Author(s): Engel, Christoph; Ahadova, Aysel; Seppälä, Toni; Aretz, Stefan; Bigirwamungu-Bargeman, Marloes; Bläker, Hendrik; Bucksch, Karolin; Büttner, Reinhard; de Vos tot Nederveen Cappel, Wouter; Endris, Volker; Holinski-Feder, Elke; Holzapfel, Stefanie; Hüneburg, Robert; Jacobs, Maarten A.J.M.; Koornstra, Jan J.; Langers, Alexandra M.; Lepistö, Anna; Morak, Monika; Möslein, Gabriela; Peltomäki, Päivi;

Title: Associations of Pathogenic Variants in MLH1, MSH2, and MSH6 With Risk of Colorectal Adenomas and Tumors and With Somatic Mutations in Patients With Lynch Syndrome

Year: 2020

Version: Accepted version (Final draft)

Please cite the original version:

Engel, C., Ahadova, A., Seppälä, T., Aretz, S., Bigirwamungu-Bargeman, M., Bläker, H., Bucksch, K., Büttner, R., de Vos tot Nederveen Cappel, W., Endris, V., Holinski-Feder, E., Holzapfel, S., Hüneburg, R., Jacobs, M. A., Koornstra, J. J., Langers, A. M., Lepistö, A., Morak, M., Möslein, G., . . . Vasen, H. F. (2020). Associations of Pathogenic Variants in MLH1, MSH2, and MSH6 With Risk of Colorectal Adenomas and Tumors and With Somatic Mutations in Patients With Lynch Syndrome. Gastroenterology, 158(5), 1326-1333. https://doi.org/10.1053/j.gastro.2019.12.032

Journal Pre-proof

Associations of Pathogenic Variants in *MLH1*, *MSH2*, and *MSH6* With Risk of Colorectal Adenomas and Tumors and With Somatic Mutations in Patients With Lynch Syndrome

Christoph Engel, M.D., Aysel Ahadova, Ph.D., Toni Seppälä, M.D., Stefan Aretz, M.D., Marloes Bigirwamungu-Bargeman, M.D., Hendrik Bläker, M.D., Karolin Bucksch, M.Sc., Reinhard Büttner, M.D., Wouter de Vos tot Nederveen Cappel, M.D., Volker Endris, M.D., Elke Holinski-Feder, M.D., Stefanie Holzapfel, M.D., Robert Hüneburg, M.D., Maarten A.J.M. Jacobs, M.D., Jan J. Koornstra, M.D., Alexandra M. Langers, M.D., Anna Lepistö, M.D., Monika Morak, Ph.D., Gabriela Möslein, M.D., Päivi Peltomäki, M.D., Kirsi Pylvänäinen, C.R.A., Nils Rahner, M.D., Laura Renkonen-Sinisalo, M.D., Karsten Schulmann, M.D., Verena Steinke-Lange, M.D., Albrecht Stenzinger, M.D., Christian P. Strassburg, M.D., Paul C. van de Meeberg, M.D., Mariette van Kouwen, M.D., Monique van Leerdam, M.D., Deepak B. Vangala, M.D., Juda Vecht, M.D., Marie-Louise Verhulst, M.D., Magnus von Knebel Doeberitz, M.D., Jürgen Weitz, M.D., Silke Zachariae, Ph.D., Markus Loeffler, M.D., Jukka-Pekka Mecklin, M.D., Matthias Kloor, M.D., Hans F. Vasen, M.D., on behalf of the German HNPCC Consortium, the Dutch Lynch Syndrome Collaborative Group, and the Finnish Lynch Syndrome Registry

PII: S0016-5085(20)30014-7
DOI: https://doi.org/10.1053/j.gastro.2019.12.032
Reference: YGAST 63115

To appear in: *Gastroenterology*
Accepted Date: 24 December 2019

Original Article GASTRO-D-19-01875.R1

Associations of Pathogenic Variants in *MLH1*, *MSH2*, and *MSH6* With Risk of Colorectal Adenomas and Tumors and With Somatic Mutations in Patients With Lynch Syndrome

Short Title: Colorectal cancer development in Lynch syndrome

Christoph Engel, M.D.*[,1], Aysel Ahadova, Ph.D.*[,2,3], Toni Seppälä, M.D.*[,4,5,6], Stefan Aretz, M.D.[7,8], Marloes Bigirwamungu-Bargeman, M.D.[9], Hendrik Bläker, M.D.[10], Karolin Bucksch, M.Sc.[1], Reinhard Büttner, M.D.[11], Wouter de Vos tot Nederveen Cappel, M.D.[12], Volker Endris, M.D.[13], Elke Holinski-Feder, M.D.[14,15], Stefanie Holzapfel, M.D.[7,8], Robert Hüneburg, M.D.[16,17], Maarten A.J.M. Jacobs, M.D.[18], Jan J. Koornstra, M.D.[19], Alexandra M. Langers, M.D.[20], Anna Lepistö, M.D.[21,22], Monika Morak, Ph.D.[14,15], Gabriela Möslein, M.D.[23], Päivi Peltomäki, M.D.[24], Kirsi Pylvänäinen, C.R.A.[25], Nils Rahner, M.D.[26], Laura Renkonen-Sinisalo, M.D.[21,22], Karsten Schulmann, M.D.[27,28], Verena Steinke-Lange, M.D.[14,15], Albrecht Stenzinger, M.D.[13], Christian P. Strassburg, M.D.[16,17], Paul C. van de Meeberg, M.D.[29], Mariette van Kouwen, M.D.[30], Monique van Leerdam, M.D.[18], Deepak B. Vangala, M.D.[31], Juda Vecht, M.D.[12], Marie-Louise Verhulst, M.D.[32], Magnus von Knebel Doeberitz, M.D.[2,3], Jürgen Weitz, M.D.[33], Silke Zachariae, Ph.D.[1], Markus Loeffler, M.D.[1], Jukka-Pekka Mecklin, M.D.[#,34,35], Matthias Kloor, M.D.[#,2,3], Hans F. Vasen, M.D.[#,20]

on behalf of the German HNPCC Consortium, the Dutch Lynch Syndrome Collaborative Group, and the Finnish Lynch Syndrome Registry

