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Cancer susceptibility gene mutations in type I and II endometrial cancer☆,☆☆

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HIGHLIGHTS

- *BRCA1/2* mutations were identified in 0.93% and 0.12% of type I and type II EC, respectively.
- Cancer predisposition gene mutations were found in 4–6% of patients with EC.
- Frequency of LS mutations was similar in both type I and type II EC.

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ABSTRACT

Objectives. To determine the incidence of germline cancer predisposition gene mutations in patients with endometrial cancer (EC) subtypes.

Methods. Germline DNA was extracted from whole blood collected from consenting patients undergoing primary surgery for EC between 5/2005 and 11/2016. DNA samples were evaluated by product sequencing from a targeted multiplex PCR panel including 21 known/suspected cancer predisposition genes. Variants were classified as pathogenic/likely pathogenic based on allele frequency (<0.003), effects on protein function, and ClinVar assertions.

Results. Germline panel testing was performed on 1170 cases of EC; 849 (72.6%) were type I, and 321 (27.4%) were type II EC, including 135 (11.5%) uterine serous cancers (USC). *BRCA1* mutations were enriched in Type II EC compared to Type I EC (0.93% vs. 0.12%, $p = 0.07$). Lynch Syndrome (LS) mutations were identified in 1.4% of type I and 1.6% of type II EC ($p = 0.79$), including 1.5% for USC. In total, predisposition gene mutations were present in 4.2% of type I and 5.3% of type II EC, as well as 6.7% of patients with USC).

Conclusions. BRCA1/2 and Lynch mutations were rare in this cohort of unselected patients with type I and II EC, including USC. However, the presence of predisposition gene mutations in 4.2% of EC type I, 5.3% of EC type II, and 6.7% of USC suggests that somatic mutation testing should be considered for all EC patients.

1. Introduction

Endometrial cancer (EC) is composed of type I and type II subgroups, with the type I subtype accounting for over 80% of EC and type II EC representing the remaining 10–20%. However, the more aggressive type II histologic subtype accounts for at least 40% of EC-related deaths [1,2]. To date, only six genes included on clinical hereditary cancer panel tests have a proven association with EC (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, and *PTEN*) [3], with referrals for clinical panel testing generally limited to patients suspected of having Lynch syndrome (LS). Furthermore, genetic predisposition to type II EC, and risk factors for the disease

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are not well defined. Several small, retrospective studies have suggested a link between *BRCA1* mutations and uterine serous cancer, while others have found no such risk. These studies are difficult to interpret, as many include only Ashkenazi Jewish populations or are confounded by tamoxifen exposure [4–6]. However, a recent prospective study found an increased risk of serous/serous-like EC in a large cohort of *BRCA1*-positive women undergoing risk reducing salpingo-oophorectomy (RRSO) without hysterectomy. In this investigation, 4 out of 627 women with *BRCA1* mutations developed serous/serous-like EC after 3781.0 woman-years of follow-up. The observed rate of serous/serous-like EC was 22.2 times the expected rate and translated to a 2.6–4.7% risk of developing serous/serous-like EC through age 70 [7]. However, these estimates were based on only four cases of serous-like EC, three of which were associated with a history of tamoxifen use. Although observed rates of serous/serous-like EC remained high when stratified for tamoxifen exposure, it is difficult to make recommendations regarding risk-reducing hysterectomy in *BRCA* carriers based on limited sample sizes.

Sample size has also limited estimation of type II EC risk in patients with Lynch Syndrome (LS). In one investigation of 50 patients with serous or mixed serous endometrial cancer, no mismatch repair (MMR) defects were identified [8]. In another study of 40 cases of MMR-deficient endometrial cancer, only 26% were grade 3, and most were of endometrioid subtype. Only 5 type II cases were identified, including 1 uterine serous cancer. Another study including 134 patients with USC identified an MMR mutation in a single case with primarily endometrioid histology and only 1% serous component [9]. Given these limited data, the risk of type II EC in patients with LS is unknown.

