5-2013

Mismatch Repair Deficient Tumors Lacking Known Sporadic Causes: Are They All Due to Lynch Syndrome?

Katherine M. Dempsey

Mismatch Repair Deficient Tumors Lacking Known Sporadic Causes: Are They All Due to Lynch Syndrome?

by

Katherine Margaret Dempsey, B.A.

APPROVED:

Dr. Karen Lu, M.D.,
Supervisory Professor

Molly S. Daniels, M.S., C.G.C.

Dr. Russell Broaddus, M.D., Ph.D.

Dr. Y. Nancy You, M.D.

Sarah Jane Noblin, M.S., C.G.C.

APPROVED:

Dean, The University of Texas
Graduate School of Biomedical Sciences

MISMATCH REPAIR DEFICIENT TUMORS LACKING KNOWN SPORADIC CAUSES: ARE THEY ALL DUE TO LYNCH SYNDROME?

A

THESIS

Presented to the Faculty of the University of Texas Health Science Center at Houston

and The University of Texas MD Anderson Cancer Center

Graduate School of Biomedical Sciences

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

by

Katherine Margaret Dempsey, B.A.

Houston, TX

May 2013

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisory committee for their contributions to this project: Dr. Karen Lu, M. D. and supervisory chair, Dr. Y. Nancy You, M.D., Dr. Russell Broaddus, M. D., Ph.D., Sarah J. Noblin, M.S., C.G.C. and Molly Daniels, M.S., C.G.C. Thank you for your help, guidance, and immense wealth of knowledge about Lynch syndrome. Thank you to Maureen Mork, M.S., C.G.C., for her willingness to contribute to this project as well as an honorary committee member. This project would not have been possible without Bryan Fellman, M.S. and Diana Urbauer, M.S. Thank you for all your help with the statistics and the data generated by this project.

Thank you to all of the University of Texas Genetic Counseling Program's supervisors, faculty and staff. I would like to extend special thanks the program director Claire N. Singletary, M.S., C.G.C., the program assistant director, Sarah J. Noblin, M.S., C.G.C. and my mentor Marianna Raia, M.S., C.G.C. The guidance, encouragement, support and education I have received from all of you throughout my time as a genetic counseling student has been invaluable. To the UTGCP administrative assistant and "GC mom", Norma Leal: thank you for always having an open ear and open arms when we needed them.

To my classmates, Allison Copeland, Jessica Davis, Michelle McDougle, Nicole Mohrbacher, and Bradley Rolf: thank you all for taking this crazy journey with me and for providing me with constant support and encouragement along the way.

Finally, I would like to thank my friends and family, both near and far, for their love, support and understanding. I would especially like to thank my parents, Kevin and Natalie Dempsey, for always encouraging me to follow my dreams, even when those dreams take me across the country. To my younger brother Stephen, I am so grateful to have another "science nerd" in the family.

MISMATCH REPAIR DEFICIENT TUMORS LACKING KNOWN SPORADIC CAUSES: ARE THEY ALL DUE TO LYNCH SYNDROME?

Publication No. _____

Katherine Margaret Dempsey, B.A.

Supervisory Professor: Dr. Karen Lu, M.D.

BACKGROUND: Mismatch repair deficient (MMRD) colorectal (CRC) or endometrial (EC) cancers in the absence of *MLH1* promoter hypermethylation and *BRAF* mutations are suggestive of Lynch syndrome (LS). Positive germline genetic test results confirm LS. It is unclear if individuals with MMRD tumors but no identified germline mutation or sporadic cause (MMRD+/germline-) have LS.

HYPOTHESIS: Since LS is hereditary, individuals with LS should have a stronger family history of LS-related cancers than individuals with sporadic tumors. We hypothesized that MMRD+/germline- CRC and/or EC patients would have less suggestive family histories than LS CRC and/or EC patients.

METHODS: 253 individuals with an MMRD CRC or EC who underwent genetic counseling at 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 PREMM1,2,6. Kruskal-Wallis tests were used to compare family history scores. Logistic regression was used to determine what factors were predictive of LS.

RESULTS: MMRD+/germline- individuals had significantly lower median family history scores (PREMM1,2,6=7.3, MMRpro=8.1) than LS individuals (PREMM1,2,6=26.1, MMRpro=89.8, $p<0.0001$) and had significantly higher median family history scores than sporadic MSI-H (PREMM1,2,6 =5.0, $p=0.0013$, MMRpro=0.7, $p<0.0001$). Family history scores were positively correlated with likelihood of testing germline positive ($p<0.0001$).

CONCLUSION: MMRD+/germline- individuals have less suggestive family histories of LS than individuals with LS, but more suggestive family histories than sporadic MSI-H individuals. CRC and/or EC patients with abnormal tumor studies are more likely to have a germline LS mutation if they have a family history suggestive of hereditary cancer. These results imply that the MMRD+/germline- group may not all have LS. This finding highlights the need to determine other somatic, epigenetic or germline causes of MMRD tumors so that these patients and their families can be accurately counseled regarding screening and management.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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]. The natural history of LS has been well established and screening and treatment guidelines have been created based on this natural history for affected individuals.

Currently, the most effective way to identify individuals with possible LS is through tumor testing, which includes microsatellite instability (MSI) and immunohistochemistry (IHC). Approximately 95% of LS-related colorectal cancers are found to be MSI-high (MSI-H). It is important to note that approximately 10-15% of sporadic CRCs [10, 11] and 20-30% of sporadic ECs [12] are also MSI-high. 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). A recent study out of MD Anderson Cancer Center in Houston, TX found that there was a 97% concordance rate between MSI and IHC testing [13]. MMRD tumors can be indicative of a germline mutation and warrant further genetic counseling and testing. MMRD tumors can also be due to epigenetic and somatic causes, such as *MLH1* hypermethylation or a *BRAF* V600E mutation, two well documented sporadic causes of MMRD tumors exhibiting high MSI and loss of MLH1 and PMS2 on IHC. *MLH1* hypermethylation or *BRAF* mutations are extremely rare in individuals with LS and the presence of either indicates the tumor is most likely sporadic [15]. Further tumor studies are necessary to rule out both these known sporadic causes in CRC. *MLH1* hypermethylation has been seen in ECs, but *BRAF* V600E mutations have not been implicated as a cause of sporadic MSI-high ECs [16].

The gold standard for diagnosing individuals with LS continues to be germline testing. Individuals with MMRD tumors should undergo comprehensive germline testing, including sequencing and deletion/duplication analysis of the appropriate MMR gene(s). With positive germline testing, one can not only confirm a diagnosis of LS, but can also offer family members targeted mutation analysis. Germline testing has a specificity of greater than 99%, but has a low sensitivity ranging from 24% to 67%, depending on genes implicated, type of germline testing used and population studied [7, 17, 18].

As tumor studies have become more widespread, there is an emerging cohort of individuals who have MMRD tumors, but no identifiable mutation (MMRD+/germline-). For example, in a recent study, 23 of 59 patients (38.9%) with MMRD tumors who pursued genetic testing had uninformative negative genetic test results [19]. There are two possible explanations as to why this cohort has emerged: (1) these individuals do have LS, but our current genetic testing technology is not sensitive enough to detect the germline mutations in these individuals; or (2) these individuals do not have LS and there is another explanation for the phenotype of these individuals, such as epigenetic or somatic changes or modifier genes. Other potential rare heritable causes of MMRD tumors include constitutional *MLH1* hypermethylation and complex rearrangements of the MMR genes that cannot currently be detected by germline testing technology [20]. In addition to *MLH1* hypermethylation and *BRAF* V600E mutations, it has recently been discovered that, while rare, biallelic somatic mutations in the MMR genes are also possible [21, 22], as well as somatic mosaicism [22].