* Drs. Engel, Ahadova and Seppälä contributed equally to the article (first authors)
\# Drs. Vasen, Kloor and Mecklin contributed equally to the article (senior authors)

Authors' affiliations

1 Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany
2 Department of Applied Tumour Biology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany
3 Cooperation Unit Applied Tumour Biology, German Cancer Research Center (DKFZ), Heidelberg, Germany
4 Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland
5 University of Helsinki, Helsinki, Finland
6 Johns Hopkins Hospital, Surgical Oncology, Baltimore, MD, USA
7 Institute of Human Genetics, University of Bonn, Bonn, Germany
8 National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany
9 Department of Gastroenterology & Hepatology, Medisch Spectrum Hospital, Enschede, The Netherlands
10 Institute of Pathology, University Hospital Leipzig, Leipzig, Germany
11 Institute of Pathology, University of Cologne, Cologne, Germany
12 Department of Gastroenterology & Hepatology, Isala Zwolle, Zwolle, The Netherlands
13 Department of General Pathology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany
14 Medizinische Klinik und Poliklinik IV, Campus Innenstadt, Klinikum der Universität München, Munich, Germany
15 Center of Medical Genetics, Munich, Germany
16 National Center for Hereditary Tumor Syndromes, University Hospital Bonn, Bonn, Germany
17 Department of Internal Medicine I, University Hospital Bonn, Bonn, Germany
18 Department of Gastroenterology & Hepatology, Amsterdam University Medical Center, Amsterdam, The Netherlands
19 Department of Gastroenterology & Hepatology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands
20 Department of Gastroenterology & Hepatology, Leiden University Medical Centre, Leiden, The Netherlands
21 Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland
22 Research Programs Unit, Genome-Scale Biology, University of Helsinki, Helsinki, Finland
23 Center for Hereditary Tumors, HELIOS Klinikum Wuppertal, University Witten-Herdecke, Wuppertal, Germany
24 Department of Medical and Clinical Genetics, University of Helsinki, Helsinki, Finland
25 Department of Education and Science, Central Finland Hospital District, Jyväskylä, Finland
26 Institute of Human Genetics, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany
27 Department of Hematology and Oncology, Klinikum Hochsauerland, Meschede, Germany
28 MVZ Arnsberg, Medical Practice for Hematology and Oncology, Arnsberg, Germany
29 Department of Gastroenterology & Hepatology, Slingeland Hospital, Doetinchem, The Netherlands
30 Department of Gastroenterology & Hepatology, Radboud University Medical Centre, Nijmegen, The Netherlands
31 Department of Medicine, Knappschaftskrankenhaus, Ruhr-University Bochum, Bochum, Germany
32 Department of Gastroenterology & Hepatology, Maxima Medical Centre, Eindhoven, The Netherlands

[33] Department of Visceral, Thoracic and Vascular Surgery, University Hospital Carl Gustav Carus of the Technical University Dresden, Dresden, Germany
[34] Department of Surgery, Central Finland Central Hospital, Jyväskylä, Finland
[35] Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

Grant support:
The study was supported by the German Cancer Aid (grant number 111008) and the Wilhelm Sander Foundation (grant number 2016.056.1). The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Correspondence:
Dr. Christoph Engel, M.D.
Institute for Medical Informatics, Statistics and Epidemiology
University of Leipzig
Haertelstrasse 16-18
04107 Leipzig
Germany
Phone: +49 341 9716124
Fax: +49 341 9716109
Email: christoph.engel@imise.uni-leipzig.de

Disclosures: None of the authors has a potential conflict of interest relevant to the manuscript.

Writing assistance: None

Author contributions: The study was designed by CE, AA, TS, MK and HFV. CE and AA analysed the data, had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors interpreted the data. The manuscript was written by CE, AA, and HFV. The decision to submit the manuscript for publication was made by all authors. All authors contributed to the review of the manuscript. No other persons were involved in manuscript writing.

ABSTRACT

Background & Aims: Lynch syndrome is caused by variants in DNA mismatch repair (MMR) genes and associated with an increased risk of colorectal cancer (CRC). In patients with Lynch syndrome, CRCs can develop via different pathways. We studied associations between Lynch syndrome-associated variants in MMR genes and risks of adenoma and CRC and somatic mutations in *APC* and *CTNNB1* in tumors in an international cohort of patients.

Methods: We combined clinical and molecular data from 3 studies. We obtained clinical data from 2747 patients with Lynch syndrome associated with variants in *MLH1*, *MSH2*, or *MSH6* from Germany, the Netherlands, and Finland who received at least 2 surveillance colonoscopies and were followed for a median time of 7.8 years for development of adenomas or CRC. We performed DNA sequence analyses of 48 colorectal tumors (from 16 patients with mutations in *MLH1*, 29 patients with mutations in *MSH2*, and 3 with mutations in *MSH6*) for somatic mutations in *APC* and *CTNNB1.*

Results: Risk of advanced adenoma in 10 y was 17.8% in patients with pathogenic variants in *MSH2* vs 7.7% in *MLH1* (*P*<.001). Higher proportions of patients with pathogenic variants in *MLH1* or *MSH2* developed CRC in 10 y (11.3% and 11.4%) than patients with pathogenic variants in *MSH6* (4.7%) (*P*=.001 and *P*=.003 for *MLH1* and *MSH2* vs *MSH6*, respectively). Somatic mutations in *APC* were found in 75% of tumors from patients with pathogenic variants in *MSH2* vs 11% in *MLH1* (*P*=.015). Somatic mutations in *CTNNB1* were found in 50% of tumors from patients with pathogenic variants in *MLH1* vs 7% in *MSH2* (*P*=.002). None of the 3 tumors with pathogenic variants in *MSH6* had a mutation in *CTNNB1*, but all had mutations in *APC*.

