HIGH COLORECTAL AND LOW ENDOMETRIAL CANCER RISK IN *EPCAM* DELETION-POSITIVE LYNCH SYNDROME: A COHORT STUDY

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Summary

BACKGROUND—Lynch syndrome is caused by germline mutations in mismatch repair genes (*MSH2, MLH1, MSH6* or *PMS2)*, which lead to a high risk of predominantly colorectal and endometrial cancer. Recently, we found that also constitutional 3′ end deletions of *EPCAM* can cause Lynch syndrome through epigenetic silencing of *MSH2* in *EPCAM* expressing tissues. This results in a tissue specific MSH2-deficiency, which may evoke a different cancer risk and spectrum. To optimize the care for *EPCAM* deletion carriers we studied their cancer risk and spectrum.

METHODS—Clinical data of 194 carriers from 41 *EPCAM* families were systematically collected and compared to those of 431 carriers from 91 families with mutations in *MLH1, MSH2*, or *MSH6*.

FINDINGS—*EPCAM* deletion carriers exhibited a 75% [95%CI 65–85%] cumulative risk of colorectal cancer before the age of 70 years, with a mean age at diagnosis of 43 years, which is comparable to that of carriers of a combined *EPCAM-MSH2* deletion (69% [95%CI 47-91%], p=0·8609) or of a mutation in *MSH2* (77% [95%CI 64-90%], p=0·5892) or *MLH1* (79% [95%CI 68-90%], p=0·5492) and higher than that of *MSH6* mutation carriers (50% [95%CI 38-62%], p<0·0001). In contrast, women with *EPCAM* deletions (n=87) exhibited a 12% [95%CI 0-27%] cumulative risk of endometrial cancer, which is significantly lower than in carriers of a combined *EPCAM-MSH2* deletion (55% [95%CI 20-90%], p<0·0001) or of a mutation in *MSH2* (51% [95%CI 33-69%], p=0.0006) or *MSH6* (34% [95%CI 20-48%], p=0.0309) and lower than in *MLH1* (33% [95%CI 15-51%] p=0·1193) mutation carriers. This risk seems to be restricted to large deletions that extend close to the *MSH2* gene promoter. Overall, a relatively high incidence of duodenal (n=3) and pancreatic (n=4) cancers was observed.

INTERPRETATION—*EPCAM* deletion carriers do have a high risk of colorectal cancer. Only those with deletions extending close to the *MSH2* promoter have an increased risk of endometrial cancer. These results underscore the impact of mosaic MSH2-deficiency on cancer risk and are indicative for a protocol revision for surveillance and preventive surgery in *EPCAM* deletion carriers.

Keywords

Lynch syndrome; cancer risk; TACSTD1; EPCAM; MSH2; genotype-phenotype correlation

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INTRODUCTION

Lynch syndrome, or hereditary nonpolyposis colorectal cancer, is caused by pathogenic germline mutations in one of the DNA mismatch repair genes *MLH1, MSH2, MSH6* and *PMS2*. Lynch syndrome is characterized by a high risk of early onset colorectal cancer and several extra-colonic malignancies, in particular endometrial cancer (1). Carriers of

mutations in *MLH1, MSH2*, or *MSH6* have a 30-80% risk of developing colorectal carcinoma by age the age of 70 years. Women with Lynch syndrome have an additional 27-71% risk for developing endometrial cancer at this age (2-4). In asymptomatic mutation carriers from Lynch syndrome families surveillance for colorectal cancer starting at an early stage is recommended in order to improve survival. Similarly, surveillance and prophylactic surgery for endometrial cancer are widely applied (4). As yet, it is unclear for which other extra-colonic malignancies surveillance would be beneficial, but based on the occurrence of Lynch syndrome-associated extra-colonic malignancies within a specific family, additional surveillance is often considered (2;5).

