of tumors was concordant with the MSI status determined by NGS and/or pentaplex (180/190 and 145/156; Supplementary Table 1; Supplementary Figure 1, E, available online). For a substantial part of renal pelvis carcinomas (54%, 7/13), the MMR IHC could not be interpreted without knowledge of MSI status (Supplementary Figure 2, A-H, available online) because staining was partly retained in the neoplastic cells. For somatic second hit analysis, 196 tumors were available. A somatic second hit was identified in 81% of MSI-H/dMMR tumors (96/117), 46% of MSI-intermediate tumors (6/13), 6% of MSS tumors (4/65), and 1 tumor with an MSI-inconclusive status (1/1) (Supplementary Figure 1, C and D, available online). In total, 28% (54/196) of malignancies lost the wild-type allele, whereas the allele with the gPV was lost in only 2% (4/196) of malignancies.

MSI-H/dMMR in malignancies outside the Lynch spectrum

The majority of Lynch-spectrum tumors were MSI-H/dMMR (85%, 85/100), with the largest fraction for pancreas (100%, 4/4), small bowel (96%, 27/28), and renal pelvis carcinomas (92%, 12/13). Interestingly, also a substantial fraction of non–Lynch-spectrum malignancies was MSI-H/dMMR (37%, 50/136), including more than one-half of esophageal (57%, 4/7) and urinary bladder carcinomas (53%, 18/34). MSI-H/dMMR was found in a fraction of all analyzed tumor types for which more than 1 tumor was available, except melanoma (Figure 1, C right; Table 1; Supplementary Table 1; Supplementary Figure 1, B-E, available online).

To investigate possible associations between the MSI status and histological subtypes, we assessed the fraction of MSI-H/dMMR in various histological subtypes of ovarian and breast carcinomas. We observed that all ovarian carcinoma of the endometrioid subtype were MSI-H/dMMR. Remarkably, endometrioid ovarian carcinomas were the predominant subtype (69%; 9/13), whereas the serous subtype was observed in only 15% of ovarian carcinomas (2/13) in this study (Figure 2, A; Supplementary Table 1, available online). A SIR indicated an increased risk of endometrioid ovarian carcinoma (32.2, 95% CI = 17.6 to 54.0; Figure 2, B) with a median age of onset of 47 years (range = 37-66 years). Furthermore, we observed a remarkably high percentage of breast carcinomas with medullary features (33%, 12/36; Figure 2, A; Supplementary Figure 2, I, available online). Moreover, a larger fraction of these carcinomas than of invasive adenocarcinoma no special type (NST) presented with MSI-H/dMMR (75% [9/12] vs 14% [3/22], $P = .0266$; Figure 2, A). A SIR analysis showed an increased risk of breast carcinomas with medullary features (38.8, 95% CI = 16.7 to 76.5; Figure 2, B) with a median age of onset of 66 years (range = 47-86 years).

