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RESEARCH ARTICLE

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Sporadic deficient mismatch repair in colorectal cancer increases the risk for non-colorectal malignancy: A European multicenter cohort study

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Abstract

Background and Objectives: Disparities between tumors arising via different sporadic carcinogenetic pathways have not been studied systematically. This retrospective multicenter cohort study evaluated the differences in the risk for non-colorectal malignancy between sporadic colorectal cancer (CRC) patients from different DNA mismatch repair status.

Methods: A retrospective European multicenter cohort study including in total of 1706 CRC patients treated between 1996 and 2019 in three different countries. The proficiency (pMMR) or deficiency (dMMR) of mismatch repair was determined by immunohistochemistry. Cases were analyzed for tumor *BRAF*^{V600E} mutation, and *BRAF* mutated tumors were further analyzed for hypermethylation status in the promoter region of MLH1 to distinguish between sporadic and hereditary cases. Swedish and Finish patients were matched with their respective National Cancer Registries. For the Czech cohort, thorough scrutiny of medical files was performed to identify any non-colorectal malignancy within 20 years before or after the diagnosis of CRC. Poisson regression analysis was performed to identify the incidence rates of non-colorectal malignancies. For validation purposes, standardized incidence ratios were calculated for the Swedish cases adjusted for age, year, and sex.

Results: Of the 1706 CRC patients included in the analysis, 819 were female [48%], median age at surgery was 67 years [interquartile range: 60–75], and sporadic dMMR was found in 188 patients (11%). Patients with sporadic dMMR CRC had a higher incidence rate ratio (IRR) for non-colorectal malignancy before and after diagnosis compared to patients with a pMMR tumor, in both uni- (IRR = 2.49, 95% confidence interval [CI] = 1.89–3.31, $p = 0.003$) and multivariable analysis (IRR = 2.24, 95% CI = 1.67–3.01, $p = 0.004$). This association

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applied whether or not the non-colorectal tumor developed before or after the diagnosis of CRC in both uni- (IRR = 1.91, 95% CI = 1.28–2.98, $p = 0.004$), (IRR = 2.45, 95% CI = 1.72–3.49, $p = 0.004$) and multivariable analysis (IRR = 1.67,95% CI = 1.05–2.65, $p = 0.029$), (IRR = 2.35, 95% CI = 1.63–3.42, $p = 0.005$), respectively.

Conclusion: In this retrospective European multicenter cohort study, patients with sporadic dMMR CRC had a higher risk for non-colorectal malignancy than those with pMMR CRC. These findings indicate the need for further studies to establish the need for and design of surveillance strategies for patients with dMMR CRC.

KEYWORDS
colorectal cancer, non-colorectal malignancy, sporadic deficient mismatch repair

1 | INTRODUCTION

Colorectal cancer (CRC) remains the third most common malignancy worldwide (1.85 million new cases/year; 10.2% of all malignancies).[1–3] Some dietary factors, obesity, inflammatory bowel disease, and smoking are known risk factors.[1] Hereditary diseases such as familial adenomatous polyposis and Lynch Syndrome are associated with genetic predisposition to cancer and constitute about 5%–10% of all CRC cases.[1]

There are two main pathways leading to the transformation of normal colonic epithelium to histologically distinct precursor adenomatous lesions, and ultimately to CRC.[4] The first one, known as the chromosomal instability (CIN) pathway, is often associated with mutational inactivation of the APC-gene and found in approximately 85% of sporadic CRCs. The second pathway, known as the deficient mismatch repair (dMMR) pathway, involves defective/inactivation of DNA mismatch repair (MMR) genes that are responsible for correcting DNA replication errors and is found in approximately 15%–20% of all CRC cases. Inherited mutations in the MMR genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* are the main causes of Lynch Syndrome constituting 3%–5% of all dMMR cases. In contrast, sporadic dMMR tumors are mainly caused by epigenetic silencing of the *MLH1* gene through hypermethylation of the *MLH1* promoter and is found in approximately 15% of CRC cases.[5,6]

In recent decades, important advances have been made in our understanding of familial CRC and its clinical implications for diagnosis, prevention, and therapy. This applies specifically to Lynch-associated carcinogenesis, with its markedly increased lifetime risk for CRC and endometrial cancer, as well as cancers of the ovary, stomach, urogenital tract, small bowel, pancreas, and tumors from the biliary tract.[7,8] However, there is little information on whether sporadic dMMR is associated with a higher risk for cancer in other organs before or after the CRC diagnosis. Previous reports have revealed dMMR in approximately 15%–30% of gastric cancer cases, with only a fraction having a hereditary background, as well as approximately 20% of endometrial cancer cases.[9,10] Interestingly, endometrial cancer patients with dMMR tumors have better disease-free and disease-specific survival than patients with proficient MMR (pMMR) tumors.[10] Malignancies coming from the upper gastrointestinal tract reveal dMMR status only in a small fraction, and studies assessing the prognosis among these patients are lacking today.[11,12] Despite these findings, there has been little change in the algorithms for cancer surveillance and prevention strategies in patients with sporadic dMMR CRC.

