

Prognostic relevance of *MLH1* and *MSH2* mutations in hereditary non-polyposis colorectal cancer patients

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ABSTRACT

Aims and background. Colorectal carcinoma patients from hereditary non-polyposis colorectal cancer families are suggested to have a better prognosis than sporadic colorectal carcinoma cases. Since the majority of hereditary non-polyposis colorectal cancer-related colorectal carcinomas are characterized by microsatellite instability due to germline mutations in DNA mismatch repair genes, this is consistent with the prolonged survival observed in sporadic microsatellite instability-positive colorectal carcinoma compared to microsatellite stable cases. However, a fraction of colorectal carcinoma cases belongs to families that, despite fulfilling the clinical criteria for hereditary non-polyposis colorectal cancer, do not carry mismatch repair gene mutations. Our aim was to verify to what extent the genotypic heterogeneity influences the prognosis of hereditary non-polyposis colorectal cancer patients.

Methods. A survival analysis was performed on 526 colorectal carcinoma cases from 204 Amsterdam Criteria-positive hereditary non-polyposis colorectal cancer families. Enrolled cases were classified as *MLH1*-positive, *MSH2*-positive and mutation-negative, according to the results of genetic testing in each family.

Results. Five-year survival rates were 0.73 (95% CI, 0.66-0.80), 0.75 (95% CI, 0.66-0.84) and 0.62 (95% CI, 0.55-0.68) for *MLH1*-positive, *MSH2*-positive and mutation-negative groups, respectively (logrank test, $P = 0.01$). Hazard ratio, computed using Cox regression analysis and adjusted for age, sex, tumor site and stage, was 0.71 (95% CI, 0.51-0.98) for the mutation-positive compared to the mutation-negative group. Moreover, in the latter group, patients with microsatellite instability-positive colorectal carcinomas showed a better outcome than microsatellite stable cases (5-year survival rates, 0.81 and 0.60, respectively; logrank test, $P = 0.006$).

Conclusions. Our results suggest that the prognosis of hereditary non-polyposis colorectal cancer-related colorectal carcinoma patients depends on the associated constitutional mismatch repair genotype.

Key words: hereditary non-polyposis colorectal cancer, *MLH1*, *MSH2*, survival.

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Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disorder characterized by a high risk of colorectal cancer (CRC) in the absence of diffuse gastrointestinal polyposis. The prevalence of the syndrome is not yet clear, but it may account for 1-5% of all CRC¹.

Prior to the identification of its genetic bases, the disease was diagnosed in the presence of families with aggregation of early onset CRC (frequently located in the proximal colon and often multiple), and with an excess of extracolonic cancers, including carcinomas of the endometrium, ovary, small intestine, biliary tract, ureter, kidneys, pelvis, stomach and pancreas². Such features have been incorporated into the clinical criteria that define HNPCC, referred to as the 'Amsterdam criteria'³⁻⁴.

The observation that a large fraction of CRC arising in HNPCC patients are characterized by microsatellite instability (MSI), i.e. by alterations in short tandem repeat DNA sequences, established a link between the disease and defects of the DNA mismatch repair (MMR) system⁵. To date, germline mutations in four different MMR genes have been reported in association with HNPCC. These include *MLH1*, *MSH2* (which together account for almost 90% of all identified mutations), *MSH6* and *PMS2*¹.

MSI is also present in approximately 12-15% of sporadic CRC cases, where it is due to somatic genetic or epigenetic alterations, such as methylation of the *MLH1* gene promoter⁶. The tumors differ from most CRC, which are characterized by chromosomal instability⁷, and it has been postulated that they may have a different clinical behavior. In particular, several studies have evidenced a better prognosis for MSI-positive CRC than for CRC with microsatellite stable (MSS) genotypes. This has been recently confirmed by a meta-analysis of more than 7,600 CRC cases characterized for the presence of MSI⁸.