In order to more accurately characterize mutations associated with EC, we performed germline testing in a large, prospectively collected cohort of patients with all EC subtypes. The objective of this study was to determine the incidence of germline *BRCA* and hereditary breast and/or ovarian cancer gene mutations in all EC subtypes. These data may determine the need for mutation screening in this population and help inform practical recommendations regarding hysterectomy for *BRCA*-positive women undergoing prophylactic salpingo-oophorectomy.

2. Methods

Consenting patients with a pre-operative diagnosis of EC or complex atypical hyperplasia (CAH) between 5/2005 and 11/2016 were enrolled to a prospectively maintained database at the time of primary surgery at a single institution (Mayo Clinic, Rochester, MN). Information regarding personal and family history of cancer was obtained from a patient-completed questionnaire. Histologic diagnosis was based on the final pathology report of the hysterectomy specimen. Tumors were considered serous if >10% serous histology was reported. Type I EC included tumors with grades 1–2 endometrioid histology, whereas Type II EC included grade 3 endometrioid and all other non-endometrioid tumors. Histologic information was abstracted from clinical pathology reports.

Germline DNA was extracted from whole blood collected at the time of enrollment. DNA samples were evaluated by sequencing of products from a targeted multiplex amplicon-based QIAseq panel (Qiagen) including 21 known or suspected cancer predisposition genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDKN2A*, *CHEK2*, *NBN*, *NF1*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*). Primers covering the coding regions and essential splice sites for each of the genes of interest were included on the panel. Amplicon products were dual barcoded for sample identification. The resulting DNA libraries were sequenced using 150 bp paired-end reads on an Illumina HiSeq4000. Up to 768 samples were sequenced per lane with a median sequence read depth of 200×. Cutadapt v1.10 was used to trim adaptor sequences, and the remaining reads were aligned to the human reference (GRCh37; bwa-mem v0.7.10). Variants were joint-called with haplotype caller and evaluated for depth of coverage using the Genome Atlas Toolkit (GATK) v.3.4–4.6 [10]. Variants were excluded if fewer than 5 alternate reads or 20 total reads were available. Variants were also called using a single-sample caller (Vardict v1.5.1) using default settings except that variant calls were limited to those with <1% alternate allele frequency [11]. The union of variants between the two variant callers was used for downstream analysis. Eight samples (4 pairs) had a high degree of relatedness (Identity By Descent values > 0.5). None were mutation carriers. For each of the 4 pairs, the relatives with the youngest age at diagnosis were retained for the analyses.

Analyses were focused on pathogenic variants in mismatch repair genes associated with LS (*MSH2*, *MSH6*, *PMS2*, and *MLH1*) as well as other hereditary breast and/or ovarian cancer predisposition genes (*ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *NBN*, *NF1*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53*) defined as "increased risk" of either breast or ovarian cancer with recommendations for clinical management in NCCN Clinical Practice Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian [12] in both EC cases and gnomAD non-Finnish European (NFE) public reference controls [13]. The Genome Aggregation Database (gnomAD) is a database of aggregated and harmonized exome and genome sequencing data. With the exception of TCGA, all studies contributing to the database are non-cancer studies. The database includes 55,860 unrelated non-Finnish European (NFE) exomes which were used as reference controls in our study. Variants were classified as pathogenic/likely pathogenic based on allele frequency (<0.003), effects on protein function, and ClinVar assertions. Rare variants (AF <0.003 in gnomAD) were included in the analyses along with known pathogenic recurrent or founder mutations (e.g. CHEK2 c.1100delC).Stop-gain, frameshift, and essential splice site (+/− 1–2 consensus region) variants were considered pathogenic unless functional evidence or ClinVar assertions from clinical groups (SCRP, Invitae, Ambry Genetics, GeneDx, Emory, InSiGHT) suggested otherwise. If the ClinVar assertions from clinical groups were not in agreement, the more conservative assertion was retained. In addition, nonsense mediated mRNA decay effects were considered for *BRIP1*, with variants in the last exon or last 55 nucleotides of the penultimate exon excluded. Variants in exons 9 and 11–15 of *PMS2* were not included in analyses due to homology with the *PMS2L* pseudogene in these regions. All variants classified as pathogenic in EC cases underwent visual inspection in IGV 2.4.4 [14]. All variants in EC cases with an alternate allele fraction <0.20 or >0.80 were excluded due to possible mosaicism or clonal hematopoiesis.

