Cancer risk in MLH1, MSH2 and MSH6 mutation carriers; different risk profiles may influence clinical management

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Abstract

Background: Lynch syndrome (LS) is associated with a high risk for colorectal cancer (CRC) and extracolonic malignancies, such as endometrial carcinoma (EC). The risk is dependent of the affected mismatch repair gene. The aim of the present study was to calculate the cumulative risk of LS related cancers in proven MLH1, MSH2 and MSH6 mutation carriers.

Methods: The study population consisted out of 67 proven LS families. Clinical information including mutation status and tumour diagnosis was collected. Cumulative risks were calculated and compared using Kaplan Meier survival analysis.

Results: MSH6 mutation carriers, both males and females had the lowest risk for developing CRC at age 70 years, 54% and 30% respectively and the age of onset was delayed by 3-5 years in males. With respect to endometrial carcinoma, female MSH6 mutation carriers had the highest risk at age 70 years (61%) compared to MLH1 (25%) and MSH2 (49%). Also, the age of EC onset was delayed by 5-10 years in comparison with MLH1 and MSH2.

Conclusions: Although the cumulative lifetime risk of LS related cancer is similar, MLH1, MSH2 and MSH6 mutations seem to cause distinguishable cancer risk profiles. Female MSH6 mutation carriers have a lower CRC risk and a higher risk for developing endometrial carcinoma. As a consequence, surveillance colonoscopy starting at age 30 years instead of 20-25 years is more suitable. Also, prophylactic hysterectomy may be more indicated in female MSH6 mutation carriers compared to MLH1 and MSH2 mutation carriers.

Background

Lynch syndrome (LS), also known as hereditary non-polyposis colorectal cancer, is the most common hereditary colorectal cancer (CRC) syndrome and accounts for 2-5% of all colorectal cancer cases [1]. Germline mutations in any of the four mismatch repair (MMR) genes, MLH1[2],
**Methods**

**Study population**

During the period 1994-2007, an MMR gene mutation was detected in 67 families who were counselled at the Department of Clinical Genetics of the Erasmus MC University Medical Center, because of a clinical suspicion for Lynch syndrome. Clinical data of family members including sex, age, mutation status, age at diagnosis of both LS-associated and other cancers were collected. LS-associated cancer included colorectal, endometrial, stomach, ovaries, upper uroepithelial tract, biliary tract, skin and brain cancer. Also, the site of the tumour, age at death and cause of death were collected. With consent of the patients or (in case the patient was deceased) of a close relative the cancer diagnosis was confirmed by pathology and/or medical reports. All pathology and medical reports were reviewed by the first author (DR) in order to confirm the diagnosis. If a subject reported the occurrence of cancer in the family and no pathology or medical report was available, the cancer was excluded from analysis. In addition, data regarding colonoscopic surveillance of affected and unaffected family members were collected.

Only subjects with a proven MMR gene mutation were included in this study.

**Mutation analysis**

Mutation analysis was performed by denaturing gradient gel electrophoresis, sequencing and multiplex ligation-dependent probe amplification (MRC-Holland kits P003 and P008). Mutation nomenclature was used according to international guidelines [http://www.hgvs.org](http://www.hgvs.org). A variant was considered a mutation when leading to a predicted truncated protein or based on previously published data. Silent or missense variants which were previously unreported or of unclear status were labelled unclassified variants (UV) and not considered as an MMR gene mutation.

**Statistical analysis**

Data were submitted for statistical testing using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL), version 12.0.1. Data are given as median and range or as mean with standard deviation when appropriate. The chi square test, Student’s t test and log rank test were used to compare differences between MLH1, MSH2 and MSH6 mutation carriers. Penetrance for age was calculated using the Kaplan Meier survival analysis method and included the 67 index cases. In case of multiple or recurrent colorectal carcinoma or endometrial adenocarcinoma, only the first diagnosis of either cancer was included in the analysis. The observation time for the different cancers was from birth until the date of first cancer diagnosis, death, date of hysterectomy (only for the observation time of endometrial carcinoma) or the end of the study (31 December 2007). A p value below .05 was considered statistically significant.

