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Article

Comprehensive analysis of germline drivers in endometrial cancer

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Abstract

Background: We sought to determine the prevalence of germline pathogenic variants (gPVs) in unselected patients with endometrial cancer (EC), define biallelic gPVs within tumors, and describe their associations with clinicopathologic features.

Methods: Germline assessment of at least 76 cancer predisposition genes was performed in patients with EC undergoing clinical tumor-normal Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) sequencing from January 1, 2015, to June 30, 2021. In patients with gPVs, biallelic alterations in ECs were identified through analysis of loss of heterozygosity and somatic PVs. Clinicopathologic variables were compared using nonparametric tests.

Results: Of 1625 patients with EC, 216 (13%) had gPVs, and 15 patients had 2 gPVs. There were 231 gPVs in 35 genes (75 [32%] high penetrance; 39 [17%] moderate penetrance; and 117 [51%] low, recessive, or uncertain penetrance). Compared with those without gPVs, patients with gPVs were younger ($P = .002$), more often White ($P = .009$), and less obese ($P = .025$) and had differences in distribution of tumor histology ($P = .017$) and molecular subtype ($P < .001$). Among 231 gPVs, 74 (32%) exhibited biallelic inactivation within tumors. For high-penetrance gPVs, 63% (47 of 75) of ECs had biallelic alterations, primarily affecting mismatch repair (MMR) and homologous recombination related genes, including *BRCA1*, *BRCA2*, *RAD51D*, and *PALB2*. Biallelic inactivation varied across molecular subtypes with highest rates in microsatellite instability-high (MSI-H) or copy-number (CN)–high subtypes (3 of 12 [25%] POLE, 30 of 77 [39%] MSI-H, 27 of 60 [45%] CN-high, 9 of 57 [16%] CN-low; $P < .001$).

Conclusions: Of unselected patients with EC, 13% had gPVs, with 63% of gPVs in high-penetrance genes (MMR and homologous recombination) exhibiting biallelic inactivation, potentially driving cancer development. This supports germline assessment in EC given implications for treatment and cancer prevention.

Recent studies have highlighted the molecular heterogeneity of endometrial cancer (EC) and associations with outcomes (1), leading to universal assessment of mismatch repair (MMR) deficiency and microsatellite instability (MSI), with implications for treatment and inherited risk, as MMR-deficient and/or MSI-high (MSI-H) tumors are a hallmark of Lynch syndrome (LS) (1-4). Although most ECs are sporadic, 2%-6% develop in the setting of LS, occurring in patients with germline pathogenic variants (gPVs) in MMR genes (*MLH1*, *MSH2*, *PMS2*, *MSH6*, *EPCAM*) (5,6).

Increased risk of EC has also been suggested in individuals with *PTEN*-associated autosomal dominant syndromes (eg, Cowden syndrome) (7-10), *POLE* and *POLD1* mutations (11,12), and biallelic mutations in *MUTYH* (13). Germline mutations in *BRCA1* and *BRCA2*, *CHEK2*, and other DNA repair genes have been identified in patients with high-grade ECs (14,15), and there are increasing data to suggest an elevated risk of high-grade ECs with *BRCA1* and *BRCA2* gPVs (16,17).

We previously reported on 156 patients with newly diagnosed EC undergoing tumor-normal sequencing from April 2016 to May

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2017; 14% of patients had gPVs (6). Other studies have reported gPVs in 10%-15% of patients with EC undergoing genetic testing (18-20). These studies are limited by ascertainment bias and lack of tumor-level data, which can infer the contribution of gPVs to tumor development through assessment of biallelic inactivation within the tumor at the gPV locus (21,22). We previously identified high levels of biallelic inactivation in EC tumors of patients with gPVs in *BRCA1* and *BRCA2*, with tumors harboring genomic features of homologous recombination deficiency (23).

Outside of LS and MMRdeficient/MSI-H EC, association of other gPVs with EC development has not been well established. Currently, it is unclear if all identified gPVs, particularly those in low-penetrance or recessive genes, are associated with disease. We sought to determine the prevalence of gPVs in unselected patients with EC, define associations with clinicopathologic features, and assess for biallelic inactivation of gPVs in tumors.

Methods

Patient selection

Patients with histologically confirmed EC (excluding uterine sarcomas) consented to clinical tumor-normal sequencing using Memorial Sloan Kettering Cancer Center (MSK)-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) from January 1, 2015, to June 30, 2021, targeting 341-505 cancer-related genes were included (24,25). Beginning in 2015, our institution standardized care to offer MSK-IMPACT sequencing to all patients with EC; thus, the cohort was unselected. Germline analysis included 76-90 genes (26). Molecular pathologists reviewed results to define pathogenic or likely pathogenic variants (27). gPVs were classified as high (relative risk [RR] > 4), moderate ($2 \leq RR \geq 4$), low (RR < 2), or uncertain penetrance, or recessive (28-30). Variants of uncertain significance were not reported. This study was approved by the institutional review board of MSK.

Clinicopathologic data

Clinical information, including age at diagnosis, body mass index (BMI), disease stage, self-reported race (Asian, Black, White, and Unknown), and Ashkenazi Jewish ancestry, were abstracted from the medical record. Pathology reports were reviewed for histology, grade, MMR immunohistochemistry, and *MLH1* promoter hypermethylation status. Further reviews of medical and family history were performed by genetic counselors for select cases.

Molecular data and biallelic gPV inactivation

MSI status based on MSIsensor score, tumor mutational burden, tumor purity, and variant allele frequencies were obtained from MSK-IMPACT, as previously described (31,32). Tumors with a MSIsensor score of at least 10 were considered MSI-H, less than 10 and at least 3 MSI-indeterminate, and less than 3 microsatellite stable (33).