Data are summarized using mean and standard deviation (SD) for continuous variables and frequency and percentages for categorical variables and mutations. Comparisons between mutation carriers and non-carriers were evaluated using the two-sample *t*-test for age and BMI, the Fisher's exact test for Caucasian race, and the chi-square test for stage, grade and histology. Mutation frequencies were compared between subtypes and between EC cases and gnomAD reference controls using the Fisher's exact test. All calculated *p*-values were two-sided. Ninety-five percent confidence interval (95% CI) were constructed using a) an exact method for a binominal parameter for the incidence of each mutation and b) an exact method for the odds ratios using an algorithm by Fay [15]. Statistical analysis was performed using the SAS version 9.4 software package or with R package 3.4.2.

3. Results

Of the 1549 consented patients with a pre-operative diagnosis of EC or CAH, 372 were excluded either due to lack of blood samples ($n = 205$), non-endometrial primary cancer ($n = 40$), or CAH based on final pathology ($n = 127$). In addition, three samples were not submitted for sequencing due to failed quality control following library preparation, and four patients were excluded due to a high degree of relatedness to another individual identified in the bioinformatics quality control process. Among the remaining 1170 patients, there were 849 (72.6%) with type I EC and 321 (27.4%) with type II EC, of which 135 were uterine serous cancers (11.5%). Demographic and clinical characteristics are summarized in Table 1. Among the 321 patients with type

Table 1
Summary of demographic and clinical characteristics.

Characteristic	All ($N = 1170$)	Type I EC ($N = 849$)	Type II EC ($N = 321$)
Age at surgery (years), mean (SD)	63.6 (10.2)	62.2 (9.9)	67.5 (9.9)
Caucasian, n (%)	1164 (99.5)	846 (99.6)	318 (99.1)
BMI (kg/m^2), mean (SD)	35.0 (9.4)	36.2 (9.5)	31.9 (8.3)
Menopausal status, n (%)			
Pre	53 (4.5)	49 (5.8)	4 (1.2)
Peri	47 (4.0)	41 (4.8)	6 (1.9)
Post	1058 (90.4)	751 (88.5)	307 (95.6)
Not documented	12 (1.0)	8 (0.9)	4 (1.2)
FIGO 2009 Stage, n (%)			
IA	839 (71.7)	681 (80.2)	158 (49.2)
IB	145 (12.4)	97 (11.4)	48 (15.0)
II	22 (1.9)	10 (1.2)	12 (3.7)
IIIA	21 (1.8)	11 (1.3)	10 (3.1)
IIIB	3 (0.3)	2 (0.2)	1 (0.3)
IIIC	95 (8.1)	39 (4.6)	56 (17.4)
IVA	7 (0.6)	0 (0)	7 (2.2)
IVB	28 (2.4)	5 (0.6)	23 (7.2)
Unstaged	10 (0.9)	4 (0.5)	6 (1.9)
FIGO grade, n (%)			
1	579 (49.5)	579 (68.2)	n/a
2	270 (23.1)	270 (31.8)	n/a
3	321 (27.4)	n/a	321 (100.0)
Histology, n (%)			
Endometrioid[a]	956 (81.7)	849 (100)	107 (33.3)
Serous	135 (11.5)	n/a	135 (42.1)
Carcinosarcoma	43 (3.7)	n/a	43 (13.4)
Clear cell	36 (3.1)	n/a	36 (11.2)

Abbreviations: BMI, body mass index; EC, endometrial cancer; FIGO, International Federation of *Gynecology* and Obstetrics; n/a, not applicable; SD, standard deviation.
[a] Includes endometrioid/mucinous histology.