Recently, we identified germline deletions in the *EPCAM* gene, previously known as *TACSTD1*, as a novel cause of Lynch syndrome (6;7). These deletions disrupt the 3′ end of *EPCAM*, leading to transcriptional read-through into, and subsequent epigenetic silencing of, its neighbouring gene *MSH2*, thus causing Lynch syndrome (6). Since this silencing phenomenon is restricted to cells expressing *EPCAM*, subjects with *EPCAM* deletions show mosaic patterns of *MSH2* inactivation which, compared to carriers of a mutation in *MSH2*, may lead to differences in tumour incidence and/or spectrum. The relatively high expression of *EPCAM* in colorectal cancer stem cells (8;9) explains why subjects with an *EPCAM* deletion have a significantly increased risk of colorectal cancer. Since very little is known about the expression of *EPCAM* in stem cells of extra-colonic malignancies, the risk of developing other Lynch syndrome-associated tumours in *EPCAM* deletion carriers is as yet unclear. Also, since EpCAM can modulate both cell adhesion and proliferation (10;11), the inactivation of *EPCAM* itself may affect tumour risk.

Multiple families with such deletions have been reported by others (7;12-15). Determination of the possibly specific tumour spectrum and age-specific cancer risk in families carrying *EPCAM* deletions is required to generate optimal recognition and surveillance strategies. Here, we employed deletion scanning in conjunction with clinical inventories to establish *EPCAM* deletion-associated cancer risks and compared these risks with those of Lynch syndrome patients carrying either a mutation in *MLH1*, *MSH2*, *MSH6*, or a deletion affecting both *EPCAM* and its neighbouring gene *MSH2 (EPCAM-MSH2)..*

PATIENTS and METHODS

Study population and data collection

Families with *EPCAM* deletions—All 41 families with a 3′ end *EPCAM* deletion that were known at the department of Human Genetics of the Radboud University Nijmegen Medical Centre by November 2009, were eligible for this study. In all families the deletion was confirmed not to include the defined promoter region and open reading frame of the *MSH2* gene (R. Kuiper et al, manuscript in preparation). The deletion in 14 of these 41 families has been reported before (6;7;12;14;16). Collection of the remaining families was based on the occurrence of as yet unexplained MSH2-deficient tumours in the Netherlands and Germany, and by analysis of germline DNA samples of subjects with unexplained MSH2-deficient tumours that were referred to the Radboud University Nijmegen Medical Centre. Only subjects tested positive for a deletion and obligate carriers were included in the current study. Genetic counsellors collected the following variables: gender, year of birth, year of death and year of tumour diagnosis, and clinicopathological and molecular data, including location of the tumour, microsatellite instability status, immunohistochemical status of mismatch repair proteins and methylation status of the *MSH2* gene promoter.

At the Radboud University Nijmegen Medical Centre clinical data of deletion carriers were collected until February 1, 2010. In total the data of 16 families harbouring 105 carriers of a Dutch founder deletion (6), 2 families harbouring 42 carriers with an identical Swiss

deletion (14) and 23 families harbouring 47 carriers with various different deletions from Germany (n=9), Hungary (n=5), USA (n=4), Hong Kong (n=2), Canada (n=1), United Kingdom (n=1) and, the Netherlands (n=1) were included. In total, information on 194 *EPCAM* deletion carriers representing 16 different deletions was collected. Ethics: The Committee on Research Involving Human Subjects Region Arnhem-Nijmegen approved the study (project approval: 2009/167).

Families with *MLH1*, *MSH2* or *MSH6* mutations—The collection of clinical data of a cohort of 95 Lynch syndrome families has been described before (5). From this cohort 4 families with an *EPCAM* deletion were excluded, as they were already incorporated as *EPCAM* deletion families, and 7 families with a deletion involving both *EPCAM* and the 5′ part of *MSH2*, reported as *EPCAM-MSH2*, were considered separately. Only data on subjects tested positive for a given mutation and obligate carriers were included in the analyses. This resulted in a set of 91 families with an *EPCAM-MSH2* (n=7), *MSH2* (n=32), *MSH6* (n=26), or *MLH1* (n=26) mutation representing 42, 143, 160, and 128 subjects, respectively.

Immunohistochemistry for EpCAM

Immunohistochemistry was performed on formalin fixed, paraffin embedded tissues with the antibody Ep-CAM Ab-1(Clone VU-ID9; Thermo Fisher) using standard procedures.

