Is it all Lynch Syndrome? An assessment of family history in individuals with mismatch repair deficient tumors

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Abstract

Background & Aims—Mismatch repair deficient (MMRD) colorectal (CRC) and endometrial (EC) cancers may be suggestive of Lynch syndrome (LS). LS can only be confirmed by positive germline testing. It is unclear if individuals with MMRD tumors but no identifiable cause (MMRD+/germline−) have LS. As LS is hereditary, individuals with LS are expected to

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have family histories of LS-related tumors. Our study compared the family histories of MMRD+/germline− CRC and/or EC patients to LS CRC and/or EC patients.

Methods—253 individuals with an MMRD CRC or EC from one institution were included in analysis in 1 of 4 groups: LS, MMRD+/germline−, MMRD+/VUS, sporadic MSI-H (MMRD tumor with *MLH1* promoter hypermethylation or *BRAF* mutation). Family histories were analyzed utilizing MMRpro and PREMM$_{1,2,6}$. Kruskal-Wallis tests were used to compare family history scores.

Results—MMRD+/germline− individuals had significantly lower median family history scores (MMRpro=8.1, PREMM$_{1,2,6}$=7.3) than LS individuals (MMRpro=89.8, PREMM$_{1,2,6}$=26.1, p<0.0001).

Conclusion—MMRD+/germline− individuals have less suggestive family histories of LS than individuals with LS. These results imply that MMRD+/germline− individuals may not all have LS. This finding highlights the need to determine other causes of MMRD tumors so that these patients and their families can be accurately counseled regarding screening and management.

Keywords

lynch syndrome; mismatch repair deficient tumor; genetic testing; tumor studies

INTRODUCTION

Lynch syndrome (LS), also known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant hereditary cancer syndrome that confers a significantly increased lifetime risk of colorectal cancer (CRC) and endometrial cancer (EC) as well as an increased risk of a number of other cancers.[1–5] LS accounts for 2–4% of CRCs [6] and approximately 2% of ECs.[7] It is caused by germline mutations in the mismatch repair (MMR) genes *MLH1, MSH2, MSH6* and *PMS2*, as well as *EPCAM/TACSTD1*.[8,9]

Tumor studies, consisting of microsatellite instability (MSI) and immunohistochemistry (IHC) have proven to be effective at identifying individuals at risk of having LS. A universal tumor testing approach has been shown to have a sensitivity of up to 100% and a specificity of up to 93%.[10] Approximately 95% of LS-related colorectal cancers are found to be MSI-high (MSI-H). Loss of staining on IHC of one or more proteins is indicative of a somatic or germline defect in the MMR genes. Tumors that exhibit high MSI and/or loss of staining on IHC are considered to be MMR deficient (MMRD) and warrant further investigation.

In the case of a tumor that is MSI-H with loss of hMLH1 and hPMS2 on IHC, sporadic causes, including *MLH1* hypermethylation and/or a *BRAF* V600E mutation in CRC and *MLH1* hypermethylation in EC, must be ruled out. *BRAF* V600E mutations are indicative of *MLH1* hypermethylation in individuals with CRC. *BRAF* V600E mutations are not related to *MLH1* hypermethylation in individuals with EC. Individuals with MMRD tumors lacking these known sporadic causes should undergo LS germline testing, including sequencing and deletion/duplication analysis of the appropriate MMR gene(s), which, if positive, confirms a diagnosis of LS.

As tumor studies have become more widespread, there is an emerging cohort of individuals who have MMRD tumors lacking known sporadic causes (*MLH1* promoter hypermethlation and/or *BRAF* V600E mutations in CRC, *MLH1* promoter hypermethylation in EC), but no identifiable mutation (MMRD+/germline−). For example, in a recent study, 23 of 59 patients (38.9%) with MMRD tumors lacking known sporadic cause who pursued genetic testing had uninformative negative genetic test results.[11] It is unclear if these individuals truly have LS. There are two possible explanations as to why this cohort has emerged. The first is that these individuals do have LS, but our current genetic testing technology is not sensitive enough to detect the germline mutations in these individuals. There are documented other rare heritable causes of LS, including constitutional *MLH1* hypermethlation and complex rearrangements of the MMR genes that cannot currently be detected by clinically available genetic testing technology. [12] The second possible explanation is that these individuals do not have LS and there is another explanation for the MMRD tumor phenotype, such as epigenetic or somatic changes or modifier genes. In addition to *MLH1* hypermethylation and *BRAF* V600E mutations, it has recently been discovered that, biallelic somatic mutations in the MMR genes are also possible [13,14] and can account for up to 50% of unexplained MMRD tumors.[15] In addition, while rare, somatic mosaicism has been observed.[14]

From a clinical standpoint, a family history can be a very powerful risk assessment tool, and can either significantly increase the concern or significantly decrease the concern for a hereditary cancer syndrome. As LS is a hereditary condition, we would generally expect individuals with LS to have a family history of LS-related cancers. Therefore, in order to examine the likelihood of Lynch syndrome in MMRD+/germline− individuals, we analyzed and compared the family histories of MMRD+/germline− individuals, LS individuals, MMRD+/VUS individuals and sporadic MSI-H individuals, to determine if there was a difference in suggestiveness of family history between the groups.