Conclusions: In an analysis of clinical and DNA sequence data from patients with Lynch syndrome from 3 countries, we associated pathogenic variants in MMR genes with risk of adenoma and CRC, and somatic mutations in *APC* and *CTNNB1* in colorectal tumors. If these findings are confirmed, surveillance guidelines might be adjusted based on MMR gene variants.

Keywords: prognostic factor, genetic analysis, outcome, cancer risk

INTRODUCTION

Lynch syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome, accounting for approximately 3% of all cases of CRC.[1] LS is an autosomal dominant inherited disorder caused by pathogenic germline variants in mismatch repair (MMR) genes, including *MLH1*, *MSH2* (*EPCAM*), *MSH6* and *PMS2*. Carriers of such gene defects are at high risk of developing primarily CRC and endometrial cancer, but also other malignancies.[2] The main features of LS include an early age of cancer onset, a high risk of developing multiple cancers, and microsatellite instability and loss of MMR expression in the tumors.[3] The risk of developing a specific cancer type in LS depends on the underlying germline MMR defect.[4-6]

Carriers of pathogenic MMR gene variants show an increased frequency of adenoma development compared to non-carriers undergoing intensive colonoscopic surveillance.[7] In addition, a Finnish study demonstrated that colonoscopy with polypectomy reduces CRC incidence and CRC mortality by >50%.[8] Together these observations confirmed the role of adenomas in the development of CRC in LS and constitute the basis for colonoscopic surveillance programs. [4, 9]

Nevertheless, a substantial number of carriers develop CRC despite colonoscopic surveillance.[10-12] Recent publications report risks of CRC of up to 46% in patients under surveillance, with much higher risks found for *MLH1* and *MSH2* carriers (43-46%) than for *MSH6* (15%) and *PMS2* carriers (0%).[13] Another recent prospective study evaluated the risk of incident CRC in a large series of *MLH1*, *MSH2*, or *MSH6* carriers from Germany, Finland and the Netherlands.[14] Despite intensive surveillance, 4-18% of the carriers developed CRC after 10 years of follow-up, independently of the screening interval.

In 2016, Ahadova et al. hypothesized that LS-CRC might develop through a pathway characterized by a lack of adenomatous tissue and by immediate invasive growth under the mucosal surface.[15] The authors suggested that these LS-CRCs may emerge from MMR-deficient crypt foci that grow under the mucosal surface and cannot be detected by colonoscopy at a pre-invasive stage,

potentially explaining CRC development despite colonoscopic surveillance and polypectomies.[16] It was subsequently demonstrated that LS-CRCs are heterogeneous and can develop via different molecular pathways with distinct initiating events: whereas some LS-CRCs develop from MMR-proficient adenomas, the majority develop from MMR-deficient lesions, either via an adenomatous phase or in the absence of a detectable precursor lesion.[17] Nonetheless, the impact of certain MMR gene mutations on the development of particular CRC subtypes remains unclear.

The development of CRC despite surveillance is an important problem in the clinical management of LS and deserves closer examination to clarify the underlying mechanisms. A better understanding of carcinogenesis in each specific group of pathogenic MMR variant carriers will have important consequences for decision-making on appropriate surveillance intervals.

The purpose of this study was to assess possible differences in the pathways of CRC development between *MLH1*, *MSH2* and *MSH6* carriers. Our specific aims were (1) to compare risks for (advanced) adenoma and CRC, and (2) to compare the frequencies of *CTNNB1* and *APC* mutations in CRCs between *MLH1*, *MSH2* and *MSH6* carriers.

METHODS

Study population

This report combines clinical and molecular data obtained in the course of three earlier studies. The clinical part is based on data from a prospective cohort study conducted to compare CRC incidence and stage in LS patients from three different countries (Germany, the Netherlands, Finland) recommending different colonoscopy intervals.[14] The study population has been described in detail elsewhere.[14] Briefly, the cohort consisted of 2,747 LS patients included in the LS registries of Germany, the Netherlands, and Finland. In all three registries, LS patients were followed prospectively within a framework of intensive colonoscopic surveillance programs. Written informed consent was obtained from all LS patients enrolled in the registries and who participated in prospective surveillance studies. Patients were eligible for the present analysis if they i) were carriers of a pathogenic germline variant in either the *MLH1*, *MSH2* or *MSH6* gene, and ii) had completed at least two surveillance colonoscopies after registry inclusion. For each colonoscopy, age at examination and worst finding (normal, adenoma, advanced adenoma, CRC) were noted, and for each CRC the age at diagnosis was recorded. An advanced adenoma was defined by a size of >1cm or the presence of either villous histology or high-grade dysplasia.

In the present analysis, we used this study population to compare the cumulative incidences of (advanced) adenomas and CRC between the *MLH1*, *MSH2*, and *MSH6* carriers.

