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Double somatic mismatch repair gene pathogenic variants as common as Lynch syndrome among endometrial cancer patients

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Abstract

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Author contribution

HH, RP, AdlC, PG, and DC conceived and planned the experiments. A.B., B.C., C.D. and D.E. carried out the experiments. CC, FB, LC, JF, DOM, RS, and DC contributed to patient enrollment. WC, WLF, AAS performed the pathological review and immunohistochemical staining assessment. CCP, WZ, DJ, AY, WC performed the molecular analyses. HH, RP, and PPS provided genetic counseling care and result interpretation to the patients. JPM performed the statistical analysis. HH, RP, PG, and DEC contributed to the interpretation of the results. HH and RP took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Declaration of Competing Interest

Professor Hampel is on the scientific advisory board for Genome Medical, InVitae Genetics, and Promega, and has had research support from Myriad Genetics Laboratories, Inc. Ms. Pearlman has had research support from Myriad Genetics Laboratories, Inc. Dr. Pritchard consults for Promega and AstraZeneca. Dr. Stanich receives research support from Emtora Biosciences, Janssen Pharmaceuticals Inc., Pfizer Inc. and the PTEN Research Foundation. Drs. Backes, Chen, Cohn, Copeland, Cosgrove, de la Chapelle, Frankel, Fowler, Jones, McElroy, O'Malley, Salani, Suarez, Yilmaz, and Zhao have no conflicts of interest to disclose.

Objective.—Lynch syndrome is the most common cause of inherited endometrial cancer, attributable to germline pathogenic variants (PV) in mismatch repair (MMR) genes. Tumor microsatellite instability (MSI-high) and MMR IHC abnormalities are characteristics of Lynch syndrome. Double somatic MMR gene PV also cause MSI-high endometrial cancers. The aim of this study was to determine the relative frequency of Lynch syndrome and double somatic MMR PV.

Methods.—341 endometrial cancer patients enrolled in the Ohio Colorectal Cancer Prevention Initiative at The Ohio State University Comprehensive Cancer Center from 1/1/13–12/31/16. All tumors underwent immunohistochemical (IHC) staining for the four MMR proteins, MSI testing, and *MLH1* methylation testing if the tumor was MMR-deficient (dMMR). Germline genetic testing for Lynch syndrome was undertaken for all cases with dMMR tumors lacking *MLH1* methylation. Tumor sequencing followed if a germline MMR gene PV was not identified.

Results.—Twenty-seven percent (91/341) of tumors were either MSI-high or had abnormal IHC indicating dMMR. As expected, most dMMR tumors had *MLH1* methylation; (69, 75.8% of the dMMR cases; 20.2% of total). Among the 22 (6.5%) cases with dMMR not explained by methylation, 10 (2.9% of total) were found to have Lynch syndrome (6 *MSH6*, 3 *MSH2*, 1 *PMS2*). Double somatic MMR PV accounted for the remaining 12 dMMR cases (3.5% of total).

Conclusions.—Since double somatic MMR gene PV are as common as Lynch syndrome among endometrial cancer patients, paired tumor and germline testing for patients with non-methylated dMMR tumor may be the most efficient approach for LS screening.

Keywords

Endometrial; Neoplasm; Cancer; Mismatch repair; Lynch syndrome; Somatic; Double somatic

1. Introduction

Approximately 25% of endometrial cancers (EC) exhibit high microsatellite instability (MSI-H), reflecting mismatch repair deficiency (dMMR) [1–3]. Universal EC tumor screening for MMR deficiency in order to identify patients who might have Lynch syndrome, the most common inherited form of EC and colorectal cancer, has been recommended by the American College of Obstetricians and Gynecologists and the Society of Gynecologic Oncology [4]. Lynch syndrome (LS) is caused by a germline pathogenic variant (PV) in *MLH1, MSH2 (EPCAM), MSH6* or *PMS2*. Individuals with LS have a significantly increased risk for developing cancers of the colon, endometrium, ovary, stomach, and others [5,6]. Identifying EC patients with dMMR tumors (both caused by Lynch syndrome and those with somatic dMMR) is important because they could benefit from treatment with immune checkpoint inhibitors [7–9]. Identifying EC patients with LS is important because they can then participate in intensified surveillance programs which may prevent additional primary cancers or diagnose these additional primary cancers early when they have better outcomes. In addition, once an EC patient has been diagnosed with LS, their relatives can be offered cascade genetic counseling and testing. Family members who have inherited LS can benefit from life-saving intensified surveillance and risk-reducingsurgeries.

The primary cause of dMMR EC is acquired methylation of the *MLH1* promoter [3]. Other causes of dMMR EC include LS due to germline PVs in the MMR genes and double somatic MMR gene PVs in the tumor. It has been reported that >50% of tumors for which the MMR deficiency was not explained by germline mutation or *MLH1* methylation are caused by double somatic MMR gene PVs when both sequencing and LOH are evaluated [10–12].

