Int. J. Cancer (Pred. Oncol.): **95,** 323–328 (2001)

Publication of the International Union Against Cancer

CLINICAL AND BIOLOGIC HETEROGENEITY OF HEREDITARY NONPOLYPOSIS COLORECTAL CANCER

Piero BENATTI[1]*, Luca RONCUCCI[1], Dorval GANAZZI[1], Antonio PERCESEPE[1], Carmela DI GREGORIO[2], Monica PEDRONI[1], Francesca BORGHI[1], Elisa SALA[1], Alessandra SCARSELLI[1], Mirco MENIGATTI[1], Giuseppina ROSSI[1], Maurizio GENUARDI[3], Alessandra VIEL[4] and Maurizio PONZ DE LEON[1]

[1]*Department of Internal Medicine, Università di Modena e Reggio Emilia, Modena, Italy*
[2]*Pathology Unit, Hospital of Carpi, Modena, Italy*
[3]*Department of Medical Genetics, Università Cattolica del Sacro Cuore, Rome, Italy*
[4]*Division of Experimental Oncology, Centro di Riferimento Oncologico, Aviano, Italy*

MMR gene mutations and MSI are not found in all clinically diagnosed HNPCC families. We evaluated whether MMR genotyping and tumor MSI analysis could identify distinct clinical subgroups among HNPCC families. Twenty-nine clinical HNPCC families were divided into 3 groups: A, families with *hMLH1* or *hMSH2* gene mutations; B, MMR gene mutations not present but MSI present in at least 50% of tumors tested; C, mutational and MSI analyses negative. We evaluated tumor spectrum, age at onset, risk of cancer in the follow-up and survival for CRC in the 3 groups. Tumors of the target organs in HNPCC (colon and rectum, endometrium, ovary, small bowel, stomach, renal pelvis and ureter) were more frequent in the first 2 groups than in the latter. Colon cancer was more frequently located in the proximal colon and showed an earlier age at onset in families with MMR gene mutation or with MSI than in families with stable tumors. Comparing the occurrence of tumors in the follow-up, in the first 2 groups patients younger than 50 years had a higher RR, which was particularly marked for CRC (RR = 18.6 for group A *vs.* group C, RR = 16.7 for group B *vs.* group C). CRC patients in the first 2 groups had a better clinical prognosis. The results of molecular analysis could distinguish, within clinically defined HNPCC families, different subgroups to which specific programs of surveillance could be addressed.

Key words: *hereditary nonpolyposis colorectal cancer; microsatellite instability; mismatch repair genes; diagnosis; follow-up; survival*

HNPCC is an autosomal dominant inherited syndrome caused by mutations in one of the DNA MMR genes, mostly *hMSH2* and *hMLH1*.[1,2] The disease is characterized by an elevated lifetime risk of developing colorectal and other extracolonic malignancies, such as endometrial, gastric, small bowel, ovary, renal pelvis and ureter cancers.[3]

Identification of HNPCC can be problematic owing to the lack of clinical or histologic distinctive features, the frequent unavailability of genetic analyses and the interpretation of biomolecular findings.[4,5] The diagnosis is basically reached on a clinical ground and relies on criteria, formally introduced in 1991 (AC-I)[6] and later modified[7] with the inclusion of those extracolonic tumors (*i.e.*, endometrium, small bowel, renal pelvis and ureter) that are considered part of the syndrome (AC-II).

From the molecular point of view, almost 90% of cancers in HNPCC families show MSI,[8] suggesting that MMR deficiency plays a crucial role in the pathogenesis of the disease. Despite this, MMR germline mutations are found in only 30–70% of HNPCC families,[9] thus raising questions on the nature of the disease and the most appropriate management for cases without a well-known genetic defect.[10]

Increasing evidence supports CRC surveillance as useful in individuals at risk in general and in AC-I HNPCC families in particular,[11,12] but the cost-effectiveness of this strategy has been demonstrated mostly for subjects with MMR mutations.[13,14] Consequently, whether HNPCC families with undefined mutational status have the same cancer risk and need the same strategies of surveillance and counseling as those with well-demonstrated MMR defects remains unknown.