Molecular analysis and histology assessment

Separately from the clinical part, the molecular part of the present report represents a re-analysis of data from two studies reported previously.[15, 17] Briefly, formalin-fixed paraffin-embedded (FFPE) tissue blocks from LS-CRCs were collected within the Department of Applied Tumor Biology, Institute of Pathology, University Hospital Heidelberg. Tumor tissue was microdissected from FFPE tissue sections and DNA was isolated for the downstream analyses. Histopathology review

revealed a tumor cell content of more than 50% in all studied samples. Mutational analysis was performed either by targeted Sanger sequencing (for determination of *CTNNB1* mutation status) or by Illumina panel sequencing of mutation HotSpot regions in 30 genes, including *CTNNB1* and *APC*.[18] The data were analyzed by Sequencing Analysis Software and Ion Torrent Suite Software, respectively. Only variants with an allele frequency >5% and minimum coverage >100 reads were taken into account. For the purposes of the present study, molecular data obtained from CRCs were sorted depending on the underlying MMR defect. All patients provided informed, written consent and the study was approved by the relevant Institutional Ethics Committee.

Statistical analysis

In the clinical cohort, prospective observation started with the first colonoscopy conducted after enrollment in the LS register (index colonoscopy) and ended with the last colonoscopy or the occurrence of a primary CRC diagnosis. CRCs detected at the index colonoscopy were defined as prevalent CRCs. All other CRCs detected during prospective observation were defined as incident cancers. The occurrence of incident extra-colonic tumors was ignored if surveillance colonoscopies were continued after such an event. Time to incident (advanced) adenoma or CRC was analyzed using the Kaplan-Meier method, with time zero at the index colonoscopy and group comparisons made using the log-rank test. Additionally, we performed multivariate Cox regression analyses adjusting for age at index colonoscopy and country as confounders, the latter reflecting the differences in colonoscopy intervals and the differences in the proportions of patients with prior CRC. Comparisons of categorical data between groups were performed using the chi-square test, or Fisher's exact test where appropriate. P values below 0.05 were considered statistically significant. All analyses were carried out using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Risk of (advanced) adenomas and CRC

The clinical cohort comprised 2,747 LS patients in total (1,027 from Germany, 806 from the Netherlands, and 914 from Finland). Basic patient characteristics can be found in Table 1. A total of 1,038 patients (38%) already had a prior CRC before the index colonoscopy. Due to the presence of two *MLH1* founder mutations in the Finnish population, the proportion of *MLH1* carriers was higher in Finland (79%) compared to Germany (39%) and the Netherlands (35%). Patients had a median of five consecutive colonoscopies (16,327 colonoscopies in total). The median per-patient observation time was 7.8 years (interquartile range 4.2 to 12.0). The cumulative prospective observation time amounted to 23,309 person-years in total. At the index colonoscopy, the frequency of prevalent adenomas was 10.2% and the frequency of prevalent CRC was 2.3%.

Figure 1 shows the comparison of the cumulative risk of incident adenoma between *MLH1*, *MSH2* and *MSH6* carriers. Ten years after the index colonoscopy, the highest risk was observed in *MSH2* carriers (44.2%, 95%CI 40.0-48.4%), followed by *MSH6* carriers (38.4%, 95%CI 30.8-45.9%), and was lowest in *MLH1* carriers (32.2%, 95%CI 29.2-35.2%). The differences in risk between *MLH1* and *MSH2* carriers ($p<0.001$) and between *MLH1* and *MSH6* carriers ($p=0.029$) were statistically significant, but not between *MSH2* and *MSH6* carriers ($p=0.400$). Figure 2 shows the comparison of the cumulative risk of incident advanced adenoma between the three groups. Ten years after the index colonoscopy, the risk of advanced adenoma was similar for *MLH1* and *MSH6* carriers (7.7%, 95%CI 6.0-9.4% and 9.4%, 95%CI 5.4-13.4%, respectively, $p=0.543$), but both had a significantly ($p<0.001$ and $p=0.010$, respectively) lower risk than *MSH2* carriers (17.8%, 95%CI 14.6-21.0%). Figure 3 shows the cumulative risks for incident CRC. Ten-year CRC risks were almost identical for *MLH1* and *MSH2* carriers (11.3%, 95%CI 9.4-13.2% and 11.4%, 95%CI 8.9-14.0%, respectively, $p=0.468$). In contrast, CRC risk in *MSH6* carriers was significantly lower (4.7%, 95%CI 1.8-7.7%) compared to *MLH1* ($p=0.001$) and *MSH2* ($p=0.003$) carriers. Multivariate Cox regression analyses

adjusting for age at index colonoscopy and country revealed similar results regarding significant group differences except for adenoma risk between *MLH1* and *MSH6* carriers, which was not significant (p-adjusted=0.265) compared to the unadjusted analysis.

Molecular features of LS-CRC

Molecular analysis was performed on CRCs from 16 *MLH1*, 29 *MSH2*, and 3 *MSH6* carriers. Allele frequencies of the observed somatic mutations ranged between 21% and 73%. The results are summarized in Table 2. Of the 16 *MLH1*-associated CRCs, eight displayed somatic *CTNNB1* mutations (50%, 95%CI: 28.0-72.0%), whereas only two somatic *CTNNB1* mutations were detected in the 29 *MSH2*-associated CRCs (7%, 95%CI: 0.9-23.0%), demonstrating a significantly higher proportion of somatic *CTNNB1* mutations in *MLH1*- compared to *MSH2*-associated CRCs (p=0.002). In contrast, somatic *APC* mutations were detected in only one of nine *MLH1*-associated CRCs (11%, 95%CI: 0-45.7%), whereas *MSH2*-associated CRCs carried somatic *APC* mutations in six out of eight analyzed CRCs (75%, 95% CI: 40.1-93.7%, p=0.015). None of the three *MSH6*-associated CRCs analyzed carried a somatic *CTNNB1* mutation, but all three presented with a somatic *APC* mutation.