As this cohort has emerged, the question: “are all MMRD tumors due to Lynch syndrome?” has been raised by many. The purpose of this study is to contribute to the answer to this overarching question by establishing if there is a difference between the family histories of LS individuals and the family histories of MMRD+/germline- individuals. Because LS is hereditary, individuals with LS should, on average, have more family history of LS-related cancers than individuals with a sporadic CRC and/or EC. A caveat to this assumption are families with *MSH6* and *PMS2* mutations. Individuals with *MSH6* and *PMS2* mutations have lower lifetime risks of CRC and EC than individuals with *MLH1* and *MSH2* mutations. They also tend to be diagnosed at a later age than individuals with *MLH1* and

MSH2 mutations [17, 23]. We hypothesize that MMRD+/germline- CRC and/or EC patients have family histories that are less suggestive of LS than LS CRC and/or EC patients. The specific aims of the project are as follows: (1) to assemble a cohort of individuals who have or have had CRC and/or EC and were treated at the University of Texas MD Anderson Cancer Center; (2) to utilize MMRpro and PREMM1,2,6 to quantify the family histories of these individuals; and (3) to examine the relationship between family history and germline testing results.

MATERIALS AND METHODS

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. Prior to 2009, individuals referred for genetic counseling had a suggestive family history or young age of diagnosis that raised suspicion for LS. After 2009, reflex tumor studies for colorectal tumors began. After August 2012, reflex tumor studies for endometrial tumors began. The vast majority of the study population, therefore, was considered high risk and is not a reflection of the general population. 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. If multiple members of a family were seen for genetic counseling, only the individual who presented initially was analyzed. 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 were collected, but 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 both the University of Texas Health Science Center at Houston on September 11, 2012 and the University of Texas MD Anderson Cancer Center on November 1, 2012.

Personal and tumor related information was collected for all probands. Pedigrees for all probands were obtained from LOCUS, the Clinical Cancer Genetics MD Anderson database.

Risk Assessment Models

Pedigree information was quantified by both PREMM1,2,6 (available from: http://dana-farber.prod.dfcidev.org/pat/cancer/gastrointestinal/crc-calculator/default.asp) 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.

PREMM1,2,6 is based on a population of unrelated probands who submitted blood samples for MMR genetic testing. PREMM1,2,6 will take into account the following factors for the proband: gender, personal history of CRC, personal history of EC, age of diagnosis of EC or CRC, and personal history of other LS-related tumor(s). The model will also take into account the following factors for affected first- and second- degree relatives: number of individuals affected by CRC, number of individuals affected by EC, age of diagnosis of CRC or EC, and history of LS-related tumor(s) [24]. LS-related tumors are considered to be: ovarian, gastric, small bowel, hepatobiliary, renal/urinary tract, pancreatic, glioblastoma multiforme, and sebaceous gland tumors. PREMM1,2,6 uses a multivariate logistic regression to perform the risk analysis. The most important factors for PREMM1,2,6 are: proband with personal history of CRC and family history of CRC; proband with personal history of 2 or more CRCs; and any individual (proband, first- or second-degree relative) with EC [24].

MMRpro is based on theoretical modeling, not a specific study population. It attempts to answer the following question: what is the probability the proband carries a mutation in one of the MMR genes given the pattern of affected individuals and unaffected individuals in the family? MMRpro will take into account the following information for the family, which consists of the proband, first-degree relatives and second-degree relatives: CRC and location (proximal, distal or unknown), EC, age of onset, age of all unaffected relatives, ancestry and ethnicity, results of MSI/IHC testing, results of germline testing, and carrier status of family members [25]. MMRpro utilizes Bayesian analysis with mutation frequency, penetrance estimates, and non-carrier incidence based on literature reviews [25].

If exact ages 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. While MMRpro can take results from tumor studies into account in the risk calculation, we chose not to use this in our risk assessment. When results from tumor studies are included, they predominate in the calculated risk assessment, thus blunting the ability of the model to summarize the suggestiveness of the family history.

Statistical Considerations

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

Demographics

Information for 274 individuals who fell into one of four groups was collected: 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. An additional 21 individuals with MSI-low and tumors with intact IHC were excluded (figure 1).

Demographic information for the final population (n=253) is summarized in table 1 by comparing the four groups. Statistically significant differences between groups (alpha = 0.05) were identified for age at diagnosis (p=<0.0001), ethnicity (p=0.0402), type of cancer (p=0.0476) and additional polyps at time of cancer diagnosis (p=0.0034). Overall, mean age of diagnosis was 51.5 years (SD=13.2). Our overall population was predominantly Caucasian (n=201, 79.8%). 64.7% of the overall population (n=116) had no additional polyps at the time of diagnosis.

Average age of diagnosis for the LS group was 48.3 years (SD=12.6), younger than the sporadic MSI-H group (p<0.008). 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). Average age of diagnosis for the MMRD+/germline- group was 51.3 years (SD=12.7), significantly younger than the sporadic MSI-H group (p<0.008). Average age of diagnosis for the MMRD+/VUS group was 46.2 years (SD=8.9), significantly younger than the sporadic MSI-H group (p<0.008). 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). The MMRD+/VUS group had significantly more ethnic diversity than the sporadic MSI-H group (p <0.008). Average age of diagnosis for individuals in the sporadic MSI-H group was 60.5 years (SD=13.0, p <0.008), significantly older than the three other groups. EC was more common in the sporadic MSI-H group (27.3%). 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.

Tumor Characteristics

Tumor characteristics for CRCs 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). Overall, there were 106 males with CRC

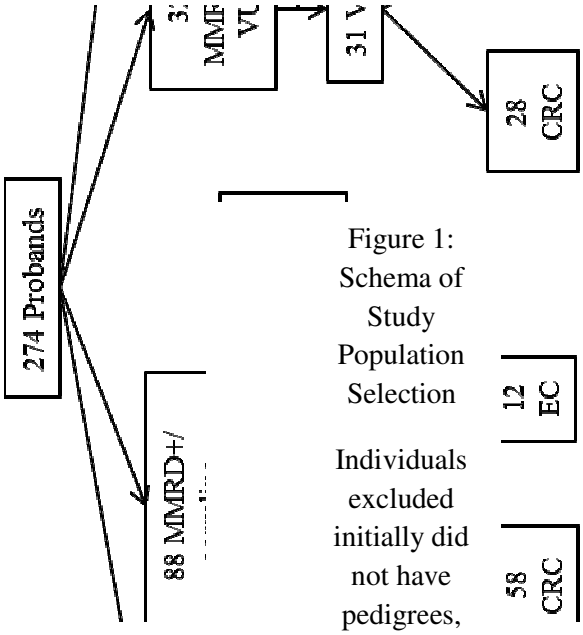

Figure 1: Schema of Study Population Selection

Individuals excluded initially did not have pedigrees, germline testing results or tumor studies results available; were not the proband in their family; had tumor studies performed on tissues aside from CRC or EC, or had a history consistent with another genetic condition. Individuals excluded later had MSI-low tumors with intact staining.

Table 1: Demographic Information

	Germline Testing Group										
	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 [c, e, f]											**<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 [f]											**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	
Additional Polyps in Colon [b]											**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	
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	5	5.2	0	0.0	0	0.0	2	3.6	7	2.8	
4	3	3.1	1	1.4	1	3.2	1	1.8	6	2.4	
5	2	2.1	0	0.0	0	0.0	1	1.8	3	1.2	
7	0	0.0	1	1.4	0	0.0	0	0.0	1	0.4	
8	1	1.0	0	0.0	0	0.0	0	0.0	1	0.4	

Significant pairwise comparisons (alpha < 0.008): [a] LS vs. MMRD+/germline-; [b] LS vs. MMRD+/VUS; [c] LS vs. Sporadic MSI-H; [d] MMRD+/germline- vs. MMRD+/VUS; [e] MMRD+/germline- vs Sporadic MSI-H; [f] MMRD+/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)		
	N	%	N	%	N	%	N	%	N	%	p-value
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	
IHC											--[+]
MLH1&PMS2*	20	25.3	34	61.8	14	50.0	39	100.0	107	53.2	
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 staining for MLH1 alone and 1 individual overall (MMRD+/germline-) who had 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.