Statistical analysis

Differences in mean age of cancer occurrence between the five mutation groups were analyzed using the one-way ANOVA method. The follow-up time for each carrier was calculated as time lapse between date of birth and date of cancer diagnosis, date of last contact or date of death, whichever came first. Kaplan-Meier (KM) survival analyses were used to calculate the risk (plus 95% confidence interval) of cancer until specific ages. The age of 70 years was chosen as censoring age. The log-rank test was used for comparisons of risks. The SPSS version 16.0 software package was used for analyses.

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RESULTS

Onset and risk of colorectal cancer

Clinical data were collected from 667 mutation carriers representing 132 independent Lynch syndrome families. Amongst these were 41 families encompassing 194 *EPCAM* deletion carriers (Table 1). During follow-up, 93 *EPCAM* deletion carriers were diagnosed with colorectal cancer at a mean age at first diagnosis of 43 ± 12 years (range 18-79 yrs), being 43 years for men and 42 years for women. This mean age was not significantly different from that of Lynch syndrome patients with an *EPCAM-MSH2*, *MSH2*, or *MLH1* mutation, but it was significantly lower than that of *MSH6* mutation carriers (p<0·0001). The cumulative risk of colorectal cancer among *EPCAM* deletion carriers until age 70 was 75%

[95% CI 65-85], being 75% for men [95%CI 63-87] and 74% for women [95%CI 56-92], which was again similar to carriers of *EPCAM-MSH2*, *MSH2*, or *MLH1* mutations, but higher than that of *MSH6* mutation carriers (p<0·0001, Table 1, Figure 1A).

Onset and risk of endometrial cancer

Among the 92 women carrying an *EPCAM* deletion, 3 endometrial cancers were diagnosed (Table 1). Two of the endometrial cancers occurred in the family originally described by Chan et al (16): patient II-1 first developed colorectal cancer at age 30 and subsequently endometrial cancer at age 56; patient II-3 was diagnosed with endometrial cancer at age 43. Both were confirmed as MSH2-deficient. The other endometrial cancer was reported by family history as the only tumor in an obligate carrier at age 47. The age at diagnosis of these 3 endometrial cancers fell within the range observed for that of the other four mutation groups. However, the incidence of endometrial cancer among women with an *EPCAM* deletion was found to be >12-fold lower compared to colorectal cancer, which is in sharp contrast to the other mutation groups (Table 1). Overall, based on a Kaplan Meier analysis *EPCAM* deletion carriers had a 12% [95% CI 0–27%] cumulative risk of endometrial cancer by the age of 70 years (Table 1, Figure 1B), which was significantly lower than that of carriers of an *EPCAM-MSH2* (p<0·0001), *MSH2* (p=0.0006), or *MSH6* (p=0·0309) mutation.

Endometrial cancer risk and *EPCAM* deletion size

We previously showed a direct correlation between *EPCAM* expression and *MSH2* promoter methylation in *EPCAM* deletion carriers (9). The low incidence of endometrial cancer in this group of patients may, therefore, be related to lower expression levels of the *EPCAM-MSH2* fusion transcript in the tumour-initiating endometrial cells. In mature endometrial carcinomas EpCAM appeared to be present, as detected by immunohistochemistry of 72 sporadic and 12 Lynch syndrome-related endometrial carcinomas (*MSH2-EPCAM* n=3; *MSH2* n=2; *MSH6* n=5; *MLH1* n=2). In line with this, methylation of the *MSH2* promoter was detected in the one endometrial carcinoma that was available for methylation testing.