DISCUSSION

Previous studies in LS have shown that up to 1 in 5 patients develop CRC despite intensive colonoscopic surveillance.[8, 10-12, 14] *MLH1* and *MSH2* carriers under surveillance are at high risk of CRC, whereas *MSH6* carriers have a much lower risk, and *PMS2* carriers may even have zero CRC risk under surveillance.[13] The main goal of the present study was to evaluate whether the molecular pathways of carcinogenesis are different between *MLH1*, *MSH2* and *MSH6* carriers and therefore explain the observed differences in adenoma and CRC risk and the effectiveness of screening programs.

The prospective clinical data of our study demonstrates that the risk of adenomas is significantly greater in *MSH2* and *MSH6* carriers compared to *MLH1* carriers, and that the risk of advanced adenomas is higher in *MSH2* carriers compared to both *MLH1* and *MSH6* carriers. However, incident CRC was more frequently observed in *MLH1* and *MSH2* carriers than in *MSH6* carriers. This is in contrast to a recently published single center study involving 242 *MLH1*, *MSH2* and *MSH6* carriers over 1739 years of follow-up, which could not detect significant differences in (advanced) adenoma incidence between MMR genes, probably due to the low sample size.[19]

Our study also demonstrates molecular differences in the carcinogenesis between *MLH1*- and *MSH2*-associated LS-CRCs. Whereas *MSH2*-associated CRCs presented with a higher frequency of somatic *APC* mutations compared to *MLH1*-associated CRCs, a significantly higher frequency of *CTNNB1* mutations was observed in *MLH1*-associated CRCs compared to *MSH2*-associated ones.[20, 21]

Interestingly, incident CRC risk in *MLH1* carriers was as high as in *MSH2* carriers, but *MLH1* carriers presented with a substantially lower (advanced) adenoma incidence than *MSH2* carriers. Moreover, the cumulative risk for advanced adenoma in *MSH2* carriers was higher than the CRC risk, which agrees with the notion that not all adenomas develop into cancer. In *MLH1* carriers, however, the risk for advanced adenoma was lower than the incident cancer risk. Assuming similar

progression rates for *MLH1*- and *MSH2*-associated advanced adenomas into CRC, this observation might suggests different pathways of CRC development in *MLH1* versus *MSH2* carriers. Seen in the light of the enhanced CRC risk in both *MLH1* and *MSH2* carriers and their molecular characteristics, it is conceivable that somatic *CTNNB1* and *APC* mutations, in combination with MMR deficiency, may both contribute to tumor progression. Whereas *MSH2*-associated cancers may have an accelerated adenoma-carcinoma sequence with somatic *APC* mutations often following MMR deficiency, a substantial proportion of *MLH1*-associated cancers may progress without prior polyp formation through an immediate invasive pathway, presumably arising from MMR-deficient crypts due to acquired somatic *CTNNB1* mutations.

The higher frequency of somatic *APC* mutations in CRCs of *MSH2* vs. *MLH1* carriers is in line with the observed higher incidence of (advanced) adenomas in *MSH2* vs. *MLH1* carriers, as *APC* mutations are often associated with adenoma development in the colonic epithelium.[22]

Both *MSH2*- and *MSH6*-associated CRCs presented with a high proportion of somatic *APC* mutations and a low proportion of somatic *CTNNB1* mutations. While this finding should be validated in a larger cohort in the case of *MSH6*-associated CRCs, this outcome is intriguing since *MSH6* carriers show a lower risk of advanced adenoma development compared to *MSH2* carriers. One explanation for this observation could be an incomplete MMR deficiency caused by MSH6 loss, with predominantly mononucleotide repeats affected, which potentially lowers the likelihood of driver mutations secondary to MMR deficiency.[23] This is further corroborated by the observation of MSH6-deficient cancers that lack MSI[24] and possibly indirectly by a lower proportion of MMR-deficient adenomas (approximately 27%) in *MSH6* carriers compared to *MSH2* or *MLH1* carriers (75-80%), as demonstrated previously.[17, 20, 25, 26]

In view of the low frequency of MMR-deficient adenomas in *MSH6*, it is conceivable that MMR-deficient crypt foci are either less common or less likely to progress in *MSH6* carriers. Progression according to the classic adenoma-carcinoma sequence, with MMR deficiency occurring after

adenoma development, could therefore be the more frequent pathway of carcinogenesis in this group of carriers. This hypothesis is supported by the observation of low grade adenomas presenting with an MSS phenotype in *MSH6* carriers, in contrast to *MLH1* carriers.[27, 28] As a consequence, surveillance colonoscopy with polypectomy might be more effective in *MSH6* carriers, as described in a recent study.[13]

A similar hypothesis might explain the high efficacy of colonoscopic screening in *PMS2* carriers. Adenomas in these patients are usually MMR-proficient and *PMS2*-associated CRCs do not commonly present with somatic *CTNNB1* mutations.[21] Thus, the lower CRC risks observed in *MSH6* and *PMS2* carriers under surveillance might be explained by inherently lower risks of CRC development in these carriers, but may also be partly due to a different pathogenesis and thereby more effective prevention by polypectomy.

The current study had several strengths, as well as some limitations. A major strength of the clinical part was the large prospective cohort with long duration of follow-up. A limitation was the low number of CRCs from *MSH6* carriers available for molecular studies, which does not allow drawing definitive conclusions. It is also important to note that the molecular data were not obtained from the CRCs of the clinical cohort, but from separate CRC samples. Since the selection of these samples did not differ from the selection of patients for the clinical part of the study, this should not have influenced the results of our study in a major way. However, prospective validation in a larger number of cancers is warranted.