ECs were classified into 4 molecular subtypes (31,32,34). Tumors 1) harboring hotspot *POLE* exonuclease domain mutations (35) were designated POLE; 2) showing lack of immunohistochemical expression of *MLH1*, *MSH2*, *MSH6*, and/or *PMS2* and/or having an MSIsensor score of at least 10 (33,36) were defined as MSI-H; 3) harboring *TP53* pathogenic variant or homozygous deletions were defined as copy number high (CN-H); and 4) lacking defining features of other subtypes were defined as CN-low (CN-L). Tumors with purity less than 10% and/or variant allele frequencies less than 5% were excluded.

Loss of heterozygosity (LOH) in tumors at the gPV locus was assessed using the Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing (FACETS) algorithm (37). Biallelic inactivation was defined as a loss of the wild-type allele in the tumor at the locus of a pathogenic variant, presence of a second somatic pathogenic alteration, or in the case of *MLH1* gPVs, promoter hypermethylation. Patients with more than 1 gPV and discordant LOH status were considered biallelic if present in the high penetrance gPV (n = 3).

Statistical analyses

Clinicopathologic variables were reported using summary statistics and stratified by gPV status. Associations between continuous clinicopathologic variables and gPV and biallelic inactivation at the patient level were performed using Wilcoxon rank sum and Kruskal-Wallis tests. Fisher exact test was used for categorical variables. We assessed LOH at the variant level (n = 231) and conducted comparisons at the patient level (n = 213). A 2-sided *P* value less than .05 was considered statistically significant. All statistical analyses were performed using R version 4.1.2 (https://cran.r-project.org/).

Results

Patient characteristics

Of 1945 patients with presumed EC consented to MSK-IMPACT sequencing, we excluded 291 (15%) who declined consent for germline results, 23 with nonendometrial primary cancer on review, and 6 with missing data, resulting in 1625 patients for this analysis (Figure 1).

Median age at EC diagnosis was 63 (range = 24-96) years, and 170 patients (10%) were diagnosed at age younger than 50 years (Table 1). Of the patients, 1193 (80%) identified as White; 202 (18%) reported Ashkenazi Jewish ancestry. Median BMI was 29.6 (range = 15.3-67.6) kg/m^2, and 1229 patients (76%) had a BMI of at least 25 kg/m^2. Stage I and II disease comprised 68% of cases, with tumors exhibiting endometrioid (grade 1 and 2 [G1 and 2]) (52%), endometrioid (G3) (9.8%), serous (14%), carcinosarcoma (11%), and clear cell (2.9%) histologies.

Compared with patients without gPV, those with gPV were younger (median age = 61 vs 63 years; $P = .002$), less likely to be overweight or obese (70% vs 77%; $P = .025$), more likely to identify as White (87% vs 79%; $P = .009$), and of Ashkenazi Jewish ancestry (29% vs 17%; $P < .001$) with no differences in stage at diagnosis (Table 1).

Germline landscape and biallelic inactivation

Among 1625 patients with EC, 216 (13%) had gPVs of whom 15 had 2 gPVs. There were 231 gPVs in 35 genes: 75 (32%) high penetrance, 39 (17%) moderate penetrance, 43 (19%) low penetrance, 40 (17%) recessive, and 34 (15%) of uncertain penetrance (Supplementary Tables 1 and 2, available online).

Most gPVs (136 of 231, 59%) were associated with monoallelic loss; however, 74 (32%) exhibited biallelic inactivation of gPVs in tumors, and 21 (9%) were unclassifiable. We found statistically significant differences in biallelic inactivation by penetrance ($P < .001$); 63% (47 of 75) of high-penetrance gPVs exhibited biallelic inactivation within tumors, primarily in MMR and homologous recombination–related genes, compared with 28% (11 of 39) of moderate-penetrance gPVs (Figure 2, Table 2). Among 44 patients with MSI-H tumors and gPVs in non-MMR genes, only 6 (14%) had biallelic inactivation in the tumor (Figure 3).

Patients with biallelic vs monoallelic loss in EC tumors were younger at diagnosis (median age = 58 vs 62 years, respectively; $P = .002$) and more likely to be diagnosed with stage III or IV than