Materials and Methods

Patient and Data Collection

The study sample included probands who presented to the University of Texas MD Anderson Cancer Center for genetic counseling for an MMRD CRC and/or EC from January 1995 to October 2012. Personal and tumor related information was collected for all probands from the MD Anderson electronic medical record. Pedigrees for all probands were obtained from the Clinical Cancer Genetics MD Anderson database. Individuals were excluded if tumor study results, germline testing results or a pedigree were not available. Individuals with tumor studies performed only on tissues other than colon or endometrium were excluded. MMRD tumors were defined as one of the following: MSS with loss of staining on IHC; MSI-H with staining intact on IHC; MSI-H with loss of staining on IHC. Individuals who were MSS with intact staining were not included in the initial query of the database. If multiple members of a family were seen for genetic counseling, only the individual who presented initially was included for analysis. Individuals who had a personal or family history indicative of another hereditary cancer syndrome were excluded. Individuals with tumors exhibiting low MSI and normal IHC who underwent germline

testing are not included in statistical analysis due to the lack of consensus that these tumor study results should be considered suggestive of LS and the lack of consistent referral of this patient population for genetic counseling and testing. The study protocol was approved by the University of Texas MD Anderson Cancer Center on November 1, 2012.

Risk Assessment Models

Pedigree information was quantified utilizing both PREMM$_{1,2,6}$ (available through the Dana Farber Cancer Institute at: http://premm.dfci.harvard.edu/) and MMRpro 5.1 (available through University of Texas Southwestern's *CancerGene© Version 5.1,* available from http://www4.utsouthwestern.edu/breasthealth/cagene/). Both models are clinically validated risk assessment tools that provide the likelihood of identifying a germline mutation in one of the MMR genes (*MLH1, MSH2,* or *MSH6*) in the proband by taking into account personal and family history information.

If exact ages of family members were not available, conservative estimation based on available information was utilized. Individuals for whom limited information was available were excluded from pedigree analysis. Half-siblings were not used in pedigree analysis. MMRpro input can include MSI and IHC results, but not *MLH1* hypermethylation and/or *BRAF* V600E mutation results. MMRpro has been validated for use both with and without inclusion of tumor study results.[16] Because everyone in the study population has abnormal tumor study results, and our purpose in calculating the MMRpro score was to summarize the suggestiveness of the personal and family history without reference to tumor study results, we chose to not include the tumor study results when running MMRpro. Additionally, PREMM$_{1,2,6}$ does not incorporate tumor study results.

Statistical Analysis

Summary statistics were performed to analyze the demographic, clinical and genetic characteristics of the patients. Chi-squared test, Fisher's exact test, or Kruskal-Wallis test were conducted to compare demographic characteristics between germline testing groups and pairwise comparisons were performed to determine statistical significance between groups. To control for multiple comparisons, a Bonferroni correction was used with alpha defined as 0.008. A Kruskal-Wallis test was conducted to compare family history scores. A Wilcoxon rank sum test was conducted to compare all pairwise comparisons. To control for multiple comparisons, a Bonferroni correction was used for the pairwise comparisons where statistical significance was defined at the alpha = 0.008 level. Wilcoxon rank sum test were also conducted to compare germline testing groups by colon/endometrial, gender and age. For these pairwise comparisons, a Bonferonni correction was used where statistical significance was defined at the alpha = 0. 01 level. A logistic regression model was also conducted with a term for family history as a predictor to predict the odds of testing germline positive. The model controlled for the following variables: age, gender, cancer type and ethnicity. All analyses were performed using STATA/SE 12.1.

Results

The information for 274 individuals was collected. These individuals were classified into one of four groups: LS, MMRD+/germline−, variant of uncertain significance (MMRD+/VUS) and known sporadic (sporadic MSI-H), defined as tumors with the presence of *MLH1* hypermethylation and/or *BRAF* V600E mutation in CRC and *MLH1* hypermethylation in EC. 21 individuals with MSI-low and tumors with intact IHC were excluded from further analysis. Of the final population of 253 individuals with MMRD tumors, 97 (80 with CRC, 12 with EC, 5 with both) had a mutation identified on germline testing (38.3%), 70 (58 with CRC, 12 with EC) had no mutation identified on germline testing and no sporadic cause identified (27.7%), 31 (28 CRC, 3 EC) were found to have a VUS on germline testing (12.3%), and 55 (40 CRC, 15 EC) were found to have a sporadic tumor (21.7%).

