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Clinical Characteristics of Colorectal Cancer Patients with Double Somatic Mismatch Repair Mutations Compared to Lynch Syndrome

Rachel Pearlman[1], **Sigurdis Haraldsdottir**[3], **Albert de la Chapelle**[2], **Jon G. Jonasson**[7,8], **Sandya Liyanarachchi**[2], **Wendy L. Frankel**[4], **Thorunn Rafnar**[6], **Kari Stefansson**[6], **Colin C. Pritchard**[5], **Heather Hampel**[1]

[1]Department of Internal Medicine, Division of Human Genetics, The Ohio State University Comprehensive Cancer Center, [2]Department of Cancer Biology and Genetics, [3]Stanford University, Department of Internal Medicine, Division of Medical Oncology; [4]Department of Pathology, The Ohio State University Wexner Medical Center, Columbus, Ohio; [5]Department of Laboratory Medicine, University of Washington, Seattle, Washington; [6]deCODE Genetics/Amgen, Reykjavik, Iceland, [7]Landspitali University Hospital, Reykjavik, Iceland, [8]University of Iceland, Reykjavik, Iceland

Abstract

Background: Colorectal cancer (CRC) patients with mismatch repair-deficient (dMMR) tumors without *MLH1* methylation or germline MMR pathogenic variants (PVs) were previously thought to have Lynch syndrome (LS). It's now appreciated that they can have double somatic (DS) MMR PVs. We explored clinical characteristics between patients with DS tumors and LS in two population-based cohorts.

Methods: We included CRC patients from Ohio 2013–2016 and Iceland 2000–2009. All had microsatellite instability testing and/or immunohistochemistry of MMR proteins, and *MLH1*

Corresponding author: Rachel Pearlman, MS, LGC, Ohio State University Comprehensive Cancer Center, Department of Internal Medicine, Division of Human Genetics, 2012 Kenny Rd, Room 255, Columbus, OH 43221, Phone: 614-293-5740, Fax: 614-293-2314, Rachel.Pearlman@osumc.edu.

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Conflict of Interest: Ms. Hampel is on the scientific advisory board for InVitae Genetics and Genome Medical, has conducted collaborative research with Myriad Genetics Laboratories, Inc, Ambry Genetics, and InVitae Genetics, and has stock in Genome Medical. Ms. Pearlman has done collaborative research with Myriad Genetics Laboratories, Inc, and InVitae Genetics. Drs. Rafnar and Stefansson are employees of deCODE genetics/Amgen. Drs. Haraldsdottir, de la Chapelle, Jonasson, Frankel, Pritchard and Ms. Liyanarachchi have no conflicts to disclose.

methylation testing when indicated. Germline next-generation sequencing was performed for all with dMMR tumors; tumor sequencing followed for patients with unexplained dMMR. Clinical characteristics of DS and LS patients were compared.

Results: Of the 232 and 51 patients with non-methylated dMMR tumors in the Ohio and Iceland cohorts, respectively, 57.8% (n=134) and 45.1% (n=23) had LS, 32.8% (n=76) and 31.4% (n=16) had DS PVs, 6% (n=14) and 9.8% (n=5) were unexplained, and 4.3% (n=10) and 13.7% (n=7) had incorrect IHC. Age of diagnosis for DS patients was older than LS patients ($p=3.73\times10^{-4}$) in the two cohorts. LS patients were more likely to meet Amsterdam II criteria (OR=15.81, $p=8.47\times10^{-6}$) and have multiple LS-associated tumors (OR=6.67, $p=3.31\times10^{-5}$). Absence of MLH1/PMS2 was predictive of DS PVs; isolated MSH6 and PMS2 absence was predictive of LS in both cohorts.

Conclusions: Individuals with LS are 15x more likely to meet Amsterdam II criteria and >5x more likely to have multiple cancers as compared to those with DS tumors. Furthermore, isolated loss of MSH6 or PMS2 protein predicts LS.

Keywords

Lynch-like syndrome; DNA repair system; somatic mutation; tumor testing

INTRODUCTION

Mismatch repair (MMR)-deficient colorectal cancer (CRC) accounts for 12%−15% of all localized CRC and 3–4% of metastatic CRC[1 2]. The primary cause of MMR-deficient CRC is caused by acquired methylation of the *MLH1* promoter[3 4]. Identification of patients with MMR-deficient CRC via universal tumor screening is critical, as MMR deficiency provides insight into hereditary cancer risk for the patient and family members, as well as indicating which patients might benefit from immunotherapy[5–7]. Individuals with MMR-deficient CRC can have Lynch syndrome (LS), caused by a germline pathogenic variant (PV) in any of the MMR genes; *MLH1, MSH2 (EPCAM), MSH6* or *PMS2*. Individuals with LS have a significantly increased risk for developing cancers of the colon, endometrium, ovary, stomach, and others[8 9]. Identifying individuals with LS prevents future cancers, and provides information for family members by facilitating cascade testing and life-saving intensive surveillance and prophylactic surgeries among those who inherited LS.

Previous studies have shown that 2.5%−3.9% of CRC has unexplained MMR deficiency[4 5 10]. Until recently, those patients were thought to have LS caused by an undetected germline MMR PV. In 2013, the term “Lynch-like syndrome” (LLS) was coined to describe this indeterminate group of patients. The Spanish EPICOLON Consortium assessed 1,705 unselected CRC patients for family history of CRC and found that the incidence of CRC was lowest in sporadic CRC families, or those with proficient MMR (0.48), highest in LS families (6.04), and moderately elevated in LLS families (2.12)[10]. It was suggested that the LLS group was likely heterogeneous, including those with sporadic CRC and those with undetected LS, given the intermediate risk for CRC[11].

It is now appreciated that many patients with LLS actually have double somatic (DS) MMR PVs in their tumor. While it has not been definitively proven that these tumors are biallelic, it has been presumed given the tumor phenotype in these patients (microsatellite instability-high [MSI-H] and abnormal immunohistochemistry [IHC]). In 2013, one study showed that 16.7% of patients with unexplained MMR-deficient CRC had DS MMR PVs, with an additional 27.8% of patients having one MMR PV identified but loss of heterozygosity (LOH) was not assessed so many of the cases with one somatic MMR PV were likely DS with LOH as the second hit[12]. This study also identified one patient (5.5%) with somatic mosaicism[12]. In 2014, three additional studies were performed which determined that 52% −69% of unexplained MMR-deficient CRC was caused by DS MMR PV when both sequencing and LOH were evaluated[13–15]. Other identified causes included previously undetected germline MMR PVs and incorrect tumor screening [13–15]. To further characterize those with DS tumors, Mesenkamp *et al.* reported that the age of patients with DS PVs was similar to that of patients with LS (p=0.055), and lower than that of patients with *MLH1* methylation (p<0.0001)[13]. Other studies have shown that patients with DS MMR PVs can still have hereditary CRC caused by *MUTYH*-associated polyposis and other genes involved in DNA repair since they can lead to acquired PVs in the MMR genes[16–18]. A recent study showed that the histology of DS CRC tumors is indistinguishable from those caused by LS[19].