This European multicenter cohort study aimed to retrospectively examine the association between sporadic dMMR status and the risk of non-colorectal malignancy among patients with known CRC. To adjust for variation in observation time, the incidence rate ratio (IRR) for non-colorectal malignancy over time, comparing patients with sporadic dMMR and pMMR CRC, was presented.

Our study was approved by the Regional Ethics Review Board, Umeå, Masaryk Memorial Cancer Institute, Helsinki University Hospital Surgical Ethics Committee (registration numbers 2014/371–31, 2015/838/MOU and Dnro THL/2137/5.05/00/2017 HUS 226/E6/06).

2 | MATERIALS AND METHODS

2.1 | Study population

The present study included 1116 CRC patients treated between October 17, 1996, and March 31, 2009, at one university hospital and two regional hospitals in Västerbotten County, Sweden, and 577 CRC patients treated at Helsinki University Hospital, Finland, between September 1, 1998, and December 31, 2005. A further 414 colon cancer (CC) patients treated at the University Hospital of Pilsen, Czech Republic, between January 1, 2018, and December 31, 2019, were added to the Scandinavian cohorts. Inclusion criteria were: (i) histologically confirmed CRC; (ii) stage confirmed and transformed to the American Joint Committee on Cancer (AJCC) TNM classification; and (iii) available clinical and pathological data

regarding the course of the disease and demographic data.[13] Patients younger than 18 years were excluded. The following data were collected from the hospital records: age, sex, site and stage at diagnosis.

All cases with conclusive immunohistochemistry (IHC) were evaluated for sporadic MMR-status as described below. Cases showing protein loss of *MSH2* or *MSH6* or isolated loss of *PMS2* were considered positive for Lynch Syndrome. Patients with loss of *MLH1* were further analyzed for *BRAF*-mutation status. *MLH1*-loss cases with wild-type $BRAF^{V600}$ were also considered positive for Lynch Syndrome. Patients with loss of *MLH1* but with detected $BRAF^{V600}$ mutations were further tested for hypermethylation of the *MLH1* promoter. Cases with positive hypermethylation were considered to represent sporadic dMMR, and cases with no hypermethylation were classified as Lynch Syndrome (Figure 1).

Of the 2107 patients, 141 had inconclusive immunohistochemistry or missing data, and 67 had inconclusive *BRAF* status and were thus excluded from further analysis (Figure 2). The remaining patients were matched against the National Cancer Registries in Sweden and Finland, while medical file scrutiny was performed for the Czech population to identify non-colorectal malignancies within 20 years before or after CRC diagnosis. From this population, 62 patients with a second or recurrent CRC and 40 cases registered as non-melanoma skin cancer were excluded (Figure 2). A further 44 patients with $BRAF^{V600}$ wild-type and 47 cases with $BRAF^{V600E}$ mutation and no hypermethylation of the *MLH1* promoter were categorized as positive for Lynch Syndrome using the algorithm shown in Figure 1 and were thus excluded (Figure 2).

2.2 | Determination of MMR status

The proficiency or deficiency of the MMR system in tumor tissues was determined by IHC analysis of four protein products of genes involved in the MMR system. Considering the dependent expression of specific MMR protein heterodimers *MSH2/MSH6* and *MLH1/PMS2*, loss of expression of one or more of these proteins indicates dMMR.[14–16] All specimens were assessed under the supervision of a pathologist with special interest in CRCI.[17]

2.3 | BRAF tumor tissue analysis

Formalin-fixed, paraffin-embedded tumor tissues were collected at the departments involved. In the Swedish cohort, *BRAF* status was determined using a $BRAF^{V600E}$ mutation-specific TaqMan allelic discrimination assay.[18,19] In the Czech Republic cohort, *BRAF*