[+] Statistical significance could not be established, as numerous individuals were missing this demographic characteristic

(51.5%) and 100 females with CRC (48.5%). In general, the sporadic MSI-H group had more females (67.5%) and the MMRD+/VUS group had more males (64.3%). Location, histology, grade and MSI results were similar among all groups. Overall, the majority of tumors were either stage II (35.6%) or stage III (29.2%). Individuals in the LS group were more likely to have a stage I tumor (29.1%). Overall, most tumors exhibited loss of *MLH1* and *PMS2* (n=86, 42.8%) followed by loss of *MSH2* and *MSH6* (27.4%) on IHC. In the positive group, there were more tumors with loss of *MSH2* and *MSH6* (48.1%) and *MSH6* only (15.2%) on IHC staining than the overall population.

Tumor characteristics for ECs 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. Location and IHC results were significantly different between groups (p=0.0407 and p=0.002 respectively). 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. Overall, IHC revealed loss of *MLH1* and *PMS2* in 23 tumors (48.9%) and loss of *MSH2* and *MSH6* in 16 tumors (34.0%). In general, the LS group had more tumors with loss of *MSH2* and *MSH6* (58.8%) and *MSH6* only (11.8%). As expected, the sporadic MSI-H group had significantly more individuals with loss of MLH1/PMS2 on IHC (100%) than the other groups ($p<0.008$). Histology, grade, stage and MSI results were similar among all groups.

Germline Testing

As expected, germline test results and IHC results were concordant for both the LS group and the MMRD+/VUS group. 46.4% (n=45) of LS individuals overall had a germline *MSH2* mutation. 48.1% (n=38) of LS CRC individuals had loss of MSH2 and MSH6 on IHC and 43.8% (n=35) had germline *MSH2* mutations identified. 58.8% (n=10) of LS EC patients had loss of MSH2 and MSH6 on IHC and 66.7% (n=8) had germline *MSH2* mutations identified. 40% (n=2) of women who had both CRC and EC had *MSH2* mutations. The two most common genes in which MMRD+/VUS were identified were *MLH1* (n=16, 50%) and *MSH2* (n=12, 37.5%). 50% (n=14) of MMRD+/VUS CRC)

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)		
	N	%	N	%	N	%	N	%	N	%	p-value
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	
Grade											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 [c]											**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	
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. MMRD+/germline-; [b] LS vs. MMRD+/VUS; [c] LS vs. Sporadic MSI-H; [d] MMRD+/germline- vs. MMRD+/VUS; [e] MMRD+/germline- vs Sporadic MSI-H; [f] MMRD+/VUS vs. Sporadic MSI-H

individuals had loss of MLH1 and PMS2 on IHC and 55.2% (n=16) had a MMRD+/VUS in *MLH1*. 28.6% (n=8) had loss of MSH2 and MSH6 on IHC and 31% (n=9) had a MMRD+/VUS in *MSH2*. No EC MMRD+/VUS individuals had a MMRD+/VUS in *MLH1*. 66.7% (n=2) had loss of MSH2 and MSH6 on IHC and 100% (n=3) had a MMRD+/VUS in *MSH2*. Overall, the majority of the mutations for the positive group were truncating mutations (n= 57, 58.8%). The majority of mutations for the MMRD+/VUS group were missense mutations (n= 29, 93.5%).

Family History Assessments

Summary statistics for family history scores for both MMRpro and PREMM1,2,6 can be found in table 4. The median family history scores for the LS group were higher on both modalities (MMRpro median =89.8; PREMM1,2,6 median =26.1) than the other three groups. There was a wide range of scores for both modalities (MMRpro range=0-100; PREMM1,2,6 range=5.0-97.6, figures 2 and 3). PREMM1,2,6 family history scores were lower than MMRpro scores for all groups except sporadic MSI-H. Significant measures between germline groups are summarized in table 5. Statistical significance (alpha = 0.0008) was reached for all comparisons for both MMRpro and PREMM1,2,6 except for MMRD+/germline- versus MMRD+/VUS (MMRpro p=0.1924; PREMM1,2,6 p=.0249).

Table 4: Family History Assessment Summary Statistics

	Germline Testing Group	N	Min	Med	Max
MMRpro	LS	97	0.0	89.8	100.0
	MMRD+/germline-	70	0.0	8.1	100.0
	MMRD+/VUS	31	0.0	28.0	99.8
	sporadic MSI-H	55	0.0	0.7	94.0
	Total	253	0.0	13.9	100.0
PREMM1,2,6	LS	97	5.0	26.1	97.6
	MMRD+/germline-	70	5.0	7.3	93.1
	MMRD+/VUS	31	5.0	11.1	82.5
	sporadic MSI-H	55	5.0	5.0	37.4
	Total	253	5.0	9.2	97.6