What are the implications of our findings for clinical practice? Our results suggest that the high CRC risk in *MLH1* and *MSH2* carriers under surveillance might be largely attributed to the molecular characteristics of the CRCs, possibly contributing to a fast adenoma-carcinoma sequence or initial submucosal growth in the absence of adenomatous tissue. The development of CRC despite intensive surveillance colonoscopy in these groups of carriers should therefore be considered as part of the phenotype. Fortunately, most CRCs detected by screening are local,

without metastatic disease, and almost all patients have a favorable outcome.[6, 29] These considerations should be discussed with the patients, and it should be emphasized that detection of a CRC during intensive colonoscopic surveillance is not surprising and does not necessarily indicate a failure of the surveillance program. In contrast, colonoscopy and polypectomy are effective in almost all *MSH6* carriers, as also reported for *PMS2* carriers.[6]

In conclusion, this is the first study to compare clinical and molecular findings between carriers of alterations in the MMR genes *MLH1*, *MSH2* and *MSH6*. Our results suggest that growth characteristics and accumulation of certain molecular alterations may explain the high CRC risk in both *MLH1* and *MSH2* carriers. Despite similar CRC risks there were remarkable differences in *CTNNB1* and *APC* mutation frequencies between *MLH1* and *MSH2* carriers. The previously reported low frequency of MMR deficiency in *MSH6*-associated adenomas, in combination with the low frequency of *CTNNB1* mutations and the higher frequency of *APC* mutations, suggests that an MMR-deficient polypous pathway in *MSH6* carriers typically arises after the development of adenomas. If these findings are confirmed in future studies, surveillance guidelines might be adjusted based on the underlying MMR gene variant.

REFERENCES

1. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851-60.
2. Jasperson KW, Tuohy TM, Neklason DW, et al. Hereditary and familial colon cancer. Gastroenterology 2010;138:2044-58.
3. Lynch HT, Snyder CL, Shaw TG, et al. Milestones of Lynch syndrome: 1895-2015. Nat Rev Cancer 2015;15:181-94.
4. Vasen HF, Blanco I, Aktan-Collan K, et al. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. Gut 2013;62:812-23.
5. Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-society Task Force on colorectal cancer. Am J Gastroenterol 2014;109:1159-79.
6. Moller P, Seppala T, Bernstein I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut 2017;66:464-472.
7. De Jong AE, Morreau H, Van Puijenbroek M, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 2004;126:42-8.
8. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118:829-34.
9. Stoffel EM, Mangu PB, Limburg PJ. Hereditary colorectal cancer syndromes: American Society of Clinical Oncology clinical practice guideline endorsement of the familial risk-

colorectal cancer: European Society for Medical Oncology clinical practice guidelines. J Oncol Pract 2015;11:e437-41.

10. Engel C, Rahner N, Schulmann K, et al. Efficacy of annual colonoscopic surveillance in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 2010;8:174-82.

11. Mecklin JP, Aarnio M, Laara E, et al. Development of colorectal tumors in colonoscopic surveillance in Lynch syndrome. Gastroenterology 2007;133:1093-8.

12. Vasen HF, Abdirahman M, Brohet R, et al. One to 2-year surveillance intervals reduce risk of colorectal cancer in families with Lynch syndrome. Gastroenterology 2010;138:2300-6.

13. Moller P, Seppala TT, Bernstein I, et al. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. Gut 2018;67:1306-1316.

14. **Engel C, Vasen HF, Seppala T**, et al. No Difference in Colorectal Cancer Incidence or Stage at Detection by Colonoscopy Among 3 Countries With Different Lynch Syndrome Surveillance Policies. Gastroenterology 2018;155:1400-1409 e2.

15. Ahadova A, von Knebel Doeberitz M, Blaker H, et al. CTNNB1-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in Lynch syndrome. Fam Cancer 2016;15:579-86.

16. Kloor M, Huth C, Voigt AY, et al. Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study. Lancet Oncol 2012;13:598-606.

17. Ahadova A, Gallon R, Gebert J, et al. Three molecular pathways model colorectal carcinogenesis in Lynch syndrome. Int J Cancer 2018;143:139-150.

18. Jesinghaus M, Pfarr N, Endris V, et al. Genotyping of colorectal cancer for cancer precision medicine: Results from the IPH Center for Molecular Pathology. Genes Chromosomes Cancer 2016;55:505-21.

19. Goverde A, Eikenboom EL, Viskil EL, et al. Yield of Lynch Syndrome Surveillance for Patients With Pathogenic Variants in DNA Mismatch Repair Genes. Clin Gastroenterol Hepatol 2019.

20. Sekine S, Mori T, Ogawa R, et al. Mismatch repair deficiency commonly precedes adenoma formation in Lynch Syndrome-Associated colorectal tumorigenesis. Mod Pathol 2017;30:1144-1151.

21. Ten Broeke SW, van Bavel TC, Jansen AML, et al. Molecular Background of Colorectal Tumors From Patients With Lynch Syndrome Associated With Germline Variants in PMS2. Gastroenterology 2018;155:844-851.

22. Goss KH, Groden J. Biology of the adenomatous polyposis coli tumor suppressor. J Clin Oncol 2000;18:1967-79.

23. Kolodner R. Biochemistry and genetics of eukaryotic mismatch repair. Genes Dev 1996;10:1433-42.

24. Lagerstedt Robinson K, Liu T, Vandrovcova J, et al. Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics. J Natl Cancer Inst 2007;99:291-9.