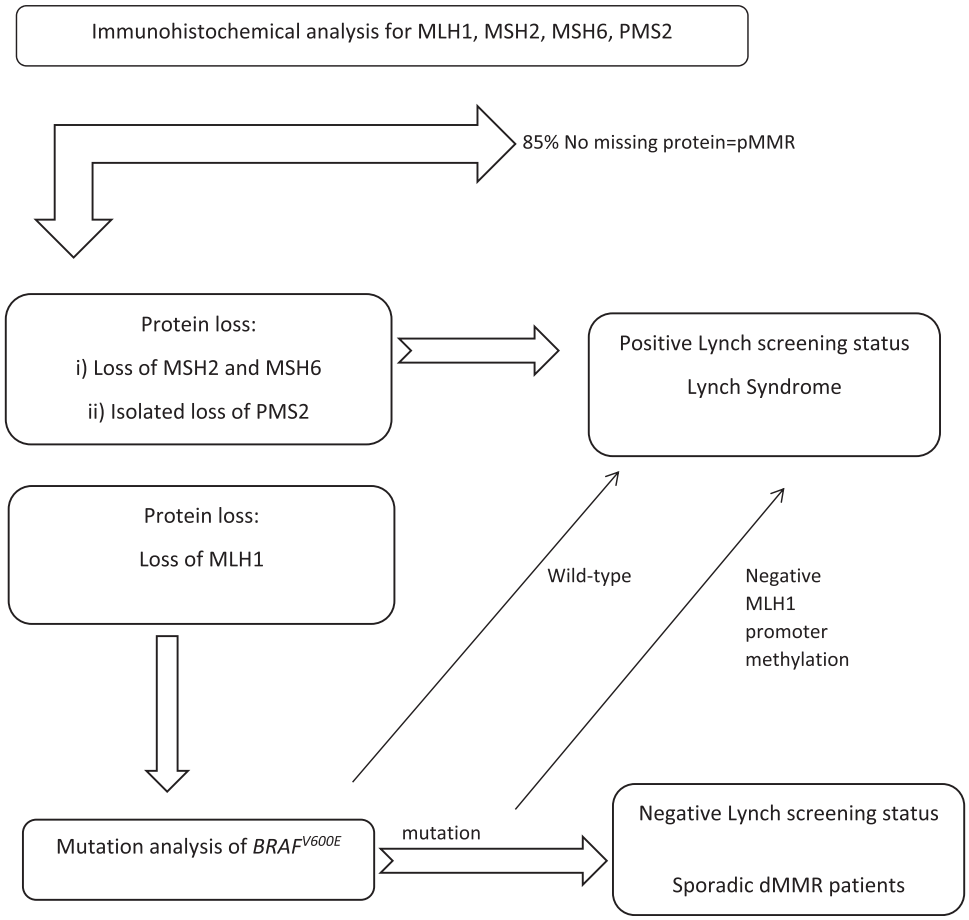

FIGURE 1 Algorithm for selection of sporadic dMMR CRC cases. CRC, colorectal cancer.