Our study was aimed at evaluating whether HNPCC families in which a mutation has been identified are clinically different from families in which a mutation has yet to be found. In particular, we used the results of molecular analyses, *i.e.*, *hMLH1* and *hMSH2* genotyping and MSI tumor testing, to stratify HNPCC families, selected with AC-II, in 3 distinct subgroups (families with MMR mutation, families without mutation, families without mutation but with MSI tumors). Finally, these 3 groups have been compared to assess whether the biomolecular heterogeneity of HNPCC is associated with clinical heterogeneity, which could influence the strategies of management, surveillance and prevention.

MATERIAL AND METHODS

Families and clinical variables

Through a previously described[15] multistep clinical approach, we identified HNPCC families from the data of a population-based CRC registry instituted in the area of Modena (northern Italy) in 1984. Briefly, the nuclear pedigrees (limited to first-degree relatives) of all incident CRC cases diagnosed in the period 1984–1997 were collected and then stratified according to the presence of 6 clinical features, all suggestive of increased risk of hereditary disease: verticality (parent or offspring affected by CRC or other tumors featuring HNPCC, such as endometrium, small bowel, ovary, urinary tract, stomach), aggregation (in the sibship of the proband, at least 50% of siblings affected by cancer at any site), early onset (cancers at any sites developed before the age of 50), localization in the right colon (caecum, ascending, transverse and flexures), multiple tumors (at any sites, both synchronous and metachronous) and mucinous histologic type (presence of mucus in 50% or more of the colorectal tumor at histology). When a patient had 2 or more of these features, an extended genealogic pedigree was traced and then analyzed for the presence of diagnostic criteria for HNPCC. This approach led us to estimate the

Abbreviations: AC-I and AC-II, Amsterdam criteria I and II for the diagnosis of HNPCC; CI, confidence interval; CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer; MMR, mismatch repair; MSI, microsatellite instability; MSS, microsatellite-stable; RR, relative risk; SSCP, single-strand conformation polymorphism.

Grant sponsor: Italian Association for Cancer Research; Grant sponsor: Italian Ministry of Education; Grant sponsor: Consiglio Nazionale delle Ricerche; Grant number: 98.00 S01.CT04; Grant sponsor: Region Emilia Romagna; Grant number: 15.3.1986; Grant sponsor: European Commission; Grant number: BMH4-CTS6-0072.

*Correspondence to: Dipartimento Medicina Interna, Divisione Medicina III, Università di Modena e Reggio Emilia, via del Pozzo 71, 41100 Modena, Italy. Fax: +39 59 363114. E-mail: pbenatti@unimo.it

Received 29 January 2001; Revised 9 May 2001; Accepted 21 May 2001

frequency of HNPCC in the general population as 2.6% of all CRCs.[16] Further details of this clinical approach have been published in various reports.[15–17]

Once identified, families were considered eligible for the study if they met the following criteria: (*i*) fulfillment of AC-II for HNPCC diagnosis, (*ii*) MSI testing in at least 1 case of CRC or endometrial cancer in the family and (*iii*) availability of *hMLH1* and *hMSH2* mutation analysis results. According to biomolecular assays, eligible families were then subdivided in different groups: group A, mutation-positive families, if a germline mutation in 1 of the 2 MMR genes was found; group B, MSI families, if MSI was present in the majority of tumors tested (≥50%) but without mutations in *hMLH1* or *hMSH2;* group C, MSS families, when mutational analysis was negative and MSI was found in <50% of tumors tested (Table I).

We then evaluated whether tumor spectrum, age at onset, RR and cancers developed in the follow-up showed significant differences among the 3 groups. Finally, 5-year CRC-specific survival was analyzed according to molecular diagnosis (mutation-positive, MSI, MSS), TNM stage (4 categories: stages I, II, III and IV + unstaged tumors), gender, age at diagnosis (dichotomized in 2 groups: ≤50 and >50 years) and colorectal sublocalization. In the latter case, 2 groups were defined: right colon (from caecum to splenic flexure) and left colon (distal to splenic flexure) + rectum. Patients were considered eligible for survival analysis if they had a histologically verified CRC and if information on their clinical status was available by 31 December 1999. Causes of death were ascertained through clinical charts and death certificates.[18]