25. Yurgelun MB, Goel A, Hornick JL, et al. Microsatellite instability and DNA mismatch repair protein deficiency in Lynch syndrome colorectal polyps. Cancer Prev Res (Phila) 2012;5:574-82.

26. Walsh MD, Buchanan DD, Pearson SA, et al. Immunohistochemical testing of conventional adenomas for loss of expression of mismatch repair proteins in Lynch syndrome mutation carriers: a case series from the Australasian site of the colon cancer family registry. Mod Pathol 2012;25:722-30.

27. Ferreira AM, Westers H, Sousa S, et al. Mononucleotide precedes dinucleotide repeat instability during colorectal tumour development in Lynch syndrome patients. J Pathol 2009;219:96-102.

28. Maki-Nevala S, Valo S, Ristimaki A, et al. DNA methylation changes and somatic mutations as tumorigenic events in Lynch syndrome-associated adenomas retaining mismatch repair protein expression. EBioMedicine 2019;39:280-291.

29. Argillander TE, Koornstra JJ, van Kouwen M, et al. Features of incident colorectal cancer in Lynch syndrome. United European Gastroenterol J 2018;6:1215-1222.

FIGURES

Figure 1. Cumulative incidence of adenoma by affected gene (*MLH1*, *MSH2*, *MSH6*)

Figure 2. Cumulative incidence of advanced adenoma by affected gene (*MLH1*, *MSH2*, *MSH6*)

Figure 3. Cumulative incidence of incident CRC by affected gene (*MLH1*, *MSH2*, *MSH6*)

TABLES

Table 1: Clinical characteristics of patients in the clinical cohort

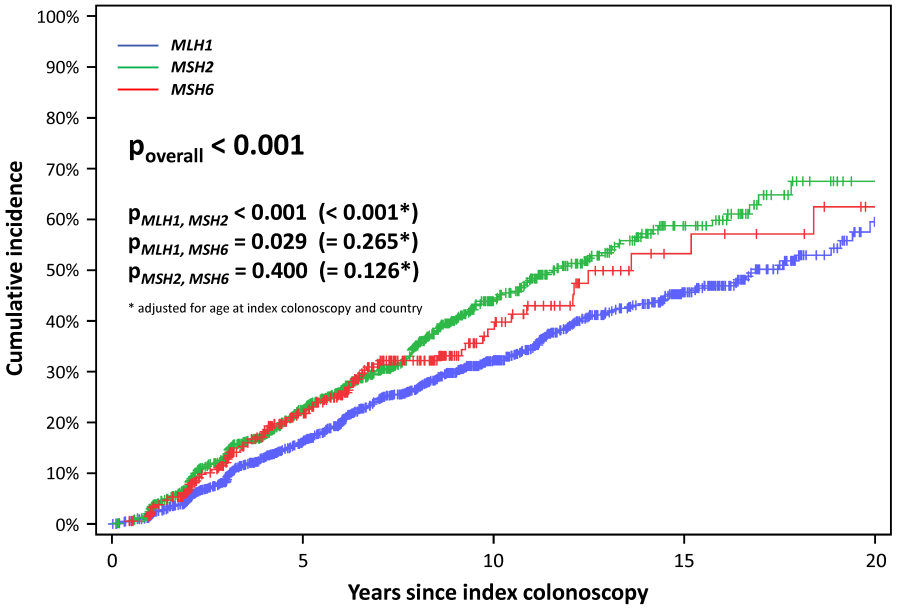
	MLH1	***MSH2***	***MSH6***	**Total**
	n = 1407	n = 986	n = 354	n = 2747
Country, no (%)				
Germany	400 (28.4)	507 (51.4)	120 (33.9)	1027 (37.4)
Netherlands	285 (20.3)	336 (34.1)	185 (52.3)	806 (29.3)
Finland	722 (51.3)	143 (14.5)	49 (13.8)	914 (33.3)
Sex, no (%)				
male	672 (47.8)	483 (49.0)	160 (45.2)	1315 (47.9)
female	735 (52.2)	503 (51.0)	194 (54.8)	1432 (52.1)
CRC before index colonoscopy, no (%)	526 (37.4)	405 (41.1)	107 (30.2)	1038 (37.8)
Age at index colonoscopy, mean (±SD)	42.7 (±13.5)	44.0 (±12.3)	48.7 (±13.7)	43.9 (±13.2)
Year of index colonoscopy, mean (±SD)	2002 (±6)	2004 (±5)	2005 (±4)	2003 (±5)
Number of colonoscopies				
per patient, median (IQR)	5 (3-8)	6 (4-8)	4 (3-6)	5 (3-8)
cumulative	8229	6300	1798	16327
Observation time, years				
per patient, median (IQR)	8.5 (4.2-13.2)	7.4 (4.4-11.3)	6.5 (4.1-9.4)	7.8 (4.2-12.0)
cumulative	12798	7961	2550	23309
Incident CRC, no of patients / CRC	167 / 169	93 / 97	12 / 13	272 / 279