Remarkably, we noticed that all 3 endometrial tumours occurred in subjects from families with an *EPCAM* deletion extending closely to the *MSH2* promoter region (Figure 2A and Supplementary Table 1, <2.5 kb upstream of the *MSH2* gene). Within these families there were only 13 confirmed female deletion carriers.These observations suggest that *EPCAM* deletions that extend close to *MSH2* may more efficiently inactivate *MSH2*. In order to explore this suggestion, we divided the *EPCAM* deletion families into two subgroups (Figure 2A): subgroup 1 containing carriers with deletions located at least 10 kb upstream of the *MSH2* gene (69 male and 62 female carriers), and subgroup 2 containing carriers with deletions extending to 5·5 kb upstream of the *MSH2* gene (33 male and 30 female carriers). The risk of colorectal cancer until the age of 70 years was similar for both subgroups being 78% [95% CI: 67-90%] and 66% [95% CI: 46-85], respectively, and did not significantly differ from that in carriers of an *EPCAM-MSH2* deletion or a *MSH2* mutation (Figure 2B). The risk of endometrial cancer in subgroup 2 being 31% [95% CI 0-65%] seems still lower than that of carriers of an *EPCAM-MSH2* deletion or a *MSH2* mutation (Figure 2C), suggesting that either not all carriers in subgroup 2 have an increased endometrial cancer risk or that the risk per individual is lower. These findings suggest that an increased risk of endometrial cancer is dependent on the size and location of the *EPCAM* deletion.

Occurrence of other extra-colonic malignancies

Among *EPCAM* deletion carriers, 16 malignancies other than colorectal or endometrial cancer were detected (Table 2), of which 2 occurred in a single patient. Duodenal cancer

was detected in 3 such carriers. Two of these cancers were available for analysis, and showed microsatellite instability (MSI-high), negative immunohistochemical staining for MSH2 and methylation of the *MSH2* promoter, indicative of a role of the *EPCAM* deletion in the development of the DNA mismatch repair deficiency. Pancreatic cancer was reported in 4 *EPCAM* deletion carriers. Unfortunately, no tumour specimens were available for further analysis. No duodenal cancer and only one pancreatic cancer were detected among 473 carriers of an *EPCAM-MSH2*, *MSH2*, *MLH1*, or *MSH6* mutation.

DISCUSSION

To our knowledge, this is the first study describing the cancer profile and risk estimate in a large cohort of Lynch syndrome families exhibiting *EPCAM* deletions. We observed a high (75%) risk of colorectal cancer among the deletion carriers, which was similar to that of carriers with a mutation in the *MSH2* gene or a deletion affecting both the *EPCAM* and *MSH2* genes. In addition, a relatively high risk of duodenal and pancreatic cancer was observed. In contrast, the overall cumulative risk by the age of 70 years of endometrial cancer was only 12%, and appeared to be consistently low in carriers with *EPCAM* deletions located further upstream of the *MSH2* gene, as all 3 endometrial cancers were found in women with the two *EPCAM* deletions that extend closest to the *MSH2* gene. Together, these results indicate that carriers of *EPCAM* deletions in families with Lynch syndrome have a distinct cancer risk, and that this risk is dependent on the size and location of the deleted region.

In our study for all different types of mutations (*EPCAM*, *EPCAM-MSH2*, *MSH2*, *MSH6* and *MLH1*) the index patients are included in the risk estimates. Because of ascertainment bias this will have led to an overestimation of the actual cancer risk for each of the mutations. Indeed in our cohort of Lynch syndrome families with an *MSH2* mutation, the colorectal cancer risk appeared somewhat higher than reported by others, whereas the endometrial cancer risk for *MSH2* mutation carriers and both the colorectal and endometrial cancer risks of *MLH1* and *MSH6* mutation carriers were in conformity with that reported by others (20-24)., Remarkably, we observed several duodenal and pancreatic cancers in *EPCAM* deletion carriers, while no duodenal and only one pancreatic cancer was observed in carriers with a mutation in one of the mismatch repair genes, which is in line with the very low incidence of duodenal and pancreatic cancer in families harbouring a mismatch repair gene mutation reported by others (23;25;26). It remains to be established whether the risk for these cancers is indeed higher in individuals with an *EPCAM* deletion as compared to individuals with a mismatch repair gene mutation. Comparison of a larger cohort of families with an *EPCAM* deletion, a combined *EPCAM-MSH2* deletion or a mutation in *MSH2* may unravel whether the inactivation of *EPCAM* is important for the apparently increased risk of these latter cancers.