We sought to define the relative frequency of LS and double somatic tumors in a large, single institution cohort of EC patients as a step toward determining the role tumor sequencing might play in LS screening.

2. Methods

347 women newly diagnosed with primary invasive EC in Ohio between 1/1/2013–12/31/2016 were prospectively enrolled into the Ohio Colorectal Cancer Prevention Initiative (OCCPI; ClinicalTrials.gov identifier: NCT01850654). Written informed consent was obtained from all participants. Institutional Review Board (IRB) approval for the OCCPI was obtained by the Ohio State University (OSU) IRB (2012C0123). Of the 347 patients enrolled, 6 were deemed ineligible. Primary reasons for ineligibility included ineligible pathology type, insufficient tumor material, and not being diagnosed at Ohio State. The remaining 341 active and eligible patients had all of their testing completed successfully (Table 1). Methods have previously been described [13,14], but briefly, all tumors were screened for MMR deficiency by microsatellite instability (MSI) testing and/or immunohistochemical (IHC) analysis. Microsatellite instability testing was completed using the Promega MSI Analysis System (Version 1.2), which includes five repeat markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27). Tumors with ≥2/5 markers showing instability were classified as MSI-high (MSI-H). IHC staining for all four MMR proteins was done as routine clinical care for all but four patients. Antibodies included MLH-1 Clone: Leica ES05 (Mouse: NCL-L-MLH1), MSH-2 Clone: Calbiochem FE11 (Mouse: NA27), MSH-6 Clone: Epitomics EP49 (Rabbit: AC-0047), PMS-2 Clone: BD Pharmingen A16–4 (Mouse: 556415). Proteins with convincing stain in >1% of cells, or equivocal staining, were considered “present”. Equivocal and weak IHC in this study was treated as present/intact, rather than absent, since both MSI and IHC were performed and it was assumed that any case with true MMR deficiency would have a MSI-H tumor. For the four cases that did not get IHC performed clinically, the two-stain method of IHC was utilized as has been previously described [13]. Methylation of the *MLH1* promoter was assessed using pyrosequencing [15] when tumors were MSI-high and/or absent MLH1 and PMS2 proteins on IHC, with ≥15% methylation (averaging across four CpG sites) classified as *MLH1* hypermethylation. Patients with MMR deficiency (without *MLH1* hypermethylation if MLH1 was absent on IHC) underwent germline next-generation sequencing (NGS) (ColoSeq or BROCA, University of Washington). Cases sent before 8/1/2016 received ColoSeq; cases sent after 8/1/2016 received BROCA. Tumor sequencing of the MMR genes with ColoSeq Tumor followed for patients with unexplained MMR deficient tumors. Loss of heterozygosity analysis (LOH) in ColoSeq Tumor is performed by analysis of b-allele variant fractions of heterozygous single nucleotide polymorphisms (SNPs). Heterozygous SNPs are identified by annotation of data by dbSNP, and filtering out homozygous variants

(>98% variant fraction). Genomic regions in which three consecutive SNPs have skewing of b-allele fraction of more than 10% from baseline non-tumor values are considered to have LOH. LOH calls are manually interpreted and confirmed following expert molecular pathologist review (Pritchard) in the correct molecular context. The Clinical Laboratory Improvement Amendments–approved laboratories adjudicated the pathogenicity of all germline mutations using criterion established by the American College of Medical Genetics and International Agency for Research on Cancer guidelines [16,17]. Our approach to MMR variant interpretation has been described previously [12,13,18,19]. For tumor sequencing, cases were considered double somatic if two pathogenic and/or likely pathogenic somatic variants were identified or if one pathogenic or likely pathogenic somatic variant was identified with associated loss of heterozygosity (LOH). Clinical and pathologic data were abstracted from the intake form and electronic medical record. Genetic counseling was provided to all EC patients diagnosed with LS as part of the research study and free genetic counseling and testing was offered to any of their at-risk relatives. Genetic counseling could be provided in person or via the telephone and was performed either by a study genetic counselor or Informed DNA (www.informeddna.com).

2.1. Statistics

For association between continuous measures with categorical variables, ANOVA was employed. For associations between categorical variables, two-sided Fisher's exact test was used. The R statistical computing software [20] was used for all analyses. Herein, we consider comparison-wise $p < 0.05$ as statistically significant.

