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ORIGINAL REPORT

Genotype-Phenotype Comparison of German *MLH1* and *MSH2* Mutation Carriers Clinically Affected With Lynch Syndrome: A Report by the German HNPCC Consortium

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ABSTRACT

Purpose
Lynch syndrome is linked to germline mutations in mismatch repair genes. We analyzed the genotype-phenotype correlations in the largest cohort so far reported.

Patients and Methods
Following standard algorithms, we identified 281 of 574 unrelated families with deleterious germline mutations in *MLH1* (n = 124) or *MSH2* (n = 157). A total of 988 patients with 1,381 cancers were included in this analysis.

Results
We identified 181 and 259 individuals with proven or obligatory and 254 and 294 with assumed *MLH1* and *MSH2* mutations, respectively. Age at diagnosis was younger both in regard to first cancer (40 *v* 43 years; *P* < .009) and to first colorectal cancer (CRC; 41 *v* 44 years; *P* = .004) in *MLH1* (n = 435) versus *MSH2* (n = 553) mutation carriers. In both groups, rectal cancers were remarkably frequent, and the time span between first and second CRC was smaller if the first primary occurred left sided. Gastric cancer was the third most frequent malignancy occurring without a similarly affected relative in most cases. All prostate cancers occurred in *MSH2* mutation carriers.

Conclusion
The proportion of rectal cancers and shorter time span to metachronous cancers indicates the need for a defined treatment strategy for primary rectal cancers in hereditary nonpolyposis colorectal cancer patients. Male *MLH1* mutation carriers require earlier colonoscopy beginning at age 20 years. We propose regular gastric surveillance starting at age 35 years, regardless of the familial occurrence of this cancer. The association of prostate cancer with *MSH2* mutations should be taken into consideration both for clinical and genetic counseling practice.

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INTRODUCTION

Lynch syndrome, also called hereditary nonpolyposis colorectal cancer (HNPCC), is an autosomal dominant condition caused by germline mutations of mismatch repair genes (for review, see Peltomaki and Vasen[1] and Liu et al[2]). HNPCC accounts for 2% to 5% of colorectal cancers (CRCs).[3-5] HNPCC patients have a lifetime risk of 60% to 80% for developing CRC. In addition they are at increased risk for developing extracolonic cancers (endometrium, stomach, hepatobiliary tract, ovary, small bowel, upper urinary tract, CNS, skin).[8-13] Early age of onset, predominantly right-sided colon cancers, and syn- or metachronous cancers are other features of the syndrome.[7,14-16] Jarvinen et al[17] demonstrated that a surveillance colonoscopy program reduces the incidence and mortality of HNPCC-associated CRC. The identification of genotype-phenotype correlations could provide an attractive basis for more specific surveillance program focused on the individualized risk.

Approximately 85% of genetically defined HNPCC patients have germline mutations in *MLH1* and *MSH2*.[2,18] Several investigators have tried to correlate the phenotype with the affected gene.[14,19-24] At present, there is only one relevant genotype-phenotype correlation for *MSH2* mutations and the Muir-Torre syndrome.[11,25-27] To overcome the limitations of small sample sizes of

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Table 1. Baseline Characteristics of the Study Cohort

	Amsterdam Criteria		Bethesda Guidelines		Total	
No. of families fulfilling inclusion criteria and with completed *MLH1* and *MSH2* analysis	288		286		574	
No. of families with deleterious mutation	170		111		281	
MLH1	83		41		124	
MSH2	87		70		157	
No. of selected individuals with proven or obligate mutation carrier status*	134	156	47	103	181	259
No. of selected first-degree relatives of index cases†	119	107	28	45	147	152
No. of selected second-degree relatives of index cases†	97	112	10	30	107	142

NOTE. The proportions of *MLH1* and *MSH2* mutation-positive families in Amsterdam and Bethesda families are not significantly different ($P = .07$).
*Includes affected index cases as well as individuals who had a predictive test but had not yet developed cancer, and cases with an obligate carrier status deduced from the pedigree. These cases may not necessarily have cancer.
†Includes relatives only if affected by at least a colorectal, endometrial, small bowel, or upper urinary tract cancer; assumed to most likely represent mutation carriers.

previously published studies, here we analyzed possible phenotype-genotype correlations in 988 patients from 281 *MLH1* and *MSH2* mutation-positive families, representing the largest cohort to date of Lynch syndrome patients worldwide.