Although the cumulative risk at age 70 of endometrial cancer in *EPCAM* deletion carriers of 12% is still higher than the population risk of 1,6% (17), this 12% risk is much lower than that of *MSH2* mutation carriers (51%) and the combined *MSH2-EPCAM* deletion carriers (55%). This most likely relates to the mosaic tissue-specific pattern of *MSH2* inactivation in these carriers which is dependent on the tissue-specific level of *EPCAM* expression. As we previously reported, transcriptional read-through of *EPCAM* results in *in cis* epigenetic silencing of the *MSH2* gene, whereas in tissues that lack *EPCAM* expression, *MSH2* remains active (6). We, therefore, assume that the low incidence of endometrial cancer could be explained by an insufficient level of *EPCAM* expression in the endometrial cells during early stages of tumour development, resulting in a normal activity of *MSH2* and, consequently, a normal risk for tumour development.

It is unlikely that the relatively low incidence of endometrial cancer in *EPCAM* deletion carriers can be attributed to a selection bias for colorectal cancer families. All *EPCAM* deletion carriers included in this study were derived from cohorts of patients with a clinical picture suggestive of Lynch syndrome, similar to the cohort from which the families with *MSH2*, *MLH1*, and *MSH6* mutations were selected. It is also unlikely that this low incidence of endometrial cancer is affected by an unintended selection of the tumour type carried by the index patients, as 74% of the women included in this study were either derived from one large Dutch family (55% of women) or two large Swiss families (19% of women), in which relatives up to the 5th degree of the original index patient have been tested for the presence of a mutation. Although we cannot exclude that a modifying genetic factor acts *in cis* with either the Dutch or the Swiss founder deletion, this seems unlikely as, to the best of our knowledge, a lack of endometrial cancers in families with specific *MSH2* mutations has not been reported before. Moreover, inactivation of the *EPCAM* gene is not a protective factor in itself, as the risk of endometrial cancer of individuals with a combined *EPCAM-MSH2* deletion is similar to that of individuals with a single mutation in *MSH2.*

The only three early-onset endometrial cancers that we found occurred in women with a deletion that extends close to the *MSH2* promoter region (27;28). There are several possible scenarios that may contribute to this phenomenon. Firstly, the efficiency of *MSH2* inactivation could, for example be associated with the distance of the *EPCAM* and *MSH2* promoters on the allele carrying the deletion. Larger *EPCAM* deletions extending close to the *MSH2* gene will put the two promoters into closer proximity, thus enabling endometrial cells to drive *MSH2* methylation, despite the weaker *EPCAM* promoter activity in these cells. Secondly, in subjects with deletions that extend close to the *MSH2* gene the inactivation of *MSH2* may be less dependent on high levels of *EPCAM* expression due to loss of a regulatory element. The presence of such an element in this region has thus far not been reported, but we did notice that the region overlaps with a punctuate site of enriched di- and tri-methylation of histone H3 on lysine 4 (H3K4Me2 and H3K4Me3) in HepG2 cells (29;30), which strongly correlate with active promoters or enhancers (31;32).

Whatever the mechanism may be, our data indicate that the risk for endometrial cancer in carriers of *EPCAM* deletions is dependent on the size and location of the deletion. The exact criteria of deletions that confer a low endometrial cancer risk remain to be defined by further assessments of endometrial cancer incidences in carriers of different *EPCAM* deletions and analyses of the *EPCAM-MSH2* intergenic region for transcription-mediating capacity.

Surveillance programs for Lynch syndrome families are typically aimed at early detection of colorectal and endometrial tumours, sometimes supplemented with surveillance for other Lynch syndrome-associated malignancies that occur within the family (24;33). Recently, for example, surveillance for urinary tract cancer in *MSH2* mutation carriers has been recommended (5). However where the predicted incidence of cancer is low, a targeted cancer prevention programme is less likely to offer clinical benefit, especially where evidence for its efficacy is limited. Therefore, our current findings suggest that surveillance and preventive surgery for endometrial cancer could reasonably be omitted for carriers of a smaller *EPCAM* deletion extending further away from the *MSH2* promoter.