Based on these observations, a survival advantage could be speculated for patients with HNPCC-associated CRC, which are mostly MSI-positive, compared with patients with sporadic CRC, which are prevalently MSS. This hypothesis is in accord with early descriptive studies reporting crude survival data that indicated a better outcome for CRC in HNPCC than for sporadic CRCs⁹⁻¹². In addition, Watson *et al.*¹³ reported a hazard ratio (HR), adjusted for stage and age differences, of 0.67 for HNPCC patients with CRC compared to sporadic CRC. However, three independent studies did not demonstrate any advantage for HNPCC patients after multivariate analysis¹⁴⁻¹⁶. Moreover, Farrington *et al.*¹⁷ found that MSI status was associated with a better prognosis in older patients, but not in those diagnosed before 30 years of age, who are more likely to carry germline mutations of MMR genes.

A possible explanation for these conflicting results relates to the genetic heterogeneity of HNPCC, which, as mentioned before, is associated with mutations in dif-

ferent genes. In addition, molecular screenings failed to identify the underlying genetic lesion in 20-70% of HNPCC families, depending on the inclusion criteria adopted for DNA testing¹. Indeed, two studies that considered the genotypic status of HNPCC patients reported that carriers of MMR gene mutations had a better survival than CRC patients unselected for family history¹³⁻¹⁸. However, these findings were not confirmed by a recent study carried out on 870 consecutively ascertained CRC patients, in which no statistically significant difference was observed between survival rates of carriers and non-carriers of MMR gene mutations¹⁹.

The aim of the present study was to investigate whether among HNPCC patients with CRC those who carry mutations in MMR genes have a survival advantage compared to non-carriers, and, consequently, whether genotype may be considered a prognostic indicator in these subjects.

Patients and methods

Patients

The study was based on 526 ascertained CRC cases from 204 verified independent HNPCC families reported in the framework of a collaborative study involving various Italian institutions. The diagnosis of HNPCC was dependent on family history and fulfillment of the Amsterdam criteria I and II^{3,4}.

Clinical and pathological data

A minimum data set was established for the pooled analysis. Information about clinico-pathological variables recorded at CRC diagnosis and personal history of cancers was collected. Adenocarcinomas were typed and graded according to the World Health Organization criteria²⁰. Histological examination of the operative specimen provided information about tumor extension in the bowel wall, adjacent organs and lymph nodes. We created a classification algorithm that took into consideration the primary treatment received by the patients. Carcinomas were classified according Dukes stage. We defined the right colon as the tract from the cecum to the splenic flexure. The left colon included the descending and sigmoid colon. The rectum was defined as the rectosigmoid junction and rectum.

Patients were stratified into three age groups – less than 40 years, from 40 to 59 years, and older than 59 years – in order to compare the prevalence of carcinomas in the different groups considered.

Molecular analysis

Blood samples were collected from individuals eligible to mutation screening after receiving written informed consent for genetic testing. Approval of the local Ethics Committees at participating institutions was obtained.

The occurrence of germline nucleotide alterations in the *MLH1* and *MSH2* genes was investigated in index cases of the families included in the study, using a combination of different screening methods. More precisely, 109 cases were analyzed by single-strand conformation polymorphism assay and 43 by direct sequencing as described²¹⁻²⁴, whereas 15 cases were screened by denaturing gradient gel electrophoresis and 37 by denaturing high performance liquid chromatography according to published protocols with minor modifications²⁵⁻²⁷. All mutations identified by indirect methods were confirmed by sequence analysis.

Cases who tested negative for the above analyses were investigated for the presence of genomic rearrangements, including the deletion or duplication of one of more exons in both the *MLH1* and *MSH2* genes by southern blotting analysis²⁸, multiplex ligation-dependent probe assay²⁹, or non-fluorescent multiplex polymerase chain reaction coupled with high-performance liquid chromatography³⁰. Analyzed individuals were classified as mutation-positive if they were carriers of nucleotide variants that a) introduced a premature stop codon (nonsense or frameshift), b) affected the consensus sequences at the/a mRNA splice site, or c) caused a missense change not classifiable as a common polymorphism, or if they were carriers of deletions/insertions involving one or more exons or the entire gene. In mutation-positive families, if the constitutional DNA of CRC patients other than the index case was available for testing, the presence of the identified alterations was verified by direct sequence analysis or using one of the methods for the screening of genomic rearrangements.