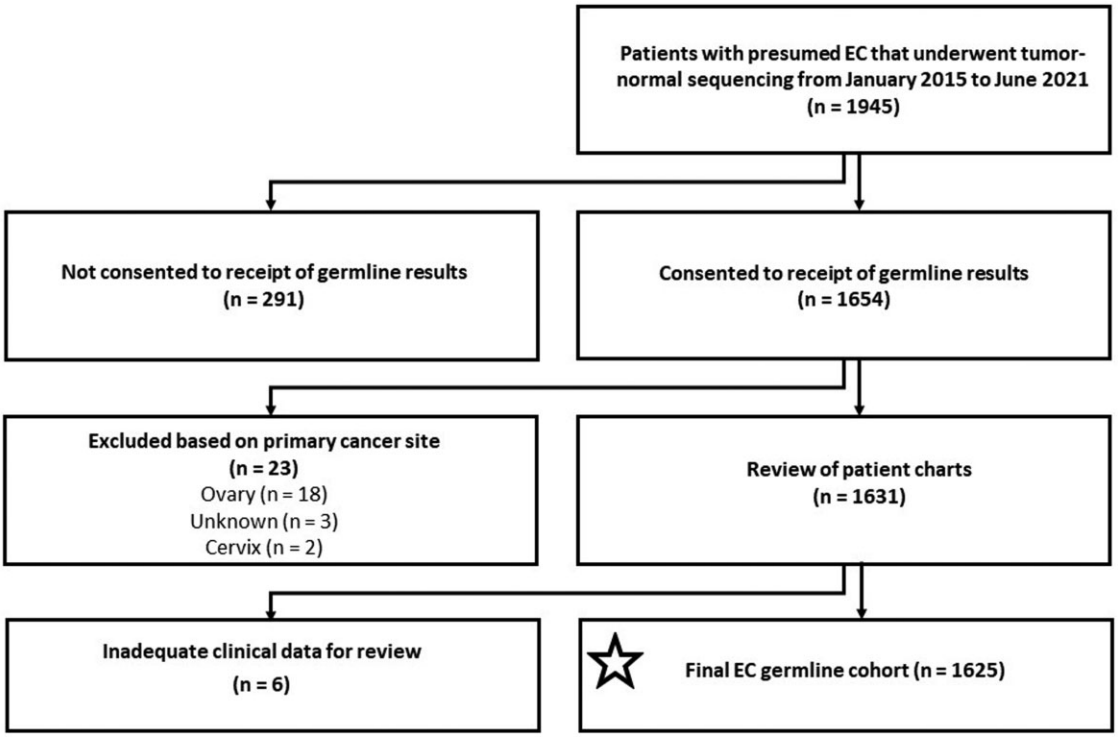

Figure 1. Patient selection. The figure depicts selection of final cohort of 1625 patients with endometrial cancer (EC) who underwent clinical tumor-normal sequencing with germline assessment of at least 76 genes who were analyzed in this study.

stage I or II disease (48% vs 32%, respectively; $P = .003$; Figure 2 and Table 2) with no differences in obesity rates, race, and Ashkenazi Jewish ancestry. When examining only those with gPV in high- or moderate-penetrance genes, results were similar (Supplementary Table 3, available online).

Histologic vs molecular subtypes

Histology differed between ECs occurring in patients with gPVs compared with those without gPVs ($P = .017$); gPVs were present in 12.4% (99 of 800) of G1 and G2 endometrioid, 19.1% (29 of 152) of G3 endometrioid, 8.7% (19 of 218) of serous, 23.9% (11 of 46) of clear cell, 11.3% (19 of 168) of carcinosarcoma, 15.2% (5 of 33) of de- or undifferentiated, and 14.5% (19 of 131) of mixed or high-grade carcinoma not otherwise specified (NOS) tumors (Table 1). Histology also varied in tumors with and without biallelic inactivation ($P = .020$; Table 2) with biallelic inactivation observed in 19.2% (19 of 99) G1 and G2 endometrioid, 44.8% (13 of 29) G3 endometrioid, 26.3% (5 of 19) serous, 57.9% (11 of 19) carcinosarcoma, 27.3% (3 of 11) clear cell, 60.0% (3 of 5) de- or undifferentiated, and 26.3% (5 of 19) of mixed or high-grade carcinoma NOS tumors (Table 2; Supplementary Figure 1, available online).

When stratified by molecular subtype, 546 (34%) ECs were classified as CN-L, 542 (33%) as CN-H, 413 (25%) as MSI-H, and 106 (6.5%) as POLE; 18 (1.1%) were unclassifiable. Distribution of molecular subtype varied for ECs from those with and without gPVs ($P < .001$) (Table 1); gPVs were present in 11.3% (12 of 106) of POLE, 18.6% (77 of 413) of MSI-H, 11.1% (60 of 542) CH-H, and 10.4% (57 of 546) CN-L tumors. Patients with biallelic vs monoallelic gPVs also had distinct distributions of molecular subtypes ($P < .001$). Biallelic inactivation was observed in 25% (3 of 12) of POLE, 39% (30 of 77) of MSI-H, 45% (27 of 60) of CN-H, and 16% (9 of 57) of CN-L tumors (Figure 3 and Table 2). Within the MSI-H subgroup, patients with gPVs and biallelic loss were younger at diagnosis (median age = 55 vs 62 years; $P < .001$) and less likely to have *MLH1* promoter hypermethylation within tumors (13% vs 65%; $P < .001$) compared with patients with monoallelic loss. A similar age trend was observed in patients with CN-H tumors (median age = 63 vs 68 years; $P = .054$) (Supplementary Tables 4 and 5, available online).

MMR genes

A total of 39 patients had LS, representing 18% of patients with gPVs and 2.4% of all patients with EC in the cohort. The majority of LS was *MSH6*- (49%) and *MSH2*-associated (28%). Median age at EC diagnosis was 53 (range = 31-70) years. *PMS2*-associated ECs had an older age at diagnosis (median = 60.5 years; range = 51-70 years; $P < .01$). None of the patients harbored a 3' deletion in *EPCAM*. Although most tumors were endometrioid histology (67%), we also observed clear cell, mixed or high-grade carcinoma NOS, and carcinosarcoma histologies but no serous carcinomas (Supplementary Table 6, available online). All except 6 patients with LS had MSI-H tumors. Among these, 2 ECs were classified as POLE with biallelic loss at the gPV and 1 as CN-L with no biallelic loss at the *MSH6* gPV, suggesting a sporadic EC, and 3 could not be classified (Supplementary Table 7, available online).

Homologous recombination genes

In homologous recombination genes, gPVs in *BRCA1* (n = 10) and *BRCA2* (n = 11) were most common, followed by *PALB2* (n = 3) and *RAD51D* (n = 2); no gPV in *RAD51C* was observed. None of the ECs in patients with *BRCA1* gPVs were of serous histology but included G3 endometrioid (n = 5), carcinosarcoma (n = 3), and mixed or high-grade carcinoma NOS (n = 2) histologies. All 10 ECs in patients with *BRCA1* gPVs and both evaluable patients with *RAD51D* gPVs had biallelic inactivation. A subset of ECs with evaluable gPVs in *BRCA2* (6 of 10, 60%) and *PALB2* (2 of 3, 67%) had biallelic inactivation. Most ECs with gPVs affecting homologous recombination genes were CN-H (16 of 26, 62%) or CN-L (5 of