In conclusion, we report that *EPCAM* deletion carriers have a high risk of developing colorectal cancer that is comparable to that of *MLH1* or *MSH2* mismatch repair gene mutation carriers. The risk of endometrial cancer, however, is significantly lower. This low risk may be due to an insufficient level of *EPCAM* expression in endometrial cancer progenitor cells. Our data are indicative for an optimized protocol for the recognition and targeted prevention of cancer in *EPCAM* deletion carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
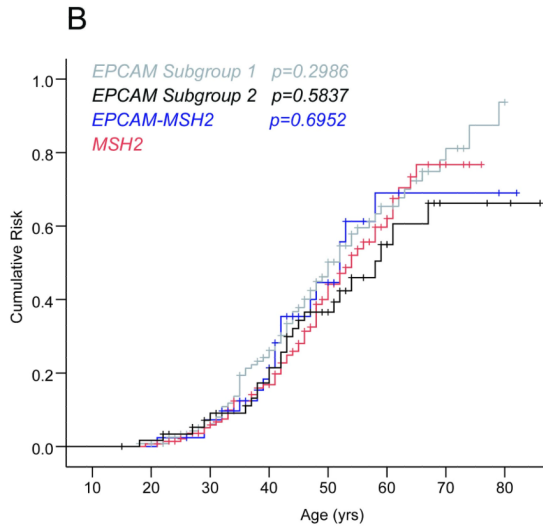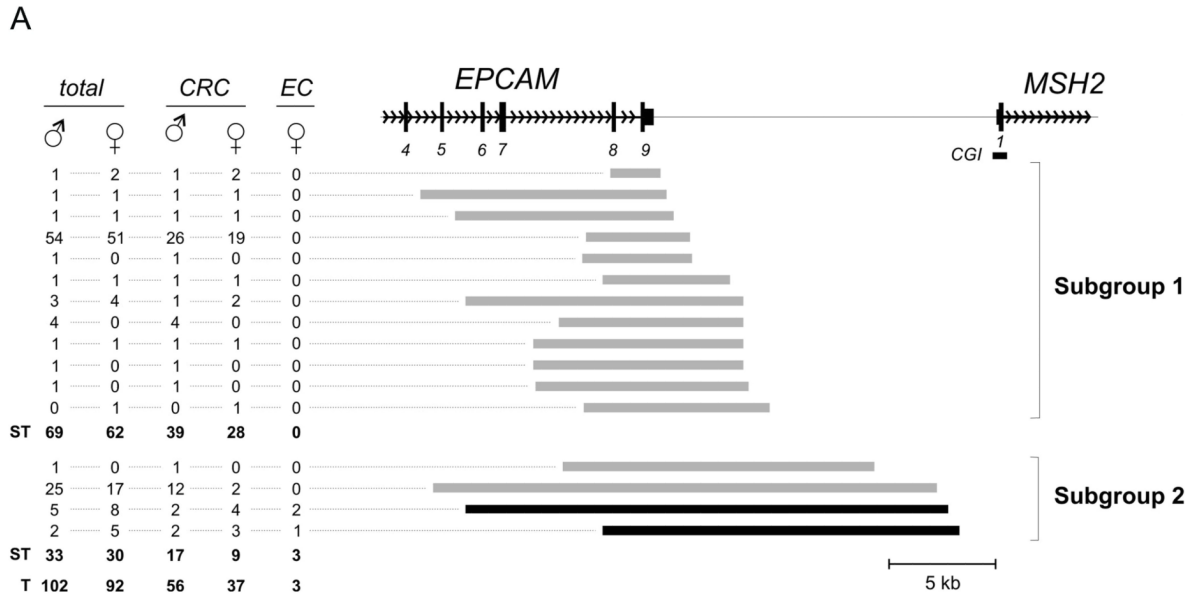
Acknowledgments