Demographic, Clinical and Pathological Characteristics

Demographic information for the final population (n=253) is summarized in table 1. Statistically significant differences between groups (alpha = 0.05) were identified for age at cancer diagnosis (p=<0.0001), ethnicity (p=0.0402), and type of cancer (p=0.0476). Overall, mean age of diagnosis was 51.5 years (SD=13.2). Average age of diagnosis was 48.3 years (SD=12.6) for the LS group, 51.3 years (SD=12.7) for the MMRD+/germline− group, and 46.2 years (SD=8.9) for the MMRD+/VUS group, significantly younger than the sporadic MSI-H group (average age of diagnosis = 60.5 years, SD =13.0, p<0.008).

Tumor characteristics for individuals with CRC overall (n=211) and between groups (LS n=85; MMRD+/germline− n=58; MMRD+/VUS n=28; sporadic MSI-H n=40) are summarized in table 2. Statistical significance was reached for gender (p=0.0228) and additional polyps identified at time of diagnosis (p=0.0034). 64.7% of the overall population (n=116) had no additional polyps at the time of diagnosis. Individuals in the LS group were more likely to have additional polyps at time of cancer diagnosis (47%) than the sporadic MSI-H group (p<0.008) and individuals in the MMRD+/VUS group were significantly less likely to have additional polyps at the time of diagnosis (9.1%) than the LS group (p<0.008).

Tumor characteristics for individuals with EC overall (n=47) and between groups (LS n=17; MMRD+/germline− n=12; MMRD+/VUS n=3, sporadic MSI-H n=15) are summarized in Table 3. There was a significant difference between groups for location of tumor (p=0.0407). Overall, the majority of tumors were located in the uterine body (78.7%). 100% of sporadic MSI-H tumors were located in the uterine body. There was also a significant difference between the IHC results of the various groups (p=0.002). Overall, IHC revealed loss of hMLH1 and hPMS2 in 23 tumors (48.9%) and loss of hMSH2 and hMSH6 in 16 tumors (34.0%). In general, the LS group had more tumors with loss of hMSH2 and hMSH6 (58.8%) and hMSH6 only (11.8%). As expected, the sporadic MSI-H group had significantly more individuals with loss of hMLH1/hPMS2 on IHC (100%) than the other groups (p<0.008). Average body mass index (BMI) of individuals with EC varied significantly between groups (p=0.0138). Overall average BMI for the EC group was 28.8. Individuals in the LS group on average had a BMI of 23.7, lower than the overall group and the three other groups (MMRD+/germline− =30.5 BMI; MMRD+/VUS =26.8 BMI; sporadic MSI-H =31.9 BMI).

Germline Testing

Of the 97 individuals identified to have a mutation in one of the MMR genes, 26 had mutations in *MLH1* (26.8%), 45 had mutations in *MSH2* (46.4%), 18 had mutations in *MSH6* (18.6%), 7 had mutations in *PMS2* (9.3%) and 1 had a mutation in *EPCAM* (1.0%). Of the 31 individuals identified to have a VUS in one of their MMR genes, 16 had a VUS in *MLH1* (51.6%), 12 had a VUS in *MSH2* (38.7%), 2 had a VUS in *MSH6* (6.5%) and 1 had a VUS in *PMS2* (3.2%).

Family History Assessment

Median family history scores of LS, MMRD+/germline−, MMRD+/VUS, and sporadic MSI-H were assessed and compared (Figure 1). The median family history scores were 89.8 (range 0.0–100) for MMRpro and 26.1 (range 5.0–97.6) for $PREMM_{1,2,6}$ for the LS group, 8.1 (range 0.0–100) for MMRpro and 7.3 (range 5.0–93.1) for $PREMM_{1,2,6}$ for the MMRD +/germline− group, 28.0 (range 0.0–99.8) for MMRpro and 11.1 (range 5.0–82.5) for $PREMM_{1,2,6}$ for the MMRD+/VUS group, 0.7 (range 0.0–94.0) for MMRpro and 5.0 (range 5.0–37.4) for $PREMM_{1,2,6}$ for the sporadic MSI-H group. In light of the reduced penetrance in *MSH6*- and *PMS2*-associated LS as compared to *MLH1*- and *MSH2*-associated LS, the LS group also was split into *MLH1/MSH2* positive and *MSH6/PMS2* positive. The median family history scores were 95.1 (range 0.3–100.0) for MMRpro and 38.7 (range 5.0–97.6) for $PREMM_{1,2,6}$ for the *MLH1/MSH2* positive group; and 7.7 (range 0.0–92.1) for MMRpro and 7.3 (range 5.0–65.0) for $PREMM_{1,2,6}$ for the *MSH6/PMS2* positive group.