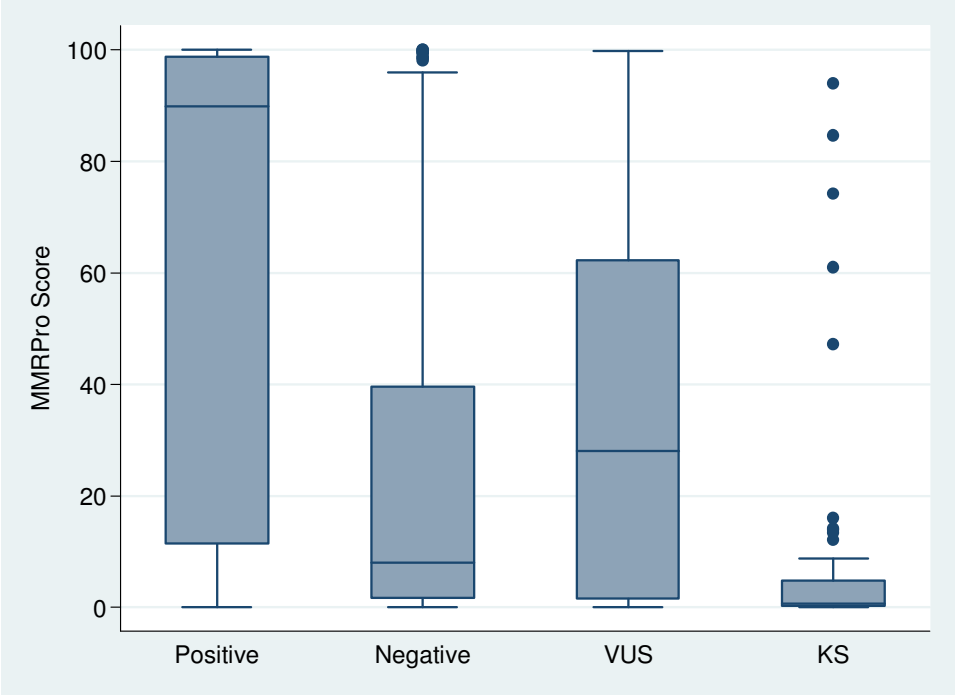

Figure 2: Median MMRpro Family History Scores

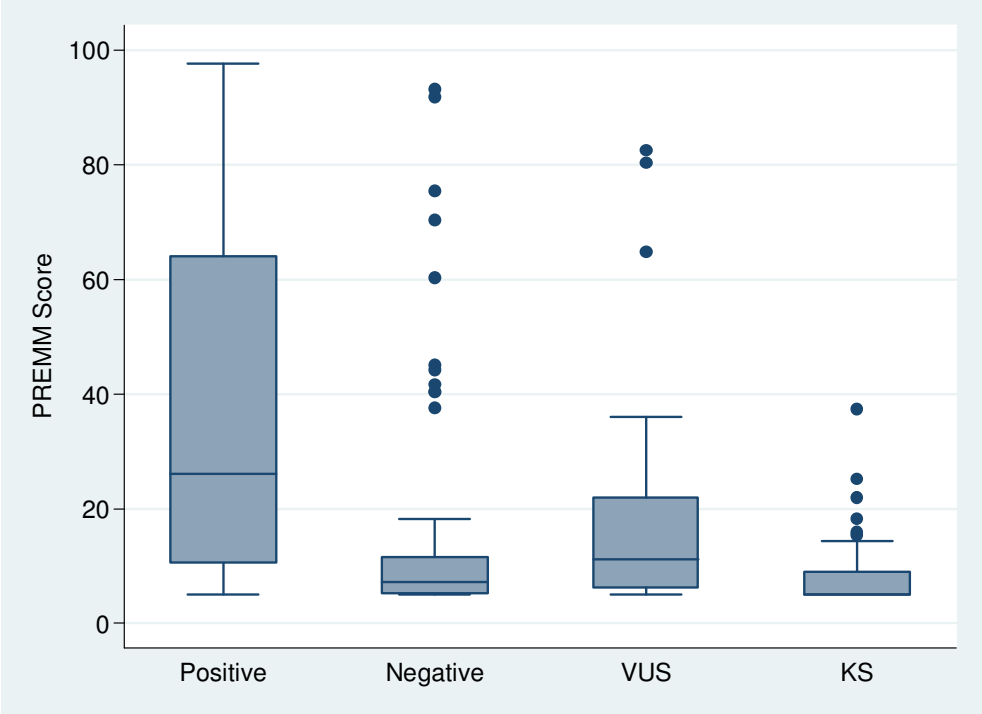

Figure 3: Median PREMM1,2,6 Family History Scores

Table 5: Significance of Family History Scores between Germline Groups

	p-value
MMRpro	**<0.0001**
LS vs. MMRD+/germline-	**<0.0001**
LS vs. MMRD+/VUS	**0.0063**
LS vs. sporadic MSI-H	**<0.0001**
MMRD+/germline-vs. MMRD+/VUS	0.1924
MMRD+/germline-vs. sporadic MSI-H	**<0.0001**
MMRD+/VUS vs. sporadic MSI-H	**<0.0001**
PREMM1,2,6	**<0.0001**
LS vs. MMRD+/germline-	**<0.0001**
LS vs. MMRD+/VUS	**0.0038**
LS vs. sporadic MSI-H	**<0.0001**
MMRD+/germline-vs. MMRD+/VUS	0.0249
MMRD+/germline-vs. sporadic MSI-H	**0.0013**
MMRD+/VUS vs. sporadic MSI-H	**<0.0001**

Family history scores were further stratified by location of cancer, age of diagnosis (<50 versus ≥50), gender for CRC only, and gene mutated in the LS group only. Summary statistics for CRC versus EC can be found in table 6 and comparison statistics can be found in table 7. Overall, the LS group had family history scores that were higher on both modalities for both CRC (MMRpro median =88.3; PREMM1,2,6 median =23.6) and EC (MMRpro median =96.3; PREMM1,2,6 median =36.5) than the other three groups. Statistical significance was reached for CRC sporadic MSI-H versus EC sporadic MSI-H on both modalities (MMRpro p=0.0039; PREMM1,2,6 p=0.0016). EC individuals in the sporadic MSI-H group had higher median family history scores (MMRpro median =2.7; PREMM1,2,6 median=8.7) than CRC individuals in the sporadic MSI-H group (MMRpro median=0.3; PREMM1,2,6 median=5.0).

Summary statistics for age of diagnosis can be found in table 8 and comparison statistics can be found in table 9. Overall, the LS group had the highest median family history scores between groups on both modalities for diagnosed <50 (MMRpro median =91.7; PREMM1,2,6 median=23.5) and diagnosed ≥50 (MMRpro median=64.7, PREMM1,2,6 median=30.6) than the other three groups. Statistical significance was reached for diagnosed <50 sporadic MSI-H versus diagnosed ≥50 sporadic MSI-H on both modalities. Individuals diagnosed <50 had higher median family history scores (MMRpro

median=5.2; PREMM1,2,6 median =7.9) than diagnosed ≥50 (MMRpro median =0.4; PREMM1,2,6 median =5.0).

Table 6: Colorectal Cancer versus Endometrial Cancer Summary Statistics

		MMRpro				PREMM1,2,6			
	Germline Group	N	Med	Min	Max	N	Med	Min	Max
CRC	LS	80	88.3	0.0	99.9	80	23.6	5.0	96.0
	MMRD+/germline-	58	9.7	0.0	100.0	58	7.3	5.0	93.1
	MMRD+/VUS	28	37.5	0.0	99.8	28	10.9	5.0	82.5
	sporadic MSI-H	40	0.3	0.0	84.6	40	5.0	5.0	25.1
EC	LS	12	96.3	1.8	100.0	12	36.5	5.0	97.6
	MMRD+/germline-	12	2.8	0.3	87.3	12	5.7	5.0	45.0
	MMRD+/VUS	3	20.9	6.1	28.0	3	14.1	8.3	16.7
	sporadic MSI-H	15	2.7	0.2	94.0	15	8.7	5.0	37.4

Table 7: Significance of Family History Scores between Germline Groups and Location of Tumor

	p-value
MMRpro	
CRC LS vs. EC LS	0.3446
CRC MMRD+/germline- vs. EC MMRD+/germline-	0.1878
Colon MMRD+/VUS vs. EC MMRD+/VUS	0.6884
CRC sporadic MSI-H vs. EC sporadic MSI-H	**0.0039**
PREMM1,2,6	
CRC LS vs. EC LS	0.3130
CRC MMRD+/germline- vs. EC MMRD+/germline-	0.6832
CRC MMRD+/VUS vs. EC MMRD+/VUS	0.7888
CRC sporadic MSI-H vs. EC sporadic MSI-H	**0.0016**

Table 8: Age of Diagnosis Summary Statistics

			MMRpro			PREMM1,2,6			
	Germline Group	N	Med	Min	Max	N	Med	Min	Max
Age < 50	LS	53	91.7	1.8	100.0	53	23.5	5.0	97.6
	MMRD+/germline-	32	15.1	0.9	100.0	32	7.3	5.0	91.8
	MMRD+/VUS	18	47.5	0.7	99.8	18	15.6	5.0	82.5
	sporadic MSI-H	11	5.2	0.5	74.2	11	7.9	5.0	25.1
Age ≥ 50	LS	44	64.7	0.0	100.0	44	30.6	5.0	93.6
	MMRD+/germline-	38	3.4	0.0	100.0	38	7.2	5.0	93.1
	MMRD+/VUS	13	14.8	0.0	88.8	13	9.5	5.0	27.0
	sporadic MSI-H	44	0.4	0.0	94.0	44	5.0	5.0	37.4

Table 9: Significance of Family History scores between Germline Groups and Age of Diagnosis

	p-value
MMRpro	
< 50 LS vs. ≥ 50 LS	0.0605
< 50 MMRD+/germline- vs. ≥ 50 MMRD+/germline-	0.0358
< 50 MMRD+/VUS vs. ≥ 50 MMRD+/VUS	0.0263
< 50 sporadic MSI-H vs. ≥ 50 sporadic MSI-H	**0.0015**
PREMM1,2,6	
< 50 LS vs. ≥ 50 LS	0.8933
< 50 MMRD+/germline- vs. ≥ 50 MMRD+/germline-	0.5646
< 50 MMRD+/VUS vs. ≥ 50 MMRD+/VUS	0.0370
< 50 sporadic MSI-H vs. ≥ 50 sporadic MSI-H	**0.0072**

Summary statistics for gender for CRC only can be found in table 10 and comparison statistics can be found in table 11. Overall, individuals in the LS groups had higher median family history scores for both modalities for males with CRC (MMRpro median=37.9; PREMM1,2,6 median=23.6) and females with CRC (MMRpro median=90.7; PREMM1,2,6 median=21.8) than the other three groups. Statistical significance was reached for PREMM1,2,6 for the following comparisons: males MMRD+/germline- versus females MMRD+/germline- (p=0.0067) and males sporadic MSI-H versus females sporadic MSI-H (p=0.0059). Males in the MMRD+/germline- group had higher median family history scores (median=8.3) than females in the MMRD+/germline- group (median=6.4). Males in the sporadic MSI-H group had higher median family history scores (median =5.5) than females in the sporadic MSI-H group (median=5.0).