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A

B
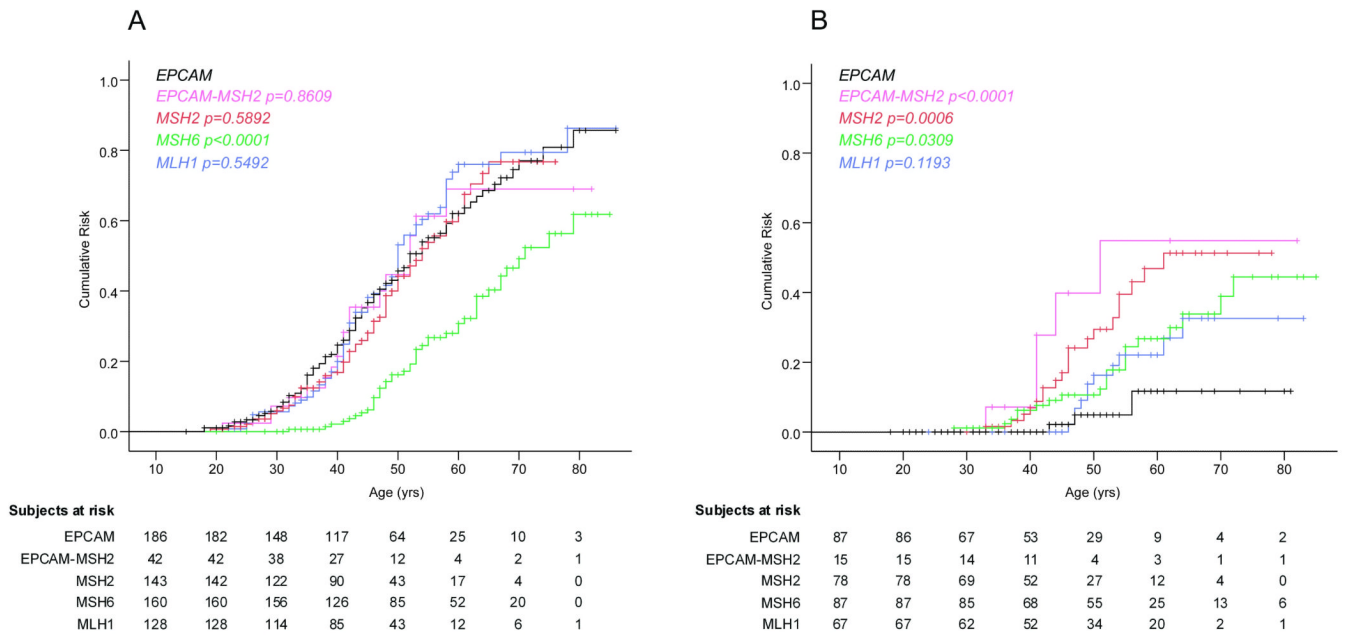
Figure 1.
Cancer risk in *EPCAM* deletion carriers. Cumulative risk until the age of 70 of colorectal cancer (A) and endometrial cancer (B) in *EPCAM* (black lines), *EPCAM-MSH2* (pink lines), *MSH2* (red lines), *MSH6* (green lines), and *MLH1* (blue lines) mutation carriers. Indicated log-rank p-values are comparisons relative to *EPCAM* deletion carriers. The number of subjects in the table below the graphs indicate the number of mutation carriers, that are at risk for their first colorectal (A) or endometrial cancer (B) at the given age. Eight *EPCAM* deletion carriers were excluded from the Kaplan Meier curves because the exact age at colorectal cancer diagnosis (n=2) or at follow up (n=6) were unknown.
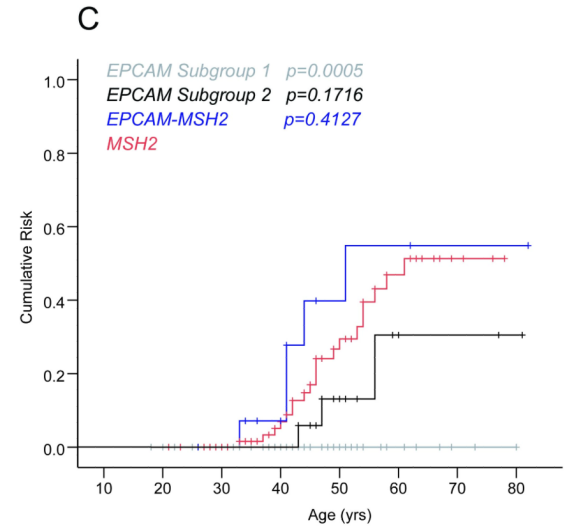
Figure 2.
Cancer risk in *EPCAM* deletion carriers in relation to deletion breakpoint and size. (A) Schematic representation of the size of each *EPCAM* deletion (depicted by bars) and its position relative to the *MSH2* CpG island (CGI) promoter. The deletions found in the endometrial cancer patients are depicted in black, the others are presented in grey. For each deletion the number of carriers as well as the number of patients with colorectal cancer (CRC) or endometrial cancer (EC) are indicated on the left. ST: subtotal; T: total. The cumulative colorectal (B) and endometrial (C) cancer risk of subgroup 1 (grey lines), subgroup 2 (black lines) in comparison to those of *MSH2* (red lines) and *MSH2-EPCAM* (bleu lines) mutation carriers. Indicated log-rank p-values are comparisons relative to *MSH2*