PATIENTS AND METHODS

Study Design

In 1999, after ethical approval, a multicenter study was established in six German centers. After obtaining written, informed consent, patient and family data were collected in a central database. In the interdisciplinary counseling process, at least a three-generation pedigree was targeted, and families were classified according to our inclusion criteria for the study. Whenever possible, the cancer diagnoses were verified through pathology reports. Individuals already affected or at risk were offered surveillance.

Inclusion Criteria

Inclusion criteria were as follows: (1) affected patients from families with at least three members affected by histologically verified colorectal, endometrial, small bowel, upper urinary tract cancer in whom one affected member is a first-degree relative of the other two and at least two generations are affected; (2) individuals with two HNPCC-associated cancers (colon, rectum, endometrium, ovary, stomach, biliary system, small bowel, upper urinary tract); (3) individuals with CRC and a first-degree relative with CRC and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma, in whom one of the cancers was diagnosed at age younger than 45 years, and the adenoma was diagnosed at age younger than 40 years; (4) individuals with CRC or endometrial cancer diagnosed at age younger than 45 years; (5) individuals with adenoma diagnosed at age younger than 40 years; and exclusion of familial adenomatous polyposis. These criteria include the Amsterdam II criteria and the original Bethesda guidelines.[6,7,28]

Microsatellite Instability Testing, Immunhistochemistry, and Germline Mutation Testing

MLH1 and *MSH2* germline mutation testing was performed after identification of high-level microsatellite instability (MSI-H) and/or immunohistochemical reduction or loss of expression of *MLH1* or *MSH2* in tumor tissue. Due to unavailability of tumor specimens, 37 Amsterdam II patients were subjected directly to germline mutation testing. Mutation analyses were performed either by direct sequencing (three centers) or after prescreening using high performance liquid chromatography (three centers). Three centers that identified 55% of the included families applied assays (Southern blot analysis, semiquantitative multiplex method,[29] or multiplex ligation-dependent probe amplification) to detect large genomic deletions.[30] Only exon deletions, frame shifts, splice site mutations (mutations at positions −1 and −2 or +1 and +2

of the splice acceptor or donor sites, respectively, or after demonstration of an effect on splicing using mRNA-analyses) and nonsense mutations were considered as deleterious. Missense mutations were classified most conservatively as unclassified variants or as polymorphisms unless proven otherwise.[30] Relatives of index cases with a deleterious mutation were offered predictive genetic testing.

Study Population

Families who[1] fulfill the inclusion criteria,[2] carry a deleterious germline alteration in *MLH1* or *MSH2* and[3] have a complete data set were included.

Table 2. Frequencies Distribution of Tumor Occurrences in *MLH1* and *MSH2* Mutation Carriers

	%		
Tumor Localization	*MLH1* (n = 600)	*MSH2* (n = 781)	Adjusted P
Colon, colon rectosigmoid	69.8	58.9	**.0005**
Rectum	7.7	5.9	.99
Endometrium	4.5	5.4	.99
Stomach	4.3	5.2	.99
Skin	0.8	4.2	**.001**
Small bowel	2.7	1.7	.99
Ovary	1.3	1.7	.99
Upper urothelial tract*	0.5	2.3	.99
Pancreas	1.3	1.3	.99
Brain	0.7	1.4	.99
Breast	1.0	0.9	.99
Bladder	0.3	1.4	.63
Kidney	0.3	1.3	.95
Prostate		1.3	.11
Testis	0.2	0.6	.99
Hepatobiliary tract	0.2	0.4	.99
Other gynecologic malignancies†	2.8	2.8	.99
Other or unspecified malignancies‡	1.5	3.3	.53
Total	100.0	100.0	