Microsatellite testing and MMR gene mutation analysis

For microsatellite analysis, DNA was extracted from neoplastic and paired normal mucosa specimens of CRC and endometrial cancer according to the standard procedure.[19] Five markers, 3 mononucleotides (BAT25, BAT26 and BAT40) and 2 dinucleotides (D17S787 and D18S58), were used; tumors showing instability at 2 or more loci were scored as unstable.[20] This set of microsatellite markers does not strictly correspond to the first panel proposed by the Bethesda guidelines[20] mainly because, in most cases, MSI testing was begun prior to their introduction. The choice of the panel used in our study was based on the high sensitivity and consistency of these primers at detecting high-frequency MSI. In particular, mononucleotides BAT25, BAT26 and BAT40 have been shown to be highly sensitive and specific at identifying MSI in HNPCC patients in our sample[21] and in other studies.[22–24]

As previously reported,[25] germline mutations in *hMSH2* and *hMLH1* were studied by SSCP and sequencing on DNA derived from blood leukocytes. Samples showing an altered SSCP mobility pattern were sequenced by means of the Sequenase PCR product-sequencing kit (Amersham, Aylesbury, UK).

Statistical analysis

The χ^2 test was used to assess the statistical significance of differences among groups except for mean age at cancer diagnosis, mean length of follow-up and mean family size, which were analyzed by ANOVA (univariate models). RRs and 95% CIs were used to evaluate differences in cancer development in the follow-up considering persons/year at risk as the reference population category in each group. For survival analysis, categorical variables were created for all parameters evaluating CRC-specific survival through the method of Kaplan-Meier[26] and estimating differences with the log-rank test.[27] The independent effect on survival of each variable was evaluated through Cox regression multivariate analysis, using the stepwise backward conditional method.[28] Statistical significance was set at $p < 0.05$. All analyses were carried out using SPSS (Chicago, IL) Windows software.

TABLE I – MAIN FEATURES OF FAMILIES AT THE TIME OF HNPCC DIAGNOSIS

Family code	Number of family members	Number of affected members	Colon cancer		Extracolonic HNPCC organs	
			Cases	Age (range)	Cases	Age (range)
Group A						
1	34	7	5	41–47	3	31–59
2	29	10	7	27–73	4	43–66
4	34	7	5	41–55	3	41–47
10	36	8	5	47–65	5	41–60
20	51	12	7	44–56	4	49–58
27	32	10	12	40–69	1	49
29	37	8	8	41–61	2	44–53
33	38	7	7	33–50	—	—
35	42	11	6	20–57	4	34–51
39	28	5	4	48–79	3	33–49
Group B						
3	22	3	3	41–78	—	—
6	24	4	5	28–57	1	70
7	21	3	4	44–56	1	42
9	20	4	4	36–56	—	—
24	28	3	3	46–65	—	—
25	36	6	7	34–67	1	83
34	19	3	2	45–46	1	68
36	26	4	3	55–70	2	41–62
40	22	3	4	48–67	—	—
41	23	4	3	37–42	—	—
Group C						
11	39	8	10	48–72	—	—
12	37	4	3	50–65	—	—
17	47	7	5	47–86	1	65
21	36	4	3	34–70	1	76
23	60	15	9	40–78	1	54
26	35	7	6	45–85	1	85
37	38	5	6	41–90	—	—
38	33	4	2	37–73	1	48
42	30	3	3	49–82	—	—

The third column reports the total number of patients affected by cancer at any site, whereas the fourth and sixth columns report the total number of colorectal and extracolonic HNPCC cancers.

RESULTS

Twenty-nine families met the criteria for inclusion: 10 carried MMR gene mutations (group A), 10 were characterized by MSI phenotype but not by MMR gene mutations (group B) and 9 did not show mutations or MSI (group C). In group A, 6 families were characterized by germline mutations in *hMLH1* while 4 harbored pathogenic mutations in *hMSH2*.

Overall, 76 tumors (70 colorectal and 6 endometrial) could be analyzed for MSI. In group A, all 25 tumors tested (20 colorectal and 5 endometrial, range of tumors tested per family 1–5) showed MSI. Twenty-three (22 colorectal and 1 endometrial) of 25 tumors (92%) were unstable in families with MSI (range of tumors tested per family 2–4). In group C, only 2 of 26 colorectal tumors tested (7.7%) showed instability (range of tumors tested per family 2–5). Considering the relation between MSI and colorectal tumor sublocalization, the percentage of MSI left-sided colorectal tumors was 100% (7 tested cases) in group A, 75% (6 of 8 tested cases) in group B and 5% (1 of 20) in group C. The corresponding percentages of MSI right colon cancer were 100% in group A (13 cases), 93% (13 of 14 cases) in group B and 17% (1 of 6) in group C.