The overall LS group had significantly higher family history scores on both MMRpro and $PREMM_{1,2,6}$ than the MMRD+/germline− group (MMRpro p<0.0001; $PREMM_{1,2,6}$ p<0.0001), the MMRD+/VUS group (MMRpro p=0.0063; $PREMM_{1,2,6}$ p=0.0038) and the sporadic MSI-H group (MMRpro p<0.0001; $PREMM_{1,2,6}$ p<0.0001). The MMRD+/ germline− group had significantly higher median family history scores on both MMRpro and $PREMM_{1,2,6}$ than the sporadic MSI-H group (MMRpro p<0.0001; $PREMM_{1,2,6}$ p<0.0001). There was no significant difference between the MMRD+/germline− group and the MMRD+/VUS group for both MMRpro (p=0.1924) and $PREMM_{1,2,6}$ (p=0.0249). The MMRD+/VUS group had significantly higher family history scores than the sporadic MSI-H group for both MMRpro (p<0.0001) and $PREMM_{1,2,6}$ (p<0.0001). When the LS group was split into *MLH1/MSH2* positive and *MSH6/PMS2* positive and compared with the MMRD +/germline− group, it was observed that the *MLH1/MSH2* positive group had significantly higher family history scores than the MMRD+/germline− group (MMRpro p<0.001; $PREMM_{1,2,6}$ p<0.001). The *MSH6/PMS2* positive group had similar family history scores to the MMRD+/germline− group (MMRpro p=0.5933; $PREMM_{1,2,6}$ p=0.6938).

When the family history scores were compared by gene implicated for the LS group and the MMRD+/germline− group, it was revealed that the difference between these two groups was driven by the family history scores of individuals with *MLH1* and *MSH2* mutations (table 5). There was a significant difference between the family history scores of LS individuals with an *MLH1* mutation versus MMRD+/germline− individuals with loss of hMLH1/ hPMS2 on IHC for both MMRpro (p<0.0001) and $PREMM_{1,2,6}$ (p<0.0001). The difference between the LS group with *MSH2* mutations and the MMRD+/germline− group with loss of

hMSH2 /hMSH6 on IHC was trending towards significance for both MMRpro (p=0.0812) and PREMM$_{1,2,6}$ (p=0.0536).

Family history scores were also compared based on cancer site. There was no significant difference in the family history scores between CRC LS and EC LS (MMRpro p=0.3446; PREMM$_{1,2,6}$ p=0.3130), CRC MMRD+/germline− and EC MMRD+/germline− (MMRpro p=0.1878; PREMM$_{1,2,6}$ p=0.6832) or CRC MMRD+/VUS and EC MMRD+/VUS (MMRpro p=0.6884; PREMM$_{1,2,6}$ p=0.7888). Individuals with EC in the KS group had significantly higher family history scores than individuals with CRC in the KS group on both MMRpro (p=0.0039) and PREMM$_{1,2,6}$ (p=0.0016).

Both family history modalities were significant predictors of testing germline positive in the logistic regression analysis. For every one unit increase in MMRpro, the odds of being germline positive increase by a factor of 1.02 (95% CI: 1.01 – 1.03; p < 0.001). For every one unit increase in PREMM$_{1,2,6}$, the odds of being germline positive increase by a factor of 1.04 (95% CI: 1.02 – 1.05; p = < 0.001).

Discussion

Individuals with MMRD+/germline− tumors have significantly lower median family history scores than individuals with LS, indicating that they have family histories that are less suggestive of a hereditary cancer syndrome. Based on these results, and the growing body of literature surrounding MMRD tumors, it is plausible to consider that these individuals have a distinct disease from classic LS. Tumor studies may not be considered diagnostic of LS in the absence of an unidentifiable germline mutation, particularly when family history is entirely absent. Interestingly, individuals with MMRD+/germline− tumors have significantly higher median family history scores than individuals with a MSI-H sporadic tumor, suggesting that it would also be incorrect to assume that all MMRD+/germline− tumors are secondary to sporadic or epigenetic causes.

In addition, other data support the notion that MMRD+/germline tumors may be due to a combination of hereditary and sporadic causes. Endometrial cancer patients with LS had an average BMI in the normal range, while the remaining 3 groups had average BMIs in the overweight to obese range. Obesity is a known risk factor for sporadic EC. Therefore, the high BMIs in the MMRD+/germline− group, similar to what was seen in the sporadic group, point towards the possibility of sporadic causes for the MMRD+/germline− ECs. In addition, individuals in the overall MMRD+/germline− group had an average age of diagnosis that was similar to the average age of diagnosis in the LS group. The average age of diagnosis was significantly younger than the sporadic MSI-H group and what has been observed in the general population.[17] It was similar to what has been described as average age of LS-related cancer onset in the literature [18], suggesting the possibility of a hereditary component for the MMRD+/germline− group.