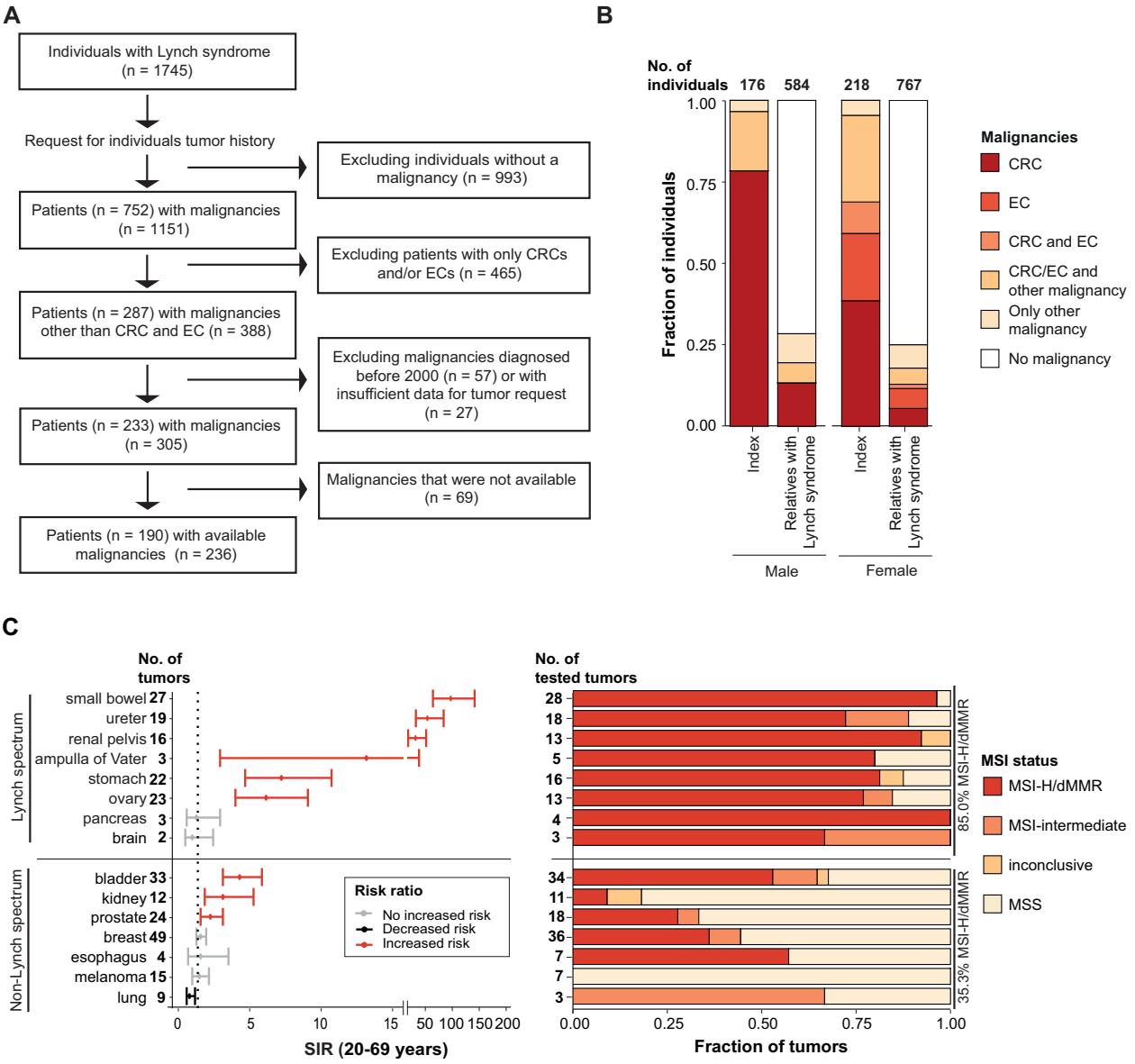


Figure 1. Malignancy development in the Lynch syndrome cohort. **A**) Flowchart of the cohort and tumor selection. **B**) Fraction of individuals with or without malignancy per sex and identification history. Malignancy groups include all individuals with only a colorectal carcinoma (CRC), only endometrial carcinoma (EC), both CRC and EC development, CRC or EC with another type of malignancy, individuals with a malignancy other than CRC or EC, and no malignancy. Number of individuals is given per group above the figure. **C**) **Left:** Standardardized incidence ratio (SIR) for the age of 20-69 years for all malignancies developed in both index individuals and relatives with Lynch syndrome compared with the general Dutch population. No statistically significant increased risk, statistically significant decreased risk, and statistically significant increased risk are shown per malignancy. Numbers of tumors are given per malignancy type on the left of the figure. **Right:** The fraction of microsatellite instability (MSI-H) or mismatch repair-deficiency (dMMR) according to a consensus, in which 2 out of the 3 following techniques needed to be concordant: MSI next generation sequencing (NGS), MSI GeneScan, and immunohistochemistry of the MMR proteins. Data were analyzed for microsatellite stable (MSS), MSI-inconclusive results, MSI-intermediate results, and MSI-H/dMMR. The number of tested tumors is given per malignancy type on the left of the figure. Twenty other tumor types are included in Supplementary Figure 1, B, available online.