Table II summarizes the main clinical features of the groups at baseline (*i.e.*, time of HNPCC diagnosis in the index case). Families of the 3 groups did not differ according to gender and proportion of members alive; although not significant, family size was slightly smaller in group B. The number of affected members was higher in group A than in groups B and C ($p < 0.05$); moreover, multiple malignancies tended to occur more frequently in patients of the first 2 groups. Regarding tumor spectrum, HNPCC target organs (colon-rectum, endometrium, stomach, small bowel, ureter and renal pelvis, ovary) were more often involved in groups A and B (84.8% and 90%, respectively) than in C (73.2%, $p < 0.005$). Colon cancer was more often proximally localized in patients with MMR gene mutations, while progressive involvement of the distal colon and rectum was evident in the other 2 groups. Gastric cancer represented the most frequent extracolonic malignancy in each group. Finally, CRC patients in the first 2 groups were significantly younger than patients in group C. For extracolonic HNPCC-related tumors, age at onset was earlier in group A than in groups B and C, whereas no significant differences were observed considering only malignancies not included in the HNPCC spectrum.

Follow-up

For each family member at risk, time of follow-up was calculated from the date of HNPCC diagnosis in the index case to 31 December 1999. Although not statistically significant, duration was shorter for group B (mean 6.7 years) compared to A (8.2 years) and C (9.4 years).

Follow-up revealed significant clinical differences especially comparing groups A and B with group C (Table III). In the first 2 groups, >85% of tumors occurred in HNPCC-related organs (colon, rectum, endometrium, stomach, renal pelvis and ureter, ovary), whereas the percentage was lower (64.5%) in group C ($p = 0.03$). In the follow-up, among HNPCC-related neoplasms, endometrial cancer was more frequent than gastric carcinoma in groups A and B, whereas in group C the stomach was the only extracolonic organ involved. Patients developing CRC in the follow-up were significantly younger ($p = 0.001$) in groups A and B than in group C.

Table IV shows the RRs of cancer at different sites resulting from comparison between groups. RRs were calculated for all patients and separately for those younger than 50 years. Major differences were observed when comparing groups A and C: in any of the conditions considered, except extra-HNPCC organs, patients in group A had a significantly higher cancer risk. In contrast, groups A and B were not different for cancer risk in the follow-up. Finally, patients in group B had significantly higher risk for CRC and, when younger than 50 years, for tumors at any site compared to group C.

TABLE II – CLINICAL FEATURES OF THE 3 GROUPS EXAMINED AT THE TIME OF BASELINE REGISTRATION (*I.E.*, TIME OF HNPCC DIAGNOSIS IN THE PROBAND)

	Group A	Group B	Group C	*p*
Number of families	10	10	9	
Subjects	361	241	355	ns[1]
(M/F)	(188/173)	(129/112)	(169/186)	
Family size (mean ± SE)	36.1 ± 6.7	24.1 ± 5.0	39.4 ± 9.01	ns
Alive (older than 18 years)	181	109	189	ns
Members affected	85	37	57	<0.05[2]
(% of total subjects)	(23.5)	(15.3)	(16.1)	
Affected by multiple tumors	21	10	11	ns
(% of total affected members)	(24.7)	(27.0)	(19.3)	
Total tumors	112	49	71	<0.001[2]
Tumor spectrum				<0.005[3,4]
Colon	66	38	47	
(% of total)	(58.9)	(78.0)	(66.2)	
Right colon	25	14	7	
Left colon/rectum	15	12	23	
Not assessable[5]	26	12	17	
Extracolonic HNPCC-related sites	29	6	5	
(% of total)	(25.9)	(12.0)	(7.0)	
Stomach	18	5	3	
Endometrium	9	—	1	
Small bowel	1	—	—	
Ureter and renal pelvis	1	—	1	
Ovary	—	1	—	
Extra-HNPCC sites	17	5	19	
(% of total)	(15.2)	(10.0)	(26.8)	
Mean age at diagnosis (years)				
Colon	47.6 ± 11.0	49.0 ± 12.1	63.4 ± 13.7	<0.05[4]
HNPCC-related sites	48.4 ± 8.7	61.0 ± 16.5	65.6 ± 15.2	<0.05[2]
Extra-HNPCC sites	52.5 ± 12.9	53.2 ± 12.6	57.6 ± 14.1	ns

[1]ns, not statistically significant.–[2]Group A *vs.* groups B and C.–[3]HNPCC-related sites (colon + extracolonic HNPCC-related sites) *vs.* extra-HNPCC sites.–[4]Groups A and B *vs.* group C.–[5]Colorectal subsites were not assessable for cancers verified through death certificates or reported by family history.