3. Results

3.1. Patients

The clinical characteristics of the 341 EC patients are presented in Table 1. The overall testing schema utilized in this study is shown in Fig. 1. The average age of diagnosis was 59 (range 29–87) and the majority of patients (96.2%) were white. The cohort was on average obese, with a mean BMI of 36.5 kg/m^2 (range 19.0–66.6). The majority of patients had endometrioid histology (82.7%) followed by serous, mixed endometrioid/serous, and carcinosarcoma. The majority of patients were FIGO stage IA, grade 1, had myometrial invasion but no lymphovascular invasion. Prior or synchronous malignancies were reported by 16.7% of patients, with breast cancer being the most common. Most subjects reported cancers in first-degree relatives with 53.1% of patients having at least one first-degree relative with a Lynch syndrome-associated tumor such as colorectal, endometrial, ovarian, gastric, pancreatic, small intestine, hepatobiliary, brain, bladder, kidney, or ureter cancers.

3.2. Mismatch repair deficiency

All 341 tumors were evaluated by IHC. Sixteen of the tumors (4.7%) did not have sufficient tumor DNA to complete MSI testing; of which one had abnormal IHC. Seventy-three tumors were MSI-high (2/73 had normal IHC), 12 were MSI-low (3/12 had normal IHC), and 240 were microsatellite stable (232/240 had normal IHC). In total 91 cases (26.7%) had defective mismatch repair including: 73 cases with MSI-high tumors, 9 cases with abnormal IHC and MSI-low tumors, 8 cases with abnormal IHC and MSS tumors, and 1 case with

abnormal IHC which had insufficient tumor for MSI testing. In the 325 cases where both IHC and MSI were completed, concordance was seen in 94.2% of cases (Table 2). Of the 91 patients with dMMR tumors, 71 (78.9%) had absence of MLH1 and PMS2, 8 (8.8%) had absence of MSH2 and MSH6, 8 (8.8%) had isolated absence of MSH6, 2 (2.2%) had isolated absence of PMS2, and 2 (2.2%) had normal IHC.

3.3. MLH1 hypermethylation

MLH1 methylation testing was performed on 85 cases (all 73 MSI-high cases and any MSS [5] or MSI-low [7] cases with absence of MLH1 on IHC). It was not performed on the 6 dMMR cases that were MSI-low, MSS, or insufficient for MSI testing cases with absence of MSH6 alone or together with MSH2. In total, 69 (75.8%) of the 91 dMMR endometrial tumors were found to have *MLH1* promoter hypermethylation. Sixty-seven (94.3%) of the 71 cases with absence of MLH1 and PMS2 on IHC were found to have *MLH1* promoter hypermethylation. In addition, one case with PMS2 only absent and one MSI-high case with normal IHC were found to have *MLH1* promoter hypermethylation.

3.4. Germline mutation testing

There were 22 EC patients with dMMR tumors, with *MLH1* hypermethylation ruled out when indicated, who underwent germline genetic testing. Ten patients (2.9% of all ECs; 45.5% of the patients with non-hypermethylated dMMR tumors) were found to have Lynch syndrome due to a germline MMR PV (6 *MSH6*, 3 *MSH2*, 1 *PMS2*; see Table 3).

3.5. Tumor sequencing

There were 12 tumors with unexplained dMMR. Coloseq Tumor NGS identified double somatic MMR PVs in all 12 tumors. Four cases had double somatic *MLH1* PV, five cases had double somatic *MSH2* PV, and three cases had double somatic *MSH6* PV (see Table 4).

3.6. IHC, LS, and double somatic PV in the MMR genes

The proportion of LS cases versus double somatic MMR variant cases varied based on IHC findings (Table 5). More EC patients with isolated absence of MSH6 were attributed to LS than double somatic MMR. PMS2 absence or MSI-high with normal IHC were only found in LS patients but there was only one of each. Germline *MSH6* mutations were the most common cause of LS. Absence of MLH1 and PMS2 without *MLH1* hypermethylation was only found in EC patients with double somatic MMR. MSH2 and MSH6 absence was more frequent in double somatic MMR variants than LS, with *MSH2* being the most common somatically mutated MMR gene. While the numbers of patients in Table 5 are too low to reach any conclusions, they are useful for hypothesis generation.

3.7. Clinicopathologic characteristics of LS and double somatic MMR PV cases

Age at diagnosis was significantly associated with MMR class ($p = 0.0002$, Table 1). Patients with LS were diagnosed with EC at significantly younger ages (48.3 years) than those with double somatic MMR PV (61.5 years, $p = 0.00015$; 95% CI of difference = [7.3–19.1]), proficient MMR tumors (58.6 years, $p = 0.002$; 95% CI of difference = [3.8–16.8]) or *MLH1* hypermethylation (62.3 years, $p = 0.000018$; 95% CI of difference = [7.9–20.2]).