II EC, 34 (11.2%) had a personal history of breast cancer and 15 (4.7%) had previous tamoxifen exposure. Among the 135 patients with USC, 18 (13.3%) had a personal history of breast cancer and 8 (5.9%) reported previous tamoxifen exposure (Table 1).

Among these 1170 patients, 53 mutations were identified in 12 cancer predisposition genes (*MSH2*, *MSH6*, *PMS2*, *ATM*, *BRCA1*, *BRCA1*, *BRIP1*, *CHEK2*, *NBN*, *RAD51C*, *P53*, *NF1*). Overall, cancer predisposition gene mutations were identified in 4.2% (36/849) of type I EC, 5.3% (17/321) of type II EC, and 6.7% (9/135) of USC. When further stratified by histology, cancer predisposition gene mutations were identified in 5.6% (2/36) with clear cell histology, 4.6% (2/43) with carcinosarcoma, and 3.7% (4/107) with grade 3 endometrioid cancer. Non-LS cancer predisposition gene mutations occurred in 2.8% (24/849) patients with type I EC compared to 3.7% (12/321) patients with type II EC, including 5.2% (7/135) patients with USC (Fig. 1).

Demographic and clinical characteristics were compared between cancer predisposition gene mutation carriers and non-carriers in Table 2. Mean age at surgery was significantly lower among patients with cancer predisposition gene mutations (mean (SD), 60.1 (9.7) vs. 63.8 (10.2); $p = 0.01$). However, this difference is likely explained by the younger age at diagnosis among patients with MMR/Lynch syndrome gene mutations (mean (SD), 56.6 (7.2) years). There was no statistically significant age difference between cancer predisposition gene mutation carriers and wildtype patients when the 17 patients with LS gene mutations were excluded (61.8 (10.3) vs. 63.8 (10.2), $p = 0.24$). Overall, 9% (9/100) of pre- or peri-menopausal women had a cancer predisposition gene mutation compared with 4.1% (43/1058) of post-menopausal women ($p = 0.038$). For type I EC, mean BMI was significantly lower in cancer predisposition gene mutation carriers compared to non-carriers (mean (SD), 31.3 (7.7) vs. 36.4 (9.5); $p = 0.002$). Stage at diagnosis was not significantly different between patients with and without cancer predisposition gene mutations.

The incidence of germline *BRCA1/2* mutations was low in type I EC, type II EC, and USC with rates of 0.47% (95% CI, 0.13–1.20%), 0.93% (95% CI, 0.19–2.71%), and 0.74% (95% CI, 0.02–4.06%), respectively. The rate of *BRCA1* mutations was higher among patients with type II compared to type I EC, though the difference was not statistically significant. *BRCA1* mutations were found in only 0.12% (1/852) of type I EC (95% CI 0–0.65%) compared to 0.93% (3/321) of type II EC (95% CI 0.19–2.71%) ($p = 0.07$) (Fig. 1 and Table 2). Of the three cases of type II EC, one was grade 3 endometrioid, one carcinosarcoma, and one USC. Family history of breast cancer was present in all four *BRCA1* mutation carriers, but there was no family history of EC or ovarian cancer. One *BRCA1* mutation carrier also had a personal history of breast cancer. All *BRCA1* mutation carriers were post-menopausal at EC diagnosis. *BRCA2* mutations were identified in three patients with type I EC (0.35%), including two diagnosed with grade 2 endometrioid and one diagnosed with grade 1 endometrioid. No type II EC cases had *BRCA2* mutations. None of the *BRCA2* mutation carriers had a personal history of breast cancer or a family history of breast, ovarian, or endometrial cancer. In addition, none of the patients with USC and a history of breast cancer and/or tamoxifen exposure were found to have *BRCA1/2* mutations.