**Results**

**Study population**

In the 67 families with an MMR gene mutation, 26 (39%) were detected with an MLH1 mutation, 20 (30%) with an MSH2 mutation and 21 (31%) with an MSH6 mutation. Of the 67 families, 46 (69%) met the Amsterdam II criteria. Mutation analysis was performed in 725 subjects (296 men and 429 women) and a mutation was identified in 246 subjects (92 men, 154 women) (Table 1). At the time of mutation analysis the mean age of the 246 mutation carriers was 49 ± 16 years. Of the 246 mutation carriers, 115 (47%) were diagnosed with a Lynch syndrome associated tumour. One hundred and four (42%) mutation

MSH2[3], MSH6[4] and PMS2[5], are the underlying cause of LS. Subjects carrying a mutation in one of the MMR genes have a higher risk for developing colorectal cancer, but also for endometrial carcinoma and malignancies of the stomach, small bowel, ovaries, upper uroepithelial tract, biliary tract, skin and brain [6-9].

The colorectal cancer risk in LS is dependent on sex and the MMR gene involved. The reported lifetime risk for colorectal cancer in the literature varies from 28-100% in males and 25-83% in females [7,10-18]. The risk of developing endometrial carcinoma ranges from 30-71% and the risk of other LS-associated cancers is less than 10-15% [9]. Furthermore, some studies have suggested that extracolonic cancers are more often observed in MSH2 mutation families compared to MLH1 mutation families [13,19]. MSH6 mutation families probably have a milder clinical phenotype with a later onset of both CRC and EC and clustering of endometrial carcinoma [17]. The risks in PMS2 mutation families are largely unknown. One study reported that PMS2 mutation families have a milder phenotype compared to MLH1 and MSH2 families [20].

Unfortunately, the precise lifetime risk for CRC and endometrial carcinoma may be biased because the families selected in previous studies were mainly selected on basis of clustering of CRC or fulfilment of clinical criteria (Amsterdam II criteria). Furthermore, it was not always clear whether the affected subjects were proven mutation carriers. In addition, most studies have only evaluated lifetime risks for MLH1 and MSH2 mutations, while studies evaluating MSH6 mutation families are sparse. The most efficient way to calculate the lifetime risks of CRC and EC in Lynch syndrome would be to calculate these risks based on a cohort of proven mutation carriers. Therefore, the aim of the present study was to calculate the cumulative lifetime risks for CRC and EC in Lynch syndrome using a cohort of proven MLH1, MSH2 and MSH6 mutation carriers.
carriers already had been diagnosed with a Lynch syndrome associated tumour before mutation analysis was performed. Colorectal cancer was diagnosed in 83 (34%) mutation carriers, including 17 (7%) mutation carriers who developed 2 or more CRCs during their lifetime. Endometrial carcinoma was diagnosed in 37 (24%) of the 154 female mutation carriers, including 13 mutation carriers who also developed CRC during their life. Of the six families with a strong family history of endometrial carcinoma (two or more cases within the family), five (83%) were diagnosed with an MSH6 mutation. With respect to the other LS-associated cancers, 19 (8%) mutation carriers developed another LS-associated cancer during their life (Table 1). Seven of these nineteen mutation carriers were also diagnosed with CRC, one mutation carrier also with endometrial carcinoma and four mutation carriers with both CRC and EC. In total, 194 mutation carriers were under colonoscopic surveillance, including 69 subjects who had already been diagnosed with colorectal cancer before mutational testing was performed.

One of the 69 mutation carriers had previously been diagnosed with EC and developed CRC while being under colonoscopic surveillance. The other 68 mutation carriers were included in a colonoscopic surveillance program after being diagnosed with colorectal cancer. These 68 subjects were treated surgically (partial colectomy) for colorectal cancer and colonoscopic surveillance of the remaining colon was performed. Of the remaining 125 mutation carriers none developed colorectal cancer and in 23 (18%) adenomatous polyps had been detected and removed. The person-years of follow up was 1414 years and the mean follow up time of the subjects under colonoscopic surveillance was 7 ± 4 years.