We sought to define clinical characteristics differentiating patients with DS tumors from two population-based cohorts, and compare them to patients with LS.

METHODS

Ohio:

3,471 adults newly diagnosed with primary invasive CRC in Ohio between 1/1/2013–12/31/2016 were prospectively enrolled into the Ohio Colorectal Cancer Prevention Initiative (OCCPI; ClinicalTrials.gov identifier:). Written informed consent was obtained from all participants. Institutional Review Board (IRB) approval for the OCCPI was obtained by the individual participating hospitals, Community Oncology Programs, or by ceding review to the Ohio State University (OSU) IRB (2012C0123). Of the 3,471 patients enrolled, 118 were deemed ineligible and 7 withdrew. Primary reasons for ineligibility included insufficient tumor material, ineligible pathology type, diagnosed outside of the qualifying study period, and not being diagnosed in Ohio. Of the 3,346 active and eligible patients, testing was successful for 3,310. Methods have previously been described[17], but briefly, all tumors were screened for MMR deficiency by MSI testing and/or IHC analysis. Microsatellite instability testing was completed using the Promega MSI Analysis System (Version 1.2), which includes five repeat markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27). Tumors with ≥2/5 markers showing instability were classified as MSI-H. Immunohistochemistry of the MMR proteins was performed using the two-stain method as previously described[20]. Staining for all four MMR proteins was done as routine clinical care for some patients, and attempted for all patients if MSI could not be performed or if the MSI and two-stain IHC results were discordant. Antibodies included MLH-1 Clone: Leica ES05 (Mouse: NCL-L-MLH1), MSH-2 Clone: Calbiochem FE11 (Mouse: NA27), MSH-6

Clone: Epitomics EP49 (Rabbit: AC-0047), PMS-2 Clone: BD Pharmingen A16–4 (Mouse: 556415). Proteins with convincing stain in >1% of cells, or equivocal staining, were considered "present". Methylation of the *MLH1* promoter was assessed at four CpG sites using pyrosequencing[21] when tumors were MSI-high and/or absent MLH1 and PMS2 proteins on IHC, with ≥15% methylation classified as positive. Patients with MMR deficiency without *MLH1* methylation underwent germline next-generation sequencing (NGS) (ColoSeq or BROCA, University of Washington [UW]). Genomic regions were captured using biotinylated RNA oliognucleotides (SureSelect) and sequenced on an Illumina HiSeq2000 instrument[22]. Large rearrangements were detected[23]. Tumor sequencing with ColoSeq Tumor of the MMR genes followed for patients with unexplained MMR-deficient tumors. Data was created by the NGS Laboratory and Analytics group. Pathology reports were reviewed for all patients. The patients' previous cancer history and first-degree relative cancer history was obtained at study enrollment, and three-generation pedigrees were obtained (when possible) after result disclosure. Each pedigree was assessed for clinical characteristics and if Amsterdam II and Revised Bethesda criterion were met. PREMM5 scores were obtained for each patient using current age and known family history as of 2/2018. The following stipulations were also applied: For deceased patients, age at death was used as the current age in the calculation. For cases without an exact age of diagnosis (range provided), the middle number was used (ex: 40s-50s was calculated using 50). If age of diagnosis wasn't known, the average diagnosis age for CRC and EC from the American Cancer Society was used (age 60 for EC, age 72 for women with CRC, age 68 for men with CRC). Some clinical characteristics of fifty-one cases have been previously reported[15 17]

Iceland:

1,182 patients with CRC diagnosed from 2000–2009 in Iceland were included. The study was approved by the Icelandic National Bioethics Committee (VSNb2013010033/03.15), the Icelandic Data Protection Authority (2013010109TS), and the OSU IRB (2013C0144). Written informed consent was obtained from all subjects participating in research studies at deCODE Genetics. Methods have been previously published[24], but briefly, IHC for all four MMR proteins was performed on tissue microarrays (two cores of 1mm per tumor) using primary antibodies for MLH1 (Novacastra, Buffalo Grove, IL; NCL-L-MLH-1; Clone:ESO5; diluted 1:500), MSH2 (Calbiochem, [Merck Biosciences AG], Basel-Land, Switzerland; NA-27; Clone:FE11; diluted 1:3,000), MSH6 (Epitomics Inc, Burlingame, CA; AC-0047; Clone:EP49; diluted 1:800) and PMS2 (BD Pharmingen, San Jose, CA; 556415; clone:A16–4; diluted 1:300). If the tumor was absent for MLH1/PMS2 immunostaining, *MLH1* methylation testing was performed by pyrosequencing using the Pyromark Q96 CpG MLH1 kit (QIAGEN, Hilden, Germany) with ≥15% methylation classified as positive. All patients with abnormal IHC and no *MLH1* methylation underwent germline testing for MMR variants found by genome sequencing (GS) of 8,435 Icelanders. If no MMR PVs were identified, WGS was performed on blood samples with Illumina technology. In cases where blood DNA was not available, DNA from archived formalin-fixed paraffin embedded normal tissue was subjected to Sanger sequencing of the MMR genes. Tumor sequencing using ColoSeq Tumor was performed in MMR-deficient cases that remained unexplained after negative germline testing and *MLH1* methylation analysis. All Icelandic cases were

previously reported[24]. In cases of equivocal MMR staining with an identified germline PV, ColoSeq Tumor was done to determine the second pathogenic hit to the same MMR gene. The patient's previous cancer history and first-degree relative cancer history was obtained from deCODE Genetics and the Icelandic Cancer Registry, and three-generation pedigrees were created. Each pedigree was assessed for clinical characteristics and if Amsterdam II and Revised Bethesda criterion were met. PREMM5 scores were obtained for each patient using current age at study enrollment or age at death if patient was deceased and known family history as of 2/2018.

Classification of mutations for both cohorts:

Our approach to MMR variant interpretation has been described previously[15 17 25]. The Clinical Laboratory Improvement Amendments-approved laboratory (UW) adjudicated the pathogenicity of all germline mutations using criterion established by the American College of Medical Genetics and the International Agency for Research on Cancer guidelines[26 27]. All variants were reviewed by at least two clinical lab directors prior to interpretation. For tumor sequencing, cases were considered DS if two pathogenic or likely pathogenic somatic mutations were identified or if one pathogenic or likely pathogenic somatic mutation was identified with associated LOH. For patients with MMR-deficient tumors and a germline MMR variant of uncertain significance (VUS), tumors were assessed for additional MMR mutations or LOH to attempt to clarify the pathogenicity of the variant. Variants were reclassified as likely pathogenic when tumor screening results supported pathogenicity and one additional pathogenic mutation was identified in the tumor using methods previously described[15].