NOTE. Relative proportions of all tumors that occurred in proven or assumed mutation carriers. Percentages do not represent risk figures. Includes proven and obligate mutation carriers and first or second degree relatives affected by colorectal, endometrial, small bowel, or upper urinary tract cancers. Boldfacing indicates significant *P* values (Bonferroni-Holm adjusted). For sex-dependent tumors, sex-specific frequencies were compared.
*Renal pelvis/ureter.
†Cervix, corpus uteri not coded as endometrial cancer, female genitalia.
‡Head, neck, face, nose, larynx, lung, thyroid, leukemia, esophagus, liver, bone, cartilage, melanoma, genitourinary tract unspecified, and localization unspecified.

All tumors of individuals with identified or obligatory mutation status as well as from first- and second-degree relatives of the index cases if affected by either colorectal, endometrial, small bowel, or upper urinary tract cancers were considered in the study. These relatives were assumed to represent mutation carriers. Tumors of proven noncarriers were excluded.

Statistical Analysis

Categoric outcome data were reported as absolute or relative frequencies and compared between groups using Fisher's exact test. Continuous outcome data were described using means, medians and percentiles where appropriate and compared between groups using the Mann-Whitney *U* test. The Bonferroni-Holm procedure was applied to adjust significance levels when multiple comparisons of tumor frequencies between *MLH1* and *MSH2* were performed. Disease-free intervals were analyzed using the Kaplan-Meier product limit method and compared between groups using the log-rank test. SPSS Release 10.0.7 (SPSS Inc, Chicago, IL) was used for all analyses. *P* values less or equal than 0.05 were considered significant.

RESULTS

A total of 281 deleterious mutations in *MLH1* or *MSH2* were identified among 574 unrelated German families fulfilling the inclusion criteria. These 281 families comprised 418 individuals with proven and 22 individuals with obligatory mutation carrier status. In addition, their first- and second-degree relatives with either colorectal, endometrial, small bowel, or upper urinary tract cancers were included for this analysis, accounting for a total of 988 (*MLH1*: 435; *MSH2*: 553) patients (Table 1). An additional 64 and 50 families with an unclassified variant in *MLH1* and *MSH2*, respectively, were not considered in this study. There were no significant differences in numbers of documented first- and second-degree relatives between *MLH1* and *MSH2* mutation-positive index patients (*P* = .82).

Tumor Frequencies and Distribution

Among 1,381 tumors, CRC was the most frequent malignancy, accounting for 78% versus 65% of tumors in *MLH1* as opposed to *MSH2* mutation carriers (*P* < .0001) (Table 2). The relative proportion of CRC was significantly lower in females compared with males (*P* < .001).

Expectedly, there was a high proportion of right-sided CRCs of 60% in both groups. Surprisingly, the rectum was affected in 21% (*MLH1*) and 20% (*MSH2*) as the first colorectal tumor manifestation. The localization of CRCs as well as anatomic distribution between first and second CRCs was similar between *MLH1* and *MSH2* carriers (Fig 1). Interestingly, patients with a left-sided CRC (splenic flexure to rectum) developed a metachronous colon tumor in a statistically significant shorter time span than patients with a first right-sided CRC (Fig 2).

The frequency of nonmelanomatous skin tumors (11 sebaceous adenomas, four sebaceous carcinomas, seven squamous cell carcinomas, five epitheliomas, eight miscellaneous tumors) differed significantly between *MLH1* and *MSH2* mutation carriers (*P* = .0001). Thirty-three (86%) of 38 skin tumors were seen in patients with *MSH2* mutations.

Prostate cancer was the most common cancer type not belonging to the established HNPCC spectrum. In one patient it was the first primary, and in nine patients it occurred as a metachronous tumor. All cases occurred in *MSH2* mutation-positive families; however, this observation did not reach statistical significance (Table 2). Eight of the 10 cases were proven or obligatory mutation carriers. Bladder cancer was observed with a relative proportion of 1.4% in *MSH2* and 0.3% *MLH1* mutation carriers. Ten of 13 patients had a proven or obligate carrier status.