Table 1. Clinicopathologic information by germline findings

Characteristic	All patients, No. (%) (N = 1625)	Germline negative, No. (%) (n = 1409)	Germline pathogenic variant, No. (%) (n = 216)	P
Age at diagnosis				
Median (range), y	63 (24-96)	63 (24-96)	61 (31-86)	.002
Bivariate				.19
Younger than 50 y	170 (10)	142 (10)	28 (13)	
50 y and older	1455 (90)	1267 (90)	188 (87)	
Self-identified race				.009
Asian	125 (8.4)	110 (8.5)	15 (7.4)	
Black	178 (12)	166 (13)	12 (5.9)	
White	1193 (80)	1018 (79)	175 (87)	
Unknown	129	115	14	
AJ ancestry				<.001
Yes	202 (18)	162 (17)	40 (29)	
No	914 (82)	818 (83)	96 (71)	
Unknown	509	429	80	
BMI				
Median (range), kg/m^2	29.6 (15.3-67.6)	29.8 (15.3-67.6)	28.9 (17.2-60.1)	.053
Overweight or obese				.025
BMI <25 kg/m^2	379 (24)	315 (23)	64 (30)	
BMI ≥25 kg/m^2	1229 (76)	1078 (77)	151 (70)	
Unknown	17	16	1	
FIGO stage				.26
I and II	1036 (68)	903 (69)	133 (65)	
III and IV	481 (32)	409 (31)	72 (35)	
Unknown	108	97	11	
Histology				.017
Endometrioid G1 and G2	800 (52)	701 (52)	99 (49)	
Endometrioid G3	152 (9.8)	123 (9.1)	29 (14)	
Serous	218 (14)	199 (15)	19 (9.5)	
Carcinosarcoma	168 (11)	149 (11)	19 (9.5)	
Clear cell	46 (2.9)	35 (2.6)	11 (5.5)	
De- or undifferentiated	33 (2.1)	28 (2.1)	5 (2.5)	
Mixed or high-grade carcinoma NOS	131 (8.5)	112 (8.3)	19 (9.5)	
Unknown	77	62	15	
Molecular subtype				<.001
POLE	106 (6.5)	94 (6.7)	12 (5.6)	
MSI-H	413 (25)	336 (24)	77 (36)	
CN-H	542 (33)	482 (34)	60 (28)	
CN-L	546 (34)	489 (35)	57 (26)	
Unclassifiable	18 (1.1)	8 (0.6)	10 (4.6)	

AJ = Ashkenazi Jewish; BMI = body mass index; CN-H = copy number high; CN-L = copy number low; FIGO = International Federation of Gynecology and Obstetrics; MSI-H = microsatellite instability high; NOS = not otherwise specified; POLE = polymerase epsilon.

26, 19%). Of note, in 2 patients with *BRCA2* gPVs and monoallelic loss, the EC was MSI-H, suggesting cancer developed independently of the *BRCA2* gPV. All patients with gPVs in *PALB2* or *RAD51D* had CN-H or CN-L EC (Figure 3; Supplementary Table 8, available online). Notably, gPVs in other DNA repair genes, including *CHEK2* (7 of 27), *ATM* (3 of 9), *BARD1* (0 of 3), and *BRIP1* (0 of 2), exhibited variable rates of biallelic inactivation in tumors, and interestingly, the more penetrant *CHEK2* variants had higher levels of biallelic inactivation compared with the I157T variant (Supplementary Table 1, available online).

Given novel associations of *RAD51D* and *PALB2* gPVs with EC, family histories in patients whose tumors exhibited biallelic loss were explored further (Figure 4). In patient case 1, because of a family history of ovarian cancer, the proband underwent risk-reducing bilateral salpingo-oophorectomy (RRSO) without hysterectomy at age 51 years. Her sister was later diagnosed with triple-negative breast cancer at age 49 years, and genetic testing identified a *RAD51D* gPV, which the proband also carried. The proband was subsequently diagnosed with stage IIIC serous EC at age 69 years. In patient case 2, the proband had a family history of stomach cancer and was diagnosed with stage IVA EC at age 56 years.

In patient case 3, the proband presented with abnormal uterine bleeding at age 49 years and was diagnosed with G1 endometrioid adenocarcinoma on endometrial curettage. She underwent total laparoscopic hysterectomy, RRSO, and sentinel lymph node biopsy and was diagnosed with stage II, G1 endometroid EC. MSK-IMPACT sequencing revealed a *PALB2* gPV. The proband's sister, who had a history of hormone receptor–positive breast cancer at age 50 years, underwent cascade testing and was found to share the same *PALB2* gPV. The sister subsequently underwent risk-reducing total laparoscopic hysterectomy and RRSO at age 58 years and was found to have complex atypical hyperplasia bordering on well-differentiated endometrioid EC. In patient case 4, the proband was diagnosed with stage IA EC at age 62 years, with no family history of gynecologic cancers.

Discussion

In our cohort of patients with EC, 13% harbored gPVs across 35 genes. Through integration of somatic and germline data, we found that although only 32% of ECs exhibited biallelic loss, biallelic inactivation was more common among high-penetrance