Affected MMR genes and fractions of MSI-H/dMMR malignancies

The cohort included individuals with a gPV affecting *MLH1* (n = 307), *MSH2* (n = 370, including 49 individuals with a 3' *EPCAM* deletion), *MSH6* (n = 630), and *PMS2* (n = 438). Individuals with a gPV in *MLH1* or *MSH2* more often developed malignancies other than CRC and EC (19% [57/307 individuals] and 22% [82/370 individuals; Figure 2, C]) than individuals with a gPV in *MSH6* (15%, 97/630 individuals) or *PMS2* (12%, 51/438 individuals; $P = .0016$; Figure 2, C). Moreover, for malignancies other than CRC and EC, the fraction of MSI-H/dMMR malignancies was statistically significantly larger for patients with a gPV in *MLH1* (72% [42/58]) or *MSH2* (81% [58/72]) than for patients with a gPV in *MSH6* (36% [28/77]) or *PMS2* (24% [7/29], $P = 3.625e\text{-}10$; Figure 2, D; Supplementary Figure 1, E, available online).

Discussion

Individuals with Lynch syndrome are at high risk to develop MSI-H/dMMR CRCs and ECs, which can be treated with PD-1 inhibitors (9). Here we show that in patients with Lynch syndrome, also more than one-half of the tested malignancies other than CRC