For MSI analysis, the five-marker panel recommended by the international guidelines³¹ was analyzed. Tumor and matched normal DNA were amplified by polymerase chain reaction using either a) radio-labeled primers followed by electrophoresis on denaturing polyacrylamide gels and autoradiography, or b) fluorescently labeled primers, followed by gel-electrophoresis on a 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) and detection using the GeneScan and Genotyper softwares, as reported³². A sample was considered MSI-positive when at least two microsatellites were altered.

Follow-up procedures and survival calculation

The follow-up procedures of CRC patients were as previously reported³³, without substantial differences among the different institutions involved in the study. In general, surveillance programs were fitted also to take into account the increased risk of extracolonic manifestations associated with HNPCC. The index date for survival calculation was defined as the date of first diagnosis of CRC. Clinical records of patients provided information on their vital status. Death certificates were obtained for all deceased patients.

Statistical analysis

The observed survival was estimated using the Kaplan and Meier product limit method. Cumulative survival probability was calculated at 5 years within each of the different groups considered and stratified according to various clinical and pathological variables. Differences were tested using the logrank test. To assess the relative excess risk of death among different patient groups and to control for confounding factors, proportional hazards models (including all available prognostic factors – sex, age at diagnosis, tumor site and stage) were fitted computing HR and the corresponding 95% confidence intervals (95% CI). The proportional assumption was examined with log-log survival plots or by adding time-dependent interaction terms into the model.

Results

The clinical, pathological and genetic characteristics of the 526 HNPCC-related CRC cases considered are shown in Table 1. Overall, the group included 288 males

Table 1 - Distribution of 526 hereditary non-polyposis colorectal cancer patients with colorectal cancers according to gender, clinical/pathological characteristics and mutation status

	Mutation			P*
	<i>MLH1</i> (n = 162)	<i>MSH2</i> (n = 96)	Not found (n = 268)	
Gender				
Males	92 (56.8)	62 (64.6)	134 (53.5)	0.04
Females	70 (43.2)	34 (35.4)	134 (46.5)	
Age (yr) at CRC diagnosis				
<40	53 (32.7)	30 (31.3)	62 (23.1)	0.001
40-59	90 (55.6)	56 (58.3)	139 (51.9)	
≥60	19 (11.7)	10 (10.4)	67 (25.0)	
Site				
Right	82 (50.6)	42 (43.7)	125 (46.6)	0.0004
Left	25 (15.4)	19 (19.8)	63 (23.6)	
Rectum	15 (9.3)	16 (16.7)	50 (18.7)	
Multiple	14 (8.6)	9 (9.4)	12 (4.4)	
Not reported	26 (16.1)	10 (10.4)	18 (6.7)	
Stage (Dukes)				
A	22 (13.6)	13 (13.6)	28 (10.5)	0.4
B	53 (32.7)	37 (38.5)	99 (36.9)	
C	28 (17.3)	13 (13.6)	51 (19.0)	
D	17 (10.5)	13 (13.6)	43 (16.0)	
Not reported	42 (25.9)	20 (20.8)	47 (17.5)	
Grading				
Well-differentiated	21 (13.0)	13 (13.5)	32 (11.9)	0.4
Moderately differentiated	37 (22.8)	27 (28.1)	83 (31.0)	
Poorly differentiated	23 (14.2)	9 (9.4)	26 (9.7)	
Mucinous	9 (5.6)	11 (11.5)	20 (7.5)	
Not reported	72 (44.4)	36 (37.5)	107 (39.9)	

*Fisher exact test.
In parenthesis, percentage.

and 238 females. Mean age at index CRC was 48 years (range, 20-88). The anatomical site was reported in 472 cases, and the distribution was as follows: 249 (53%) in the right colon, 107 (23%) in the left colon, 81 (17%) in the rectum, and 35 (7%) at multiple locations. Of the 417 cases for which the information was available, 232 (56%) were early stage (Dukes A and B) and 165 (44%) advanced stage (Dukes C and D) tumors.