LS gene mutations were identified in 1.4% (12/849) of type I and 1.6% (5/321) of type II EC ($p = 0.79$), including 1.5% (2/135) in USC. *MSH6* ($n = 9$) and *PMS2* ($n = 2$) were the most common LS gene mutations

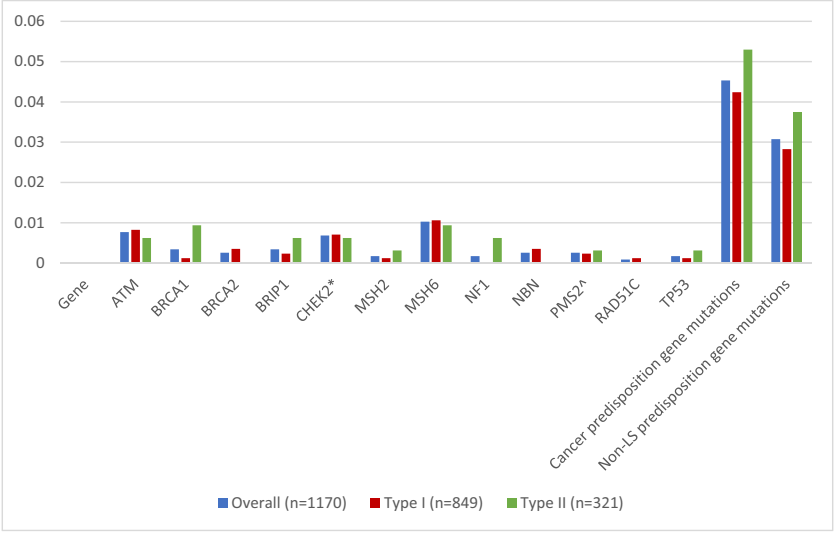

Fig. 1. Rates of cancer predisposition gene mutations in specific EC subtypes. * Does not include p.Ile157Thr. ^ Does not include variants in PMS2 pseudogene region or c.736_741del6ins11. # All USC cases and mutations are included in the Type II frequencies.

Table 2
Comparison of demographic and clinical characteristics between cancer predisposition gene mutation carriers and non-carriers.

Characteristic	All patients			Type I EC			Type II EC		
	Cancer pre-disposition mutation carriers ($N = 53$)	Non-carriers ($N = 1117$)	P[a]	Cancer pre-disposition gene mutation carriers ($N = 36$)	Non-carriers ($N = 813$)	P[a]	Cancer pre-disposition gene mutation carriers ($N = 17$)	Non-carriers ($N = 304$)	P[a]
Age at surgery (years), mean (SD)	60.1 (9.7)	63.8 (10.2)	0.010	59.8 (9.8)	62.3 (9.9)	0.14	60.8 (9.8)	67.9 (9.7)	0.004
Caucasian, n (%)	53 (100)	1111 (99.5)	1.00	36 (100)	810 (99.6)	1.00	17 (100)	301 (99.0)	1.00
BMI (kg/m^2), mean (SD)	30.6 (7.8)	35.2 (9.4)	<0.001	31.3 (7.7)	36.4 (9.5)	0.002	29.2 (8.3)	32.1 (8.3)	0.16
FIGO 2009 Stage, n (%)			0.47			0.62			0.51
IA	40 (75.5)	799 (71.5)		28 (77.8)	653 (80.3)		12 (70.6)	146 (48.0)	
IB	9 (17.0)	136 (12.2)		7 (19.4)	90 (11.1)		2 (11.8)	46 (15.1)	
II	0 (0.0)	22 (2.0)		0	10 (1.2)		0	12 (4.0)	
III	2 (3.8)	117 (10.5)		1 (2.8)	51 (6.3)		1 (5.9)	66 (21.7)	
IV	2 (3.8)	34 (2.9)		0	5 (0.6)		2 (11.8)	28 (9.2)	
Unstaged	0	10 (0.9)		0	4 (0.5)		0	6 (2.0)	
FIGO Grade, n (%)			0.49			0.35			–
1	22 (41.5)	557 (49.9)		22 (61.1)	557 (68.5)		n/a	n/a	
2	14 (26.4)	256 (22.9)		14 (38.9)	256 (31.5)		n/a	n/a	
3	17 (32.1)	304 (27.2)		n/a	n/a		17 (100)	304 (100)	
Histology, n (%)			0.47			–			0.80
Endometrioid[b]	40 (75.5)	916 (82.0)		36 (100)	813 (100)		4 (23.5)	103 (33.9)	
Serous	9 (17.0)	126 (11.3)		n/a	n/a		9 (52.9)	126 (41.4)	
Carcinosarcoma	2 (3.8)	41 (3.7)		n/a	n/a		2 (11.8)	41 (13.5)	
Clear cell	2 (3.8)	34 (3.0)		n/a	n/a		2 (11.8)	34 (11.2)	