Kidney tumors not coded as malignancies of renal pelvis were seen more often in *MSH2* mutation carriers (1.3%, *MLH1*: 0.3%). All other cancer types were very uncommon (Table 2).

For endometrium, gastric, small bowel, upper urinary tract, pancreas, hepatobiliary system, ovary, brain, and breast malignancies, no significant genotype-phenotype correlations could be observed.

Age at Diagnosis

Considering only the first tumor in a patient, there was a significant earlier age at diagnosis in *MLH1* compared with *MSH2* mutation carriers in regard to any cancer type and CRC (Fig 3A-B). The median age at diagnosis was 40 and 43 years for any cancer type (*P* = .009) and 41 and 44 years for CRC (*P* = .004) for *MLH1* and *MSH2*, respectively (Fig 3A-B). We found no significant differences in the ages at diagnosis between the two genes for all extracolonic HNPCC cancers, endometrial, small bowel malignancies, and brain tumors (Fig 3C-F).
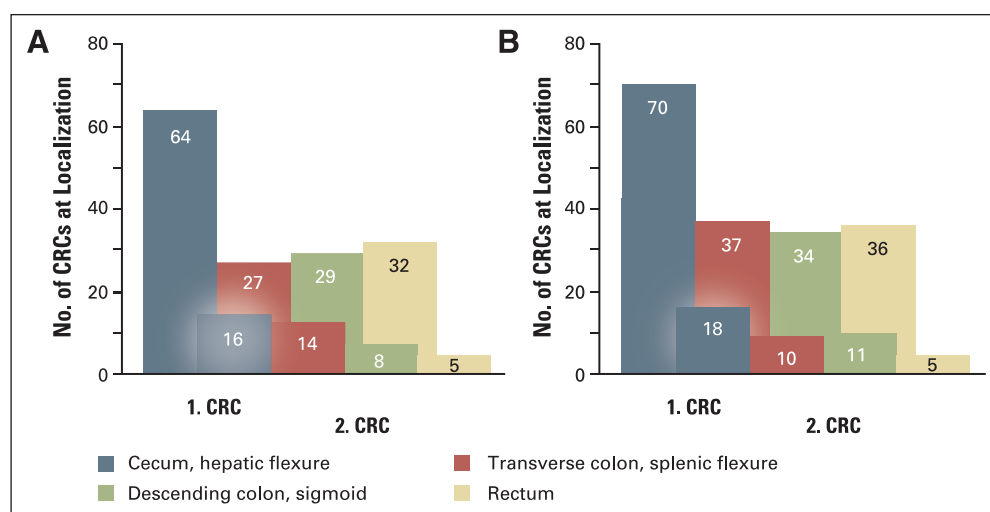
Fig 1. Anatomic distribution of first and second colorectal cancers (CRCs) in patients with pathogenic (A) *MLH1* and (B) *MSH2* mutations. The localization of the first and second colorectal tumor did not differ among *MLH1* and *MSH2* (*P* = .91; *P* = .73) or between (*P* = .72) mutation carriers.
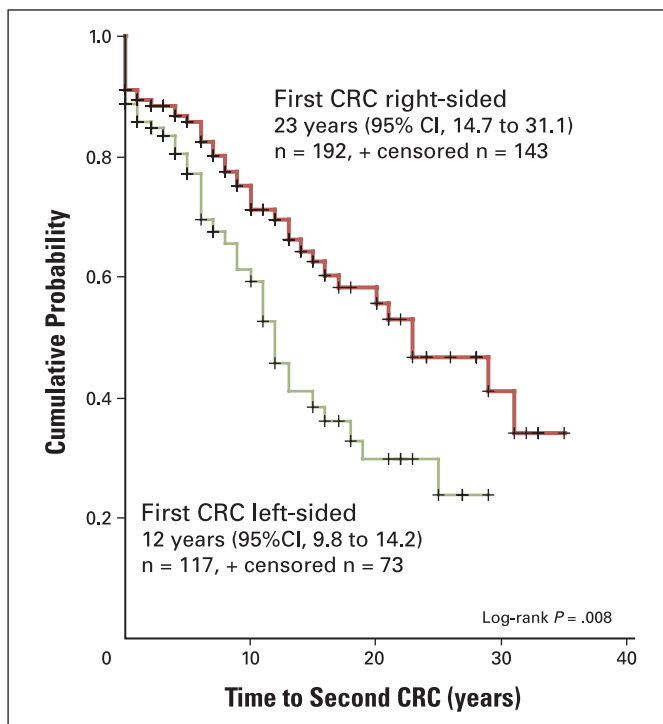
Fig 2. Time interval between first and second colorectal carcinomas. In *MLH1* and *MSH2* mutation carriers there is a shorter time interval if the first colorectal cancer (CRC) is localized left sided (right sided, cecum to transverse colon; left sided, splenic flexure to rectum).