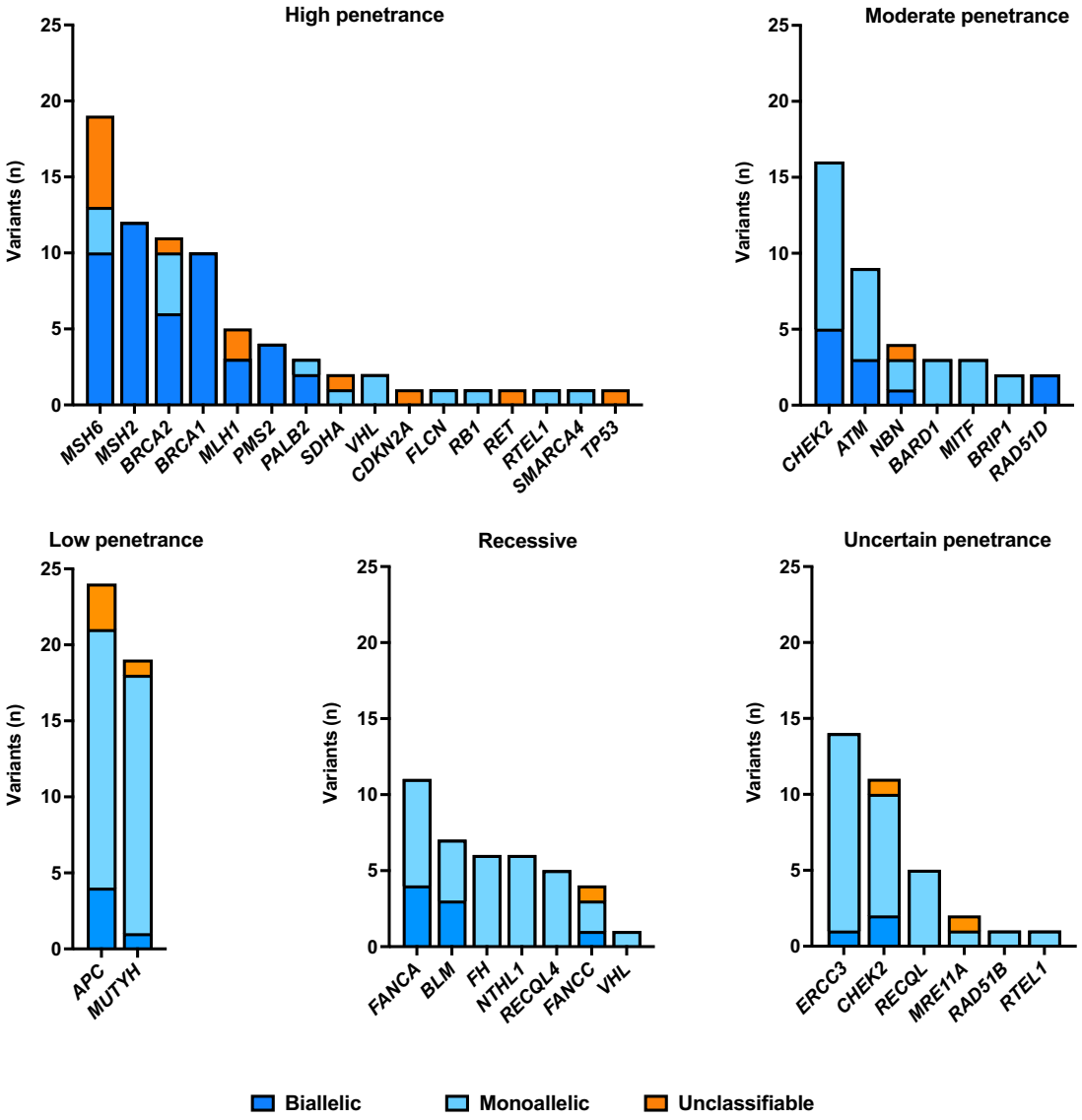

Figure 2. Germline pathogenic variants and loss of heterozygosity by gene penetrance. The figure depicts the 231 germline pathogenic variants in at least 76 genes, monoallelic vs biallelic, grouped by gene penetrance (high, moderate, low, recessive, and uncertain). Higher levels of biallelic loss were observed in high- and moderate-penetrance genes compared with low-, recessive-, and uncertain-penetrance genes.

genes (63%), including *BRCA1*, *BRCA2*, *RAD51D*, and *PALB2*. Additionally, molecular and traditional histologic subtypes varied among patients with gPVs and tumors exhibiting biallelic inactivation, with enrichment of gPVs and biallelic inactivation in MSI-H and CN-H tumors compared with CN-L and POLE tumors. Given implications for treatment and cancer risk reduction, these findings support germline assessment in EC.

Our findings are consistent with rates of gPVs reported in recent studies evaluating multigene panel genetic testing in patients with EC (9%-15%) (15,18-20). Most gPVs were observed in MMR genes associated with LS, ranging from 1.8% to 5.8% in unselected populations (15,19,38) and 8.2% to 9.4% in those referred from genetics clinics (20) or genetic testing laboratories (18). Our LS rate of 2.4% is consistent with previous findings in unselected cohorts, suggesting minimal ascertainment bias reflective of the general population. These studies have also highlighted gPVs in EC outside of MMR genes, in particular *BRCA1* and *BRCA2* and other homologous recombination genes, and associations with serous EC; however, these studies lack integrated tumor data, which are critical to determine if the gPV is a driver of disease.

Additionally, our observed gPV rate in EC is similar to those reported in colorectal (15.4%), prostate (16%), and breast (17.4%) cancers and slightly lower than rates in pancreatic (19.6%) and ovarian (25.5%) cancers from prior pan-cancer studies (29,39). There are universal recommendations for germline assessment in ovarian and pancreatic cancers, and criteria are broadening in breast and prostate cancer, mostly driven by treatment implications (40). In colon cancer, the field is moving toward universal germline assessment, despite less certainty around clinical management of non-MMR genes in this setting and more limited treatment implications compared with EC (41).