Statistics for both cohorts:

Descriptive statistics were provided. Continuous data were tested for normality with Shapiro-Wilk test and for homogeneity of variances with Bartlett test. When the above assumptions are satisfied, multiple regression analysis was used to analyze continuous data. Non-parametric Wilcoxon Rank Sum tests were used when the assumptions are violated. Pearson $\chi 2$ tests with continuity correction or Fisher exact test were used to analyze dichotomous variables. Meta-analysis, to combine results from two cohorts, was performed by applying Mantel-Haenszel test and Fixed Effect model with inverse variance method for dichotomous and continuous variables respectively. All tests were 2-sided, and level of significance was set at .05. Of note, there are two pairs of CRC patients in the Ohio cohort who are first-degree relatives (mother-daughter pair and sibling pair). Two of these patients (one from each pair) were excluded from statistical analysis as their close relation violated assumptions of the tests.

RESULTS

Ohio:

Of the 232 patients with a MMR-deficient tumor without methylation, 57.8% (n=134) had LS, 32.8% (n=76) had DS PVs, 6% (n=14) were unexplained, and 4.3% (n=10) had incorrect IHC (Table 1). Of patients with a true MMR deficient tumor without methylation or a germline MMR PV in the gene corresponding with their absent protein in cases with

abnormal IHC, 82% (73/89) have DS PVs, which is the highest percentage reported to date. The majority (60 %) of patients with absence of MLH1/PMS2 on IHC had DS PVs, and the other staining patterns (absence of MSH2/MSH6, isolated absence of MSH6, isolated absence of PMS2, normal IHC with a MSI-H tumor) were more predictive of LS (Table 1). Two patients are counted twice in Table 1: one has both LS (germline *PMS2* PV) and double somatic *MSH6* PVs (case 409149), and one has both LS (germline *MLH1* PV) and unexplained absence of MSH6 on IHC (case 417591). These two patients are only counted once in Table 2, under the LS column. The clinical characteristics of LS patients compared to DS and unexplained patients are presented in Table 2; germline and somatic mutations in the LS and DS cases are in Supplementary Tables 1 and 2. The average age of diagnosis for DS patients was older than LS patients (58.8 vs 52.4, $p=1.68\times10^{-3}$), but still younger than what would be expected in the general population[28]. Compared to patients with DS PVs, patients with LS were more likely to have synchronous or metachronous LS-associated tumors (33.9% vs 4.5%, $p= 1.64\times10^{-6}$), have a first-degree relative with CRC or EC (57.3% vs 18.2%, $p= 1.43\times10^{-7}$, meet Amsterdam criteria II (25.8% vs 1.5%, $p=4.40\times10^{-6}$) and revised Bethesda criteria (87.1% vs 48.5%, $p=2.47\times10^{-8}$), and have a higher median PREMM5 score (9.4% vs 2.45%, $p=6.97\times10^{-12}$) (Tables 2 and 3). Overall, nineteen patients had a MMR-deficient CRC plus a germline PV in a non-MMR gene (8 LS, 9 DS, 2 unexplained; Supplementary Table 3). These patients were included in Table 1 but not in Table 2, as it is possible that their germline PV may have contributed to their clinical characteristics and family history (particularly for the DS and unexplained patients). Supplementary Table 4 details the cases in the unexplained group. Case 506793 had a germline VUS in the MMR gene that was consistent with their missing proteins on IHC, plus one somatic PV in their tumor suggesting that this VUS could be pathogenic. In the DS group, case 369991 had a germline VUS in the MMR gene that was consistent with their missing proteins on IHC, plus two clearly pathogenic somatic PVs in their tumor suggesting that this VUS could be benign. MMR IHC results corresponded to the affected MMR gene in all cases except for the eleven with intact IHC and five with mutations in the unexpected member of the heterodimer pair (ex. two cases with germline *MSH6* mutations had absence of MSH2/MSH6 in their tumor, see Supplementary Tables 1 and 2).

Iceland:

Of all MMR-deficient CRCs without methylation, 45.1% (n=23) had LS, 31.4% (n=16) had DS PVs, 9.8% (n=5) were unexplained and 13.7% (n=7) had incorrect staining (see Table 1). Of patients with a MMR-deficient tumor without methylation or a germline MMR PV, 57.1% (16/28) have DS PVs. Absence of MLH1 or MSH2 on IHC was more predictive of DS than LS. Average age of diagnosis was not significantly different for DS compared to LS (69 vs 62, p=0.12; see Table 2). LS patients were significantly more likely to have higher median PREMM5 scores (6.0% vs 2.2%, $p=7.49\times10^{-2}$) and were borderline statistically more likely to fulfill Amsterdam II (34.8% vs 6.7%, $p=6.11\times10^{-2}$) and revised Bethesda criteria (82.6% vs 60%, $p=1.50\times10^{-1}$) (Table 3). There was no significant difference between metachronous (4.3% vs 0%, p=1) and synchronous tumors (8.7% vs 0%, $p=5.09\times10^{-1}$) in the two groups but a trend towards more first-degree relatives with CRC or EC was seen in the LS group (60.9% vs 40%, $p= 3.20\times10^{-1}$). All patients with MMR-deficient CRC with DS PVs underwent germline genome sequencing and one patient was

found to have a germline mutation in a non-MMR gene (*CHEK2*), as detailed in Supplementary Table 3. This patient is included in Table 1 but not in Table 2. MMR IHC results corresponded to the affected MMR gene.

DISCUSSION

It is becoming increasingly recognized that DS MMR PVs represent a group of cancers distinct from LS with a MMR-deficient phenotype that is caused by somatic PVs. In this paper we describe the clinical characteristics and family history of those with DS PVs and compare to those with LS from two large population-based studies that used similar algorithms for testing. We aimed to explore whether certain characteristics might predict LS over DS or vice versa (once *MLH1* methylation has been ruled out as appropriate in MLH1/PMS2 deficient tumors).

Because the germline mutational landscape is so different between the two populations, it is important to compare the DS cohorts to the LS cohorts from that population. Therefore, our results are presented separately for each population, as well as combined, in Tables 2 and 3. In the Ohio cohort, the most predictive characteristics of LS include meeting Amsterdam II and/or Revised Bethesda criterion, multiple primary tumors, PREMM5 score, a first-degree relative with CRC or EC, or having absence of MSH2/MSH6 on IHC or isolated absence of MSH6 or PMS2 on IHC. In the Icelandic cohort, the most predictive characteristics of LS include PREMM5 score, fulfilling Amsterdam II criteria, and having isolated absence of MSH6 or PMS2 on IHC. See Table 3 for a meta-analysis of the two cohorts. DS PVs were not as common as LS in either cohort. DS PVs were found in patients of all ages, ranging from 27–96 in the Ohio cohort and 41–88 in the Icelandic cohort with a median age higher than that of patients with LS.

In the Ohio cohort, younger age at diagnosis and having a metachronous or synchronous tumor was more predictive of LS, while the same was not true for the Icelandic cohort. Prior studies in Ohio have revealed that PV in *MSH2* are most common[5], and *MSH2* PV carriers have a higher lifetime risk of cancers as well as a younger age at diagnosis as compared to *MSH6* and *PMS2* PV carriers. In Iceland, *MSH6* and *PMS2* PV are most common with 96% of all LS-related CRC being related to these genes[24]. Therefore, it is not surprising to see less metachronous and synchronous tumors as well as a higher age at diagnosis in LS patients from Iceland given the reduced penetrance of those genes. It was rare to see synchronous or metachronous CRC in both the Ohio and Icelandic DS patients.