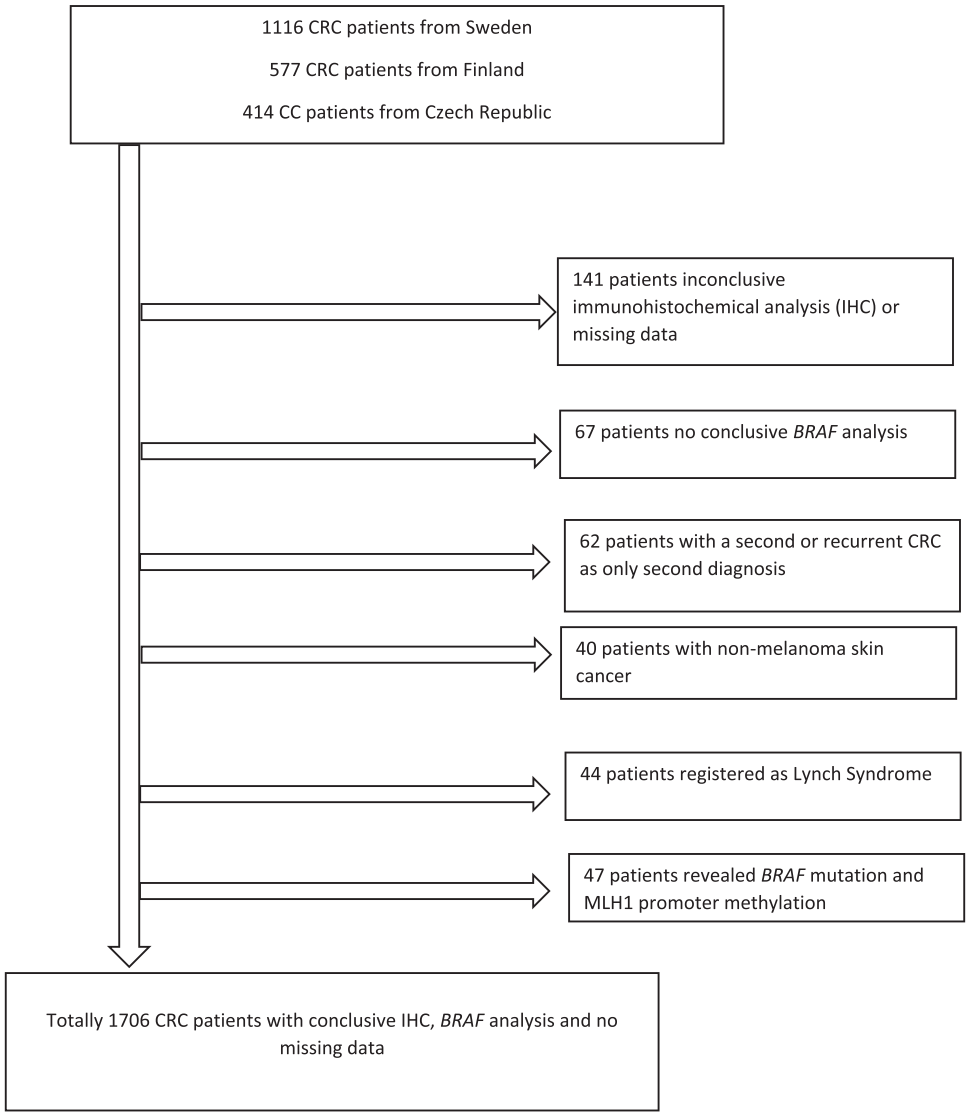

FIGURE 2 Flow diagrams of the study population.

mutation testing was performed using Cobas® *BRAF* V600 Mutation Test. In the Finnish cohort, *BRAF* mutation status was analyzed using a mutation-specific (V600E) monoclonal antibody in the same immunohistochemical setup used for MMR proteins.

2.4 | Hypermethylation analysis

DNA was extracted from FFPE tumor tissue (5 × 10 μm) using the QIAamp® DNA FFPE Tissue Kit. The samples were treated with 40 μL Proteinase K and 4 μL RNAse. DNA concentration was measured using the Qubit™ dsDNA BR kit calculated with the Spectrophotometer/Fluorometer DS-11FX+ (DeNovix). Methylation-specific PCR was performed using a PyroMark PCR Kit. The PCR was run in a Veriti Thermal Cycler (ABI). Data was analyzed using PyroMark Q24 Advanced 3.0.0 Software (Qiagen), and methylation in percentage ($C^m/(C^m+C)$) of each CpG site was calculated.

2.5 | Statistical analysis

Statistical analyses were conducted using IBM SPSS version 26. To adjust for variations in observation times, Poisson regression analysis was performed to analyze the relationships between the IRR for non-colorectal malignancy and age, sex, MMR status, site, and stage (I–IV). The observation period was from 20 years before CRC diagnosis until death from any cause or end of follow-up of individuals still alive. For validation purposes, standardized incidence ratios (SIRs) were calculated for each of the Swedish cases reported (patients with pMMR and dMMR separately) and adjusted for sex, year (in 1-year classes), and age (in 5-year classes). Incidental cancer rates were calculated based on data from the Swedish Cancer Registry with Sweden as the reference population. Incidental cancer rates were calculated by multiplying the number of person-years for each sex, year, and age group by the corresponding cancer incidence rates in the reference population. SIR with 95% confidence interval (CI) was

calculated as the ratio of the number of observed and expected cases, assuming that the observed number of cases followed a Poisson distribution.

3 | RESULTS

3.1 | Basic characteristics

In all, 1706 CRC, of whom 819 were female (48%), met the inclusion criteria. Among the cases included, 560 patients (33%) were diagnosed with a rectal tumor. The median age at diagnosis was 67 (interquartile range [IQR]: 60–75) years, and the patients were dichotomized at 70 years. Sporadic dMMR status was observed in 186 patients (11%).

A total of 324 patients (19%) were diagnosed with non-colorectal malignancies within 20 years before or after the index CRC diagnosis. A sporadic dMMR status was detected in 60 patients (18%) ($p = 0.005$) (Table 1). Non-colorectal malignancies are shown in Table 2.