MMRD+/germline− individuals lie in the middle of the family history spectrum. Possible explanations as to why this is include the following: the cohort could be a mixture of individuals with true LS, in whom our current genetic testing technology is not sensitive

enough to detect their mutation, and individuals who do not have LS, but rather have an MMRD sporadic tumor. It is also possible that this cohort represents a currently undefined hereditary cancer syndrome or subset of LS, with lower cancer risks than true LS, but increased risks over the general population.

Within the LS group, individuals with an *MLH1* or *MSH2* mutation had significantly higher median family history scores than individuals with an *MSH6* or *PMS2* mutations. This was expected, given the lower lifetime risks of cancer with *MSH6* and *PMS2* mutations. The difference between the family history scores of the MMRD+/germline− group and the LS group are driven by the family history scores of individuals with an *MLH1* or an *MSH2* mutation, both overall and when the MMRD+/germline− group and the LS group are compared by gene implicated. Overall family history scores of MMRD+/germline− individuals were more similar to family history scores of individuals with an *MSH6* or a *PMS2* mutation. This could indicate that the cancer risks for MMRD+/germline− individuals are more similar to those of individuals with an *MSH6* or a *PMS2* mutation, as shown by Rodriguez-Soler et al.[19] It could also indicate that some individuals in the MMRD+/germline− group may have MMRD tumors due to a low penetrant germline mutation in a currently unidentified cancer predisposition gene. With regards to the importance of family history, a logistic regression model showed that the likelihood of testing germline positive increases with the strength, or the suggestive-ness, of the family history, even in this population of patients who all have abnormal tumor studies. In populations with no tumor studies, such as those utilized to design the models, the same correlation is observed. [16, 22] This further strengthens the argument that the MMRD+/germline− group is likely a heterogeneous group of MMRD tumors secondary to heritable causes and MMRD tumors secondary to sporadic causes.

We did observe a wide range of family history scores on both modalities in all four groups. This range was more pronounced with MMRpro than with PREMM$_{1,2,6}$. This observation is not unexpected in the MMRD+/VUS group, as this group is likely a mix of mutations that are deleterious and mutations that are polymorphisms. It is well documented that the family history of LS is variable and thus also unsurprising that there is a wide range of scores observed in the LS group. The sporadic MSI-H group tended to have lower family history scores, however there were a number of outliers on both modalities. It is possible that the family history of cancer in these families is due to common environmental exposure, low penetrant genes, or a combination of the two. Finally, the fact that we observed a wide range of scores in the MMRD+/germline− group strengthens the argument that this group is likely a mixture of hereditary and sporadic, although it is impossible set an absolute cut off for what is LS in this group as we observed individuals with a confirmed diagnosis of LS with no family history, as well as individuals in the MMRD+/germline− group with highly suggestive family histories.

As universal tumor screening protocols have become more widespread, the MMRD+/germline− cohort has only continued to expand. While the body of evidence suggesting that not all MMRD+/germline− tumors are secondary to LS, we currently have no way of distinguishing between false positive tumor studies and true positive tumor studies, and this must be acknowledged as a limitation of universal tumor screening strategies. In addition,

clinical management of these individuals and their families becomes quite complicated. At this time, we recommend that these individuals follow the same screening guidelines as individuals with LS, as no clinical tools exist yet to distinguish LS and MMRD+/germline−. However, it is quite possible that we are subjecting individuals with MMRD+/germline− individuals and their families to unnecessary invasive screening procedures. Based on young average age of diagnosis in the probands seen in our study and the young age of cancer onset in relatives[19], it seems reasonable to begin offering cancer screenings at a younger age than recommended for the general population. But the question of exactly how to screen MMRD +/germline− individuals and their families remains. Following these individuals with general population screening recommendations when an increased risk of cancer and an increased family history of cancer have been observed could mean we would begin missing preventable cancers. However, it is also likely inappropriate to be subjecting these individuals to increased surveillance when they do not appear to have the same cancer risks as individuals and families with LS.[19] Until we are able to definitely determine who has LS, and who does not, it will continue to be difficult to make appropriate screening recommendations for MMRD+/germline− individuals. Further work needs to be done to further define the CRC and extra-colonic cancer risks in MMRD+/germline− individuals in order to develop appropriate surveillance recommendations.

Recent population based studies also support these conclusions. Rodriguez-Soler et al [19] showed that families of individuals with MMRD tumors but no identifiable germline mutation have a lower risk of developing colorectal cancer in their lifetime than LS families, but a higher risk than individuals with a known sporadic tumor. Additionally, they showed that the family histories of individuals with LS tended to be more suggestive than those with no identifiable germline mutation. Buchanan et al [20] showed that tumor studies alone have a low positive predictive value amongst endometrial cancer patients. There has been increasing research into other possible sporadic causes of MMRD tumors, [13,14, 20], with Mesencamp et al [15] showing that up to 50% of unexplained abnormal tumor studies may be due to biallelic somatic mutations in the MMR genes. The only clinical testing readily available, however, continues to be *MLH1* hypermethylation and *BRAF* V600E mutation analysis. Therefore, at this time we are unable to determine the number of MMRD+/ germline− individuals who truly have a sporadic tumor secondary to biallelic somatic mutations. As our tumor genetic testing technology continues to improve and become more widespread, this will likely change. Many of these recent population based studies provided some analysis of family history. Our study, however, is the first to our knowledge to provide an in depth analysis of the family histories of individuals with abnormal tumor studies on both endometrial and colorectal tumors.