**Lifetime risks**

The respective lifetime risks curves are shown in figure 1, figure 2, figure 3 and figure 4. For all LS-associated tumours, the cumulative risks in both male and female mutation carriers at 70 years was 71% for MLH1, 77% for MSH2 and 75% for MSH6 mutation carriers (Figure 1). Although the cumulative risks at age 70 years were similar for the three different MMR genes, the log rank test showed a significant difference for developing any Lynch syndrome associated cancer between MSH6, MLH1 and MSH2 mutation carriers (p = 0.01). This was due to the fact that before the age of 70 years the risk of developing any Lynch syndrome associated cancer in MSH6 carriers was lower compared to MLH1 or MSH2 mutation carriers (Figure 1).
In Figure 2, the age related cumulative risk for CRC is shown for male MLH1, MSH2 and MSH6 mutation carriers. At age 70 years, the cumulative risk was the highest for MLH1 mutation carriers, 78%, while the cumulative risks for MSH2 and MSH6 mutation carriers were 57% and 54% respectively. There was no significant difference in age related cumulative risk between MSH6 mutation carriers (p = 0.05) compared to MLH1 and MSH2 mutation carriers. However, the highest increase in risk in male MLH1 and MSH2 mutation carriers was observed between the ages of 40 to 50 years, while the risk in male MSH6 mutation carriers mostly increased between the ages of 50 to 60 years. Although the age related risks were not significant different between the three different MMR genes, there was a trend in male MLH1 and MSH2 mutation carriers to develop CRC at an earlier age than male MSH6 mutation carriers.

For endometrial carcinoma, the highest cumulative risk was observed in the MSH6 mutation carriers (61%), while the cumulative risks for MLH1 and MSH2 mutation carriers were 52% and 30% respectively. The cumulative risks for CRC in females were lower compared to males, 57% for MLH1, 52% for MSH2 and 30% for MSH6 mutation carriers (Figure 3), with a significantly lower age related cumulative risk in MSH6 mutation carriers (p = 0.001) compared to MLH1 and MSH2 mutation carriers.

Discussion
In this study, we evaluated 246 individuals from 67 families with a proven mismatch repair gene mutation to determine the cumulative lifetime risk of developing can-
MLH1 Endometrial carcinoma in females; Figure 4
ated the risks associated with females at age 70 years [15]. All these studies only evalu-
time risk for CRC so far, 27% for males and 22% for more recently published study reported the lowest life-
100% in males and 30-63% risk in females [7,11-13]. A reported somewhat similar risks for CRC ranging from 65-
males and 83% in females [10]. Most later studies
One of the first studies evaluating the lifetime risk for developing CRC in Lynch patients.

Figure 4
Endometrial carcinoma in females; cumulative risks for MLH1, MSH2 and MSH6 mutation carriers.

Our study indicates that, however the cumulative risks of cancer at age 70 years in MLH1, MSH2 and MSH6 mutation carriers is similar, each mutated gene has a distinguishable cancer risk profile. In MSH6 mutation carriers the risk at age 70 years for developing CRC was the lowest in both male (54%) and female (30%) when compared to carriers of MLH1 and MSH2 mutations.

Between male MSH6 and MSH2 mutation carriers also a significant difference in the age of CRC onset (48 vs. 43 years, p = 0.03) was found and there was a trend in higher age of CRC onset between male MSH6 and MLH1 mutation carriers. For female mutation carriers, no significant differences were found in the mean age of onset of CRC. This can be explained by the fact that female MLH1 and MSH2 mutation carriers still developed CRC at an older age. The lower risk of CRC onset in female MSH6 mutation carriers under the age of 50 years raises the question whether colonoscopic surveillance guidelines in these subjects can be changed. Current guidelines advise to start with biennial colonoscopy surveillance from the age of 20-25 years [25]. In our study population, the youngest affected female MSH6 mutation carrier with CRC was 34 years. Our data and the data from previous studies support that colonoscopic surveillance can be started at an age of 30 years in female MSH6 mutation carriers [17].