In both cohorts, DS PVs occurred more frequently in the *MLH1* and *MSH2* genes as compared to *MSH6* and *PMS2*. However, due to differences in LS mutational landscapes, staining pattern predictability differs between the two populations. In Ohio, an absence of MLH1/PMS2 is more likely related to DS while MSH2/MSH6 absence is more likely related to LS. In Iceland however, MLH1/PMS2 and MSH2/MSH6 absence are more likely to predict DS than LS. In both populations, sole absence of MSH6 or PMS2 is more likely to be due to LS.

Defining clinical characteristics between LS and DS patients will be helpful in the clinical setting, especially for counseling patients who are identified with abnormal IHC during routine universal tumor screening (see Table 1). For example, knowing that patients with absence of MLH1/PMS2 without *MLH1* methylation and little family history are unlikely to have LS, clinicians could consider ordering paired tumor-normal NGS since the majority of these patients will have DS PVs. Likewise, those with positive family history and absence of MSH2/6, MSH6, or PMS2 could proceed with germline testing first given the higher likelihood of LS compared to DS mutations.

The tumor location bears a somewhat different resemblance in the two populations where 76.8% of tumors in the DS groups are right-sided vs 59.1% in the LS groups. Dr. Lynch described years ago that LS-related tumors, while having a predilection for the right colon, could also arise on the left side and a rectal tumor location should not preclude the diagnosis of LS[29]. Mas-Moya et al. compared 45 patients with LS to 16 patients with LLS (presumably most of these were DS) and had similar findings with LLS patients being more likely to have a right-sided location, less likely to have isolated MSH6 loss and less likely to have synchronous and metachronous tumors[30].

PREMM5 scores were calculated for all patients with non-methylated MMR deficient tumors included in Table 2. PREMM5 scores were clearly higher in LS patients in both cohorts (Supplementary Tables 1 and 2). Of the Ohio patients, 87.9% of LS patients had at least a 2.5% predicted probability of carrying a MMR PV, which is the risk deemed appropriate for referral for genetic evaluation by the model creators[31], while 50% of DS and 42.9% of unexplained patients had a score of ≥2.5. Of the Icelandic patients, 78.3% of LS patients had a PREMM5 score ≥2.5% while 46.7% of DS and 25% of unexplained patients had a score of ≥2.5.

Although DS MMR-deficient tumors are now well described as a separate entity from LS, it is still unclear what causes these tumors and a lingering question remains as to whether these patients could have an unidentified inherited PV in an MMR or another cancer susceptibility gene. This is crucial to determine, as the cancer screening programs for those with DS MMR-deficient tumors, and their family members, could resemble those with sporadic CRC. Of note, all patients in the Ohio cohort underwent germline genetic testing with a panel of cancer susceptibility genes (ColoSeq, 12–22 genes or BROCA, 66 genes) with nine DS patients having mutations in other genes (2 biallelic *MUTYH*, 1 *RPS20*, 1 *GALNT12*, 1 *RAD51D* and *MUTYH* heterozygote, 2 *CHEK2*, 1 *NTHL1* heterozygote, 1 *POT1*). Of those nine patients, only two were diagnosed before age 50 and six did not have a family history concerning for hereditary predisposition. Similarly, all patients in the Icelandic cohort underwent genome sequencing and one DS patient was found to have a germline PV in another cancer susceptibility gene (*CHEK2*). This patient was under age 50 at diagnosis and had a PREMM5 score of 14.5. Over 10% (10/92) of DS MMR-deficient tumors in the two cohorts had a germline PV in a non-MMR cancer susceptibility gene. Aside from the two MUTYH-associated polyposis cases[16], it is not known whether these germline mutations contribute to the development of DS MMR-deficient tumors or are simply secondary findings. However, due to the high likelihood of a non-MMR cancer susceptibility PV being found in CRC patients with DS PVs, a large panel of hereditary cancer susceptibility genes

should always be considered to ensure patients with other syndromes are detected before establishing a sole diagnosis of DS PVs.

In our investigation of clinical characteristics, there are no findings that suggest that DS tumors are inherited. On the contrary, these individuals had less family history than those with LS, very low rates of synchronous and metachronous CRC and most do not fulfill Amsterdam II criteria. Of course, for DS patients who were diagnosed at a very young age or those who have a strong family history of cancer, an unknown hereditary syndrome cannot be ruled out and heightened surveillance may be warranted in those cases. The National Comprehensive Cancer Network (v1.2018) recommends that patients with MMR-deficient tumors without a germline mutation be managed based on family history[32].

In some cases, it is possible that DS tumors could be related to prior radiation or chemotherapy. A recent publication found DS MMR PVs in 70% of non-*MLH1* methylated MMR-deficient CRC in Hodgkin's lymphoma survivors from the Netherlands, which was higher than in a Dutch CRC population-based cohort[33]. Interestingly, 18.2% of the Ohio DS patients and 50% of the Iceland DS patients had a history of other malignancies. At least three of eight patients in the Iceland cohort did receive intraabdominal radiation (for prostate cancer, anal cancer and Hodgkin's disease), so it possible that these were indeed treatment related.

The remaining group of unexplained patients is likely a heterogeneous mix of individuals with missed germline MMR PVs (LS) and missed somatic PVs (DS) (see Supplementary Table 4). PREMM5 scores among the unexplained cases were higher than in DS, although we had few cases so no statistical comparisons were undertaken. We also included one patient in the unexplained group who had a germline VUS the MMR gene that matched their abnormal IHC. This patient had just one somatic MMR PV in addition to the germline MMR VUS, so it is possible that this patient actually does have LS. In addition, the Iceland cohort reported the first case of LS due to a chromosome translocation involving the *MLH1* gene, which cannot be detected by NGS [24]. There are certainly other structural rearrangements like this and the Boland inversion (*MSH2* exons 1–7[34]) that are not being detected by our current testing methodologies, which could potentially explain some of these unanswered cases.

The strengths of this study include the large size of this cohort which is the largest DS patient cohort presented to date as well as the fact that these cases were unselectively obtained from large population-based studies that screened all CRC cases for MMR deficiency. Limitations include the fact that prior cancer and family history was obtained by patient report in the Ohio study and the Icelandic study had few LS and DS cases, limiting the power of any statistical comparison between the two groups. In addition, MSI was not done on all patients in the Iceland study, so it is possible that there is an underrepresentation of unexplained MSI-H/intact IHC patients, as MSI was not assessed uniformly in that cohort.