TABLE III – CANCER OCCURRENCE IN THE FOLLOW-UP OF THE 3 INVESTIGATED GROUPS

	Group A	Group B	Group C	*p*
Length of follow-up (years ± SD)	8.2 ± 4.9	6.9 ± 4.2	9.4 ± 4.1	ns[1]
Persons/year at risk (older than 18 years)	1,403	730	1,625	
Persons/year at risk <50 years (older than 18 years)	689	469	695	
All patients				
Total tumors (% of total)	52 (100)	22 (100)	31 (100)	
Colon (% of total)	32 (61.5)	16 (72.7)	18 (58.1)	
Extracolonic HNPCC-related sites (% of total)	13 (25)	4 (18.2)	2 (6.4)	0.03[2,3]
Extra-HNPCC tumors (% of total)	7 (13.5)	2 (9.1)	11 (35.5)	
Age at diagnosis (years)				
Colon	47.6 ± 8.9	43.6 ± 9.4	62.6 ± 10.4	0.001[3]
Extracolonic HNPCC-related tumors	56.6 ± 10.2	55.5 ± 17.9	62.5 ± 19.1	ns
Extra-HNPCC sites	58.4 ± 11.1	51 ± 5.6	58.9 ± 13.4	ns

[1]ns, not statistically significant.–[2]HNPCC-related sites (colon + extracolonic HNPCC-related sites) *vs.* extra-HNPCC sites.–[3]Groups A and B *vs.* group C.

TABLE IV – RR AND 95% CI OF TUMOR DEVELOPMENT IN THE FOLLOW-UP

	Group A *vs.* C RR (95% CI)	Group A *vs.* B RR (95% CI)	Group B *vs.* C RR (95% CI)
Total cancer			
All patients	1.98 (1.26–3.11)	1.29 (0.78–2.17)	1.52 (0.87–2.67)
Patients <50 years	9.52 (3.37–26.9)	1.67 (0.90–3.08)	5.70 (1.88–17.3)
Colorectal cancer			
All patients	2.08 (1.16–3.73)	1.04 (0.57–1.91)	2.00 (1.02–3.95)
Patients <50 years	18.6 (2.48–139.8)	1.50 (0.73–3.10)	16.7 (2.14–129.5)
Extracolonic HNPCC-related sites			
All patients	7.59 (1.71–33.7)	1.70 (0.55–5.22)	4.47 (0.82–24.5)
Patients <50 years	8.15 (1.02–65.4)	1.82 (0.48–6.91)	4.47 (0.46–43.1)
Extra-HNPCC sites			
All patients	0.73 (0.28–1.90)	1.82 (0.38–8.80)	0.40 (0.09–1.82)
Patients <50 years	2.02 (0.37–11.1)	2.73 (0.30–24.5)	0.74 (0.67–8.19)

Survival

Figure 1 shows CRC-specific survival of patients according to group: the log-rank test revealed a significantly better prognosis for patients from families with a germline MMR gene mutation or MSI (log-rank = 19.5, $p = 0.0001$). Furthermore, the presence of MMR gene mutations or MSI, together with stage, appeared as an independent prognostic factor by Cox regression multivariate analysis (Table V).