The limitations of this study are as follows. There were a number of individuals in the MMRD+/germline− group who were lost to follow up or who were deceased before a complete genetic work-up could be completed. It is possible that some of these individuals truly had LS or truly had a sporadic MSI-H tumor, but the appropriate testing was never performed. 6 were lost to follow up with no sporadic tumor work up, and 24 only had MLH1-hypermethylation performed. Despite this, the MMRD+/germline− group still had less suggestive family histories than the LS group and removing these six individuals from our data analysis did not affect the final outcome of the study (data not shown). Although

rare, some individuals with LS have tumors that display *MLH1* hypermethylation[23], and there are also cases of heritable constitutional *MLH1* hypermethylation[24]. Therefore we cannot exclude that there may be individuals in the MSI-H sporadic group who truly have LS or who have constitutional *MLH1* hypermethylation, but based on the rarity of these phenomena, this would be unlikely to affect the overall conclusions of the study.

Because this was not a population-based study, there is the potential for referral bias. For colorectal cancers diagnosed or treated prior to 2009, the practice at MD Anderson Cancer Center was to perform tumor studies on and/or provide a genetics referral only for "high risk" individuals. After 2009, MD Anderson Cancer Center adopted a more universal tumor studies approach for colorectal cancers. Tumor studies for endometrial cancer, however, have only routinely been performed at MDACC since August 2012. Regardless of this potential bias, MMRD+/germline− individuals referred still had less suggestive family histories than LS individuals. It is possible that the schism between family history scores for MMRD+/germline− and LS could be more pronounced in the general population.

Possible future directions from this study include sequencing the tumor DNA of all the individuals in the MMRD+/germline− group to determine how many have biallelic somatic MMR mutations. The family histories of those with biallelic somatic MMR mutations and those whose MMRD tumor continues to be unexplained could then be compared. This could further strengthen the argument presented here.

In conclusion, individuals with MMRD+/germline− have a less suggestive family history than individuals with LS, but a more suggestive family history than individuals with a sporadic MSI-H tumor and a similar family history to individuals with a MMRD+/VUS. These results further reinforce the need to continue exploring other causes of MMRD tumors, as it does not all appear to be classical LS. As our understanding of other somatic and epigenetic causes of MMRD tumors expands, we need to reevaluate our current testing practices and develop other clinical testing to rule out all known somatic and epigenetic causes. We may also need to reconsider the current screening guidelines for individuals with MMRD+/germline−, as we may be subjecting these individuals to unnecessary invasive surveillance.

Acknowledgments

Abbreviations

CRC	colorectal cancer
EC	endometrial cancer
IHC	immunohistochemistry
MSI	microsatellite instability
MMR	mismatch repair

MMRD	mismatch repair deficient
LS	Lynch Syndrome
MMRD+/germline−	mismatch repair deficient tumor with no identifiable germline mutation
MMRD+/VUS	mismatch repair deficient tumor with variant of uncertain significance
Sporadic MSI-H	mismatch repair deficient tumor with MLH1 promoter hypermethylation or a BRAF mutation

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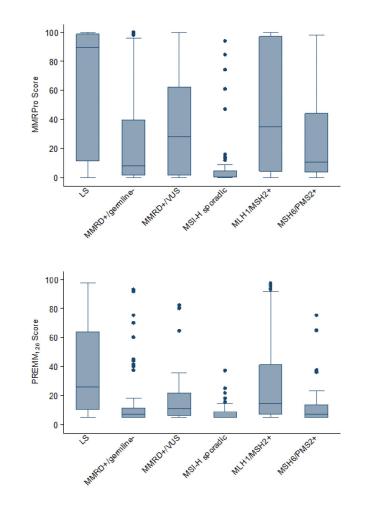
Figure 1.
Median family history scores