IRR for the occurrence of non-colorectal malignancy within 20 years before and after CRC diagnosis in uni- and multivariable analysis.

There was a significantly higher IRR for the occurrence of non-colorectal malignancy within 20 years before and after (until death or end of follow-up) the diagnosis of CRC, in both uni- (IRR = 2.49, 95% CI = 1.89–3.31, $p = 0.003$) and multivariable (IRR = 2.24, 95% CI = 1.67–3.01, $p = 0.004$) analysis for sporadic dMMR compared to pMMR tumors (Table 3). Age was seen to be an independent significant predictor for the occurrence of a non-colorectal malignancy in both uni- (IRR = 1.05, 95% CI = 1.04–1.06, $p < 0.001$) and multivariable (IRR = 1.03, 95% CI = 1.02–1.04, $p < 0.001$) analysis. No other variables showed a significant relationship (Table 3).

IRR for the occurrence of non-colorectal malignancy within 20 years before CRC diagnosis in uni- and multivariable analysis.

There was a significantly higher IRR for non-colorectal malignancy within 20 years before the diagnosis of CRC in uni- (IRR = 1.91, 95% CI = 1.28–2.98, $p = 0.004$) and multivariable (IRR = 1.67, 95% CI = 1.05–2.65, $p = 0.029$) analysis for sporadic dMMR compared to pMMR tumors (Table 4). Age was the only independent variable that manifested as a significant predictor of other malignancies in both uni- (IRR = 1.05, 95% CI = 1.03–1.07, $p = 0.001$) and multivariable (IRR = 1.05, 95% CI = 1.03–1.07, $p < 0.001$) analysis. No other variable showed a significant relationship (Table 4).

IRR for the occurrence of non-colorectal malignancy during the observation period after CRC diagnosis in uni- and multivariable analysis.

There was a significantly higher IRR for non-colorectal malignancy after the diagnosis of CRC in uni- (IRR = 2.45, 95% CI = 1.72–3.49, $p = 0.004$) and multivariable (IRR = 2.35, 95% CI = 1.63–3.42, $p = 0.005$) analyses for sporadic dMMR compared to pMMR tumors. Once again, age manifested as a significant predictor of non-colorectal malignancy in both uni- (IRR = 1.04, 95% CI = 1.02–1.05, $p < 0.001$) and multivariable (IRR = 1.03, 95% CI = 1.02–1.05, $p < 0.001$) analysis. No other variable showed a significant relationship (Table 5).

TABLE 1 Non-colorectal cancer figures according to clinical characteristics and tumor pathology: entire study population.

Covariable	Total[a] ($n = 1706$)	Other cancer[a] ($n = 324$)	No other cancer[a] ($n = 1382$)	p value
Age				NA
Years median	67	70	67	
Range	29–105	29–97	29–105	
IQR	60–75	63–77	60–75	
Dichotomized 70 years				<0.001
<70	972 (57)	146 (45)	826 (60)	
≥70	734 (43)	178 (55)	556 (40)	
Sex ratio				0.122
Male	887 (52)	159 (49)	728 (53)	
Female	819 (48)	165 (51)	654 (47)	
Mismatch repair status				0.005
(sporadic) Deficient	186 (11)	60 (18)	126 (13)	
Proficient	1520 (89)	264 (82)	1256 (87)	
Tumor				0.512
Right colon	680 (40)	137 (42)	543 (39)	
Left colon	466 (27)	83 (26)	383 (28)	
Rectum	560 (33)	104 (32)	456 (33)	
Tumor stage				0.030
I	163 (10)	35 (11)	128 (10)	
II	691 (40)	146 (45)	545 (39)	
III	652 (38)	110 (34)	542 (39)	
IV	200 (12)	33 (10)	167 (12)	

Abbreviations: IQR, interquartile range; NA, not applicable.

[a]Data are expressed as number (percentage) of patients. The percentages were rounded off to obtain a total of 100.

SIRs, patients with dMMR CRC tumors, adjusted for year, sex, and age.

For the Swedish study population, calculation of SIR for validation purpose revealed that the expected and observed cancer cases among patients with sporadic dMMR tumors showed a statistically significant higher incidence of other non-colorectal malignancies among these patients. The incidence of other non-colorectal tumors among sporadic dMMR patients was increased (SIR = 1.23, 95% CI = 1.00–1.49, $p = 0.042$), especially among female patients (SIR = 1.10, 95% CI = 1.04–1.88, $p = 0.020$) (Table 6).

SIRs, patients with a pMMR CRC tumor, adjusted for year, sex, and age.