In conclusion, we have shown that patients with DS MMR PVs do not appear to have features consistent with an inherited cancer syndrome and assessing clinical characteristics

such as family history, personal cancer history, PREMM5 scores and IHC staining patterns can help distinguish them from LS patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper:

MMR	Mismatch repair
CRC	colorectal cancer
LS	Lynch syndrome
LLS	Lynch-like syndrome
DS	double somatic
MSI	microsatellite instability
IHC	immunohistochemistry
LOH	loss of heterozygosity
OCCPI	Ohio Colorectal Cancer Prevention Initiative
IRB	Institutional Review Board
OSU	Ohio State University
NGS	next-generation sequencing
UW	University of Washington
PV	pathogenic variant
VUS	variant of uncertain significance

REFERENCES

1. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, Chadwick RB, Kaariainen H, Eskelinen M, Jarvinen H, Mecklin JP, de la Chapelle A. Incidence of hereditary

nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338(21):1481–7 [PubMed: 9593786]

2. Moreira L, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, Hopper JL, Le Marchand L, Gallinger S, Newcomb PA, Haile R, Thibodeau SN, Gunawardena S, Jenkins MA, Buchanan DD, Potter JD, Baron JA, Ahnen DJ, Moreno V, Andreu M, Ponz de Leon M, Rustgi AK, Castells A, Consortium E. Identification of Lynch syndrome among patients with colorectal cancer. JAMA 2012;308(15):1555–65 doi: 10.1001/jama.2012.13088[published Online First: Epub Date]|. [PubMed: 23073952]
3. Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM, Kolodner R. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res 1997;57(5):808–11 [PubMed: 9041175]
4. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Clendenning M, Sotamaa K, Prior T, Westman JA, Panescu J, Fix D, Lockman J, LaJeunesse J, Comeras I, de la Chapelle A. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26(35):5783–8 doi: 10.1200/JCO.2008.17.5950[published Online First: Epub Date]|. [PubMed: 18809606]
5. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, de la Chapelle A. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352(18):1851–60 doi: 10.1056/NEJMoa043146[published Online First: Epub Date]|. [PubMed: 15872200]
6. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. Genet Med 2009;11(1):35–41 [PubMed: 19125126]
7. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015;372(26):2509–20 doi: 10.1056/NEJMoa1500596[published Online First: Epub Date]|. [PubMed: 26028255]
8. Watson P, Lynch H. Cancer risk in mismatch repair gene mutation carriers. Familial Cancer 2001;1:57–60 [PubMed: 14574017]
9. Watson P, Vasen HF, Mecklin JP, Bernstein I, Aarnio M, Jarvinen HJ, Myrhoj T, Sunde L, Wijnen JT, Lynch HT. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. Int J Cancer 2008;123(2):444–9 doi: 10.1002/ijc.23508[published Online First: Epub Date]|. [PubMed: 18398828]
10. Rodriguez-Soler M, Perez-Carbonell L, Guarinos C, Zapater P, Castillejo A, Barbera VM, Juarez M, Bessa X, Xicola RM, Clofent J, Bujanda L, Balaguer F, Rene JM, de-Castro L, Marin-Gabriel JC, Lanas A, Cubiella J, Nicolas-Perez D, Brea-Fernandez A, Castellvi-Bel S, Alenda C, Ruiz-Ponte C, Carracedo A, Castells A, Andreu M, Llor X, Soto JL, Paya A, Jover R. Risk of cancer in cases of suspected lynch syndrome without germline mutation. Gastroenterology 2013;144(5): 926–32 e1; 10.1053/j.gastro.2013.01.044[published Online First: Epub Date]|. [PubMed: 23354017]
11. Boland CR. The mystery of mismatch repair deficiency: lynch or lynch-like? Gastroenterology 2013;144(5):868–70 doi: 10.1053/j.gastro.2013.03.014[published Online First: Epub Date]|. [PubMed: 23523847]
12. Sourrouille I, Coulet F, Lefevre JH, Colas C, Eyries M, Svrcek M, Bardier-Dupas A, Parc Y, Soubrier F. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. Fam Cancer 2013;12(1):27–33 doi: 10.1007/s10689-012-9568-9[published Online First: Epub Date]|. [PubMed: 22987205]
13. Mensenkamp AR, Vogelaar IP, van Zelst-Stams WA, Goossens M, Ouchene H, Hendriks-Cornelissen SJ, Kwint MP, Hoogerbrugge N, Nagtegaal ID, Ligtenberg MJ. Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in Lynch syndrome-like

tumors. Gastroenterology 2014;146(3):643–46 e8 doi: 10.1053/j.gastro.2013.12.002[published Online First: Epub Date]|. [PubMed: 24333619]