Table 1

Demographic Information

	Germline Testing Group										Total (n = 253)		
	LS (n = 97)		MMRD+/Lynch− (n = 70)		MMRD+/VUS (n = 31)		sporadic MSI-H (n = 55)				Total (n = 253)		
	N	%	N	%	N	%	N	%			N	%	p-value
Age [a, b, c]													**<0.0001**
N	97		70		31		55				253		
Mean (SD)	48.3 (12.6)		51.3 (12.7)		46.2 (8.9)		60.5 (13.0)				51.5 (13.2)		
Min (Med) Max	23 (48) 83		24 (50) 81		30 (46) 67		25 (60) 83				23 (50) 83		
Vital Status													0.5280
Alive	79	81.4	62	88.6	28	90.3	48	87.3			217	85.8	
Deceased	18	18.6	8	11.4	3	9.7	7	12.7			36	14.2	
Ethnicity [c]													**0.0402**
Caucasian	77	80.2	57	81.4	18	58.1	49	89.1			201	79.8	
African American	6	6.3	4	5.7	4	12.9	1	1.8			15	6.0	
Hispanic	9	9.4	6	8.6	2	6.5	2	3.6			19	7.5	
Asian	3	3.1	3	4.3	6	19.4	3	5.5			15	6.0	
Other	1	1.0	0	0.0	1	3.2	0	0.0			2	0.8	
Tumor Location													**0.0476**
Colon	80	82.5	58	82.9	28	90.3	40	72.7			206	81.4	
Endometrial	12	12.4	12	17.1	3	9.7	15	27.3			42	16.6	
Both	5	5.2	0	0.0	0	0.0	0	0.0			5	2.0	
Other Cancer													0.1498
No	50	51.5	47	67.1	20	64.5	36	65.5			153	60.5	
Yes	47	48.5	23	32.9	11	35.5	19	34.5			100	39.5	
Number of other cancers													
0	50	51.5	47	67.1	20	64.5	36	65.5			153	60.5	
1	26	26.8	16	22.9	9	29.0	12	21.8			63	24.9	
2	10	10.3	5	7.1	1	3.2	3	5.5			19	7.5	
3 or more	11	11.3	2	2.9	1	3.2	4	7.3			18	7.1	

Significant pairwise comparisons (alpha < 0.008):

[a]LS vs. Sporadic MSI-H;

[b]MMRD+/germline− vs Sporadic MSI-H;

[c]MRD+/VUS vs. sporadic MSI-H

Table 2

Colorectal Cancer Characteristics

		Germline Testing Group									
		LS (n = 85)		MMRD+/germline− (n = 58)		MMRD+/VUS (n = 28)		sporadic MSI-H (n = 40)		Total (n = 211)		p-value
		N	%	N	%	N	%	N	%	N	%	
Gender												
	Male	47	58.8	28	48.3	18	64.3	13	32.5	106	51.5	**0.0228**
	Female	33	41.3	30	51.3	10	35.7	27	67.5	100	48.5	
Location												--†
	Ascending	38	44.7	31	53.4	16	57.1	25	62.5	110	52.1	
	Transverse	16	18.8	11	19.0	4	14.3	12	30.0	43	20.4	
	Descending	12	14.1	6	10.3	4	14.3	3	7.5	25	11.8	
	Rectum	19	22.4	10	17.2	4	14.3	0	0.0	33	15.6	
Histology												>0.9999
	Adenocarcinoma	84	98.8	58	100.0	28	100.0	40	100.0	210	99.5	
	Tubular adenoma	1	1.2	0	0.0	0	0.0	0	0.0	1	0.5	
Grade												0.6595
	1	4	4.9	2	3.6	0	0.0	2	5.0	8	3.9	
	2	57	69.5	38	67.9	20	71.4	22	55.0	137	66.5	
	3	21	25.6	16	28.6	8	28.6	16	40.0	61	29.6	
Stage												--†
	I	23	29.1	7	12.3	3	10.7	3	7.9	36	17.8	
	II	26	32.9	21	36.8	11	39.3	14	36.8	72	35.6	
	III	19	24.1	18	31.6	9	32.1	13	34.2	59	29.2	
	IV	11	13.9	11	19.3	5	17.9	8	21.1	35	17.3	
Additional Polyps [a]												**0.0034**
	No	35	53.0	35	76.1	20	90.9	26	68.4	116	67.4	
	Yes	31	47.0	11	23.9	2	9.1	12	31.6	56	32.6	
IHC (staining absent)												--†
	MLH1&PMS2*	20	25.3	34	61.8	14	50.0	39	100.0	107	53.2	

	Germline Testing Group										
	LS (n = 85)		MMRD+/germline–(n = 58)		MMRD+/VUS (n = 28)		sporadic MSI-H (n = 40)		Total (n = 211)		p-value
	N	%	N	%	N	%	N	%	N	%	
MSH2 & MSH6 *	38	48.1	10	18.1	8	28.6	0	0.0	56	27.9	
PMS2	6	7.6	3	5.5	2	7.1	0	0.0	11	5.5	
MSH6	12	15.2	5	9.1	2	7.1	0	0.0	19	9.5	
No loss staining	3	3.8	3	5.5	2	7.1	0	0.0	8	4.0	
MSI											0.0560
High	67	100.0	40	90.9	23	95.8	33	97.1	163	96.4	
Low	0	0.0	1	2.3	0	0.0	1	2.9	2	1.2	
MSS	0	0.0	3	6.8	1	4.2	0	0.0	4	2.4	

*
There were 21 individuals overall (5 LS, 9 MMRD+/germline–, 1 MMRD+/VUS and 6 sporadic MSI-H) who had loss of staining for MLH1 alone and 1 individual overall (MMRD+/germline–) who had loss of staining for MSH2 alone before staining for PMS2 and MSH6 was available. These individuals were incorporated into the statistics for MLH1&PMS2 and MSH2&MSH6, as they would likely have stained negative for PMS2 and MSH6 if it had been available.