mutation carriers. The number of subjects in the table below the graphs indicate the number of mutation carriers, that are at risk for their first colorectal (B) or endometrial cancer (C) at the given age. Eight *EPCAM* deletion carriers were excluded from the Kaplan Meier curves because the exact age at colorectal cancer diagnosis (n=2) or at follow up (n=6) were unknown.

Table 1

Mean age at diagnosis and cumulative risk by age 70 of colorectal and endometrial cancer in Lynch mutation carriers

	EPCAM	EPCAM-MSH2[1]	MSH2	MSH6	MLH1
Families (n)	41	7	32	26	26
Mutation carriers (n)	194	42	143	160	128
Colorectal cancer:					
Carriers affected (n)	93	18	60	45	68
Mean age at diagnosis (yrs (range))	43 (18-79)[2]	41 (21-58)	44 (19-65)	54 (32-79)	44 (22-78)
Cumulative risk (%[95%CI])	75 [65-85][3]	69 [47-91]	77 [64-90]	50 [38-62]	79 [68-90]
Excess risk (%)[4]	73	67	75	48	77
Endometrial cancer:					
Female carriers (n)	92	15	78	87	67
Carriers affected (n)	3	5	20	20	11
Mean age at diagnosis (yrs (range))	49 (43-56)	42 (33-51)	47 (33-61)	50 (28-72)	52 (46-64)
Cumulative risk (%[95%CI])	12 [0-27][5]	55 [20-90]	51 [33-69]	34 [20-48]	33 [15-51]
Excess risk (%)[5]	11	54	50	33	32
Ratio of females colorectal to endometrial cancer (n/n)	12.3	0.8	1.6	1.1	2.9

[1] Combined deletion of *EPCAM* and *MSH2*

[2] Mean age at diagnosis of first colorectal cancer in *EPCAM* deletion carriers was based on the data of 91 affected carriers, because in two carriers the exact age at onset was not known

[3] The cumulative risk of colorectal cancer was based on the data of 186 *EPCAM* deletion carriers

[4] In the Netherlands, the cumulative risk at 70 years of age (for both sexes combined) of developing colorectal cancer is 2.5%. For endometrial cancer this risk is 1.6% (source Kiemeney et al (17) and Netherlands Cancer Registry at www.ikcnet.nl(18)). In the USA, these risks are 1.9% and 1.6%, respectively among caucasians (source www.seer.cancer.gov(19)).

[5] The cumulative risk of endometrial cancer was based on the data of 87 *EPCAM* deletion carriers

Table 2

Extra-colonic and extra-endometrial cancer in *EPCAM* deletion carriers

Tumor type	No of patients	MSI* status	Age at diagnosis
Duodenum	3	high/high/unknown	52/54/unknown
Pancreas	4	Unknown	46/51/65/unknown
Breast	2	Unknown	57/59
Urothelial carcinoma	1	Stable	60
Kidney	1	unknown	Unknown
Prostate	1	unknown	71
Basal cell carcinoma	1	unknown	41
Brain	1	unknown	Unknown
Gall bladder	1	unknown	69
Myelodysplastic syndrome	1	unknown	79

*
MSI = microsatellite instability