Table 10: Males with Colorectal Cancer versus Females with Colorectal Cancer Summary Statistics

		MMRpro				PREMM			
	Germline Group	N	Med	Min	Max	N	Med	Min	Max
Male	LS	47	37.9	0.3	99.8	47	23.6	5.0	96.0
	MMRD+/germline-	28	12.9	0.0	100.0	28	8.3	5.0	93.1
	MMRD+/VUS	18	31.7	0.0	99.8	18	12.3	5.0	82.5
	sporadic MSI-H	13	3.2	0.0	74.2	13	5.5	5.0	25.1
Female	LS	33	90.7	0.0	99.9	33	21.8	5.0	93.6
	MMRD+/germline-	30	9.0	0.0	99.4	30	6.4	5.0	44.2
	MMRD+/VUS	10	40.6	0.6	97.3	10	8.9	5.0	34.5
	sporadic MSI-H	27	0.3	0.0	84.6	27	5.0	5.0	11.8

Table 11: Significance of Family History Scores between Germline Groups and Gender (CRC Only)

Comparison	p-value
MMRpro	
Male LS vs. Female LS	0.2038
Male MMRD+/germline- vs. Female MMRD+/germline-	0.1556
Male MMRD+/VUS vs. Female MMRD+/VUS	0.5813
Male sporadic MSI-H vs. Female sporadic MSI-H	0.0236
PREMM1,2,6	
Male LS vs. Female LS	0.7066
Male MMRD+/germline- vs. Female MMRD+/germline-	**0.0067**
Male MMRD+/VUS vs. Female MMRD+/VUS	0.2112
Male sporadic MSI-H vs. Female sporadic MSI-H	**0.0059**

Summary statistics for gene mutated in the positive group can be found in table 12. Individuals with MLH1 or MSH2 mutations had significantly higher ($p < 0.0001$) median family history scores on both modalities (MMRpro median=95.1; PREMM1,2,6 median=38.7) than individuals with MSH6 or PMS2 mutations (MMRpro median=7.7; PREMM1,2,6 median=7.3).

Table 12: Family Histories of Gene Mutated in the LS group Summary Statistics

	MMRpro**				PREMM1,2,6**			
Gene	N	Med	Min	Max	N	Med	Min	Max
MLH1/MSH2	71	95.1	0.3	100.0	71	38.7	5.0	97.6
PMS2/MSH6	25	7.7	0.0	92.1	25	7.3	5.0	65.0

** $p<0.0001$

To establish differences between the LS group and the MMRD+/germline- group, family history scores were stratified by gene implicated by germline test result for the LS group and by IHC result in the MMRD+/germline- group. Summary statistics for overall family history scores of the MMRD+/germline- group versus *MLH1/MSH2* and *MSH6/PMS2* mutations in the LS group can be found in table 13. Individuals with *MLH1* or *MSH2* mutations had significantly higher median family history scores on both modalities (MMRpro=95.1, PREMM1,2,6=38.7) than the MMRD+/germline- group (MMRpro=7.7, $p<0.0001$; PREMM1,2,6=7.3, $p<0.0001$). There was no significant difference between individuals with *MSH6* and *PMS2* mutations and MMRD+/germline- family history scores (MMRpro p=0.5933, PREMM1,2,6 p=0.6938). Summary statistics for gene mutated in the LS group versus gene implicated in the MMRD+/germline- group can be found in table 14. Individuals with an

MLH1 mutation had significantly higher family history scores on both modalities (MMRpro=98.4, PREMM1,2,6=49.3) than MMRD+/germline- individuals with loss of MLH1/PMS2 on IHC (MMRpro=6.9, p<0.0001; PREMM1,2,6=5.6, p<0.0001).

Table 13: MMRD+/germline- versus *MLH1/MSH2* mutations and *MSH6/PMS2* mutations

	MMRpro					PREMM1,2,6				
Group	N	Min	Med	Max	p-value	N	Min	Med	Max	p-value
MMRD+/germline-	142	0.0	7.7	100.0		142	5.0	7.3	93.1	
MLH1/MSH2 mutations (LS)	71	0.3	95.1	100.0	**<0.0001**	71	5.0	38.7	97.6	**<0.0001**
MSH6/PMS2 mutations (LS)	25	0.0	7.7	92.1	0.5933	25	5.0	7.3	65.0	0.6938

Table 14: LS family history scores versus MMRD+/germline- family history scores by gene implicated

		MMRpro					PREMM1,2,6				
Group	Gene Implicated	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-	MLH1/PMS2	31	0	6.9	87.3		31	5	5.6	45	
LS	MSH2	45	0.3	90.8	100.0	0.0812	13	0.1	36.7	100.0	0.0536
MMRD+/germline-	MSH2/MSH6	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-	MSH6	5	0.0	11.1	74.5		5	5.0	7.1	10.5	
LS	PMS2	7	0.0	11.4	92.1	0.0527	7	5.0	7.3	23.5	0.0294
MMRD+/germline-	PMS2	3	20.6	96.0	98.1		3	12.6	37.6	75.4	

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 PREMM1,2,6, the odds of being germline positive increase by a factor of 1.04 (95% CI: 1.02 – 1.05; $p = < 0.001$).

DISCUSSION

Demographics and Tumor Characteristics

Demographic and tumor characteristics were consistent with what was expected. Individuals with sporadic MSI-H tumors were diagnosed at a later age (60.5 years) than the other three groups, consistent with the average age of onset of CRC in the general population (69 years) and the average age of onset of EC in the general population (61 years) [26]. Individuals in the LS, MMRD+/germline- and MMRD+/VUS groups all had similar mean ages of onset in the 40s. Average age of CRC diagnosis in LS is 42-61 years and average age of EC diagnosis in LS is 47-55 years [27]**.** A recent study showed that individuals with LS and MMRD+/germline- have similar ages of cancer onset [28].

The MMRD+/VUS group had the most ethnic diversity, as less is known about normal ethnic genetic variation outside of the Northern European population. The preponderance of CRC is easily explained by the fact that our institution has only been doing tumor studies on ECs regularly since August 2012 and since CRC has a higher incidence (46.3 per 100,000 men and women) than EC (24.1 per 100,000 women) [26]**.** Individuals in the LS group were more likely to have additional polyps identified at time of diagnosis, which was expected given that these individuals have a diagnosis of LS and are therefore at an increased risk for developing polyps that could lead to a CRC. Individuals in the LS group were also more likely to have a stage 1 CRC, consistent with the observation that while these individuals are more likely to develop a CRC during their lifetime, these CRCs are typically less aggressive and grow more slowly [29].

With regards to EC, women in the LS group had BMI in the normal range, while the remaining 3 groups had BMIs in the overweight range. Obesity is a known risk factor for sporadic EC. The preponderance of *MSH2* mutations in the study population is consistent with the American population, which has a higher rate of *MSH2* germline mutations in LS [30].