Age at diagnosis of CRCs was significantly younger in males carrying *MLH1* mutations (39 *v* 42 years; *P* < .001). There were no significant sex differences in age at diagnosis for the extracolonic tumor entities.

Number of Tumors

The mean numbers of CRCs per family was significantly higher in families with *MLH1* than *MSH2* mutations (*P* = .002). In Amsterdam as well as Bethesda families, the mean number of extracolonic HNPCC-related tumors per family was lower in *MLH1* compared with *MSH2* mutation carriers (*P* = .05). The mean number of non–HNPCC-associated cancers per family was not significantly different in *MLH1* compared with *MSH2* patients in both inclusion groups (*P* = .40; Table 3).

DISCUSSION

This study analyzed genotype-phenotype correlations in *MLH1* or *MSH2* mutation-positive families detected in the largest HNPCC-specific cohort to date. The mutation detection rate (50%) was comparable with the results from previous studies.[31-36] The identified mutations were described elsewhere.[30] We excluded families with unclassified variants because their deleterious significance is unknown. A potential bias introduced by this procedure was considered to affect both genes equally.

Obviously not all family members underwent genetic testing. Therefore, we included here first- and second-degree relatives of the index cases in whom an HNPCC-associated malignancy had occurred. Development of an HNPCC-associated malignancy resulted in a posterior probability of 60% to 99% that a patient carry the germline mutation. A similar approach has been applied before by Vasen et al.[24] However, they assigned a mutation-carrier status only to first-degree relatives with CRC or endometrial cancers. Our approach may have led to some ascertainment bias by under-representing malignancies beyond the typical HNPCC-tumor spectrum and overrepresenting CRCs. Particularly, the CRCs may have occurred in relatives not carrying a *MLH1* or *MSH2* mutation, which may have resulted in some bias toward left-sided CRCs. However, this bias should have occurred in both groups, and the frequencies of CRCs reported here are in accordance with those published previously by others.[9,37]

Median age at diagnosis of the first cancer and CRC occurrence was significantly lower in *MLH1* compared with *MSH2* mutation carriers. Also, we found an earlier age at diagnosis of CRC in males. Parc et al[20] could also detect a difference; however, this did not reach statistical significance in their series. One and one half percent and 7.5% of CRCs occurred in male *MLH1* mutation carriers before age 20 and 25 years, respectively, compared with 1% to 2% before age 25 years in females with a mutation in either gene. This finding strongly argues in favor of starting colonoscopy at age 20 years in male *MLH1* mutation carriers.

Endometrial malignancies were not diagnosed earlier in *MSH2* mutation carriers. Upper urothelial cancer was rarely diagnosed before the age of 40 years and was more often observed in *MSH2* mutation carriers, indicating that early detection may have the greatest efficacy in *MSH2* mutation carriers older than 35 years. However, an effective surveillance method still needs to be identified.