However our numbers are too small to draw definite conclusions, CRC seems to be the predominant cancer in MLH1 mutation carriers. In MSH2 and MSH6 mutation carriers extracolonic cancers appear to contribute more to the similar cumulative lifetime risk of cancer in MLH1, MSH2 and MSH6 mutation carriers. A higher risk of extracolonic-LS-associated cancer was previously reported in MSH2 mutation carriers compared to MLH1 mutation carriers [13,19]. Unfortunately, the number of extracolonic-LS associated cancer (excluding endometrial carcinoma) in our study population was too low to calculate accurate risk estimates for these cancers. In concordance with other studies [17,26] our study indicates that MSH6 carriers have the highest endometrial cancer risk followed by MSH2 and MLH1 mutation carriers. Also, this risk increases sharply after the age of 50 years. In view of the disputable effect of endometrial carcinoma surveillance [27,28], in female MSH6 carriers aged 45 years or above prophylactic hysterectomy may be suggested in order to decrease the risk for developing endometrial carcinoma [29]. In MSH2 and MLH1 female mutation carriers this option may be more questionable. In MSH2 mutation carriers the risk of other extracolonic and extraendometrial cancers may reduce faith in and benefit of risk reduc-
ing surgery. In case of surgery for another cause, additional hysterectomy should be considered also in MLH1 en MSH2 mutation carriers.
Table 2: Median age and range at diagnosis of Lynch syndrome associated cancer

| Cancer Type                  | MLH1   | MSH2   | MSH6   |
|------------------------------|--------|--------|--------|
| Colorectal cancer            | 47 (25-79) | 44 (20-82) | 53 (32-84) |
| Endometrial cancer           | 51 (46-54) | 46 (36-55) | 56 (47-67) |
| Ovarian carcinoma            | 52 (52-52) | 47 (45-48) | 49 (35-51) |
| Small bowel carcinoma        | 54 (54-54) | 36 (23-49) |        |
| Transitional cell carcinoma  |        | 58 (32-59) |        |

A strength of the present study was that the age related risks where calculated using proven mutation carriers. However, the age related risks might be somewhat lower since not all the unaffected individuals from proven mutation families opted for genetic testing and thus the total number of unaffected mutation carriers in the mutation families may be underestimated. In addition, individuals with a higher risk for mutation carriership, i.e. with an affected first degree relative, more often opt for genetic testing [30]. This may also have introduced some bias with respect to the age related risks. Also, we included the index cases in our study population. Index cases give rise to the suspicion of Lynch syndrome and they always have cancer. This may also have resulted in a slightly higher age related risk. On the other hand, the majority (77%) of not affected mutation carriers was under colonoscopy surveillance, which likely has influenced the age related risks for developing invasive CRC, since colonoscopy surveillance in Lynch syndrome patients is effective in reducing the incidence and mortality of CRC [31]. A limitation of our study was that our study population was not very large (n = 246), and the number of male carriers was 92. This could explain why we did not find a significant difference in both the mean age of CRC onset and the age related risk between male MLH1, MSH2 and MSH6 mutation carriers.

In conclusion, the present study indicates that, although the cumulative risks at age 70 years of LS related cancer in MLH1, MSH2 and MSH6 mutation carriers are similar, each mutated gene has a distinguishable cancer risk profile. It underlines that female MSH6 mutation carriers have a distinct clinical phenotype with a lower CRC risk and a higher risk for developing endometrial carcinoma. Starting with biennial colonoscopic surveillance at an age of 30 years in female MSH6 mutation carriers is more suitable and prophylactic hysterectomy may be considered from an age of 45 years.

Conclusions
The present study indicates that each mutated MMR gene has a distinguishable cancer risk profile. Female MSH6 mutation carriers have a lower CRC risk and a higher risk for developing endometrial carcinoma. Starting with biennial colonoscopic surveillance at an age of 30 years in female MSH6 mutation carriers is more suitable and prophylactic hysterectomy may be considered from an age of 45 years.

Abbreviations
CRC: colorectal cancer; EC: endometrial cancer; LS: Lynch syndrome; MMR: mismatch repair; UV: unclassified variant.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
DR participated in the data collection, performed the statistical analyses and helped to draft the manuscript. AW conceived of the study and participated in the data collection. ML helped to draft the manuscript. DD participated in the data collection. CT participated in the data collection. ES participated in the design of the study and assisted in the statistical analysis. EK helped to draft the manuscript. All authors read and approved the final manuscript.

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