TABLE 2 Non-colorectal malignancy in CRC patients with dMMR and those with pMMR cancer.

	Total (%) 363 (100)	Sporadic dMMR (%) 60 (100)	pMMR (%) 303 (100)	*p* value
Prostate cancer	91 (25.1)	18 (30)	73 (24.1)	0.338
Breast cancer	49 (13.5)	9 (15)	40 (13.2)	0.722
Endometrial cancer	46 (12.7)	4 (6.6)	42 (13.7)	0.136
Hematologic cancers	38 (10.7)	9 (15)	29 (9.6)	0.221
Urinary tract cancer	31 (8.5)	7 (11.7)	24 (7.9)	0.332
Melanoma	17 (4.9)	1 (1.6)	16 (5.3)	0.236
Endocrine cancer	14 (3.8)	3 (5)	11 (3.6)	0.627
Ovarial cancer	13 (3.6)	1 (1.6)	12 (3.9)	0.394
Esophagus/ ventrikel cancer	12 (3.3)	2 (3.3)	10 (3.4)	0.991
Hepatobiliary cancer	11 (3.0)	1 (1.8)	10 (3.4)	0.501
Lung cancer	11 (3.0)	2 (3.3)	9 (3.0)	0.870
Brain cancer	10 (2.6)	2 (3.3)	8 (2.8)	0.744
Pancreas cancer	8 (2.2)	1 (1.8)	7 (2.4)	0.766
Head and neck cancer	5 (1.2)	0 (0)	5 (1.6)	0.327
Unspecified cancer	4 (1.1)	0 (0)	4 (1.4)	0.374
Small bowel cancer	2 (0.5)	0 (0)	2 (0.7)	0.538
Skeletal cancer	1 (0.2)	0 (0)	1 (0.3)	0.651

Abbreviation: CRC, colorectal cancer.

The same calculation for the Swedish population revealed that the expected and observed cancer cases among patients with pMMR tumors showed a statistically significant lower incidence of other non-colorectal malignancies. The incidence of other non-colorectal tumors among pMMR patients was increased among these patients (SIR = 0.71, 95% CI = 0.71–0.95, $p = 0.008$), especially among male patients (SIR = 0.75, 95% CI = 0.61–0.92, $p = 0.008$) (Table 7).

4 | DISCUSSION

In this multicenter study, including a large cohort from three European medical centers, a significant association between sporadic dMMR CRC and the risk for non-colorectal malignancy before and after the diagnosis of CRC was observed. This suggests that the genetic background i.e., impaired ability to correct errors in DNA replication, may be associated with the development of tumors elsewhere, not only among the familial type but also among sporadic dMMR CRC cases. This relationship was significant in both uni- and multivariable analyses when estimating the IRR for non-colorectal malignancies. The higher IRR in sporadic dMMR cases was more obvious in the time period after CRC diagnosis. This higher risk for non-CRC in sporadic dMMR CRC cases was also seen in a validation analysis, where the number of observed cases was significantly higher than that expected among these patients. To the best of our knowledge, this is the first study to investigate the association between sporadic dMMR CRC and the risk for non-colorectal malignancy. Our results indicate that further research into the need for surveillance strategies, not only in hereditary cases, but also in sporadic cases, is warranted. Whether surveillance strategies for patients with sporadic dMMR CRC should be the same as those for hereditary dMMR cases or whether a tailored surveillance program would be more suitable is a topic of further research.

The association between sporadic dMMR status and a higher risk for non-colorectal malignancy may arise from mechanisms similar to those of familial Lynch Syndrome i.e., aberrancy in systems ensuring the fidelity of DNA replication.[20] The theory that cancer develops from increased mutation rates due to uncorrected replication errors is based on previous findings revealing that in individuals with aberrant MMR activity, the accumulation of different mutations can result in the silencing of genes that normally inhibit tumorigenesis, such as tumor suppressor genes.[21] In contrast, oncogenes that are normally turned off in healthy tissues may become activated or acquire a tumor-promoting function because of the higher mutation rate.[22] The main idea of this study was that the same mechanism for carcinogenesis, i.e. impaired ability to correct errors in DNA replication, applies to both familial and sporadic dMMR cases.