As expected, CRC was the most common malignancy. Similar frequencies were reported previously.[9,37] The frequency of CRC was

Table 3. Mean Nos. of CRCs, Extracolonic HNPCC-Related, and Non–HNPCC-Related Tumors per Family According to the Inclusion Group and the Mutated Gene

	MLH1		MSH2	
	Amsterdam Criteria	Bethesda Guidelines	Amsterdam Criteria	Bethesda Guidelines
Mean No. of CRCs per family	5.52	2.56	4.87	2.43
Mean No. of extracolonic HNPCC tumors per family	0.87	0.56	1.20	1.03
Mean No. of non-HNPCC tumors per family	1.61	1.32	1.77	1.70

NOTE. Differences in mean Nos. of tumors between *MLH1*- and *MSH2*-associated families were significant for CRCs (*P* = .002) and extracolonic HNPCC-related tumors (*P* = .05) but not for non–HNPCC-associated cancers (*P* = .38).
Abbreviations: CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer.
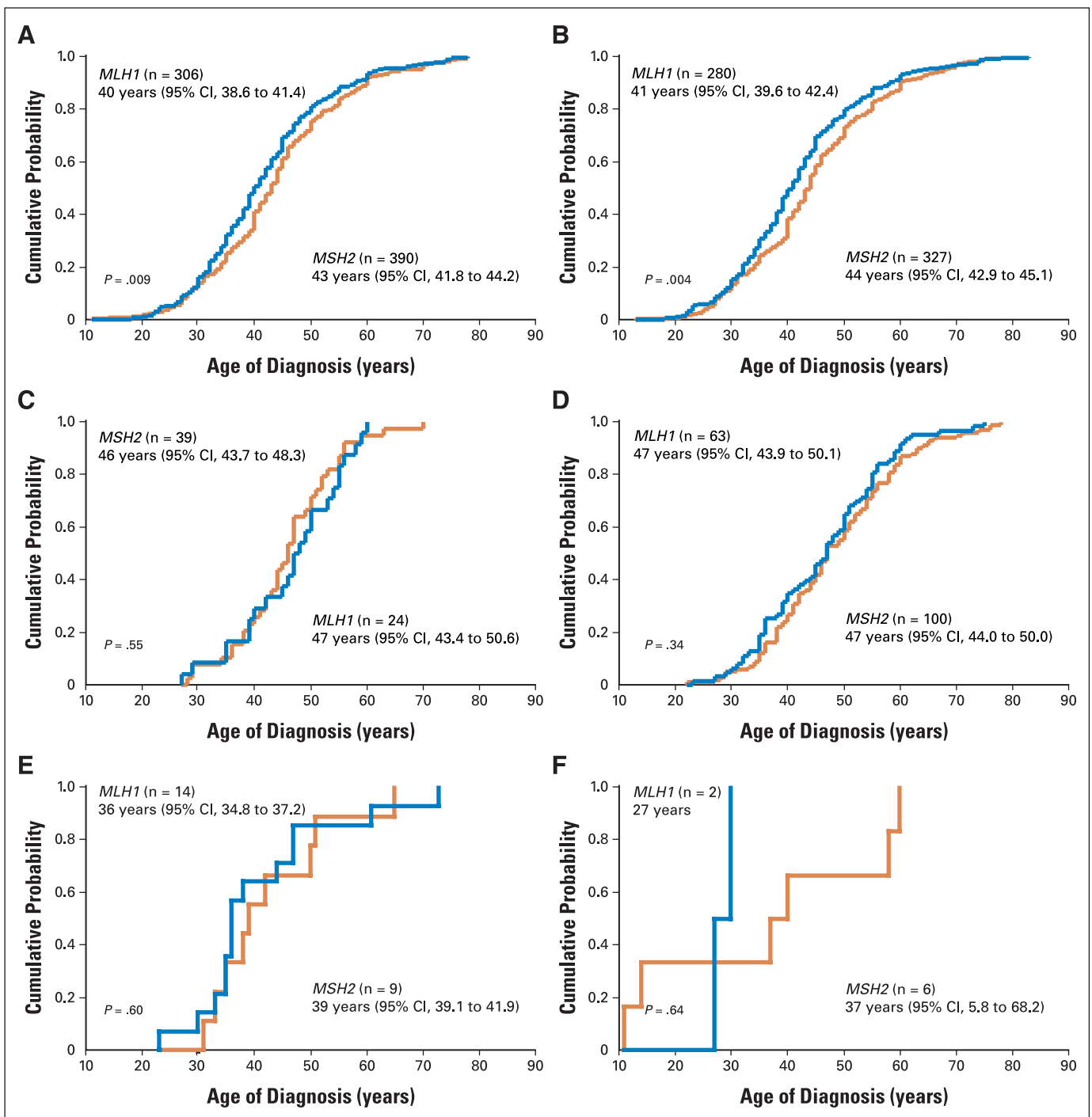
Fig 3. Cumulative probability for tumors in patients with deleterious *MLH1* or *MSH2* mutations. Only first primaries of each category were considered. For comparison of the age distribution, the Mann-Whitney *U* test was used. (A) All tumors, (B) colorectal cancers, (C) endometrial cancer, (D) all extracolonic hereditary nonpolyposis colorectal cancer–related tumors; (E) small bowel cancers; (F) brain tumors.