Significant pairwise comparisons (alpha < 0.008):

a
LS vs. MMRD+/VUS

+
Statistical significance (alpha < 0.008) could not be established, as numerous individuals were missing this demographic characteristic

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Table 3

Endometrial Cancer Characteristics

	Germline Testing Group										
	LS (n = 17)		MMRD+/germline– (n = 12)		MMRD+/VUS (n = 3)		sporadic MSI-H (n = 15)		Total (n = 47)		p-value
	N	%	N	%	N	%	N	%	N	%	
Location											**0.0407**
Lower Uterine	6	35.3	3	25.0	1	33.3	0	0.0	10	21.3	
Uterine Body	11	64.7	9	75.0	2	66.7	15	100.0	37	78.7	
Histology											0.1112
Endometrioid	14	82.4	10	83.3	2	66.7	14	93.3	40	85.1	
Serous	1	5.9	0	0.0	0	0.0	0	0.0	1	2.1	
Mixed high	2	11.8	0	0.0	0	0.0	1	6.7	3	6.4	
Clear cell	0	0.0	2	16.7	0	0.0	0	0.0	2	4.3	
Papillary	0	0.0	0	0.0	1	33.3	0	0.0	1	2.1	
Grade4											0.9867
1	3	20.0	2	18.2	0	0.0	2	13.3	7	16.3	
2	9	60.0	7	63.6	2	100.0	11	73.3	29	67.4	
3	3	20.0	2	18.2	0	0.0	2	13.3	7	16.3	
Stage											0.2890
I	7	53.8	5	55.6	0	0.0	8	61.5	20	52.6	
II	2	15.4	0	0.0	2	66.7	2	15.4	6	15.8	
III	3	23.1	4	44.4	1	33.3	2	15.4	10	26.3	
IV	1	7.7	0	0.0	0	0.0	1	7.7	2	5.3	
IHC [a] (staining absent)											**0.0002**
MLH1&PMS2 *	3	17.6	7	58.3	1	33.3	15	100.0	26	55.3	
MSH2 & MSH6 *	10	58.8	4	33.3	2	66.7	0	0.0	16	34.0	
PMS2	1	5.9	0	0.0	0	0.0	0	0.0	1	2.1	
MSH6	2	11.8	0	0.0	0	0.0	0	0.0	2	4.3	
No loss staining	1	5.9	1	8.3	0	0.0	0	0.0	2	4.3	
MSI											0.8603
High	9	90.0	8	88.9	3	100.0	11	91.7	31	91.2	

	Germline Testing Group										
	LS (n = 17)		MMRD+/germline−(n = 12)		MMRD+/VUS (n = 3)		sporadic MSI-H (n = 15)		Total (n = 47)		p-value
	N	%	N	%	N	%	N	%	N	%	
Low	1	10.0	0	0.0	0	0.0	1	8.3	2	5.9	
MSS	0	0.0	1	11.1	0	0.0	0	0.0	1	2.9	

*There were 3 individuals overall (1 LS, 1 MMRD+/germline−, 1 sporadic MSI-H) who had staining performed for MLH1 only before staining for PMS2 was available. These individuals have been added to the statistics for MLH1&PMS2, as they likely would have stained negative for PMS2 if it had been available.

Significant pairwise comparisons (alpha < 0.008):

[a]LS vs. Sporadic MSI-H

Table 4

LS family history scores versus MMRD+/germline− family history scores by gene implicated

Group	Gene Implicated	MMRpro					PREMM$_{1,2,6}$				
		N	Min	Med	Max	p-value	N	Min	Med	Max	p-value
LS	*MLH1*	26	4.3	98.4	99.8	**<0.0001**	26	5.5	49.3	96.0	**<0.0001**
MMRD+/germline−	hMLH1/hPMS2	31	0	6.9	87.3		31	5	5.6	45	
LS	*MSH2*	45	0.3	90.8	100.0	0.0812	45	0.1	36.7	100.0	0.0536
MMRD+/germline−	hMSH2/hMSH6	13	0.1	15.8	100.0		13	5.0	12.1	91.8	
LS	*MSH6*	18	0.3	7.3	91.7	0.9406	18	5.0	8.1	65.0	0.5000
MMRD+/germline−	hMSH6	5	0.0	11.1	74.5		5	5.0	7.1	10.5	