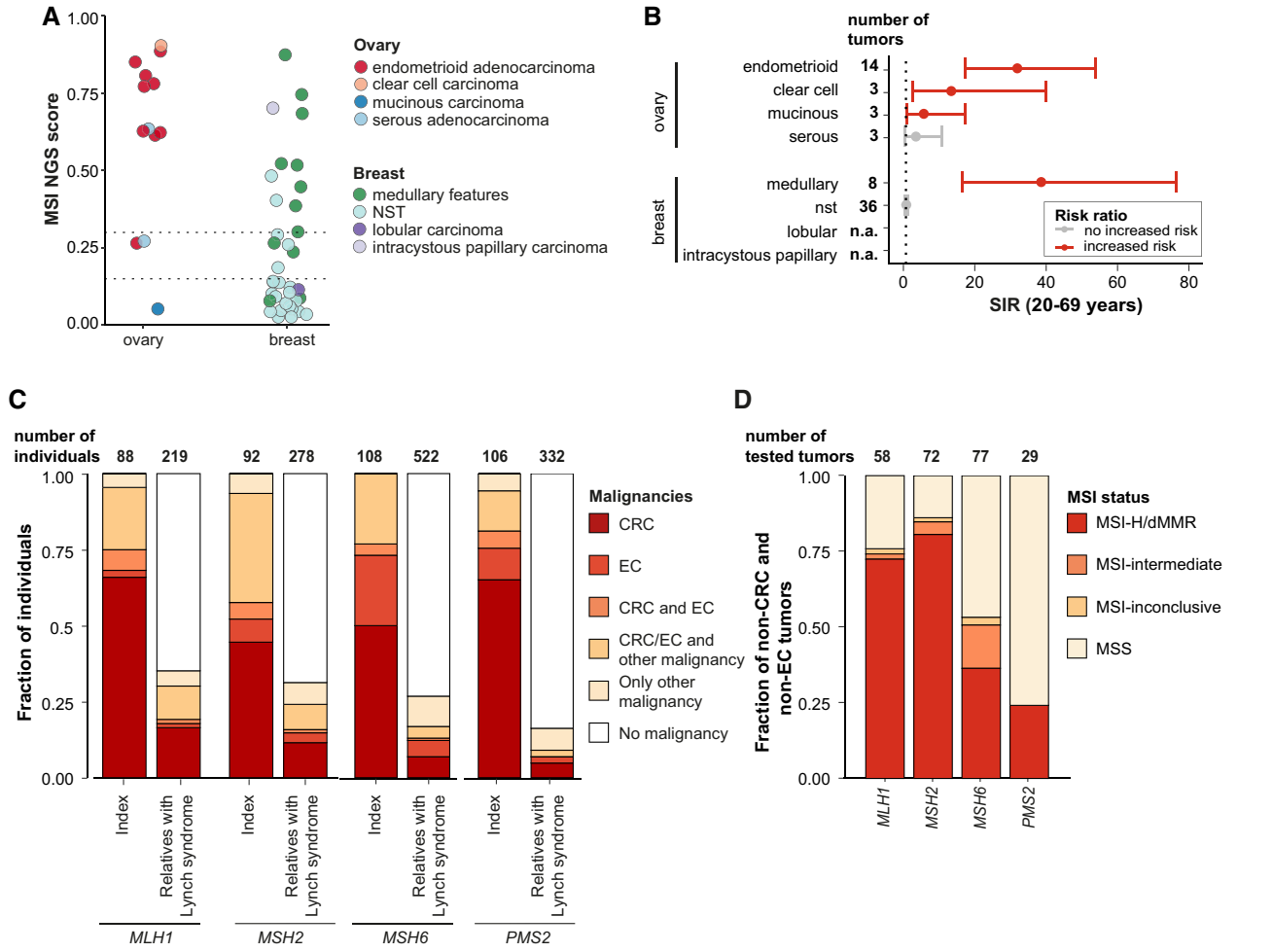


Figure 2. Malignancy development by subtype and gene. **A**) microsatellite instability (MSI) next generation sequencing (NGS) score per breast and ovarian subtypes. **Dotted lines** represent thresholds to MSI-intermediate (0.15) and MSI-H/mismatch repair-deficiency (dMMR) (0.3) results. Ovarian tumor subtypes (endometrioid adenocarcinoma, clear cell carcinoma, mucinous carcinoma, serous adenocarcinoma), and breast carcinomas (breast adenocarcinoma with medullary features, adenocarcinoma no special type [NST], lobular carcinoma, intracystous papillary carcinoma) are represented in different colours. **B**) Standardardized incidence ratios (SIR) for the age of 20-69 years for all indicated malignancies developed in index individuals and relatives with Lynch syndrome compared with the general Dutch population. No statistically significant increased risk and statistically significant increased risk are shown per malignant tumor type. n.a. not analysed. **C**) The fraction of individuals with or without malignancy per affected MMR gene and identification history. Malignancy groups included all individuals with only a colorectal carcinoma (CRC), only endometrial carcinoma (EC), both CRC and EC development, CRC or EC with another type of malignancy, individuals with a malignancy other than CRC or EC, and no malignancy. The number of individuals is given per group above the figure. **D**) Fraction of MSI-H/dMMR malignancies per affected MMR gene. Data were analyzed for microsatellite stable (MSS), inconclusive results, MSI-intermediate results, and MSI-H/dMMR. The number of tested tumors is given per affected gene above the figure.

and EC are MSI-H/dMMR. MSI-H/dMMR occurs in a substantial subset of both Lynch-spectrum and non–Lynch-spectrum malignancies. Likewise, these tumors may benefit from treatment with immune checkpoint inhibitors (10). Additionally, we found a larger fraction of MSI-H/dMMR malignancies other than CRC and EC in patients with a gPV in *MLH1* or *MSH2* compared with patients with a gPV in *MSH6* or *PMS2*. Moreover, in patients with Lynch syndrome, we observed a huge overrepresentation of endometrioid ovarium carcinomas and breast carcinomas with medullary features that mostly were MSI-H/dMMR.

The spectrum of malignancies that developed in our Lynch syndrome study cohort largely reflected the known Lynch spectrum (13). In Lynch-spectrum malignancies, a high fraction of MSI-H/dMMR tumors was observed, which was comparable with the fraction calculated in previous studies (19-25). Observed differences are probably due to stochastic variation due to small numbers or biases in patient selection. Individuals with a gPV in *MLH1* or *MSH2* have a larger fraction of MSI-H/dMMR malignancies than those with a gPV in *MSH6* of *PMS2*. Notably, IHC evaluation of renal pelvis carcinomas was often inconclusive due to partly retained staining, and we could only interpret these IHC stainings in combination with the MSI status in the tumors. Ovarian carcinomas are considered Lynch-spectrum tumors. In line with a previous study (26), we show that this increased incidence is mainly due to an increased risk for endometrioid ovarian carcinoma and possibly clear cell carcinoma, which were the most frequent ovarian cancer subtypes in our Lynch syndrome cohort (77%), whereas these subtypes are only found in 16% of unselected ovarian cancer cohorts (27). Our study confirms our previous observation (28) that urinary bladder carcinoma also may be considered as a Lynch-spectrum tumor based on a marginally increased SIR (4.0, 95% CI = 2.8 to 5.6). Bladder carcinoma risk remained increased after adjustment for a potential ascertainment bias by excluding the index patients from the analyses.

Ascertainment bias in relatives will likely be very small because surveillance measures are restricted to early detection of CRC and EC.