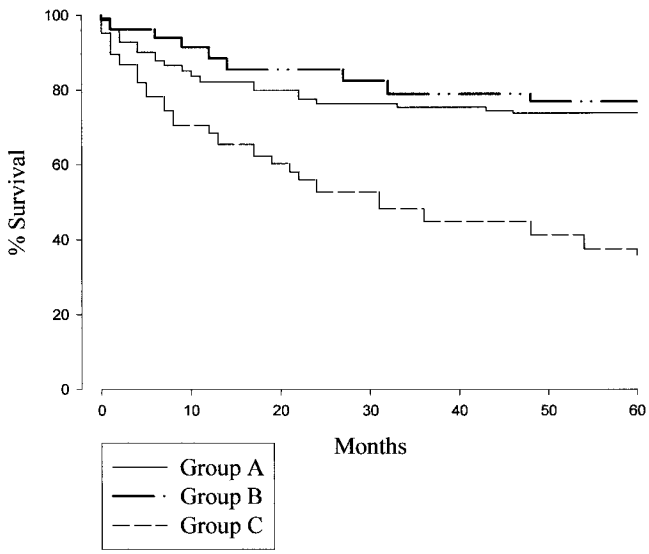

FIGURE 1 – Five-year-specific survival rates (%) in colorectal cancer patients from HNPCC families with different biomolecular features.

DISCUSSION

Our results support the hypothesis that the biomolecular heterogeneity of HNPCC is reflected in heterogeneous clinical features and confirm the value of MSI analysis as a surrogate marker for MMR gene mutations. In particular, our study shows that MSI analysis can further refine the clinical diagnosis of HNPCC by identifying, among families without known MMR mutations, those suspected of carrying genetic defects, who would be candidates for programs of prevention. This work relies on the definition of MSI, which has been applied to families and not only to tumors or patients. To define MSI families, we set as a cut-off point the presence of MSI in at least 50% of tumors tested. This value was chosen to avoid the inclusion of phenocopies in this group, considering that up to 20% of sporadic CRCs and endometrial cancers may show MSI.[29]

Many clinical similarities linked the groups with MMR mutations and only MSI, whereas MSS patients appeared to have distinct clinical features. First, the tumor spectrum of the first 2 groups was almost exclusively limited to the typical HNPCC organs, such as colon, endometrium and stomach; in contrast, the neoplastic burden of group C was characterized by a much broader organ involvement, including lung, breast, prostate and larynx. Indeed, the frequent occurrence of gastric cancer among extracolonic HNPCC neoplasms deserves some comment. This kind of tumor has been excluded from AC-II HNPCC diagnostic criteria mainly for its high frequency in the European and Asian populations.[7] In our study, gastric cancer occurred in clusters within few families with MMR mutations or MSI and as single cases in many families in group C; this is a further distinctive feature among the 3 groups. Finally, patients with CRC and HNPCC-related tumors were younger in groups A and B, underlining the need for intensive and early programs of surveillance.

RRs of cancer in the follow-up confirmed the similarities between groups A and B, highlighted some of the differences that

TABLE V – FIVE-YEAR SPECIFIC CRC SURVIVAL ACCORDING TO CLINICAL AND MOLECULAR VARIABLES IN HNPCC PATIENTS: RESULTS OF KAPLAN-MEIER UNIVARIATE ANALYSIS AND COX REGRESSION MULTIVARIATE ANALYSIS

	Cases	Deaths	Survivors	Kaplan-Meier		Cox regression	
				Log rank	*p*	Hazard ratio	95% CI
Diagnosis							
Mutation-positive (group A)	58	15	74.1			1 (referent)	—
MSI (group B)	31	7	77.4	19.5	0.0001	0.73	0.29–1.86
MSS (group C)	44	27	38.6			2.51	1.32–4.79
Sex							
Male	68	25	63.2	0.04	ns[1]	1 (referent)	—
Female	65	24	63.1			0.72	0.39–1.33
Age (years)							
≤50	67	19	71.6	4.85	0.02	1 (referent)	—
>50	66	30	54.5			1.45	0.72–2.92
Stage							
I	19	1	94.7			1 (referent)	—
II	58	8	87.9	104.9	<0.0001	2.84	0.36–22.7
III	31	15	48.4			15.7	2.05–119.3
IV/unstaged[2]	25	25	0			51.8	6.85–391.5
Site							
Right colon[3]	70	16	77.1	9.7	0.001	1 (referent)	—
Left[4]/rectum	63	33	47.6			1.46	0.69–3.09

[1]ns, not statistically significant.–[2]Patients not operated on for advanced stage of disease but without clear demonstration of metastasis.–[3]From caecum to splenic flexure.–[4]Descending and sigmoid colon.

they have compared to group C and provided important data that could influence strategies of prevention. The highest risks of CRC and HNPCC-related tumors in young patients with MMR gene mutations or MSI support the acceptance of early preventive measures focused on a few target organs. In contrast, considering the widespread tumor occurrence in families without mutations and MSI, the design of appropriate and cost-effective strategies of surveillance for family members appears particularly problematic. Even when families included in this group fulfill Amsterdam diagnostic criteria, they appear clinically different from typical HNPCC. The clusters of CRCs in this group could represent chance aggregation, especially considering the larger family size,[30] or could be due to other familial or hereditary syndromes with pathogenic defects not yet identified. At present, considering the few data available, surveillance of this type of family should not differ from the program applied to MMR mutation-positive families, but further studies on this issue are needed to validate the best strategies of surveillance and prevention.