A total of 258 patients (49%) belonged to 102 families in which a germline mutation was identified. They were classified as *MLH1*-positive ($n = 162$) or *MSH2*-positive ($n = 96$), based on the results of the molecular analyses. These included 166 individuals (index cases and relatives in whom the presence of the mutation could be experimentally verified and 92 individuals who were classified as obligate carriers based on family pedigree. The remaining 268 patients (51%) were classified as mutation negative and included 102 molecularly ascertained index cases and 162 relatives. Five CRC cases from mutation-positive families, who were found to be wild-type, were classified as phenocopies and were excluded from the study (Table 1).

No significant difference was observed for stage and histological grade among the three different groups considered (*MLH1*-positive, *MSH2*-positive and mutation negative). However, we found that whereas male and female patients were equally represented in mutation-negative families, within *MLH1*-positive and *MSH2*-positive families most CRC cases were males (57% and 65%, respectively, $P = 0.04$). In addition, we observed that age at diagnosis was significantly lower in *MLH1*-positive and *MSH2*-positive families than in mutation-negative families (46, 45 and 51 years, respectively, $P = 0.001$), thus confirming our previous findings²¹. A significant association was also observed between mutational status and CRC location ($P = 0.0004$). In particular, cases in the *MLH1*-positive group were more frequently right-sided (51%) than cases in the *MSH2*-positive and in the mutation-negative groups (44% and 47%, respectively). Moreover, the presence at diagnosis of cancers located at different anatomical sites was found to be more frequent among cases of the mutation-positive groups (approximately 9% in both *MLH1*-positive and *MSH2*-positive) than in those of the mutation-negative group (4%).

As regards clinical treatment, the fraction of patients surgically treated with a radical procedure was similar in the three groups: *MLH1*-positive, 91%; *MSH2*-positive, 98%; mutation-negative, 83%. Analogously, no difference emerged when adjuvant treatments (mainly 5-fluorouracil-based chemotherapy) were taken into account. These regimens were applied in 10%, 14% and 12% of cases in the *MLH1*-positive, *MSH2*-positive and mutation-negative groups, respectively.

The survival probabilities of *MLH1*-positive, *MSH2*-positive and mutation-negative patients are shown in Figure 1 and detailed in Table 2 according to clinical and pathological characteristics. The median time of follow-

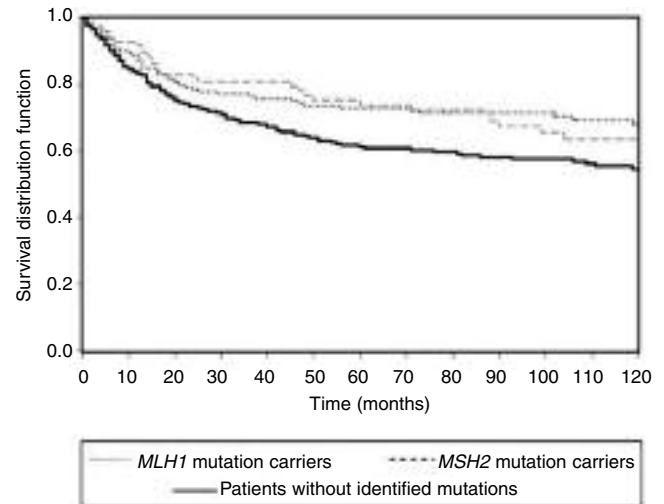


Figure 1 - Survival curves of HNPCC patients affected with colorectal cancer stratified according to mutation status.