There was no significant difference in the age of EC diagnosis between the individuals with double somatic mutations and cases with proficient MMR ($p = 0.34$). Although BMI was not significantly associated with molecular class overall ($p = 0.09$, Table 1), patients with LS had a significantly lower BMI (29 kg/m^2) than women whose tumors had proficient MMR (36.6, $p = 0.02$; 95% CI of difference = [1.2–14.2]), or *MLH1* hypermethylation (37.2, $p = 0.007$; 95% CI of difference = [2.4–14.2]). While not significant, the BMI of women with LS was still lower than that of women with double somatic MMR PVs ($p = 0.18$; 95% CI of difference = [−2.8–13.8]). Tumor histology was not significantly associated with MMR class ($p = 0.77$). FIGO grade ($p = 0.06$), lymphovascular invasion ($p = 0.001$), myometrial invasion ($p = 0.002$,) and surgical stage ($p = 0.05$) were associated with MMR class. Only 10% of LS patients had lymphovascular space invasion compared to 25%, 42%, and 18.4% in the double somatic, *MLH1* methylated and pMMR cases respectively. Myometrial invasion was present in 40% of the patients with LS, compared to 91.7%, 88.4%, and 74.8% in cases with double somatic MMR PVs, *MLH1* hypermethylation, or intact MMR respectively. There were no significant differences in the presence of synchronous and metachronous cancers ($p = 0.28$). The women with LS were not significantly more likely to have a first-degree relative with a LS-associated cancer than the women with *MLH1* methylated tumors ($p = 0.09$), MMR proficient tumors ($p = 0.19$) or double somatic MMR PV ($p = 0.20$).

3.8. Cascade testing

Families of all 10 LS probands participated in cascade testing. At-risk relatives were considered eligible for testing if they were alive, over age 18, and not previously tested. Nineteen first-degree relatives (47.5% of 40 total eligible first-degree relatives), 16 second-degree relatives (31.4% of 51 total eligible second-degree relatives), and 48 third-degree relatives and beyond underwent genetic counseling and testing. An additional 35 relatives (11 first-, 7 second-, and 17 third-degree relatives and beyond) tested positive for the familial MMR gene PV and can participate in LS intensive surveillance and prevention programs.

4. Discussion

This study confirms that the prevalence of LS among a population-based cohort of EC patients undergoing universal tumor screening for dMMR is 2.9% as previously shown [3,21,22]. In addition, germline PVs in the *MSH6* gene were the most common cause of LS among EC patients undergoing universal tumor screening for dMMR in our cohort. We now show that double somatic MMR PVs are more common than LS among EC patients (3.8%). This has implications for the genetic testing strategies for EC patients with dMMR tumors.

Prior to this study, the literature included a total of 21 EC cases with unexplained dMMR and no germline MMR gene PV that underwent tumor sequencing [10–12]. Sixteen of the 21 cases (71%) had proven or probable double somatic MMR gene PVs while one (5%) had a germline mutation that was missed in the initial LS testing. Among the four remaining cases, it proved that three were misclassified as having tumor MMR abnormalities in the initial LS screening. Repeat IHC showed the MMR proteins were intact in those cases, consistent with the absence of any MMR gene PV in the germline and tumor. Only one case with an IHC

defect remained unexplained after tumor sequencing. This study found that 100% of dMMR EC cases with no germline MMR gene PV were due to double somatic MMR gene PVs. With modern testing techniques, erroneous MSI, IHC, *MLH1* methylation, or germline testing results are less common. As a result, there were no dMMR EC cases that remained unexplained after MLH1 methylation testing, germline genetic testing, and tumor testing were performed.

Personal and family histories (first-degree relatives) of cancer are typically stronger in LS families. However, in our cohort the rates of LS-associated tumors in first-degree relatives was not significantly higher in the patients with LS. The LS probands were much younger at age of diagnosis than the rest of the cohort. Younger probands will on average have younger first-degree relatives and as such, the number of risk years for development of cancers will be less. In addition, our sample size (10 probands) is small and we may be underpowered to detect differences. Finally, our higher rate of LS-associated cancers in relatives may be due to the inclusion of all bladder and kidney cancers as LS-associated cancers. We did not have the exact pathology available for cancers reported in family members and only a subset (urothelial carcinomas) of the bladder and kidney (renal pelvis) cancers are associated with LS.

Follow-up for dMMR EC tumors has traditionally begun with germline MMR gene testing as part of a cancer gene panel. However, more than half of the cases will not have a germline mutation. Follow-up tumor sequencing is the only way to confirm that such cases are due to double somatic MMR PVs and not a germline MMR gene PVs that was missed in the initial testing. This is important in the management of the EC patients and their family members. Based on the high rate of double somatic MMR PVs, paired tumor and normal sequencing as a combined test may be an appropriate follow-up approach for an EC patient with a dMMR tumor. One-step testing instead of two may be beneficial for the patients by streamlining the testing process. As additional data become available, alternative approaches to gene testing may emerge. IHC patterns could identify cases in which germline genetic testing could be ordered first (e.g. MSH6 only absence cases that are more likely to be due to germline *MSH6* PVs than double somatic PVs). For those EC patients with strong family histories of LS cancers who lack germline or somatic MMR gene PVs, proband and family member cancer screening will remain central to cancer prevention strategies.