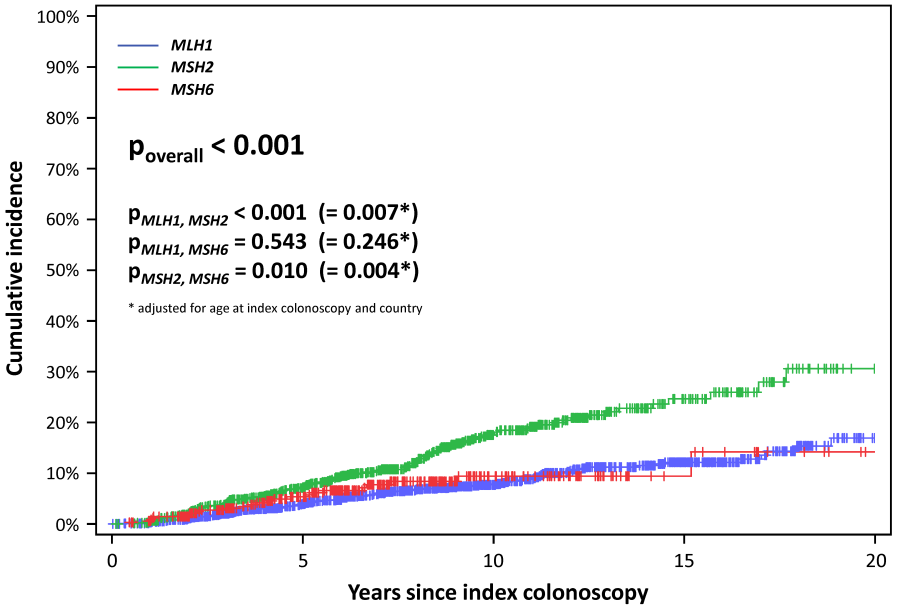
IQR, interquartile range; SD, standard deviation

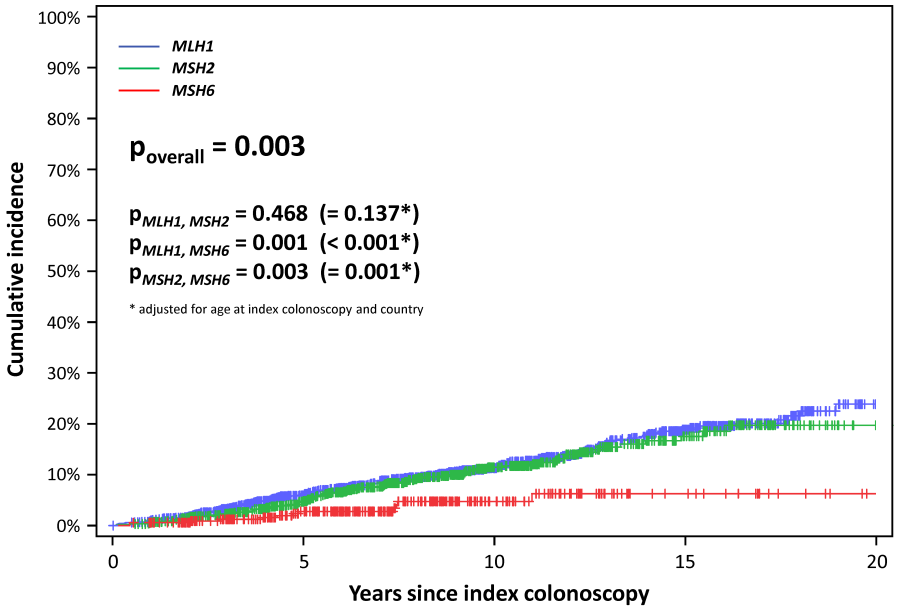
Table 2. Presence of somatic mutations in *CTNNB1* and *APC* in tumors of *MLH1*, *MSH2*, and *MSH6* carriers

	MLH1	***MSH2***	***MSH6***
	n = 16	n = 29	n = 3
Age at diagnosis, median	46	45	55
***CTNNB1* mutated**	8 of 16 (50%)	2 of 29 (7%)	0 of 3 (0%)
***APC* mutated**	1 of 9 (11%)	6 of 8 (75%)	3 of 3 (100%)

not all tumors could be analyzed for *APC* mutations


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative incidence
MLH1
MSH2
MSH6
p overall < 0.001
p MLH1, MSH2 < 0.001 (< 0.001*)
p MLH1, MSH6 = 0.029 (= 0.265*)
p MSH2, MSH6 = 0.400 (= 0.126*)
* adjusted for age at index colonoscopy and country
0
5
10
15
20
Years since index colonoscopy


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative incidence
MLH1
MSH2
MSH6
p overall < 0.001
p MLH1, MSH2 < 0.001 (= 0.007*)
p MLH1, MSH6 = 0.543 (= 0.246*)
p MSH2, MSH6 = 0.010 (= 0.004*)
* adjusted for age at index colonoscopy and country
0
5
10
15
20
Years since index colonoscopy


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cumulative incidence
MLH1
MSH2
MSH6
p overall = 0.003
p MLH1, MSH2 = 0.468 (= 0.137*)
p MLH1, MSH6 = 0.001 (< 0.001*)
p MSH2, MSH6 = 0.003 (= 0.001*)
* adjusted for age at index colonoscopy and country
0
5
10
15
20
Years since index colonoscopy

What you need to know:

BACKGROUND AND CONTEXT: Lynch syndrome is caused by variants in DNA mismatch repair (MMR) genes and is associated with an increased risk of colorectal cancer (CRC). Variants in MMR genes are likely to have different effects on CRC and adenoma risk.

NEW FINDINGS: In an analysis of adenomas and colorectal tumors from patients with Lynch syndrome from 3 countries, we associated pathogenic variants in MMR genes with risk of adenoma and CRC, and with somatic mutations in *APC* and *CTNNB1*.

LIMITATIONS: We analyzed DNA sequences of 48 tumor samples. Larger studies of CRC development, and features of tumors, are needed in patients with Lynch syndrome.

IMPACT: Surveillance guidelines for patients with Lynch syndrome might need to be adjusted based on MMR gene variants.

Lay Summary: Lynch syndrome is caused by alterations in a specific group of genes. Patients with different types of genetic alterations develop adenomas and CRC at different rates, and tumors have specific genetic features.