up was 64 months (range, 6-383). Overall, 5-year survival rates were 0.73 (95% CI, 0.66-0.80), 0.75 (95% CI, 0.66-0.84) and 0.62 (95% CI, 0.55-0.68) for *MLH1*-positive, *MSH2*-positive and mutation-negative groups, respectively (logrank test, $P = 0.01$). HR computed using Cox regression analysis and adjusted for age, sex, anatomical site and tumor stage were 0.76 (95% CI, 0.52-1.09) for *MLH1*-positive and 0.63 (95% CI, 0.39-1.01) for *MSH2*-positive groups, compared to the mutation-negative group. When the mutation-positive groups were cumulatively compared with the mutation-negative group, the 5-year survival rate was 0.74 (95% CI, 0.68-0.79; logrank test, $P = 0.005$) and the HR was 0.71 (95% CI, 0.51-0.98).

A comparative analysis was performed including only the 204 index cases. HR for *MSH2*-positive, *MLH1*-positive and all mutation-positive cases compared to mutation-negative cases were 0.87 (95% CI, 0.32-2.37), 0.56 (95% CI, 0.20-1.56) and 0.69 (95% CI, 0.31-1.52), respectively (Table 2). The presence of MSI in tumor DNA was investigated in 208 cases for whom matched normal and tumor DNA pairs were available. These included 78 mutation carriers and 130 patients from families whose index cases tested negative at mutation analyses. Although all but two CRC (97%) from mutation-positive cases showed MSI, 63 CRC (48%) from mutation-negative cases were found to be MSI-positive and 67 (52%) MSS. A significant association with a better outcome was found for MSI-positive (5-year survival rate, 0.81; 95% CI, 0.70-0.91) than for MSS patients (0.60; 95% CI, 0.48-0.72) (logrank test, $P = 0.006$).

Discussion

Following the discovery of the role of the MMR genes in the development of CRC, a new carcinogenetic path-

Table 2 - Five-year cumulative survival probabilities for 526 hereditary non-polyposis colorectal cancer patients with colorectal cancers according to gender, clinical/pathological characteristics and mutation status

	Mutation			P*
	<i>MLH1</i> (n = 162)	<i>MSH2</i> (n = 96)	Not found (n = 268)	
Gender				
Males	0.72 (0.63-0.82) ^o	0.80 (0.70-0.90)	0.58 (0.49-0.67)	0.008
Females	0.74 (0.63-0.84)	0.68 (0.52-0.85)	0.65 (0.57-0.74)	0.6
Age (yr) at CRC diagnosis				
<40	0.82 (0.71-0.93)	0.85 (0.72-0.99)	0.67 (0.54-0.79)	0.1
40-59	0.73 (0.63-0.82)	0.73 (0.61-0.86)	0.66 (0.58-0.74)	0.5
≥60	0.49 (0.25-0.73)	0.56 (0.23-0.89)	0.48 (0.36-0.61)	0.9
Site				
Right	0.79 (0.70-0.88)	0.86 (0.74-0.98)	0.63 (0.54-0.72)	0.001
Left	0.49 (0.29-0.70)	0.55 (0.32-0.78)	0.63 (0.51-0.75)	0.1
Rectum	0.73 (0.51-0.96)	0.75 (0.54-0.96)	0.55 (0.41-0.69)	0.2
Multiple	0.86 (0.67-1.00)	0.56 (0.23-0.88)	0.64 (0.36-0.92)	0.2
Not reported	0.68 (0.49-0.86)	0.83 (0.54-1.00)	0.64 (0.41-0.87)	0.5
Stage (Dukes)				
A	1.00	0.92 (0.78-1.00)	0.93 (0.83-1.00)	0.2
B	0.88 (0.79-0.97)	0.90 (0.80-1.00)	0.83 (0.75-0.91)	0.4
C	0.76 (0.59-0.93)	0.68 (0.43-0.94)	0.57 (0.42-0.72)	0.3
D	0.12 (0.00-0.27)	0.27 (0.02-0.52)	0.10 (0.01-0.19)	0.6
Not reported	0.63 (0.8-0.78)	0.74 (0.52-0.96)	0.49 (0.34-0.64)	0.2
Grading				
Well-differentiated	0.95 (0.85-1.00)	0.85 (0.65-1.00)	0.84 (0.72-0.97)	0.5
Moderately differentiated	0.72 (0.58-0.87)	0.90 (0.77-1.00)	0.53 (0.42-0.65)	0.003
Poorly differentiated	0.78 (0.61-0.95)	0.88 (0.65-1.00)	0.68 (0.49-0.86)	0.4
Mucinous	0.53 (0.19-0.87)	0.64 (0.35-0.92)	0.42 (0.19-0.64)	0.6
Not reported	0.67 (0.56-0.79)	0.61 (0.44-0.78)	0.63 (0.54-0.72)	0.8
Overall	0.73 (0.66-0.80)	0.75 (0.66-0.84)	0.62 (0.55-0.68)	0.01
Hazard ratio [§]	0.76 (0.52-1.09)	0.63 (0.39-1.01)	1 [#]	
Overall (all mutated vs not found)	0.74 (0.68-0.79)		0.62 (0.55-0.68)	0.005
Hazard ratio [§]	0.71 (0.51-0.98)		1 [#]	