Strengths of our study include use of a large, unselected cohort and integrated somatic and germline sequencing data, allowing us to comprehensively assess the contribution of gPVs to disease development. Although the overall rate of gPVs was more than 10%, the proportion exhibiting biallelic inactivation in tumors was lower, suggesting some gPVs are incidental. However, when examining high-penetrance genes with strong associations with phenotype, mostly in MMR or homologous recombination genes and enriched within the MSI-H and CN-H

Table 2. Clinicopathologic information of endometrial cancer patients with germline pathogenic variants (gPVs) by biallelic inactivation in tumors[a]

Characteristic	Monoallelic inactivation, No. (%) (n = 126)	Biallelic inactivation, No. (%) (n = 70)	Unclassifiable, No. (%) (n = 20)	*P* (3 group)	*P* (2 group, mono vs biallelic)
Age at diagnosis					
Median (range), y	62 (34-86)	58 (40-80)	60 (31-69)	.002	.002
Bivariate				.061	.027
<50 y	11 (8.7)	14 (20)	3 (15)		
≥50 y	115 (91)	56 (80)	17 (85)		
Self-identified race				.11	.28
Asian	5 (4.3)	6 (9.2)	4 (20)		
Black	6 (5.1)	5 (7.7)	1 (5.0)		
White	106 (91)	54 (83)	15 (75)		
Unknown	9	5	0		
Ashkenazi Jewish ancestry				>.99	>.99
Yes	21 (30)	14 (30)	5 (26)		
No	50 (70)	32 (70)	14 (74)		
Unknown	55	24	1		
BMI					
Median (range), kg/m^2	30.0 (17.2-60.1)	28.2 (18.1-51.9)	24.8 (18.0-50.9)	.017	.18
Overweight or obese				.10	.62
BMI <25 kg/m^2	33 (26)	21 (30)	10 (50)		
BMI ≥25 kg/m^2	93 (74)	48 (70)	10 (50)		
Unknown	0	1	0		
FIGO stage				.003	.028
I and II	81 (68)	34 (52)	18 (90)		
III and IV	38 (32)	32 (48)	2 (10)		
Unknown	7	4	0		
Histology				.020	.025
Endometrioid G1 and G2	66 (54)	19 (32)	14 (70)		
Endometrioid G3	16 (13)	13 (22)	0		
Serous	12 (9.8)	5 (8.5)	2 (10)		
Carcinosarcoma	8 (6.6)	11 (19)	0		
Clear cell	6 (4.9)	3 (5.1)	2 (10)		
De- or undifferentiated	2 (1.6)	3 (5.1)	0		
Mixed or high-grade carcinoma NOS	12 (9.8)	5 (8.5)	2 (10)		
Unknown	4	11	0		
Molecular subtype				<.001	.002
POLE	9 (7.1)	3 (4.3)	0 (0)		
MSI-H	40 (32)	30 (43)	7 (35)		
CN-H	32 (25)	27 (39)	1 (5.0)		
CN-L	45 (36)	9 (13)	3 (15)		
Unclassifiable	0 (0)	1 (1.4)	9 (45)		
Penetrance				<.001	<.001
High	15 (12)	45 (64)	13 (65)		
Mod	25 (20)	10 (14)	1 (5.0)		
Low	32 (25)	5 (7.1)	3 (15)		
Recessive	28 (22)	7 (10)	1 (5.0)		
Uncertain	26 (21)	3 (4.3)	2 (10)		

[a] Table depicts data by patient. We observed 231 gPVs in 216 patients, with 15 patients having 2 gPVs. If gPVs were discordant, patients were classified as biallelic if the biallelic gPV was high penetrance and thought to be the driver of EC development (n = 3). BMI = body mass index; CN-L = copy number low; CN-H = copy number high; FIGO = International Federation of Gynecology and Obstetrics; MSI-H = microsatellite instability high; NOS = not otherwise specified; POLE = polymerase epsilon.

tumors, levels of biallelic inactivation were higher, confirming the importance of gPVs in disease development and potential implications for targeted therapies. Additionally, even incidental findings may have implications for cancer risk reduction and cascade testing of at-risk family members given established associations of these genes with other cancers (40-42).

Among patients with LS, almost all had either an MSI-H tumor or biallelic inactivation in tumors (POLE subtype); however, we did observe 1 sporadic, CN-L EC in a patient with *MSH6*-associated LS. Although all patients with *MLH1*- or *MSH2*-associated LS had MSI-H tumors, there was more heterogeneity in molecular subgroup among *MSH6*- and *PMS2*-associated LS, reflecting phenotypic variability among LS patients (43) or limitations of MSI assessment in EC (44), which we have previously described (45).

Our work supports the use of molecular classification (eg, CN-H) in addition to traditional histological subtypes (eg, serous) in predicting presence of gPVs. We verified previously described observations of 100% biallelic inactivation in germline *BRCA1*-associated ECs and high rates of biallelic inactivation in germline *BRCA2*-associated ECs (23). Consistent with previous work, no gPVs in *BRCA1* were observed in patients with serous carcinomas, and *BRCA1*-associated ECs in high-grade histologies were better encompassed using CN-H molecular subgroups. The *BRCA2*-associated ECs exhibited lower rates of biallelic inactivation and more heterogeneity of molecular subgroups, with 2 MSI-H ECs that appear unrelated. This is consistent with epidemiological data showing differential risks of EC between *BRCA1* and *BRCA2* heterozygotes (16,17).

We identified novel associations between gPVs in *RAD51D* and *PALB2* with EC and high rates of biallelic inactivation in tumors. This has implications for treatment, as these ECs may exhibit a homologous recombination–deficient phenotype and potentially