In non–Lynch-spectrum malignancies, we find a striking 37% MSI-H/dMMR in patients with Lynch syndrome, which is in sharp contrast to the 0.8% MSI-H/dMMR in unselected patient cohorts (19). We found MSI-H/dMMR in a substantial part of nearly all non–Lynch-spectrum malignancies, including prostate, breast, and esophageal carcinomas, for which this was previously documented (19,20,29-34), but also in kidney, lung, head and neck, thyroid, liver, peritoneum, testis, sarcoma, and lymphoma. Interestingly, almost all breast carcinomas with medullary features had MSI-H/dMMR. Although breast carcinoma in general was not increased in our cohort and no or only a slight increase in breast cancer risk has been observed by others (35,36), the SIR of breast carcinoma with medullary features was increased. Therefore, breast carcinomas with medullary features should be considered as a Lynch-spectrum tumor. However, because these tumors will still rarely develop in women with Lynch syndrome, surveillance for breast carcinoma does not seem to be justified.

In 6% of tumors, we observed MSI-intermediate results. Because these tumors may represent misclassified MSS/MSI-H tumors, it is unclear whether they should be considered amenable to immunotherapy.

The presence of MSI-H in most tumors could be explained by somatic second hits, either subtle variants or loss of the wild-type allele as determined by an increased variant allele frequency of the gPV. One tumor showed *MLH1*-promoter hypermethylation. Loss of the wild-type allele occurred 13.5 times more often than loss of the allele with the gPV. This indicates that although loss of an allele of an MMR gene may occur by chance, biallelic inactivation of an MMR gene and the subsequent MSI is a driver in tumorigenesis irrespective of tumor type.

MSI-H/dMMR is used as an indicator for Lynch syndrome, especially in CRC and EC diagnosed before age 70 years (4,5,19). In these tumors, MSI-H/dMMR may be caused by a gPV (Lynch syndrome) in combination with inactivation of the wild-type allele, biallelic somatic genetic aberrations inactivating both alleles, or inactivation by *MLH1* promoter hypermethylation. The latter cause increases with age and is the predominant cause of MSI-H/dMMR over age 70 years in these tumor types. For most other tumor types, it is not efficient to actively screen for MSI-H/dMMR due to its low frequency in the general population (19). Therefore, the chance that MSI-H/dMMR in a given tumor type is due to Lynch syndrome may be as high as 77% (19). In our cohort of individuals with Lynch syndrome, we observed MSI-H/dMMR in a very wide spectrum of tumors. Therefore, if MSI-H/dMMR is detected as part of broad genetic analyses, germline evaluation of the MMR genes to assess the diagnosis Lynch syndrome should be considered.

Our study also has a few limitations. Familial cancer burden was not calculated because families were mostly small and no tumor types clustered in 1 family. The full tumor history might not be complete for each patient, because the tumor histories collected from the Dutch nationwide pathology databank include only information on all pathologically evaluated tumors since 1991; not all tumors are biopsied or removed, and thus these are not included in our study. Often the exact follow-up age was unknown, and neither risk-reducing procedures nor external risk factors were considered. As such, the observed tumor incidence and its effect size may be underestimated. Moreover, no information on external risk factors was available to include in this risk analysis. Previous studies showed that gPVs in *MSH6* and *PMS2* lead to a lower tumor risk than gPVs in *MLH1* and *MSH2* (38,39). Because the numbers of patients per gene were small in our study, a SIR per gene could not be estimated with reasonable certainty. In conclusion, in individuals with Lynch syndrome, MSI-H/dMMR occurs in more than one-half of the malignancies other than CRC and EC. To the best of our knowledge, this is the first study that shows that MSI-H/dMMR is a pan-cancer event in non–Lynch-spectrum tumors. Because MSI-H/dMMR tumors are very responsive to immune checkpoint inhibitors, this knowledge may lead to a paradigm change in the treatment of patients with Lynch syndrome, which may start with MSI-H/dMMR testing of all malignancies in patients with Lynch syndrome, independent of tumor type. Moreover, because MSI-H/dMMR malignancies other than CRC and EC, in particular those outside the Lynch spectrum, are rare in the general population, Lynch syndrome needs to be considered in all patients with such MSI-H/dMMR malignancies.

Data availability

Targeted sequencing data is available upon reasonable request from the European Genome-Phenome Archive (Study ID: EGAS00001007015).

Author contributions

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Conflicts of interest

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