Abbreviations: BMI, body mass index; EC, endometrial cancer; FIGO, International Federation of *Gynecology* and Obstetrics; n/a, not applicable; SD, standard deviation.
[a] Comparisons between the mutation carriers and non-carriers were evaluated using the two-sample *t*-test for age and BMI, the chi-square test for grade, and the Fisher's exact test for Caucasian race, stage, and histology.
[b] Includes endometrioid/mucinous histology.

among patients with type I EC. In patients with type II EC, there were three mutations in *MSH6*, one in *MSH2*, and one in *PMS2*. Two patients with clear cell histology, and one patient with serous histology had an *MSH6* mutation. The *PMS2* mutation occurred in a patient with serous histology, and the *MSH2* mutation occurred with a grade 3 endometrioid tumor. There were no *MLH1* mutations identified in this study.

The most commonly-mutated cancer predisposition genes were *CHEK2* ($n = 8$), *MSH6* ($n = 12$), and *ATM* ($n = 9$). There were no differences in incidence of these mutations when stratified for type I vs. type II endometrial cancer (Table 3). Several recurrent pathogenic variants were observed in this study including: *ATM* c.1564_1565delGA ($n = 2$), *BRIP1* c.R798X (n = 2), *MSH6* p.R298X (n = 2), *MSH6* c.3439-2A>G (n = 2), and *CHEK2* c.1100delC ($n = 6$).

Table 3
Frequency of cancer predisposition gene mutations in specific EC subtypes.

Gene	Overall (n = 1170)		Type I (n = 849)		Type II (n = 321)		USC[c] ($n = 135$)	
	n	%	n	%	n	%	n	%
ATM	9	0.77%	7	0.82%	2	0.62%	1	0.74%
BRCA1	4	0.34%	1	0.12%	3	0.93%	1	0.74%
BRCA2	3	0.26%	3	0.35%	0	0.00%	0	0.00%
BRIP1	4	0.34%	2	0.24%	2	0.62%	2	1.48%
CHEK2[a]	8	0.68%	6	0.71%	2	0.62%	1	0.74%
MSH2	2	0.17%	1	0.12%	1	0.31%	0	0.00%
MSH6	12	1.03%	9	1.06%	3	0.93%	1	0.74%
NF1	2	0.17%	0	0.00%	2	0.62%	1	0.74%
NBN	3	0.26%	3	0.35%	0	0.00%	0	0.00%
PMS2[b]	3	0.26%	2	0.24%	1	0.31%	1	0.74%
RAD51C	1	0.09%	1	0.12%	0	0.00%	0	0.00%
TP53	2	0.17%	1	0.12%	1	0.31%	1	0.74%

No mutations were observed in *MLH1*, *PALB2*, or *RAD51D*.
[a] Does not include *CHEK2* p.Ile157Thr.
[b] Does not include variants in PMS2 pseudogene region or c.736_741del6ins11.
[c] All USC cases and mutations are included in the Type II frequencies.