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HIGHLIGHTS

- 2.9% of endometrial cancers (EC) are due to Lynch syndrome.
- Double somatic mismatch repair gene mutations (3.5%) are as common as Lynch syndrome (2.9%) among EC patients.
- EC with absence of MSH6, PMS2, or microsatellite instability with normal IHC are more likely due to Lynch syndrome.
- EC with absence of MLH1 & PMS2 and no MLH1 methylation are more likely due to double somatic mismatch repair mutations.
- Endometrial cancers with absence of MSH2 & MSH6 are more likely due to double somatic mismatch repair gene mutations.

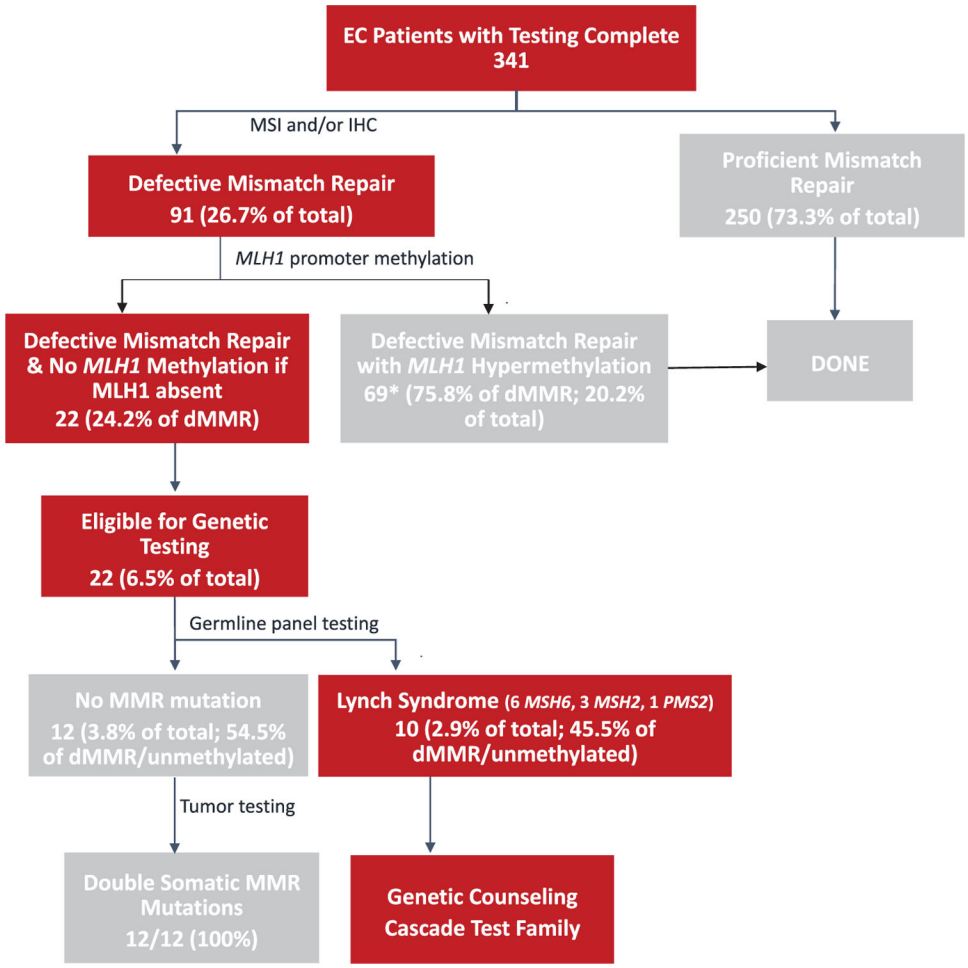

Fig. 1.
Endometrial cancer study schema.

Table 1

Clinical characteristics of patients.