*Logrank test.

^oIn parenthesis, 95% CI.[§]From Cox proportional hazard regression models including terms for age at diagnosis of colorectal cancer in continuous, gender, site and stage of disease.[#]Reference group.

way has been postulated⁷. It soon became apparent that this pathway is associated with tumors with distinct, if not specific, clinical and pathological features. In fact, CRC with defects in the MMR system, i.e., those exhibiting MSI, are more likely to have a proximal location and to show low-grade differentiation, high mucinous component and lymphocytic infiltration⁶.

Of much relevance for the clinical management of these patients is to verify whether such genotype-phenotype correlations also have an impact on the prognosis. Conflicting results have been reported on this issue. Of 32 studies examined in a large, comprehensive systematic review⁸, only 13 detected a statistically significant advantage ($P < 0.05$), in terms of overall survival and/or disease free-survival, for MSI-positive compared to MSS CRC patients, whereas the remaining 19 reports failed to detect any significant difference. Nevertheless, the pooled analysis of all the studies estimated an HR for OS associated with MSI of 0.65 (95% CI, 0.59 to 0.71)⁸.

It is less clear whether the same advantage also applies to patients with MSI-positive CRC belonging to HNPCC families³⁴. A few studies that analyzed HNPCC cases classified according to clinical and/or genetic criteria reported a better prognosis than for sporadic CRC cases^{9-13,18}, but others did not confirm these findings^{14-16,19}.

In the present analysis, we reviewed the follow-up of more than 500 HNPCC patients affected with CRC to evaluate a potential correlation between prognosis and genotype. In fact, patients belonging to families with ascertained mutations in the *MLH1* or *MSH2* gene exhibited an approximately 30% risk reduction for death compared to cases from families without identified mutations, after adjustment for sex, age at diagnosis, tumor site and stage. This advantage appeared to be slightly higher in *MLH1*- than in *MSH2*-associated cases (HR, 0.76 and 0.63, respectively).

A possible confounding factor of our analysis might be represented by the inclusion in the mutation-nega-

tive group of *MLH1*- or *MSH2*-associated cases that were not diagnosed by molecular analysis. However, one would predict the CRC of these patients to be MSI-positive, a notion supported by the detection of MSI in almost the totality (97%) of CRC samples analyzed in proven mutation carriers. Indeed, we observed that in the subgroup of apparently mutation-negative cases for which tumor samples were available, those who tested positive for MSI had a survival rate significantly higher than patients with MSS tumors (0.81 vs 0.61, respectively) and comparable to that observed in the mutation-positive group (0.74). Noticeably, the former group (MSI-positive) might include carriers of mutations in other HNPCC-related MMR genes, i.e., *MSH6* and *PMS2*, which were not investigated in the present study because they are generally less frequently mutated than *MLH1* and *MSH2* (<10%)².