Frequencies of pathogenic variants among EC cases and gnomAD non-Finnish European reference controls were compared to determine the association of specific genes with EC overall and type I EC; there were insufficient cases to determine specific gene associations with type II EC. *MSH6* was statistically significantly associated with substantially increased risk of EC (OR = 10.13, 95% 5.29–18.97, $p < 0.00001$) and type I EC (OR 10.48, 95% CI 5.04–21.20, $p < 0.0001$. In addition, *BLM* was significantly associated with Type I EC (OR 3.21, 95% CI 1.24–7.78, $p = 0.02$), but not EC overall. Despite high frequencies of *ATM* and *CHEK2* mutations in the study, no significant associations with EC were observed (*ATM* OR 1.86, $p = 0.07$; *CHEK2* OR 1.04, $p = 0.85$).

4. Discussion

Germline mutations in *BRCA* and LS genes were relatively rare in this cohort of unselected patients with EC. While the overall rate of *BRCA1* mutations was similar to that in the general population, the rate of *BRCA1* mutations eight times higher among patients with type II compared to type I EC. However, this difference was not statistically significant and was based on only four total mutations among the two groups. While our data suggest a potential association between type II EC and *BRCA1* mutations, a larger cohort will be needed to confirm this finding. It is difficult to recommend changes in clinical management of patients with *BRCA1* mutations based on these data. There was no difference in the incidence of *BRCA2* mutations between the two groups, and no *BRCA2* mutations were detected among patients with type II EC.

MSH6 mutations were significantly associated with EC overall and with both EC subtypes. However, associations between other LS gene mutations and EC were not informative due to limited sample size. We reliably ruled out *CHEK2* and *ATM* as EC predisposition mutations, as these variants were not associated with EC despite a sufficient number of mutations in our cohort. Additionally, we identified *BLM* as a potential EC predisposition gene, consistent with previous reports [16]. Interestingly, about 4–5% of all patients with endometrial cancer were found to have a mutation when panel testing for all cancer

predisposition genes was performed. While this finding does not clarify the role of prophylactic hysterectomy in BRCA- or cancer predisposition gene mutation-positive patients, it may have implications for genetic screening for patients with EC.

Other studies have reported similar rates of *BRCA* mutations in patients with USC. Pennington et al. performed germline panel testing on 151 unselected patients with USC and found a 2% rate of *BRCA1/2* mutations. While this is higher than that in the general population [17], the authors concluded that the absolute risk of USC in *BRCA*-positive patients was probably low, given the rarity of this subtype [9]. Another study of 56 patients with USC found no germline *BRCA* mutations. However, this study tested only a limited number of mutational variants, and a personal (11%) or family history (29%) of breast cancer was found in a significant proportion of patients, suggesting that some mutations may have been missed due to non-comprehensive testing [6].

In contrast, some studies have found a higher proportion of *BRCA* mutations among patients with USC. One study found a 27% rate of *BRCA* mutations in a cohort of 22 Jewish patients with USC. This rate is likely to be confounded by the increased frequency of *BRCA* mutations in Ashkenazi Jewish populations and has not been replicated in studies of unselected patients [18]. Most genetic studies in USC are also confounded by the prevalence of tamoxifen exposure in *BRCA*-positive cohorts. For instance, another investigation found an increased risk of EC in the overall cohort of *BRCA*-positive patients (standardized incidence ratio (SIR) 1.91, 95% CI 1.06–3.19, $p = 0.03$). However, the risk was greatest in the subset of *BRCA*-positive women with tamoxifen exposure (SIR = 4.14, 95% CI 1.92–7.87). Among *BRCA*-positive women who did not receive tamoxifen, the risk of USC was no higher than the standardized incidence found in age and region-matched controls (SIR 1.67, 95% CI 0.81–3.07) [19]. While most authors have specifically examined the association between *BRCA1/2* and USC, a recent prospective cohort study found an association when both serous and serous-like uterine cancers were included. These serous-like cancers included undifferentiated EC and carcinosarcoma, encompassing the majority of type II EC subtypes [7]. For this reason, we examined the association of *BRCA1/2* and other cancer predisposition gene mutations with both type II EC and USC separately.