Characteristic	All	Lynch syndrome	Double somatic	MLH1 methylated	pMMR	*p*-value
	***n* = 341**	***n* = 10**	***n* = 12**	***n* = 69**	***n* = 250**	
Age (Average, Range)	59.2 (29–87)	48.3 (39–57)	61.5 (47–76)	62.3 (36–82)	58.6 (29–87)	$p = 0.0002$
20–29	1 (0.3%)	0	0	0	1 (0.4%)	
30–39	16 (4.7%)	1 (10%)	0	2 (2.9%)	13 (5.2%)	
40–49	31 (9.1%)	3 (30%)	1 (8.3%)	2 (2.9%)	25 (10.0%)	
50–59	123 (36.1%)	6 (60%)	4 (33.3%)	20 (29.0%)	93 (37.2%)	
60–69	124 (36.4%)	0	6 (50%)	31 (44.9%)	87 (34.8%)	
70–79	38 (11.1%)	0	1 (8.3%)	12 (17.4%)	25 (10.0%)	
80–89	8 (2.3%)	0	0	2 (2.9%)	6 (2.4%)	
Self-reported race						$p = 1.000$
Caucasian	328 (96.2%)	10 (100%)	12 (100%)	67 (97.1%)	239 (95.6%)	
African-American	7 (2.1%)	0	0	1 (1.4%)	6 (2.4%)	
Other *	6 (1.8%)	0	0	1 (1.4%)	5 (2.0%)	
BMI (Average, Range)	36.5 (19.0–66.6)	29.0 (20.9–39.8)	34.4 (24.9–61.7)	37.2 (19.6–66.6)	36.6 (19.0–66.4)	$p = 0.087$
Normal: 18.5 to <25	39 (11.4%)	3 (30%)	1 (8.3%)	4 (5.8%)	31 (12.4%)	
Overweight: 25.0 to <30	61 (17.9%)	3 (30%)	5 (41.7%)	13 (18.8%)	40 (16.0%)	
Class 1 Obesity: 30.0 to <35	71 (20.8%)	2 (20%)	2 (16.7%)	15 (21.7%)	52 (20.8%)	
Class 2 Obesity: 35.0 to <40	59 (17.3%)	2 (20%)	1 (8.3%)	14 (20.3%)	42 (16.8%)	
Class 3 Obesity: ≥40	111 (32.6%)	0	3 (25%)	23 (33.3%)	85 (34.0%)	
Histology						$p = 0.766$ (Endometroid versus all other)
Endometrioid	282 (82.7%)	9 (90%)	9 (75%)	56 (81.2%)	208 (83.2%)	
Serous	18 (5.3%)	0	0	0	18 (7.2%)	
Mixed Endometrioid/Serous	14 (4.1%)	0	1 (8.3%)	4 (5.8%)	9 (3.6%)	
Carcinosarcoma	9 (2.6%)	0	0	2 (2.9%)	7 (2.8%)	
Mucinous	5 (1.5%)	0	1 (8.3%)	2 (2.9%)	2 (0.8%)	
Mixed Endometrioid/Clear Cell	3 (0.9%)	0	1 (8.3%)	0	2 (0.8%)	
Clear Cell	3 (0.9%)	1 (10%)	0	0	2 (0.8%)	
Other ≠	7 (2.1%)	0	0	5 (7.2%)	2 (0.8%)	

Characteristic	All	Lynch syndrome	Double somatic	MLH1 methylated	pMMR	*p*-value
	***n* = 341**	***n* = 10**	***n* = 12**	***n* = 69**	***n* = 250**	
FIGO Grade						*p* = 0.060
1	236 (69.2%)	9 (90%)	8 (66.7%)	40 (58.0%)	179 (71.6%)	
2	40 (11.7%)	0	1 (8.3%)	16 (23.2%)	23 (9.2%)	
3/High	65 (19.1%)	1 (10%)	3 (25%)	13 (18.8%)	48 (19.2%)	
Lymphovascular Invasion						p = 0.001
Yes	79 (23.2%)	1 (10%)	3 (25%)	29 (42.0%)	46 (18.4%)	
No	262 (76.8%)	9 (90%)	9 (75%)	40 (58.0%)	204 (81.6%)	
Myometrial Invasion						p = 0.002
Yes	263 (77.1%)	4 (40%)	11 (91.7%)	61 (88.4%)	187 (74.8%)	
No	78 (22.9%)	6 (60%)	1 (8.3%)	8 (11.6%)	63 (25.2%)	
Surgical Stage						*p* = 0.048
Low Stage (I–II)	275 (80.6%)	9 (90%)	8 (66.7%)	49 (71.0%)	209 (83.6%)	
High Stage (III–IV)	66 (19.4%)	1 (10%)	4 (33.3%)	20 (29.0%)	41 (16.4%)	
Other self-reported malignancy[§]						*p* = 0.284 (None vs Any other cancer)
Colon cancer	1 (0.3%)	0	0	1 (1.5%)	0	
Breast cancer	22 (6.5%)	0	1 (8.3%)	4 (5.8%)	17 (6.8%)	
Ovarian cancer	5 (1.5%)	1 (10%)	0	2 (3.0%)	2 (0.8%)	
Stomach cancer	0	0	0	0	0	
Small bowel cancer	0	0	0	0	0	
Urinary tract/kidney/bladder	3 (0.9%)	0	0	1 (1.5%)	2 (0.8%)	
Cervical cancer	2 (0.6%)	0	0	2 (3.0%)	0	
Other	26 (7.6%)	2 (20%)	1 (8.3%)	2 (3.0%)	21 (8.4%)	
None	284 (83.3%)	7 (70%)	10 (83.3%)	54 (78.3%)	213 (85.2%)	
≥2 LS malignancies	9 (2.6%)	1 (10%)	0	3	5 (2.0%)	
Family History[§]						*p* = 0.242 (FDR with LS-assoc cancer vs. None)
FDR with CRC	52 (15.2%)	3 (30%)	3 (25%)	8 (11.6%)	38 (15.2%)	
FDR with EC	28 (8.2%)	3 (30%)	1 (8.3%)	7 (10.4%)	17 (6.8%)	
FDR with breast cancer	65 (19%)	1 (10%)	2 (16.7%)	9 (13.0%)	53 (21.2%)	
FDR with ovarian cancer	3 (0.9%)	0	0	1 (1.5%)	2 (0.8%)	
FDR with LS-associated cancer	181 (53.1%)	8 (80%)	6 (50%)	32 (46.4%)	135 (54.0%)	