In the study population, the defect mismatch repair process, resulting in the accumulation of insertions or deletions that secondarily predispose to malignancy, was associated with older age as an independent variable. This phenomenon is probably the result of a synergic interaction between age and dMMR rather than a strictly dependent relationship between age and carcinogenesis. In other words, increasing age also increases the risk of *MLH1* promoter hypermethylation, resulting in dMMR carcinogenesis.[20] It is well-known that the incidence of cancer increases with age in both humans and animals. Different patterns of age-related distribution of tumors in different organs and tissues have been observed. Aging seems to increase the susceptibility of various tissues to the initiation of carcinogenesis and usually facilitates the promotion and progression of carcinogenesis.[23] Aging may also predispose to cancer through mechanism such as tissue accumulation of cells in the late stages of carcinogenesis, alterations in homeostasis, alterations in the immune and endocrine systems, and telomere instability.[24] For all these reasons, the effect of age on tumorigenesis cannot be restricted to dMMR cases, and the same should be the case in pMMR CRC, even though dMMR patients appear to develop malignancies at a slightly higher age than pMMR patients.[25] In contrast, reports have revealed that there are no significant differences in molecular and clinicopathologic patterns between different CRC age groups, and that cases with sporadic dMMR CRC show similar molecular alterations in different age groups.[26] This relationship between age and dMMR tumorigenesis seems to be much more complex since MMR plays a critical role in preserving the

TABLE 3 IRR for the occurrence of a non-colorectal malignancy within 20 years before and after diagnosis between sporadic dMMR and pMMR cases in the uni- and multivariable models.

	Univariable analysis			Multivariable analysis		
	IRR	95% CI	*p* value	IRR	95% CI	*p* value
Age	1.05	1.04–1.06	0.001	1.03	1.02–1.04	0.001
Sex						
Male[a]						
Female	1.03	0.82–1.30	0.798	0.90	0.71–1.34	0.358
MMR status						
Proficient[a]						
Deficient	2.49	1.89–3.31	0.003	2.24	1.67–3.01	0.004
Tumor site						
Right colon	1.07	0.83–1.36	0.610	1.09	0.67–1.13	0.291
Left colon	0.80	0.59–1.08	0.145	0.89	0.54–1.08	0.080
Rectum[a]						
Tumor stage						
I	1.44	0.88–2.35	0.144	1.41	0.86–2.32	0.168
II	1.47	0.98–2.22	0.064	1.41	0.94–2.31	0.098
III	1.21	0.79–1.84	0.376	1.25	0.82–1.90	0.301
IV[a]						

Abbreviations: CI, confidence Interval; IRR, incidence rate ratio.

[a]The referance variable.

TABLE 4 IRR of non-colorectal malignancy within 20 years before CRC diagnosis between sporadic dMMR and pMMR cases in the Uni- and multivariable analyses.

	Univariable analysis			Multivariable analysis		
	IRR	95% CI	*p* value	IRR	95% CI	*p* value
Age	1.05	1.03–1.07	0.001	1.05	1.03–1.07	0.001
Sex						
Male[a]						
Female	1.05	0.74–1.48	0.785	0.94	0.66–1.33	0.719
MMR status						
Proficient[a]						
Deficient	1.91	1.28–2.98	0.004	1.67	1.05–2.65	0.029
Tumor site						
Right colon	1.40	0.93–2.11	0.108	1.10	0.71–1.68	0.678
Left colon	1.01	0.61–1.65	0.970	0.86	0.52–1.42	0.563
Rectum[a]						
Tumor stage						
I	0.94	0.43–2.04	0.874	0.93	0.42–2.03	0.855
II	1.33	0.76–2.32	0.323	1.26	0.72–2.20	0.425
III	0.79	0.44–1.46	0.464	0.86	0.47–1.58	0.636
IV[a]						

Abbreviations: CI, confidence Interval; CRC, colorectal cancer; IRR, incidence rate ratio.

[a]The referance variable.

TABLE 5 Incidence rate ratio for other non-colorectal cancer after the diagnosis of CRC between sporadic dMMR and pMMR cases in the uni- and multivariable models.

	Univariable analysis			Multivariable analysis		
	IRR	95% CI	*p* value	IRR	95% CI	*p* value
Age	1.04	1.02–1.05	0.001	1.03	1.02–1.05	0.001
Sex						
Male[a]						
Female	1.04	0.79–1.38	0.799	0.93	0.70–1.24	0.632
MMR status						
Proficient[a]						
Deficient	2.45	1.72–3.49	0.004	2.35	1.63–3.42	0.005
Tumor site						
Right colon	1.12	0.82–1.35	0.476	0.91	0.66–1.27	0.594
Left colon	0.81	0.55–1.19	0.288	0.76	0.52–1.12	0.171
Rectum[a]						
Tumor stage						
I	0.86	0.45–1.76	0.748	0.79	0.40–1.56	0.501
II	0.97	0.53–1.77	0.913	0.84	0.47–1.54	0.573
III	0.95	0.52–1.76	0.877	0.94	0.51–1.74	0.854
IV[a]						

Abbreviations: CI, confidence Interval; CRC, colorectal cancer; IRR, incidence rate ratio.
[a]The reference variable.