significantly different between the two genes, and in addition significantly less in females than males. A lower percentage of CRC for female HNPCC patients has been reported previously.[9,21,22,24,38] We found a right-sided predominance of CRC as reported in most other HNPCC series.[15,39-43] However, rectal cancers were far more frequent than previously reported.[44] This was observed for both genes and was not related to a certain age group. Patients with an initial left-sided

CRC developed a metachronous colon tumor in a statistically significant shorter time span than patients with a first right-sided CRC (Fig 2). This indicates the need of a defined treatment strategy for primary rectal cancers in HNPCC patients. Similar to the generally accepted benefit of prophylactic (subtotal) colectomy the parallel situation of an oncological rectal resection versus proctocolectomy needs to be addressed.

Gastric cancer (GC) was the second most common GI and the third most common malignancy overall, representing up to 5% of all tumors in both gene groups. In contrast, Aarnio et al[9] reported a higher risk for GC for *MLH1* opposed to *MSH2* mutation carriers. However, in Finland there is a clear predominance of *MLH1* mutations and a higher population risk of sporadic GC than in Germany. Vasen et al[24] reported a higher risk for *MSH2* mutation carriers, although not reaching statistical significance. This may be a result of environmental and/or genetic/ethnic risk factors, which is also reflected in the higher risk of GC in Asian HNPCC populations.[45,46] The frequency of GC in our study did not differ in previous compared with current generations, excluding a time-dependent effect such as lifestyle modifications (data not shown). GC surveillance is usually limited to persons with a positive family history of GC (International Collaborative Group on HNPCC [http://www.insight-group.org/]).[47] In our series, only 26% of gastric cancer cases had a family history of gastric cancer, and 98% of the gastric cancers were diagnosed after 35 years (data not shown) indicating that in *MLH1* and *MSH2* mutation carriers in the German population, surveillance for gastric cancer should initiate at the age of 35 years regardless of a positive family history of gastric cancer. Obviously, upper GI endoscopy should always include the duodenum because approximately half of the small bowel cancers are localized in the duodenum.[48]

Upper urinary tract cancers (renal pelvis/ureter) were marginally more common in patients with *MSH2* mutations as previously reported by Vasen et al.[24] Bladder cancer was not included in the previous clinical criteria. However, we observed this site more commonly in *MSH2* mutation-positive families, indicating that bladder cancer is part of the HNPCC syndrome. It originates from the same epithelium as cancer of the ureter. In addition, the median age of bladder cancer in our series was 54 years and was therefore markedly lower than in sporadic bladder cancer.