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FDR = first-degree relative.

LS-associated cancer = colorectal, ovarian, gastric, urothelial, hepatobiliary, small bowel, or brain cancers.

[*] 1 Asian, 2 other, and 1 with race not reported.

[ǂ] Other = 2 dedifferentiated, 1 endometrioid/dedifferentiated, 1 high grade endometrial NOS, 1 mixed endometrioid/high grade, 1 mixed clear cell/serous, 1 mixed clear cell/serous/ endometrioid.

[§] Percentages may not total 100 since categories are not mutually exclusive.

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Table 2

Concordance between MSI and IHC dMMR screening in EC.

MSI	IHC	Count	Agreement
High	Abnormal	71	Concordant 94.2% (306/325)
Low	Normal/Intact	3	
Stable	Normal/Intact	232	
High	Normal/Intact	2	Discordant 5.8% (19/325)
Low	Abnormal	9	
Stable	Abnormal	8	

MSI was not possible due to small tumor size for 16/341 cases (15 with intact MMR expression and one with abnormal IHC).

Table 3

Lynch syndrome cases.

						MMR Tumor Testing						MMR Germline Mutations		
Individual ID	**Age at Dx**	**BMI**	**FDR with CRC or EC**	**Surgical Stage**	**Myometrial Invasion**	**MLH1 IHC**	**MSH2 IHC**	**MSH6 IHC**	**PMS2 IHC**	**MSI**	**MLH1 Methylation**	**Gene**	**Variant**	**Classification**
368108	39	35.9	Yes	II	Yes	Present	Absent	Absent	Present	MSI-H	Absent	MSH2	c.2006–1G > T	Likely Pathogenic
506582	41	23.79	Yes	IA	No	Present	Absent	Absent	Present	MSI-H	Absent	MSH2	c.2211–1G > C	Likely Pathogenic
450392	53	21.03	Yes	IA	No	Present	Present	Present	Present	MSI-H	Absent	MSH2	c.2132G > C, p.R711P	Pathogenic
432807	52	29.18	Yes	IA	No	Present	Present	Absent	Present	MSI-H	Absent	MSH6	c.3768 T > G, p.Y1256X	Pathogenic
410628	53	39.77	Yes	IA	No	Present	Present	Absent	Present	MSI-H	Absent	MSH6	Exon 3–6 deletion	Pathogenic
409047	57	26.78	No	IIIC1 (with LVSI)	Yes	Present	Present	Absent	Present	MSI-H	Absent	MSH6	c.3939_3957dup, p.A1320Sfs*5	Pathogenic
500138	44	33.98	No	IA	No	Present	Present	Absent	Present	Insufficient Tumor	NA	MSH6	c.1109 T > C, p.L370S	Pathogenic
368147	44	20.85	No	IA	Yes	Present	Present	Absent	Present	MSS	NA	MSH6	c.1109 T > C, p.L370S	Pathogenic
391414	50	30.26	No	IA	Yes	Present	Present	Absent	Present	MSS	NA	MSH6	c.3939_3957dup, p.A1320Sfs*5	Pathogenic
393454	50	27.92	Yes	IA	No	Present	Present	Present	Absent	MSI-H	Absent	PMS2	c.1831dup, p.I611Nfs*2	Pathogenic

All cases were Grade 1 Endometrioid adenocarcinomas except case 391414 which was a clear cell carcinoma.

Dx = Diagnosis.

BMI = Body Mass Index.