TABLE 6 Standardized incidence ratios (SIR) for patients with dMMR tumor, adjusted for year, sex, and age.

Gender	Observed	Expected	Pyrs	SIR	95% CI	*p* value
Male	51	46.2	3253	1.10	0.83–1.43	0.482
Female	44	31.0	3009	1.42	1.04–1.88	0.021
Total	95	77.2	6262	1.23	1.00–1.49	0.043

Abbreviations: CI, confidence interval; Pyrs, person-years; SIR, standardized incidence ratio.

TABLE 7 Standardized incidence ratios (SIR) for patients with pMMR tumor, adjusted for year, sex, and age.

Gender	Observed	Expected	Pyrs	SIR	95% CI	*p* value
Male	88	116.8	28249	0.75	0.61–0.92	0.008
Female	92	102.6	26098	0.90	0.73–1.10	0.296
Total	180	219.4	54346	0.71	0.71–0.95	0.008

Abbreviations: CI, confidence interval; Pyrs, person-years; SIR, standardized incidence ratio.

integrity of the genome in virtually all organisms.[27] Studies have suggested that the accumulation of DNA damage and the resulting genomic instability are not only risk factors for carcinogenesis, but also contribute to aging itself.[20,28] This hand-in-hand relationship between dMMR and age is supported by studies on mice, suggesting that the capacity for MMR diminishes with age and that MMR can process certain forms of oxidative damage to DNA, which is thought to accumulate with age.[20,28]

A broad spectrum of malignancies was observed in this study population. We excluded all non-melanoma skin neoplasms because these cancers are relatively common and have different epigenetic backgrounds due to UV radiation. Furthermore, since they are not usually fatal, the registration of non-melanoma skin cancer (ICD-10 C44) is probably not as complete as in other forms of cancer.[29] This wide spectrum of non-colorectal malignancies is also seen in the majority of studies dealing with hereditary Lynch Syndrome, but in this study, some hematological malignancies were observed that have not been reported in studies on Lynch Syndrome.[30,31] One possible explanation for this discrepancy is that hematologic malignancies are diseases that mainly affect older individuals.[32] Another difference is that endometrial cancer was less frequent in our study population than in studies on hereditary cases.[33] This may be explained by the fact that the majority of Lynch endometrial cancers carry a deleterious germline mutation in *MSH2* genes in contrast to sporadic dMMR cases where the majority carry a mutation in the *MLH1* gene.[34] As shown in previous reports, mutations in different genes lead to different penetrance and patterns of cancer expression.[35] There are significant discrepancies in cancer risk estimations in Lynch Syndrome patients, with some studies stating that the "true" risk may never be established. The main reason seems to be that current reports are biased because

estimations are based on Lynch Syndrome registers that include Lynch families with highly penetrant alleles.[36] The heterogeneity of allelic aberrations may lie behind the different etiologies of cancer syndromes encompassing a spectrum of similar clinical presentations and genetic profiles, such as Lynch-like Syndrome and familial CRC type X.[37,38] Heterogenicity could explain the differences observed between the results of previous studies on Lynch Syndrome and those in this study based exclusively on sporadic dMMR CRC, a subject that has seldom been investigated.[39]

Considerable differences exist between colonic and rectal tumors, especially regarding tumor biology, relapse patterns, and treatment modalities.[40] A limitation of this study is the inherent bias of the retrospective methodology. The strength of this study to determine the incidence of other non-colorectal malignancies in patients with sporadic dMMR CRC, is the validation results comparing expected and observed cancer cases separately for dMMR and pMMR cases, and that the study cohort consisted of populations from three different countries and different categories of hospitals, increasing the external validity and generalizability of the results.[41] Furthermore, the unique possibility of using national cancer registry data for the Scandinavian population increasing the reliability and trustworthiness of our results.[42]

5 | CONCLUSION

Patients with sporadic dMMR CRC have a higher IRR for other non-colorectal malignancies. Further studies are needed to determine the need for and design of surveillance screening programs for these patients to improve cancer diagnosis and clinical outcomes.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are not openly available in [repository name] at [DOI], reference number [reference number]. No shared data.

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