Family History Assessments and Comparisons

The results of this study indicate that individuals with LS have more suggestive family histories than individuals with MMRD+/germline-, suggesting that perhaps individuals with MMRD+/germline-

do not truly have LS. We expect individuals with a hereditary cancer syndrome such as LS to, in general, have a family history of related cancers. When the family history is less impressive, our concern for a hereditary cancer syndrome is decreased. Because individuals with MMRD+/germline- have less impressive family histories, the suspicion for a hereditary predisposition to cancer decreases. Individuals with MMRD+/germline-, however, do have more suggestive family histories than individuals with a sporadic MSI-H tumor. This indicates that individuals with MMDR+/Lynch- have higher incidence of LS-related tumors in their families. Interestingly, individuals with a MMRD+/VUS identified have similar family histories to individuals with MMRD+/germline-. There are a number of possible explanations for this: the MMRD+/VUS group could be a mix of pathogenic and non-pathogenic mutations, individuals may have an undetectable pathogenic mutation, or having a MMRD+/VUS could indicate an increased cancer risk over the general population, based on the suggestive family histories. On the spectrum of family histories, individuals with LS have the most suggestive family histories, individuals with sporadic MSI-H tumors have the least suggestive family histories and individuals with MMRD+/germline- or a MMRD+/VUS have family histories in the middle.

The results of this study are intriguing. The hypothesis was confirmed and we have demonstrated that the MMRD+/germline- individuals lie in the middle of the family history spectrum. These results are consistent with a recent population-based study by Rodriguez-Soler and colleagues [28], who determined that the families of individuals with MMRD tumors but no identifiable germline mutation have a lesser risk of developing colorectal cancer in their lifetime than LS families, but a higher risk than individuals with a known sporadic tumor. Families of individuals with MMRD tumors but no identifiable germline mutation also had less family history of LS-related tumors. In fact, 50% of these individuals were the index case of cancer in their family [28].

Possible explanations as to why the MMRD+/germline- population fall in the middle of the family history spectrum include the following. The cohort could be a mixture of individuals with true LS, 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.

As expected, individuals with an *MLH1* or *MSH2* mutation had significantly higher median family history scores than individuals with an *MSH6* or *PMS2* mutations. Lifetime CRC and EC cancer risks for individuals with an *MSH6* or *PMS2* mutation are lower than the risks associated with *MLH1* and *MSH2* mutations. Males and females with an *MSH6* mutation have a 44% and a 20% lifetime risk of developing colorectal cancer respectively [23]. Individuals with a *PMS2* mutation have a lifetime risk of 15-20% for CRC [17]. Women with a *PMS2* mutation have a 15% lifetime risk for EC [17].

It appears that the differences between the family history scores are driven by the family history scores of individuals with an *MLH1* or an *MSH2* mutation. 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 MMDR+/germline- individuals are more similar to those of individuals with an *MSH6* or a *PMS2* mutation, as shown by Rodriguez-Soler and colleagues [28]. 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.

Both MMRpro and PREMM1,2,6 scores were positively correlated with the odds of testing germline positive. These results indicate that family history is still an important piece of information in the clinical assessment. While universal tumor screening protocols are certainly more effective than other previously used protocols, there is the potential for family history to be overlooked. Even for individuals who have an MMRD tumor, family history should still play a vital role in their risk assessment. As universal tumor screening protocols become more widespread, we will likely identify just as many individuals in the MMRD+/germline- group as the LS group.

The other statistically significant comparisons can be explained by the differences in the models previously observed in other studies. MMRpro scores tend to be higher than PREMM1,2,6 scores, likely due to the fact that MMRpro can take into account more family history information than PREMM1,2,6 [31] Neither of the models have proven to be effective at predicting germline mutation status in EC, as both overestimate the likelihood [32]. Because both models place weight on the age of

diagnosis in the risk assessment, it is not unexpected that a young age of diagnosis increases the family history score. These results validate that the models were functioning as expected in our study population, indicating that the study design was sound.

Implications and Future Directions

The results of this study and the population-based study by Rodriguez-Soler and colleagues [28] confirm the need for continued research into the causes of MMRD tumors, including sporadic, epigenetic and germline causes. In the recent months, there has been increasing research into other possible sporadic causes of MMRD tumors [15, 21, 22]. At this time, however, the only clinically available testing for sporadic causes of MMRD tumors is *MLH1* hypermethylation and *BRAF* V600E mutation analysis. Many of the recently discovered somatic and epigenetic causes of MMRD tumors were found through sequencing of the tumor genome. There may be a need to further explore the utility of sequencing tumor genome in the clinical setting, especially in cases of MMRD+/germline-. It is also quite possible that there are other germline causes of MMRD tumors outside of *MLH1, MSH2, MSH6, PMS2*. There could be other modifier genes that act in a similar fashion to *EPCAM/TACSTD1,* that remain to be discovered. Deletions in *EPCAM/TACSTD1* cause heritable epigenetic silencing of *MSH2* [33]. There are also a number of other MMR genes outside of those already implicated in LS that could be further explored, such as *MSH3* and *MLH3* [34].

The need to reassess the clinical management of individuals with MMRD+/germline- also needs to be addressed. At this time, we recommend that individuals with MMRD+/germline- follow the same screening guidelines as individuals with LS because no clinical tools exist yet to distinguish LS and MMRD+/germline-. This includes: colonoscopy annually beginning at age 20-25 and upper endoscopy every 3-5 years beginning at age 30-35 for males and females, and endometrial biopsy annually for females [35]. While there is clear clinical benefit to colonoscopy, there has been no proven clinical benefit for upper endoscopy, or endometrial biopsy. In fact, the most effective way to prevent endometrial cancer is to undergo a total abdominal hysterectomy after childbearing is completed [35]. It is extremely difficult, however, to make such drastic surgical recommendations to individuals with

MMRD+/germline- and their families, as we are unsure if they truly have LS. While colonoscopy is quite effective at preventing colorectal cancer, it does not come without risks, including perforation (0.04% risk), post-polypectomy hemorrhage (0.26% risk) and adverse reactions to sedation/anesthesia [36]. If an individual with LS is found to have a CRC, there is often a discussion regarding performing more radical colon surgery than simply removing the affected area [35]. As with a prophylactic hysterectomy, these conversations are difficult to have with an MMRD+/germline- individual.

If individuals with MMRD+/germline- do not truly have LS, and have lower colorectal cancer risks as suggested by Rodriguez-Soler and colleagues [28], then they likely do not need to be undergoing invasive screening as often as individuals with LS. They do, however, need increased screening in comparison to the general population. As previously stated, the age of onset for cancer in our MMRD+/germline- population is similar to that of the LS population (51.3 years and 48.3 years, respectively), which is consistent with the results of the Rodriguez-Soler et al. study [28]. Based on this, it seems appropriate to beginning surveillance at a younger age. But the question of exactly how to screen MMRD+/germline- individuals and their families remains. As stated by Rodriguez-Soler and colleagues, we as a medical community must strike a balance in screening MMRD+/germline- individuals [28]. It would be inappropriate to follow these individuals with general population screening recommendations based on the increased cancer risks and increased family history of cancer, as we could begin missing preventable cancers. 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 [28]. Further work needs to be done to further define the CRC and extra-colonic cancer risks in MMRD+/germline- individuals to develop appropriate surveillance recommendations.

Strengths and Limitations

There are a number of strengths to this study. Firstly, we have a large cohort of individuals who all underwent genetic counseling, tumor studies, genetic testing and had pedigrees available for analysis. Secondly, we included both endometrial and colorectal cancer. Thirdly, we included the MMRD+/VUS group in our analyses.

The limitations to this study are as follows. MMRpro and PREMM1,2,6 each have their own set of limitations. Clinical judgment is often the best form of pedigree assessment, but can be subjective. MMRpro and PREMM1,2,6 provided a way to quantify family histories, but may have been an over- or under-estimate, based on the formulation for risk assessment for the respective risk models. The limitations of these models were present for all groups, thus there was still a valid comparison. The pedigrees themselves may pose a limitation, as they rely on the patient as the historian and the genetic counselor as an accurate scribe. The family history of LS can also be variable. This was confirmed in our comparison of the family history scores of MLH1/MSH2 and MSH6/PMS2. While this could be viewed as a limitation, in actuality, our study indicates that all individuals with LS, regardless of which MMR gene is mutated, have more suggestive family histories. 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. Despite this, the MMRD+/germline- group still had less suggestive family histories than the LS group. Some individuals had missing demographic and medical history information, which was not actively sought. Only CRC or EC cancers with tumor studies were included in analysis. There were other females in our cohort that had both CRC and EC, but only had tumor testing performed on one tissue. Finally, the cohort was taken from one institution.