Finally, CRC-specific survival analysis provided additional information: the better prognosis in patients with mutations or MSI confirms a previous report on the survival advantage of HNPCC patients[31] and reveals the presence of distinct biologic features between the studied groups. Indeed, 16 families in our study were also included in a previous report,[18] where we did not find significant differences in prognosis between HNPCC and sporadic CRC patients. This discrepancy with the current findings relies on the fact that, in the previous report, the HNPCC group included families with known MMR gene mutations and families without genetic defects. Grouping these patients with different outcomes and characteristics probably masked the actual prognosis of CRC arising from the MSI pathway.[32]

In conclusion, the clinical similarities between the first 2 groups indicate that MSI testing could be useful to better define clinical HNPCC families, identifying groups with different risks of carrying genetic defects. MSI testing is more accessible and available than MMR genotyping, and it has already been proposed as an effective screening method for diagnosing HNPCC in the general population[33,34] or as a prognostic factor among young patients with CRC.[35] In addition, our results are in accordance with a recent report,[36] which identified MSI status as the most reliable predictor of the presence of MMR gene mutations within families clinically suspected of having HNPCC. Studying different genes (*i.e.*, *hMSH6*)[37] or the same genes through the use of different techniques,[38] probably contributes to the increased rate of HNPCC families with known MMR mutations. At present, in clinical practice, the MSI assay could be an immediate surrogate marker of gene testing in families with CRC aggregation, especially in cases where mutational analyses are ongoing or unavailable. This should allow us to identify, in a short time, families with the highest risk of harboring germline MMR mutations and, thus, with a specific need of more intensive programs of surveillance.

REFERENCES

1. Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. J Med Genet 1999;36:801–18.
2. Peltomaki P, Vasen HFA. International Collaborative Group on HNPCC. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. Gastroenterology 1997;113:1146–58.
3. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81:214–8.
4. Syngal S, Fox EA, Li C, Dovidio M, Eng C, Kolodner RD, et al. Interpretation of genetic test results for hereditary nonpolyposis colorectal cancer: implications for clinical predisposition testing. JAMA 1999;282:247–53.
5. Genuardi M, Carrara S, Anti M, Ponz de Leon M, Viel A. Assessment of pathogenicity criteria for constitutional missense mutations of the hereditary nonpolyposis colorectal cancer genes MLH1 and MSH2. Eur J Hum Genet 1999;7:778–82.
6. Vasen HFA, Mecklin J-P, Meera Khan P, et al. The International Collaborative Group on hereditary non-polyposis colorectal cancer (ICG HNPCC). Dis Col Rectum 1991;34:424–5.
7. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC. Gastroenterology 1999;116:1453–6.
8. Lynch HT, Smyrk T, Lynch J. An update of HNPCC (Lynch syndrome). Cancer Genet Cytogenet 1997;93:84–99.
9. Lynch HT, Smyrk TC. Hereditary colorectal cancer. Semin Oncol 1999;26:478–84.
10. Tinley ST, Lynch HT. Integration of family history and medical management of patients with hereditary cancers. Cancer 1999; 86(Suppl 11):2525–32.
11. Winawer SJ, Zauber AG, Gerdes H, O'Brien MJ, Gottlieb LS, Sternberg SS, et al. National Study Workgroup. Risk of colorectal cancer in the families of patients with adenomatous polyps. N Engl J Med 1996;334:82–7.
12. Jarvinen HJ, Mecklin J-P, Sistonen P. Screening reduces colorectal