While the increased risk of type I EC in LS patients is well documented [20,21], the rate of type II EC in patients with LS is poorly defined. Some studies have found a higher percentage of endometrioid histology in LS-associated EC compared to sporadic EC [22], while others indicate a higher rate of type II cancers among patients with LS compared to young patients with sporadic EC [23]. Our study supports the use of LS screening in both type I and II EC, given similar rates of LS among patients with both subtypes. This is consistent with current Society of Gynecologic Oncology (SGO) guidelines recommending systematic clinical and/or molecular screening for LS in all EC patients regardless of histologic subtype.

Among women diagnosed with EC, the use of universal tumor testing to identify those at high risk for LS has led to increased detection compared to the use of clinical screening protocols such as the Amsterdam or Bethesda criteria [24]. Universal screening in EC patients <70 years of age at diagnosis has been shown to be cost-effective due to prevention of colorectal cancer in EC patients and their relatives [25,26]. A similar model of universal or age-related tumor testing, with validation of germline mutation status, could identify patients with cancer predisposition gene mutations, allowing for more rigorous breast and/or ovarian cancer screening after an EC diagnosis as well as early identification and prophylactic treatment in affected relatives. Shu et al. performed somatic testing in 3 patients with germline *BRCA* mutations who developed uterine serous or serous-like cancer after prophylactic oophorectomy. All serous/serous-like tumors demonstrated loss of BRCA protein expression by immunohistochemistry (IHC) analysis. One case of leiomyosarcoma retained BRCA1 protein expression [7]. This suggests that IHC protocols used for LS testing could be modified to detect cancer predisposition gene mutations in patients with EC, though larger IHC studies are needed to validate these findings.

Strengths of this study include a large cohort of patients with type I and II EC, including those with USC. We also utilized a comprehensive, 21-gene panel of clinically-relevant cancer susceptibility genes. The ability to compare patients with both type I and II EC allowed the study to confirm similar rates of LS mutations and low rates of *BRCA* mutations among patients with both subtypes. However, the study is limited by a low frequency of cancer predisposition gene mutations and lack of matched controls, which restricted statistical comparisons between patients with type I and type II EC and between specific mutations, and estimation of absolute risks of EC for individual genes. Our study is also limited by a homogeneous patient population, so it is unclear whether these results would be generalizable to all U.S. or international EC patients. Further study is needed to resolve these issues, to confirm the utility of somatic testing for identification of EC patients with cancer predisposition gene mutations, and to determine the cost-effectiveness of universal or age-related testing.

Overall, our results suggest a clinically significant rate of actionable cancer predisposition gene mutations and LS gene mutations among EC patients. Further study is needed to determine whether these mutations could be detected with IHC analysis of endometrial tumors. These findings suggest that somatic testing for an expanded panel of cancer predisposition gene mutations could identify patients in which germline testing is warranted.

Author contributions

Beverly Long: study design, manuscript writing, data preparation, data interpretation, table and figure preparation.

Jenna Lilyquist: methods section writing, data preparation, data analysis and mutation calling, manuscript editing, data interpretation.

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Chunling Hu: sample preparation, DNA extraction.

Rohan Gnanaolivu: DNA sequencing and bioinformatic analysis.

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Jamie N. Bakkum-Gamez: study design, data interpretation, editing of manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2018.10.019.

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