FDR with CRC or EC = First-degree relative with colorectal cancer or endometrial cancer.

IHC = Immunohistochemical staining.

MSI = Microsatellite instability.

Table 4

Double somatic MMR gene pathogenic variant cases.

									MMR Tumor Testing						MMR Somatic Variants		
Individual ID	**Age At Dx**	**BMI**	**FDR with CRC or EC**	**Histology**	**Surgical Stage**	**FIGO Grade**	**Myometrial Invasion**	**Lymphovascular Invasion**	**MLH1 IHC**	**MSH2 IHC**	**MSH6 IHC**	**PMS2 IHC**	**MSI**	**Methylation**	**Gene**	**Somatic Variant 1**	**Somatic Variant 2**
507193	57	46.88	No	Mixed Endometrioid/ Clear cell	IA	High	No	Not Present	Absent	Present	Present	Absent	MSI-H	Absent	MLH1	c.707_711del; p.K236Tfs*69	c.1990–3C > G
440989	58	42.97	Yes	Endometrioid	IIIA	1	Yes	Not Present	Absent	Present	Present	Absent	MSI-H	Absent	MLH1	c.1833_1842del; p.V612X	c.676C > T; p.R226X
510318	63	27.52	Yes	Endometrioid	IA	1	Yes	Not Present	Absent	Present	Present	Absent	MSI-H	Absent	MLH1	c.199G > A; p.G67R	LOH
363021	66	29.32	No	Endometrioid	IA	1	Yes	Not Present	Absent	Present	Present	Absent	MSI-H	Absent	MLH1	c.442A > C; p.T148P	LOH
500575	59	25.89	No	Mucinous	IIIC2	1	Yes	Not Present	Not done	Absent	Absent	Present	MSI-H	Absent	MSH2	c.1801C > T; p.Q601X	c.2635–24A > G
368111	47	61.67	No	Endometrioid	IA	1	Yes	Not Present	Present	Absent	Absent	Present	MSI-H	Absent	MSH2	c.1203dupA; p.Q402Tfs*15	c.1294_1298del; p.L432Gfs*9
503307	56	33.16	No	Endometrioid	IIIC1	1	Yes	Present	Present	Absent	Absent	Present	MSI-H	Absent	MSH2	c.1705_1706del; p.E569Ifs*2	LOH
410156	63	27.19	Yes	Endometrioid	IA	2	Yes	Not Present	Present	Absent	Absent	Present	MSI-H	Absent	MSH2	c.1046C > A; p.P349H	c.2089 T > C; p.C697R
402187	76	24.87	No	Endometrioid	IA	3	Yes	Not Present	Present	Absent	Absent	Present	MSI-H	Absent	MSH2	c.790C > T; p.Q264X	c.2038C > T; p.R680X
403125	65	25.09	No	Mixed Endometrioid/ Serous	IIIA	High	Yes	Present	Present	Absent	Absent	Present	MSI-L	Not done	MSH2[1]	c.1250 T > C; p.V4717A	c.2276G > T; p.G759V
500071	67	36.32	Yes	Endometrioid	IA	1	Yes	Present	Present	Present	Absent	Present	MSI-L	Not done	MSH6	c.721del; p.S241Vfs*5	c.3261dup; p.F1088Lfs*5
368105	61	32.4	Yes	Endometrioid	IA	1	Yes	Not Present	Present	Present	Absent	Present	MSS	Not done	MSH6	Exon 7 deletion	c.3261dup; p.F1088fs*5

[1]Patient 403125 has a somatic c.1376C > T (p.S459F) *POLE* mutation and hypermutated tumor with 3 variants in MSH2, 5 in MSH6, 2 in MLH1 and 4 in PMS2. These are assumed to be the relevant somatic MSH2 variants based on VAF and CADD and the IHC findings.

Dx = Diagnosis.

BMI = Body Mass Index.

FDR with CRC or EC = First-degree relative with colorectal cancer or endometrial cancer.

IHC = Immunohistochemical staining.

MSI = Microsatellite instability.

Table 5

Proportion of double domatic MMR gene mutations and Lynch syndrome by tumor MMR class.

Tumor MMR abnormality (*N* = 22)	**Mutation testing finding**		
IHC finding	**Lynch syndrome (10)**	**Double somatic mutation (12)**	**Gene in which mutation(s) found**
Absent MSH6	6 (75%)	2 (25%)	*MSH6*
Absent MSH2/MSH6	2 (25%)	6 (75%)	*MSH2*
Absent MLH1/PMS2 *	0	4 (100%)	*MLH1*
Absent PMS2	1 (100%)	0	*PMS2*
Normal IHC, MSI-H *	1 (100%)	0	*MSH6*

* *MLH1* methylation testing negative