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 individuals at a "high risk". This included: right-sided tumors, diagnosed younger than 50 years old, or a suggestive family history. All of these factors are incorporated into the risk models, resulting in an increased risk assessment for finding a germline MMR mutation. 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.

Conclusion

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 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 also need to reconsider the current screening guidelines for individuals with MMRD+/germline-, as we may be subjecting these individuals to unnecessary invasive surveillance.

BIBLIOGRAPHY

[1] Lynch, H. T., Smyrk, T. C., Watson, P., Lanspa, S. J., Lynch, J. F., Lynch, P. M., Cavalieri, R. J., Boland, C. R. (1993). Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology*, 104(5), 1535-1549.

[2] Ponti, G., Losi, L., Pedroni, M., Lucci-Cordisco, E., Di Gregorio, C., Pellacani, G., Seidenari, S. (2006). Value of MLH1 and MSH2 mutations in the appearance of Muir-Torre syndrome phenotype in HNPCC patients presenting sebaceous gland tumors or keratoacanthomas. J *The Journal of Investigative Dermatology, 126*(10), 2302-2307.

[3] South, C. D., Hampel, H., Comeras, I., Westman, J. A., Frankel, W. L., de la Chapelle, A. (2008). The frequency of Muir-Torre syndrome among Lynch syndrome families. *The Journal of the National Cancer Institute, 100*(4), 277-281.

[4] Watson, P., Vasen, H. F., Mecklin, J. P., Bernstein, I., Aarnio, M., Järvinen, H. J., Myrhoj, T., Sundle, L., Wijnen, J. T., Lynch, H. T. (2008). The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *International Journal of Cancer, 123*(2), 444-449.

[5] Barrow, E., Robinson, L., Alduaij, W., Shenton, A., Clancy, T., Lalloo, F., Hill, J., Evans, D. G. (2009). Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. *Clinical Genetics, 75*(2), 141-149.

[6] Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. (2009). 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. *Genetics in Medicine, 11*(1), 35-41.

[7] Hampel, H., Frankel, W. L., Martin, E., Arnold, M., Khanduja, K., Kuebler, P., Clendenning M., Sotamaa, K., Prior, T., Westman, J. A., Panescu, J., Fix, D., Lockman, J., LaJeunesse, J., Comeras, I., de la Chapelle, A. (2008). Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *Journal of Clinical Oncology, 26*(35), 5783-5788.

[8] Fishel, R., Kolodner, R. D. (1995). Identification of mismatch repair genes and their role in the development of cancer. *Current Opinions in Genetics and Development, 5*(3), 382-395.

[9] Ligtenberg, M. J., Kuiper, R. P., Chan, T. L., Goossens, M., Hebeda, K. M., Voorendt, M., Lee, T. Y., Bodmer, D., Hoenselaar, E., Hendriks-Cornelissen, S. J., Tsui, W. Y., Kong, C. K., Brunner, H. G., van Kessel, A. G., Yuen, S. T., van Krieken, J. H., Leung, S. Y., Hoogerbrugge, N. (2009). Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nature Genetics, 41*(1), 112-117.

[10] Aaltonen, L. A., Salovaara, R., Kristo, P., Canzian, F., Hemminki, A., Peltomäki, P., Chadwick, R. B., Kaariainen, H., Eskelinen, M., Jarvinen, H., Mecklin, J. P., de la Chapelle, A. (1998). Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *New England Journal of Medicine, 338*(21), 1481-1487.

[11] Giardiello, F. M., Brensinger, J. D., Petersen, G. M. (2001). AGA technical review on hereditary colorectal cancer and genetic testing. *Gastroenterology, 121*(1), 198-213.

[12] Zighelboim, I., Goodfellow, P.J., Gao, F., Gibb, R. K., Powell, M. A., Rater, J. S. Mutch, D. G. (2007). Microsatellite instability and epigenetic inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. *Journal of Clinical Oncology, 25*(15), 2042-2048.

[13] Bartley, A. N., Luthra, R., Saraiya, D. S., Urbauer, D. L., Broaddus, R. R. (2012). Identification of cancer patients with Lynch syndrome: clinically significant discordances and problems in tissue-based mismatch repair testing. *Cancer Prevention Research (Philadelphia, PA), 5*(2), 320-327.

[14] Moreira, L., Balaguer, F., Lindor, N., de la Chappelle, A., Hampel, H., Aaltonen, L. A., Hopper, J., L., LeMarchard, L., Gallinger, S., Newcomb, P. A., Haile, R., Thibodeau, S. N., Gunawardena, S., Jenkins, M. A., Buchanan, D. D., Potter, J. D., Baron, J. A., Ahnen, D. J., Moreno, V., Andreu, M., Ponz de Leon, M., Rustgi, A. K., Castells, A. (2012). Identification of Lynch syndrome among patients with colorectal cancer. *JAMA, 308*(15), 1555-1565.

[15] Bellizzi, A. M. & Frankel, W. L. (2009). Colorectal cancer due to deficiency in DNA mismatch repair function: a review. *Advances in Anatomic Pathology, 16*(6), 405-407.

[16] Kawaguchi, M., Yanokura, M., Banno, K., Kobayshi, Y., Kuwabara, Y., Kobayashi, M., Nomura, H., Hirasawa, A., Susumu, N., Aoki, D. (2009). Analysis of a correlation between the BRAF V600E mutation and abnormal DNA mismatch repair in patients with sporadic endometrial cancer. *International Journal of Oncology, 34*(6), 1541-1547.

[17] Senter, L., Clendenning, M., Sotamaa, K., Hampel, H., Green, J., Potter, J. D., Lindblom, A., Lagerstedt, K., Thibodeau. S. N., Lindor, N. M., Young, J., Winship, I., Sowty, J. G., White, D. M., Hopper, J. L, Baglietto, L., Jenkins, M. A., de la Chapelle, A. (2008). The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology*, *135*(2), 419-428.

[18] Weissman, S. M., Burt, R., Church, J., Erdman, S., Hampel, H., Holter, S., Jasperson, K., Kalady, M. F., Haidle, J. L., Lynch, H. T., Palaniappa, S., Wise, P. E., Senter, L. (2012). Identification of Individuals at Risk for Lynch Syndrome Using Targeted Evaluations and Genetic Testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer Joint Practice Guideline. *Journal of Genetic Counseling, 21*(4), 484-493.

[19] Heald, B., Plesec, T., Liu, X., Pai, R., Patil, D., Moline, J., Sharp, R. R., Burke, C. A., Kalady, M. F., Church, J., Eng, C. (2013). Implementation of universal microsatellite instability and immunohistochemistry screening for diagnosing Lynch syndrome in a large academic medical center. *Journal of Clinical Oncology, 31*(10), 1336-1340.

[20] Morak, M., Koehler, U., Schackert, H. K., Steinke, V., Royer-Pokora, B., Schulmann, K., Kloor, M., Hochter, W., Weingart, J., Keiling, C., Massdorf, T., Holinski-Feder, E. (2011). Biallelic *MLH1* SNP cDNA expression or constitutional promoter methylation can hide genomic rearrangements causing Lynch syndrome. *Journal of Medical Genetics, 48*(8), 513-519.

[21] Cancer Genome Atlas Network. (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature, 487*(7407), 330-337.

[22] Sourrioulle, I., Coulet, F., Lefevre, J. H., Colas, C., Eyries, M., Svrcek, M., Bardier-Dupas, A., Parc, Y., Soubrier, F. (2013). Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Familial Cancer, 12*(1), 27-33.