cancer rate in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 1995;108:1405–11.
13. Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118:829–34.
14. Vasen HFA, van Ballegooijen M, Buskens E, et al. A cost effectiveness analysis of colorectal screening of hereditary nonpolyposis colorectal carcinoma gene carriers. Cancer 1998;82:1632–7.
15. Benatti P, Sassatelli R, Roncucci L, Pedroni M, Fante R, Di Gregorio C, et al. Tumour spectrum in hereditary non-polyposis colorectal cancer (HNPCC) and in families with "suspected HNPCC". A population-based study in northern Italy. Colorectal Cancer Study Group. Int J Cancer 1993;54:371–7.
16. de Leon MP, Pedroni M, Benatti P, Percesepe A, Di Gregorio C, Foroni M, et al. Hereditary colorectal cancer in the general population: from cancer registration to molecular diagnosis. Gut 1999;45: 32–8.
17. Ponz de Leon M, Sassatelli R, Benatti P, Roncucci L. Identification of hereditary nonpolyposis colorectal cancer in the general population. Cancer 1993;71:3493–501.
18. Percesepe A, Benatti P, Roncucci L, Sassatelli R, Fante R, Ganazzi D, et al. Survival analysis in families affected by hereditary non-polyposis colorectal cancer. Int J Cancer 1997;71:373–6.
19. Pedroni M, Tamassia MG, Percesepe A, Roncucci L, Benatti P, Lanza G Jr, et al. Microsatellite instability in multiple colorectal tumors. Int J Cancer 1999;81:1–5.
20. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248–57.
21. Pedroni M, Sala E, Scarselli A, Borghi F, Menigatti M, Benatti P, et al. Microsatellite instability and mismatch-repair protein expression in hereditary and sporadic colorectal carcinogenesis. Cancer Res 2001; 61:896–99.
22. Hoang JM, Cottu PH, Thuille B, et al. BAT26 an indicator of the replication error phenotype in colorectal cancers and cell lines. Cancer Res 1997;57:300–3.
23. Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997;57:4749–56.
24. Zhou XP, Hoang JM, Cottu PH, Thomas G, Hamelin R. Allelic profiles of mononucleotide repeat microsatellites in control individuals and in colorectal tumors with and without replication errors. Oncogene 1997;15:1713–8.
25. Viel A, Genuardi M, Capozzi E, Leonardi F, Bellacosa A, Paravaton-Petsotas M, et al. Characterization of MSH2 and MLH1 mutations in Italian families with hereditary nonpolyposis colorectal cancer. Genes Chromosomes Cancer 1997;18:8–18.
26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.
27. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. (II) Analysis and examples. Br J Cancer 1977;35:1–39.
28. Cox DR. Regression models and life tables (with discussion). J R Stat Soc 1972;34:187–220.
29. Lothe RA, Peltomaki P, Meling GI, Aaltonen LA, Nystrom-Lahti M, Pylkkanen L, et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. Cancer Res 1993;53:5849–52.
30. Percesepe A, Anti M, Roncucci L, Armelao F, Marra G, Pahor M, et al. The effect of family size on estimates of the frequency of hereditary non-polyposis colorectal cancer. Br J Cancer 1995;72:1320–3.
31. Sankila R, Aaltonen LA, Jarvinen HJ, Mecklin JP. Better survival rates in patients with MLH1-associated hereditary colorectal cancer. Gastroenterology 1996;110:682–7.
32. Potter JD. Colorectal cancer: molecules and population. J Natl Cancer Inst 1999;91:916–32.
33. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338:1481–7.
34. Lamberti C, Kruse R, Ruelfs C, Caspari R, Wang Y, Jungck M, et al. Microsatellite instability—a useful diagnostic tool to select patients at high risk for hereditary non-polyposis colorectal cancer: a study in different groups of patients with colorectal cancer. Gut 1999;44:839–43.
35. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342:69–77.
36. Liu T, Wahlberg S, Burek E, Lindblom P, Rubio C, Lindblom A. Microsatellite instability as a predictor of a mutation in a DNA mismatch repair gene in familial colorectal cancer. Genes Chromosomes Cancer 2000;27:17–25.
37. Kolodner RD, Tytell JD, Schmeits JL, Kane MF, Gupta RD, Weger J, et al. Germ-line msh6 mutations in colorectal cancer families. Cancer Res 1999;59:5068–74.
38. Yan H, Papadopoulos N, Marra G, Perrera C, Jiricny J, Boland CR, et al. Conversion of diploidy to haploidy. Nature 2000;403:723–4.