14. Geurts-Giele WR, Leenen CH, Dubbink HJ, Meijssen IC, Post E, Sleddens HF, Kuipers EJ, Goverde A, van den Ouweland AM, van Lier MG, Steyerberg EW, van Leerdam ME, Wagner A, Dinjens WN. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. J Pathol 2014;234(4):548–59 doi: 10.1002/path.4419[published Online First: Epub Date]|. [PubMed: 25111426]
15. Haraldsdottir S, Hampel H, Tomsic J, Frankel WL, Pearlman R, de la Chapelle A, Pritchard CC. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. Gastroenterology 2014;147(6):1308–16 e1 doi: 10.1053/j.gastro.2014.08.041[published Online First: Epub Date]|. [PubMed: 25194673]
16. Morak M, Heidenreich B, Keller G, Hampel H, Laner A, de la Chapelle A, Holinski-Feder E. Biallelic MUTYH mutations can mimic Lynch syndrome. Eur J Hum Genet 2014;22(11):1334–7 doi: 10.1038/ejhg.2014.15[published Online First: Epub Date]|. [PubMed: 24518836]
17. Pearlman R, Frankel WL, Swanson B, Zhao W, Yilmaz A, Miller K, Bacher J, Bigley C, Nelsen L, Goodfellow PJ, Goldberg RM, Paskett E, Shields PG, Freudenheim JL, Stanich PP, Lattimer I, Arnold M, Liyanarachchi S, Kalady M, Heald B, Greenwood C, Paquette I, Prues M, Draper DJ, Lindeman C, Kuebler JP, Reynolds K, Brell JM, Shaper AA, Mahesh S, Buie N, Weeman K, Shine K, Haut M, Edwards J, Bastola S, Wickham K, Khanduja KS, Zacks R, Pritchard CC, Shirts BH, Jacobson A, Allen B, de la Chapelle A, Hampel H, Ohio Colorectal Cancer Prevention Initiative Study G. Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. JAMA oncology 2017;3(4):464–71 doi: 10.1001/jamaoncol.2016.5194[published Online First: Epub Date]|. [PubMed: 27978560]
18. Castillejo A, Vargas G, Castillejo MI, Navarro M, Barbera VM, Gonzalez S, Hernandez-Illan E, Brunet J, Ramon y Cajal T, Balmana J, Oltra S, Iglesias S, Velasco A, Solanes A, Campos O, Sanchez Heras AB, Gallego J, Carrasco E, Gonzalez Juan D, Segura A, Chirivella I, Juan MJ, Tena I, Lazaro C, Blanco I, Pineda M, Capella G, Soto JL. Prevalence of germline MUTYH mutations among Lynch-like syndrome patients. Eur J Cancer 2014;50(13):2241–50 doi: 10.1016/j.ejca.2014.05.022[published Online First: Epub Date]|. [PubMed: 24953332]
19. Hemminger JA, Pearlman R, Haraldsdottir S, Knight D, Jonasson JG, Pritchard CC, Hampel H, Frankel WL. Histology of colorectal adenocarcinoma with double somatic mismatch repair mutations is indistinguishable from those caused by lynch syndrome. Hum Pathol 2018 doi: 10.1016/j.humpath.2018.04.017[published Online First: Epub Date]|.
20. Shia J, Tang LH, Vakiani E, Guillem JG, Stadler ZK, Soslow RA, Katabi N, Weiser MR, Paty PB, Temple LK, Nash GM, Wong WD, Offit K, Klimstra DS. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. Am J Surg Pathol 2009;33(11):1639–45 doi: 10.1097/PAS.0b013e3181b15aa2[published Online First: Epub Date]|. [PubMed: 19701074]
21. Newton K, Jorgensen NM, Wallace AJ, Buchanan DD, Lalloo F, McMahon RF, Hill J, Evans DG. Tumour MLH1 promoter region methylation testing is an effective prescreen for Lynch Syndrome (HNPCC). J Med Genet 2014;51(12):789–96 doi: 10.1136/jmedgenet-2014-102552[published Online First: Epub Date]|. [PubMed: 25280751]
22. Pritchard CC, Smith C, Salipante SJ, Lee MK, Thornton AM, Nord AS, Gulden C, Kupfer SS, Swisher EM, Bennett RL, Novetsky AP, Jarvik GP, Olopade OI, Goodfellow PJ, King MC, Tait JF, Walsh T. ColoSeq provides comprehensive lynch and polyposis syndrome mutational analysis using massively parallel sequencing. J Mol Diagn 2012;14(4):357–66 doi: 10.1016/j.jmoldx.2012.03.002[published Online First: Epub Date]|. [PubMed: 22658618]
23. Nord AS, Lee M, King MC, Walsh T. Accurate and exact CNV identification from targeted high-throughput sequence data. BMC genomics 2011;12:184 doi: 10.1186/1471-2164-12-184[published Online First: Epub Date]|. [PubMed: 21486468]
24. Haraldsdottir S, Rafnar T, Frankel WL, Einarsdottir S, Sigurdsson A, Hampel H, Snaebjornsson P, Masson G, Weng D, Arngrimsson R, Kehr B, Yilmaz A, Haraldsson S, Sulem P, Stefansson T, Shields PG, Sigurdsson F, Bekaii-Saab T, Moller PH, Steinarsdottir M, Alexiusdottir K, Hitchins M, Pritchard CC, de la Chapelle A, Jonasson JG, Goldberg RM, Stefansson K. Comprehensive

population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. Nature communications 2017;8:14755 doi: 10.1038/ncomms14755[published Online First: Epub Date]|.

25. Hampel H, Pearlman R, Beightol MB, Zhao W, Jones D, Frankel WF, Goodfellow PJ, Yilmaz A, Miller K, Bacher J, Jacobson A, Paskett E, Shields PG, Goldberg RM, de la Chapelle A, Shirts BH, Pritchard CC, Study TOCCP. Assessment of tumor sequencing as a replacement for current Lynch syndrome screening tests in colorectal cancer while also identifying therapeutic targets. JAMA oncology 2018;In Press
26. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405–24 doi: 10.1038/gim.2015.30[published Online First: Epub Date]|. [PubMed: 25741868]
27. Shirts BH, Casadei S, Jacobson AL, Lee MK, Gulsuner S, Bennett RL, Miller M, Hall SA, Hampel H, Hisama FM, Naylor LV, Goetsch C, Leppig K, Tait JF, Scroggins SM, Turner EH, Livingston R, Salipante SJ, King MC, Walsh T, Pritchard CC. Improving performance of multigene panels for genomic analysis of cancer predisposition. Genet Med 2016 doi: 10.1038/gim.2015.212[published Online First: Epub Date]|.
28. Society AC. Colorectal Cancer Facts & Figures 2017–2019. Atlanta: American Cancer Society, 2017.
29. Lin KM, Shashidharan M, Ternent CA, Thorson AG, Blatchford GJ, Christensen MA, Lanspa SJ, Lemon SJ, Watson P, Lynch HT. Colorectal and extracolonic cancer variations in MLH1/MSH2 hereditary nonpolyposis colorectal cancer kindreds and the general population. Dis Colon Rectum 1998;41(4):428–33 [PubMed: 9559626]
30. Mas-Moya J, Dudley B, Brand RE, Thull D, Bahary N, Nikiforova MN, Pai RK. Clinicopathological comparison of colorectal and endometrial carcinomas in patients with Lynch-like syndrome versus patients with Lynch syndrome. Hum Pathol 2015;46(11):1616–25 doi: 10.1016/j.humpath.2015.06.022[published Online First: Epub Date]|. [PubMed: 26319271]
31. Kastrinos F, Uno H, Ukaegbu C, Alvero C, McFarland A, Yurgelun MB, Kulke MH, Schrag D, Meyerhardt JA, Fuchs CS, Mayer RJ, Ng K, Steyerberg EW, Syngal S. Development and Validation of the PREMM5 Model for Comprehensive Risk Assessment of Lynch Syndrome. J Clin Oncol 2017;35(19):2165–72 doi: 10.1200/JCO.2016.69.6120[published Online First: Epub Date]|. [PubMed: 28489507]
32. The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines™). Genetic/Familial HIgh-Risk Assessment: Colorectal (Version 1.2018). 2018 National Comprehensive Cancer Network, Inc. www.nccn.org (accessed 12/21/2018).
33. Rigter LS, Snaebjornsson P, Rosenberg EH, Atmodimedjo PN, Aleman BM, Ten Hoeve J, Geurts-Giele WR, group P, van Ravesteyn TW, Hoeksel J, Meijer GA, Te Riele H, van Leeuwen FE, Dinjens WN, van Leerdam ME. Double somatic mutations in mismatch repair genes are frequent in colorectal cancer after Hodgkin's lymphoma treatment. Gut 2018;67(3):447–55 doi: 10.1136/gutjnl-2016-312608[published Online First: Epub Date]|. [PubMed: 29439113]
34. Rhees J, Arnold M, Boland CR. Inversion of exons 1–7 of the MSH2 gene is a frequent cause of unexplained Lynch syndrome in one local population. Fam Cancer 2014;13(2):219–25 doi: 10.1007/s10689-013-9688-x[published Online First: Epub Date]|. [PubMed: 24114314]

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Table 1.