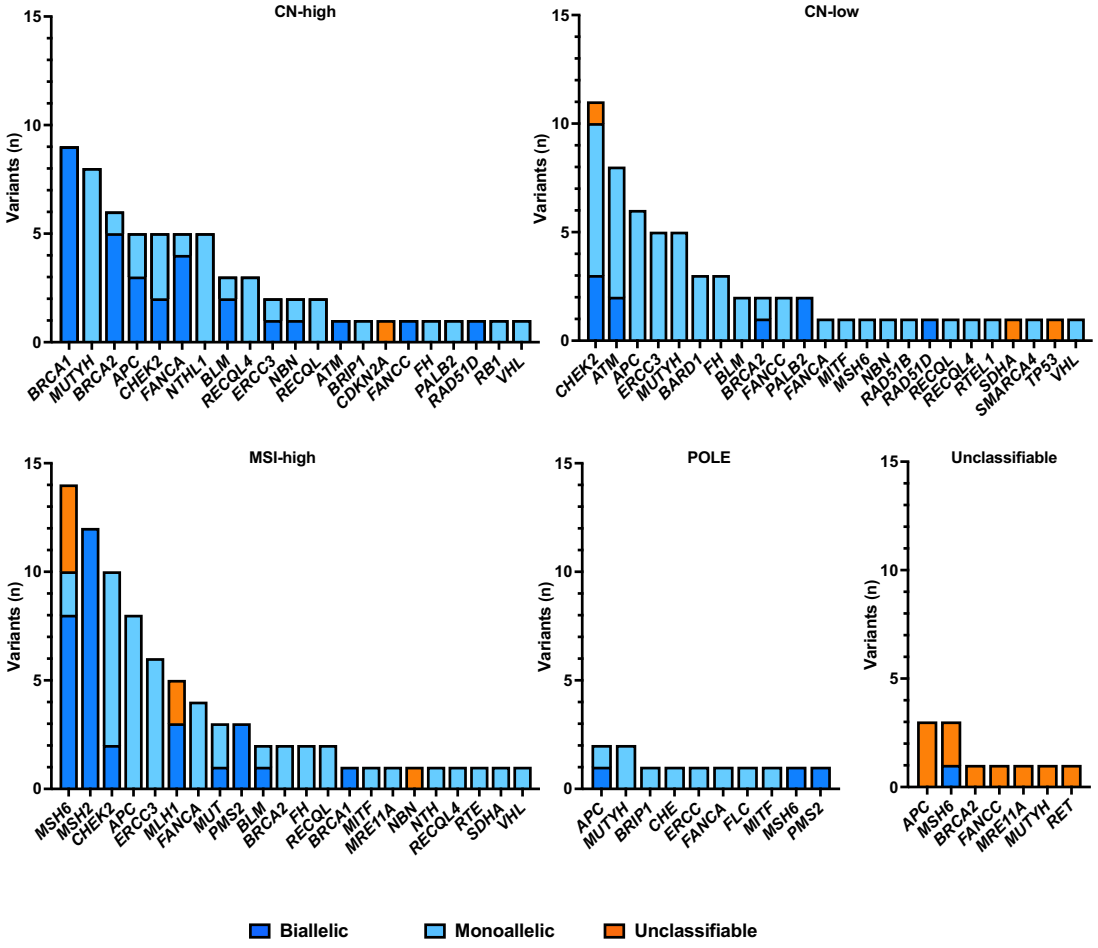

Figure 3. Germline pathogenic variants and loss of heterozygosity by molecular subtype. The figure depicts 231 germline pathogenic variants in at least 76 genes, monoallelic vs biallelic, grouped by molecular subtype. Higher levels of biallelic loss were observed in MSI-H and CN-H tumors compared with CN-L and POLE tumors. CN-H = copy number high; CN-L = copy number low; MSI = microsatellite instability; POLE = polymerase epsilon.

respond to targeted therapies, including PARP inhibitors. This may also have implications for cancer prevention, as gPVs in *RAD51D* and *PALB2* are associated with increased risk of ovarian cancer, and RRSO is recommended or considered in unaffected carriers. The role of concurrent hysterectomy to prevent EC in these situations is a topic that merits further study, particularly given recommendations to consider this in *BRCA* mutation carriers (40). Other DNA repair genes such as *ATM* and *CHEK2* were more heterogenous in terms of biallelic inactivation, suggesting tumor-specific drivers of disease. This may affect efficacy of treatments including PARP inhibitors (46) and trials of novel therapies and decreased radiation (NCT05010031), which are being explored in this population. Future studies integrating somatic and germline data with mutational and tumor homologous recombination–deficient signatures will be critical in determining which patients benefit the most from therapies.

Limitations of our study include potential ascertainment bias given our large Ashkenazi Jewish population and bias toward more aggressive, high-grade tumors, which may influence our gPV rate. Reassuringly, our rates are similar to recent publications, suggesting this bias is minimized (15,19). Our cohort was predominantly White, although 20% of patients identified as non-White. Efforts to expand genetic testing in non-White patients with EC and evaluate differences in germline findings are underway, particularly given disparities in outcomes between Black and White patients with EC (47,48). Although we found an association between gPVs in *RAD51D* and *PALB2* and EC, our sample size is small, and these findings must be verified in larger studies. Future studies should evaluate the effects of these germline findings on outcomes within specific molecular subtypes, particularly as preliminary studies show variations in survival potentially favoring germline-driven EC (49).

In conclusion, we observed a gPV rate of 13% in unselected patients with EC, supporting universal germline assessment. Although some gPVs were incidental, biallelic inactivation was observed in many tumors, particularly with high-penetrance MMR and homologous recombination gPV in MSI-H and CN-H tumors. We identified associations with novel homologous recombination genes, *RAD51D* and *PALB2*, in addition to *BRCA1* and *BRCA2* but did not find consistent biallelic inactivation in other DNA repair genes (*CHEK2* and *ATM*). Our findings may have implications for cancer risk reduction and targeted therapies and demonstrate the necessity of integrated tumor-normal evaluation to assess for true germline drivers of EC.