We found a marginal association between prostate cancer and *MSH2* mutations. It occurred in 2.4% of males exclusively carrying an *MSH2* mutation. The *MSH2* mutation status was proven or obligatory in eight and assumed in two males. Because of small overall numbers and a high incidence of sporadic prostate cancer in the general population, this finding should be interpreted with caution. It was found only infrequently in previous series of HNPCC patients.[9,20] The median age at diagnosis was 59 years in our series, which is comparatively young for this tumor. Prostate cancer was recently linked to HNPCC by a case report demonstrating MSI-H and loss of *MSH2* and *MSH6* expression in the prostate cancer of a 61-year-old *MSH2* germline mutation carrier.[27]

Skin tumors were possibly over-represented in our study because one center was particularly interested in Muir-Torre syndrome, causing a possible selection bias for skin tumors. Parc et al[20] found skin tumors with a frequency of 2% in their series. As described previously for Muir-Torre syndrome, we confirm a strong association with *MSH2* mutations.[11,25-27]

We were not able to show a significant genotype-phenotype correlation for small bowel, ovarian cancer, and CNS tumors between the two genes. This is in concordance with previous reports with smaller patient numbers.[13,20,24,49] With respect to their frequency and age distribution, we would not suggest any change regarding the surveillance recommendations for these tumors; however, proximal small bowel cancers may be detected by esophagogastroduodenoscopy.

We found a significantly higher mean number of CRCs ($P = .002$) and lower mean number of extracolonic HNPCC-related tumors ($P = .054$) in *MLH1* compared with *MSH2* mutation carriers (Table 3), a finding that was recently confirmed by Bandipalliam et al.[50] Multiple CRCs occurred in 139 patients. The tumor-free interval between first and second CRC was shorter in patients with a primary left-sided CRC (Fig 2). We argue that this is a result of the overall higher probability of right-sided tumor events. After hemicolectomy for right-sided CRCs, the lower probability of a second event in the remaining colon and rectum leads to a longer tumor-free interval.

In summary, in contrast to previous genotype-phenotype analyses in *MLH1* mutation carriers we identified[1] younger age at diagnosis both in regard to first cancer and to first CRC,[2] younger age at diagnosis of CRC in males, and[3] a higher proportion of CRC for both genders. Moreover *MSH2* mutation carriers had more extracolonic tumors, particularly upper urothelial tract cancers and skin tumors. We found some evidence that prostate cancer may be linked to *MSH2* mutations. In both *MSH2* and *MLH1* mutation carriers, we found rectal cancer as well as GC remarkably frequent. In addition, there was a shorter time interval between first and second CRC if the first tumor occurred left sided.

The high proportion of rectal cancers as an initial primary and the shorter time span to metachronous colorectal cancers indicate the need of a defined treatment strategy for primary rectal cancers in HNPCC patients (ie, oncologic resection *v* proctocolectomy). We suggest GC surveillance starting at age 35 years irrespective of the family history. In addition, our data suggest that prostate cancer may be considered an HNPCC-associated cancer. Therefore, this malignancy should be taken into consideration both clinically and in genetic counseling, particularly, of families with *MSH2* mutations.

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The authors indicated no potential conflicts of interest.

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GLOSSARY

Exon deletion: The deletion of a segment of a gene that consists of a sequence of nucleotides that encodes amino acids in the protein.

Frame shift: A frame shift is the addition or deletion in one or more bases. This kind of deletion alters the reading frame of the gene from the point of deletion forward.

Germline mutation: An inherited variation in the lineage of germ cells. Germline mutations can be passed on to offspring.

Missense mutation: A change (mutation) in one nucleotide that results in the coding of a different amino acid.

Muir-Torre syndrome: An inheritable autosomal dominant syndrome. The syndrome is caused by a mutation in the mismatch repair genes responsible for hereditary nonpolyposis colon cancer. It is characterized by a combination of sebaceous tumors of the skin and often colon cancer.

Nonsense mutation: A mutation that changes a codon that codes for an amino acid into a stop codon, therefore terminating translation.

Splice site mutation: Mutation that changes the specific sites at which the splicing of an intron takes place.

Unclassified variant: Alteration of the normal gene sequence of which the significance on the phenotype is unclear.