Outcomes of non-methylated mismatch repair deficient colorectal cancer

IHC Result	N	Lynch syndrome	Double somatic	Unexplained	False positive IHC
Total combined	283	**157**	**92**	**19**	**17**
		55.6% of dMMR	**32.5% of dMMR**	**6.7% of dMMR**	**6% of dMMR**
Ohio					
Absent MLH1/PMS2	75	28[2] (37.3%)	**45 (60%)**	1[5] (1.3%)	1 (1.3%)
Absent MSH2/MSH6	80	**55 (68.8%)**	18 (22.5%)	6[5] (7.5%)	1 (1.3%)
Absent MSH6	33	**19 (57.6%)**	5[1] (15.2%)	3[2,5] (9.1%)	6[4] (18.2%)
Absent PMS2	29	**21 (72.4%)**	5 (17.2%)	1 (3.5%)	2 (6.9%)
IHC intact (MSI-H)	17	**11[1] (64.7%)**	3 (17.6%)	3 (17.6%)	0
Total Ohio	232	**134**	**76**	**14**	**10**
		57.8% of dMMR	**32.8% of dMMR**	**6% of dMMR**	**4.3% of dMMR**
Iceland					
Absent MLH1/PMS2	10	1 (10%)	**8 (80%)**	1[5] (10%)	0
Absent MSH2/MSH6	11	2 (18.2%)	**5 (45.5%)**	4 (36.4%)	0
Absent MSH6	12	**7 (58.3%)**	1 (8.3%)	0	4 (33.3%)
Absent PMS2	16	**11 (68.8%)**	2 (12.5%)	0	3 (18.8%)
IHC intact (MSI-H)	2	**2 (100%)[3]**	NA	NA	NA
Total Iceland	51	**23**	**16**	**5**	**7**
		45.1% of dMMR	**31.4% of dMMR**	**9.8% of dMMR**	**13.7% of dMMR**

[1] One patient had both a germline MMR pathogenic variant (PMS2) and double somatic pathogenic variants (MSH6) and is counted twice in the table percentages (Lynch syndrome normal IHC and double somatic MSH6) but once in the Total Ohio denominator

[2] One patient had both a germline MMR pathogenic variant (MLH1) and unexplained absence of MSH6 on IHC and is counted twice in the table percentages (Lynch syndrome MLH1/PMS2 and unexplained MSH6) but once in the Total Ohio denominator (Lynch)

[3] These two patients had weak MSH6 stains and had a pathogenic second hit to MSH6 on tumor testing

[4] One rectal cancer post RT, biopsy was MMR proficient

[5] Four Ohio unexplained patients and one Iceland unexplained patient had insufficient material for tumor sequencing

NA; Not assessed

Table 2.

Clinical Characteristics of patients with double somatic pathogenic variants or unexplained tumors compared to Lynch syndrome

Characteristic	Ohio Lynch syndrome	Iceland Lynch syndrome	Lynch syndrome combined	Ohio double somatic	Iceland double somatic	Double somatic combined	Ohio unexplained	Iceland unexplained	Unexplained combined
OHIO	**n=1241,2,5,6**	**n=23**	**n=147**	**n=661[5]**	**n=151**	**n=81**	**n=71,4,6**	**n=44**	**n=11**
Age (Average, Range)	52.4 (20–86)	62 (31–86)	53.9 (20–86)	58.8 (27–96)	69 (45–88)	60.7 (27–96)	65 (44–84)	69.3 (30–94)	66.7 (30–94)
20–29	3 (2.4%)	0	3 (2%)	1 (1.5%)	0	1 (1.2%)	0	0	0
30–39	16 (12.9%)	1 (4.3%)	17 (11.6%)	4 (6%)	0	4 (4.9%)	0	1 (25%)	1 (9.1%)
40–49	37 (29.8%)	2 (8.8%)	39 (26.5%)	13 (19.7%)	1 (6.7%)	14 (17.3%)	1 (14.3%)	0	1 (9.1%)
50–59	34 (27.4%)	6 (26.1%)	40 (27.2%)	13 (19.7%)	4 (26.7%)	17 (21%)	1 (14.3%)	0	1 (9.1%)
60–69	22 (17.7%)	7 (30.4%)	29 (19.7%)	19 (28.8%)	2 (13.3%)	21 (25.9%)	3 (42.9%)	0	3 (27.3%)
70–79	10 (8.1%)	6 (26.1%)	16 (10.9%)	13 (19.7%)	5 (33.3%)	18 (22.2%)	1 (14.3%)	2 (50%)	3 (27.3%)
80–89	2 (1.6%)	1 (4.3%)	3 (2%)	2 (3%)	3 (20%)	5 (6.2%)	1 (14.3%)	0	1 (9.1%)
90–99	0	0	0	1 (1.5%)	0	1 (1.2%)	0	1 (25%)	1 (9.1%)
Gender									
Male	70 (56.5%)	18 (78.3%)	88 (59.9%)	33 (50%)	8 (53.3%)	41 (50.6%)	7 (100%)	1 (25%)	8 (72.7%)
Female	54 (43.5%)	5 (21.7%)	59 (40.1%)	33 (50%)	7 (46.7%)	40 (49.4%)	0	3 (75%)	3 (27.3%)
Self-reported race									
Caucasian	108 (87.1%)	23 (100%)	131 (89.1%)	60 (90.9%)	15 (100%)	75 (92.6%)	6 (85.7%)	4 (100%)	10 (90.9%)
African-American	11 (8.8%)	0	11 (7.5%)	5 (7.6%)	0	5 (6.2%)	1 (14.3%)	0	1 (9.1%)
Asian	4 (3.2%)	0	4 (2.7%)	0	0	0	0	0	0
Other	1 (0.8%)	0	1 (0.7%)	1 (1.5%)	0	1 (1.2%)	0	0	0
Not reported	0	0	0	0	0	0	0	0	0
Tumor location3	n=139	n=25	n=164	n=67	n=15	n=82	n=8	n=4	n=12
Right	82 (59%)	15 (60%)	97 (59.1%)	51 (76.1%)	12 (80%)	63 (76.8%)	2 (25%)	2 (50%)	4 (33.3%)
Left	33 (23.7%)	7 (28%)	40 (24.4%)	8 (11.9%)	2 (13.3%)	10 (12.2%)	1 (12.5%)	1 (25%)	2 (16.7%)
Rectosigmoid	3 (2.2%)	0	3 (1.8%)	3 (4.5%)	1 (6.7%)	4 (4.8%)	1 (12.5%)	0	1 (8.3%)
Rectum	21 (15.1%)	3 (12%)	24 (14.6%)	5 (7.5%)	0	5 (6.1%)	4 (50%)	1 (25%)	5 (41.7%)
Unknown	0	0	0	0	0	0	0	0	0