Another potential bias to be considered may have derived from the inference we made assuming that affected family members who could not be tested had the same genotype ascertained in their index cases. An indirect evidence that this is unlikely to represent a relevant confounding factor in our analysis is represented by the observation that the occurrence of phenocopies is a relatively rare event in HNPCC families. In fact, of the 69 CRC patients (excluding the probands) in the family set included in this study who were examined for the presence of the mutation identified in their respective index cases, only 5 (7%) tested negative. Moreover, a survival advantage for mutation carriers was observed also when the analysis was restricted to the index cases of enrolled families, although in this instance the difference did not reach significance, most likely due to the reduced number of subjects considered.

Overall, our observations suggest that among HNPCC-related CRC, those associated with or referable to germline defects of MMR genes have a better outcome, in agreement with the findings of a previous study carried out on a smaller number of families³⁵.

Our results are in contrast with those reported by Watson *et al.*¹³, who did not observe any survival difference between HNPCC patients from mutation-positive and mutation-negative families. On the one hand, this might be due to the smaller number of cases than in our study and/or to a higher proportion of families with MSI-positive CRC in the mutation-negative group. On the other hand, our analysis might have been affected by the design of the study, which was retrospective and based on data collected from different institutions. However, we believe that this is unlikely, since parameters potentially affecting disease outcome, including surgical treatment, CRC stage and adjuvant regimens, were similarly distributed in the different groups of patients considered, i.e., those from families with an *MLH1* or an *MSH2* mutation and those without identified germline alterations. Moreover, it has been reported that patients with MSI-positive CRC do not benefit

from conventional fluorouracil-based adjuvant chemotherapy³⁶⁻⁴⁰.

At present, there is no obvious explanation for our findings. However, it should be noted that CRC with MSI shows biological features that are usually correlated with a less aggressive phenotype. In particular, it has been suggested to be particularly prone to cytotoxic immune responses as indicated by the high content of activated intraepithelial T-lymphocytes, possibly resulting from the accumulation of mutations in genes encoding cell surface proteins⁴¹⁻⁴³. In addition, MSI-positive CRC displays a reduced microvessel density, due to a lower vascular endothelial growth factor expression⁴⁴⁻⁴⁶ and a reduced tendency to develop distant metastases⁴⁷. Another possibility is that MMR defects can affect the viability of tumor cells through the accumulation of mutations in genes necessary for cell survival⁴⁸. Although this hypothesis has yet to be formally demonstrated, it is noteworthy that MSI-positive CRC is less likely to carry mutations of *TP53*, *KRAS* and *DCC* genes, which are usually associated with a poor prognosis^{49,50}.

Irrespective of the precise mechanism involved, our results indicate that the same correlation observed in sporadic CRC, between a specific molecular pathway of carcinogenesis and clinical outcome, can also occur in HNPCC-associated CRC. This is in keeping with the observations of a few recent studies that examined the molecular and clinico-pathological features of patients belonging to HNPCC families without evidence of MMR deficiency. These families, for which the designation of 'familial colorectal cancer type X' has been proposed⁵¹, are characterized by a molecular pattern different from that observed in Lynch syndrome cases, i.e., in CRC patients with constitutional defects in MMR genes, with lower levels of derangement of the Wnt signaling pathway⁵². Moreover, they exhibit a later age of disease onset and a cancer incidence lower than that observed in MMR-related families^{21,51,53-56}.

Although the results of the present study need to be confirmed by prospectively built analyses, in order to control for biases that may affect retrospective investigations, such as incompleteness of data collection or selection in case referral, it is worth noting that the increase of second primary malignancies in genetically predisposed individuals, paradoxically due to the improvement of available therapeutic options, is becoming a critical issue in cancer survivorship⁵⁷. If confirmed, our findings will have important consequences on the clinical management of HNPCC families and will provide a rationale for modulating treatment and surveillance of affected cases on the basis of the result of molecular analyses.

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