[23] Baglietto, L., Lindor, N. M., Dowty, J. G., White, D. M., Wagner, A., Gomez Garcia, E. B., Vriends, A. H., Cartwright, N. R., Barnetson, R. A., Farrington, S. M., Tenessa, A., Hampel, H., Buchanan, D., Arnold, S., Young, J., Walsh, M. D., Jass, J., Macrae, F., Antill, Y., Winship, I. M., Giles, G. G., Goldblatt, J., Parry, S., Suthers, G., Leggett, B., Butz, M., Aronson, M., Poynter J. N., Baron, J. A., Le Marchand, L., Haile, R., Gallinger, S., Hopper, J. L., Potter, J., de la Chappelle, A., Vasen, H. F., Dunlop, M. G., Thibodeau, S. N., Jenkins, M. A. (2012). Risks of Lynch syndrome cancers for MSH6 mutation carriers. *Journal of the National Cancer Institute, 102*(3), 193-201.

[24] Kastrinos, F., Steyerberg, E. W., Mercado, R., Balmana, J., Holter, S., Gallinger, S., Siegmund, K. D., Church, J. M., Jenkins, M. A., Lindor, N. M., Thibodeau, S. N., Burbidge, L. A., Wenstrup, R. J., Syngal, S. (2012). The PREMM(1,2,6) model predicts risk of MLH1, MSH2 and MSH6 germline mutations based on cancer history. *Gastroenterology, 143*(5), 1399.

[25] Chen, S., Want, W., Shing, L., Nafa, K., Lee, J., Romans, K., Watson, P., Gruber, S. B., Euhus, D., Kinzler, K. W., Jass, J., Gallinger, S., Lindor, . M., Casey, G., Ellis, N., Giardiello, F. M., Offit, K., Parmigiani, G. (2006). Prediction of germline mutations and cancer risk in the Lynch syndrome. *JAMA, 296*(12), 1749-1487.

[26] Howlader, N., Noone, A. M., Krapcho, M., Neyman, N., Aminou, R., Altekruse, S. F., Kosary, C. L., Ruhl, J., Tatalovich, Z., Cho, H., Mariotto, A., Eisner, M. P., Lewis, D. R., Chen, H. S., Feuer, E. J., Cronin, K.A. (eds). (2012). SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations. Retrieved from http://seer.cancer.gov/csr/1975_2009_pops09/.

[27] Stoffel, E., Mukherjee, B., Raymond, V. M., Tayob, N., Kastrinos, F., Sparr, J., Wang, F., Bandipalliam, P., Syngal, S., Gruber, S. B. (2009). Calculation of risk of colorectal and

endometrial cancer among patients with Lynch syndrome. *Gastroenterology, 137*(5), 1621-1627.

[28] Rodriguez-Soler, M., Perez-Carbonell, L., Guarinos, C., Zapater, P., Castillejo, A., Barbera, V. M., Juarez, M., Bessa, X., Xicola, R. M., Clofent, J., Bujanda, L., Balaguer, F., Rene, J. M., de-Castro, L., Marin-Gabriel, J. C., 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, J. L., Paya, A., Jover, R. (2013). Risk of cancer in cases of suspected Lynch syndrome without germline mutation. *Gastroenterology*, *144*(5), 926-932.

[29] Watson, P., Lin, K. M., Rodriguez-Bigas, M. A., Smyrk, T., Lemon, S., Shashidharan, M., Franklin, B., Karr, B., Thorson, A., Lynch, H. T. (1998). Colorectal carcinoma survival among hereditary nonpolyposis colorectal carcinoma family members. *Cancer, 83*(2), 259-266.

[30] Stoffel, E. M. (2010). Lynch syndrome/hereditary non-polyposis colorectal cancer (HNPCC). *Minerva Gastroenterologica e Dietologica, 56*(1), 45-53.

[31] Tresallet, C., Brouquet, A., Julié, C., Beauchet, A., Vallot, C., Ménégaux, F., Mitry, E., Radvanyi, F., Malafosse, R., Rougier, P., Nordlinger, B., Laurent-Puig, P., Boileau, C., Emile, J. F., Muti, C., Penna, C., Hofmann-Radvanyi, H. (2012). Evaluation of predictive models in daily practice for the identification of patients with Lynch syndrome. *International Journal of Cancer, 130*(6), 1367-1377.

[32] Mercado, R. C., Hampel, H., Kastrinos, F., Steyerberg, E., Balmana, J., Stoffel, E., Cohn, D. E., Backes, F. J., Hopper, J. L., Jenkins, M. A., Lindor, N. M., Casey, G., Haile, R., Madhavan, S., de la Chappelle, A., Syngal, S. (2012). Performance of PREMM1,2,6, MMRpredict and MMRpro in detecting Lynch syndrome among endometrial cancer cases. *Genetics in Medicine, 14*(7), 670-680.

[33] Kempers, M. J., Kuiper, R. P., Ockeloen, C. W., Chappius, P. O., Hutter, P., Rahner, N., Schackert, H. K., Steinke, V., Holinski-Feder, E., Morak, M., Kloor, M., Buttner, R., Verwiel, E. T., van Krieken, J. H., Nagtegaal, I. D., Goossense, M., van der Post, R., S., Niessen, R. C., Sijmons, R. H., Kluijt, I., Hogervorst, F. B., Leter, E. M., Gille, A. A., Aalfs, C., M., Redeker, E. J., Hes, F. J., Topes, C. M., can Neisselrooij, B. P., van Gijn, M. E., Gomez Garcia, E. B., Eccles, D. M., Bunyan, D. J., Syngal, S., Stoffel, E. M., Culver, J. O., Palomares, M. R., Graham, T., Velsher, L., Papp, J., Olah, E., Chan, T. L., Leung, S. Y., van Kessel, A. G., Kiemeney, L. A., Hoogerbrugge, N., Lightenberg, M. J. (2011). Risk of colorectal and endometrial cancers in EPCAM deletion-positive Lynch syndrome: a cohort study. *Lancet, 12*(1), 49-55.

[34] Mismatch Repair – Reference Pathway. (2010). In *KEGG.* Retrieved from http://www.genome.jp/kegg/pathway/ko/ko03430.html.

[35] Burt, R. W., Barthel, J. S., Cannon, J. A., David, D. S, Early, D. S., Ford, J. M., Giardiello, F. M., Gruber, S. B., Halverson, A. L., Hamilton, S. R., Hampel, H., Ismail, M. K., Jasperson, K., Lazenby, A. J., Lynch, P. M., Mayer, R. J., Ness, R. M., Provenzale, D., Rao, M. S., Shike, M., Steinbach, G., Terdimen, J. P., Weinberg, D. (2012). NCCN clinical practice guidelines in oncology: colorectal cancer screening. Retrieved from: http://www.nccn.org/professionals/physician_gls/pdf/colorectal_screening.pdf.

[36] Gavin, D. R., Valori, R. M., Anderson, J. T., Donnelly, M. T., Williams, J. G., Swarbrick, E. T. (2013). The national colonoscopy audit: a nationwide assessment of the quality and safety of colonoscopy in the UK. *Gut.* Epub ahead of print.

VITA

Katherine Margaret Dempsey was born to Kevin and Natalie Hughes Dempsey on June 11, 1989 in Indianapolis, Indiana. She graduated from Zionsville Community High School in Zionsville, Indiana in June 2007 summa cum laude. She received her Bachelor of Arts in biology and psychology from Augustana College in Rock Island, Illinois in May 2011 magna cum laude, where she was a member of Beta Beta Beta, Psi Chi, Sigma Xi, and Omicron Delta Kappa. She began attending the University of Texas Genetic Counseling Program in August, 2011. She will be graduating with her Masters of Science in Genetic Counseling in May 2013.