Characteristic	Ohio Lynch syndrome	Iceland Lynch syndrome	Lynch syndrome combined	Ohio double somatic	Iceland double somatic	Double somatic combined	Ohio unexplained	Iceland unexplained	Unexplained combined
OHIO	**n=1241,2,5,6**	**n=23**	**n=147**	**n=661**[5]	**n=151**	**n=81**	**n=71,4,6**	**n=44**	**n=11**
Stage (TNM)									
I	34 (27.4%)	4 (17.4%)	38 (25.9%)	12 (18.2%)	1 (6.7%)	13 (16%)	2 (28.6%)	2 (50%)	4 (36.4%)
II	37 (29.8%)	14 (60.9%)	51 (34.7%)	28 (42.2%)	9 (60%)	37 (45.7%)	1 (14.3%)	2 (50%)	3 (27.3%)
III	36 (29%)	3 (13%)	39 (26.5%)	24 (36.4%)	4 (26.7%)	28 (34.6%)	3 (42.9%)	0	3 (27.3%)
IV	12 (9.7%)	2 (8.7%)	14 (9.5%)	2 (3%)	0	2 (2.5%)	0	0	0
Unavailable	5 (4%)	0	5 (3.4%)	0	1 (6.7%)	1 (1.2%)	1 (14.3%)	0	1 (9.1%)
Other self-reported malignancy									
Synchronous colon cancer	13 (10.5%)	2 (8.7%)	15 (10.2%)	1 (1.5%)	0	1 (1.2%)	1 (14.3%)	0	1 (9.1%)
Metachronous colon cancer	15 (12.1%)	1 (4.3%)	16 (10.9%)	0	0	0	1 (14.3%)	0	1 (9.1%)
Endometrial cancer	13 (10.5%)	1 (4.3%)	14 (9.5%)	1 (1.5%)	0	1 (1.2%)	0	0	0
Breast cancer	1 (0.8%)	0	1 (0.7%)	1 (1.5%)	0	1 (1.2%)	0	1 (25%)	1 (9.1%)
Ovarian cancer	0	0	0	0	0	0	0	0	0
Stomach cancer	2 (1.6%)	1 (4.3%)	3 (2%)	0	1 (6.7%)	1 (1.2%)	0	0	0
Small bowel cancer	3 (2.4%)	0	3 (2%)	0	0	0	0	0	0
Urinary tract	4 (3.2%)	1 (4.3%)	5 (3.4%)	1 (1.5%)	2 (13.3%)	3 (3.7%)	1 (14.3%)	0	1 (9.1%)
Hepatobiliary tract	0	0	0	0	0	0	0	0	0
Sebaceous neoplasm	4 (3.2%)	1 (4.3%)	5 (3.4%)	0	0	0	0	0	0
Brain tumor	0	0	0	0	0	0	0	0	0
Cervical cancer	2 (1.6%)	0	2 (1.4%)	1 (1.5%)	0	1 (1.2%)	0	0	0
Other	4 (3.2%)	2 (8.7%)	6 (4.1%)	9 (13.6%)	5 (33.3%)	14 (17.3%)	1 (14.3%)	0	1 (9.1%)
None	77 (62.1%)	17 (73.9%)	94 (64%)	54 (81.8%)	8 (53.3%)	62 (76.5%)	5 (71.4%)	3 (75%)	8 (72.7%)
≥2 LS malignancies	42 (33.9%)	4 (17.4%)	46 (31.3%)	3 (4.5%)	2 (13.3%)	5 (6.2%)	2 (28.6%)	0	2 (18.2%)
Clinical Criteria									
Amsterdam II	32 (25.8%)	8 (34.8%)	40 (27.2%)	1 (1.5%)	1 (6.7%)	2 (2.5%)	0	0	0
Amsterdam II using PREMM5 cancers	44 (35.5%)	8 (34.8%)	52 (35.4%)	3 (4.5%)	1 (6.7%)	4 (4.9%)	1 (14.3%)	0	1 (9.1%)

Characteristic	Ohio Lynch syndrome	Iceland Lynch syndrome	Lynch syndrome combined	Ohio double somatic	Iceland double somatic	Double somatic combined	Ohio unexplained	Iceland unexplained	Unexplained combined
OHIO	**n=1241,2,5,6**	**n=23**	**n=147**	**n=661[5]**	**n=151**	**n=81**	**n=71,4,6**	**n=44**	**n=11**
Revised Bethesda	108 (87.1%)	19 (82.6%)	127 (86.4%)	32 (48.5%)	9 (60%)	41 (50.6%)	5 (71.4%)	2 (50%)	7 (63.6%)
PREMM5 (%) (Median, IQR)	9.4 (3.9–30.1)	6 (2.9–16.5)	9 (3.6–25.7)	2.45 (1.5–5.4)	2.2 (1.7–3.7)	2.4 (1.6–4.7)	2.3 (1.8–4.2)	1.5 (1.0–3.6)	2.0 (1.6–4.2)
PREMM5 ≥2.5%	109 (87.9%)	18 (78.3%)	127 (86.4%)	33 (50%)	7 (46.7%)	40 (49.4%)	3 (42.9%)	1 (25%)	4 (36.4%)
≥1 FDRs with CRC or EC	71 (57.3%)	14 (60.9%)	85 (57.8%)	12 (18.2%)	6 (40%)	18 (22.2%)	1 (14.3%)	1 (25%)	2 (18.2%)

[1]Eight Ohio dMMR LS patients, nine Ohio dMMR DS patients, one Iceland dMMR DS patient, and two Ohio dMMR unexplained patients were excluded from this table due to having germline pathogenic variants in non-MMR genes and potential for phenotype bias

[2]Two Ohio dMMR LS patients were excluded from this table due to being first-degree relative pairs (mother-daughter pair and sibling pair). One from each pair were excluded (the proband was retained).

[3]Tumors may exceed number of patients due to synchronous tumors

[4]Four Ohio unexplained patients and one Iceland unexplained patient had insufficient material for tumor sequencing and were excluded from this table as it is not known whether they had DS PV or were truly unexplained

[5]The patient with a germline PMS2 pathogenic variant and double somatic MSH6 pathogenic variants is included in the Ohio LS column

[6]The patient with a germline MLH1 pathogenic variant and unexplained absence of MSH6 is included in the Ohio LS column

The maximum percentage of PREMM5 is >50%

Three generation pedigrees were not available for all patients. 10 LS patients, 23 DS patients, and 3 unexplained patients provided FDR cancer history only

Table 3.

Meta-Analysis of clinical characteristics of Lynch syndrome patients relative to double somatic patients

Characteristic	Ohio		Iceland		Meta-Analysis	
	OR	p-value	OR	p-value	OR	p-value
≥2 LS malignancies	10.65	1.64×10^{-6}	1.36	1	6.67	3.31×10^{-5}
Amsterdam II	22.39	4.40×10^{-6}	7.14	6.11×10^{-2}	15.81	8.47×10^{-6}
Amsterdam II using PREMM5 cancers	11.44	8.31×10^{-7}	7.14	6.11×10^{-2}	10.58	8.40×10^{-7}
Revised Bethesda	7.08	2.47×10^{-8}	3.06	1.50×10^{-1}	6.13	1.25×10^{-8}
PREMM5 ≥2.5%	7.17	4.17×10^{-8}	3.95	7.94×10^{-2}	6.44	5.47×10^{-9}
≥1 FDRs with CRC or EC	5.97	1.43×10^{-7}	2.28	3.20×10^{-1}	4.93	4.20×10^{-7}

OR=odds ratio of Lynch syndrome patients relative to double somatic patients