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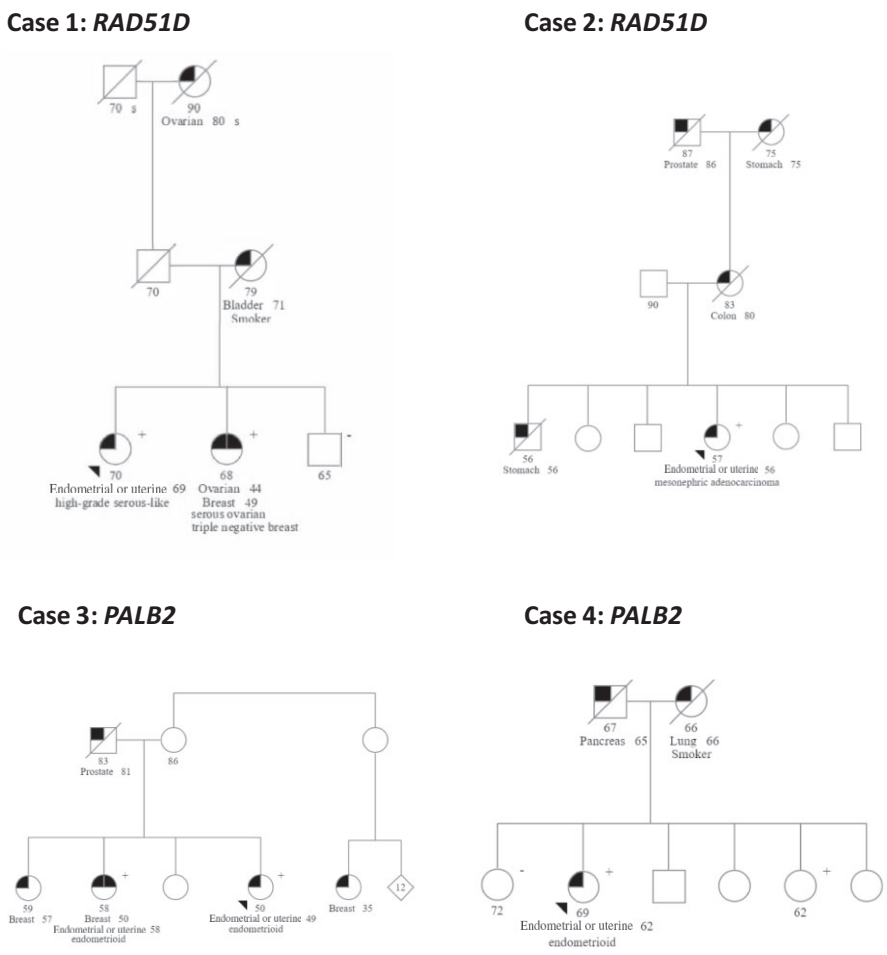

Figure 4. Clinical characteristics and family histories of patients with germline pathogenic variants (gPVs) in homologous recombination genes *RAD51D* and *PALB2* with biallelic inactivation in tumors. The **arrow** points to the proband, and **"+"** indicates presence of pathogenic variant. The figure depicts pedigrees of patients with endometrial cancer (EC) and gPVs in homologous recombination genes *RAD51D* and *PALB2* with biallelic inactivation in tumors. **Case 1)** Because of a family history of ovarian cancer, the proband underwent risk-reducing bilateral salpingo-oophorectomy (RRSO) without hysterectomy at age 51 years. Subsequently, her sister was diagnosed with triple-negative breast cancer at age 49 years, and multigene panel germline testing identified a *RAD51D* gPV, which the proband was also found to carry. At age 69 years, the proband was diagnosed with stage IIIC serous EC with biallelic loss of *RAD51D*. **Case 2)** The proband was diagnosed with stage IVA EC (mesonephric type) at age 56 years. There was a case of stomach cancer in a maternal grandmother. **Case 3**) The proband presented with abnormal uterine bleeding at age 49 years and was found to have grade 1 endometrioid adenocarcinoma on endometrial curettage. She underwent total laparoscopic hysterectomy, RRSO, and sentinel lymph node biopsy at age 50 years and was diagnosed with a stage II, grade 1 endometroid EC. Memorial Sloan Kettering Cancer Center–Integrated Mutation Profiling of Actionable Cancer Target sequencing discovered a *PALB2* gPV with biallelic loss within the tumor. The proband's sister, who had a history of estrogen- and progestin receptor–positive breast cancer at age 50 years, underwent cascade testing and was found to share the same *PALB2* gPV. Following discovery of the *PALB2* gPV, she underwent risk-reducing total laparoscopic hysterectomy and RRSO at age 58 years and had complex atypical hyperplasia bordering on well-differentiated endometrioid EC. **Case 4)** The proband was diagnosed with stage IA EC at age 62 years with no family history of gynecologic cancers. The proband's 62-year-old sister also carried the *PALB2* gPV and was cancer unaffected.

Notes

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

1. Kandoth C, Schultz N, Cherniack AD, et al.; for the Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature*. 2013;497(7447):67-73.
2. Kahn RM, Gordhandas S, Maddy BP, et al. Universal endometrial cancer tumor typing: how much has immunohistochemistry, microsatellite instability, and MLH1 methylation improved the diagnosis of Lynch syndrome across the population? *Cancer*. 2019;125(18):3172-3183.
3. Talhouk A, McConechy MK, Leung S, et al. Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. *Cancer*. 2017;123(5):802-813.
4. Latham A, Srinivasan P, Kemel Y, et al. Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. *J Clin Oncol*. 2019;37(4):286-295.
5. Lu C, Xie M, Wendl MC, et al. Patterns and functional implications of rare germline variants across 12 cancer types. *Nat Commun*. 2015;6(1):10086.
6. Cadoo KA, Mandelker DL, Mukherjee S, et al. Understanding inherited risk in unselected newly diagnosed patients with endometrial cancer. *J Clin Oncol Precis Oncol*. 2019;3(3):1.
7. Eng C. Will the real Cowden syndrome please stand up: revised diagnostic criteria. *J Med Genet*. 2000;37(11):828-830.
8. Manning-Geist BL, Gatius S, Liu Y, et al. Diagnosis and management of an endometrial cancer patient with Cowden syndrome. *Gynecol Oncol*. 2021;163(1):14-21.
9. Nelen MR, Padberg GW, Peeters EA, et al. Localization of the gene for Cowden disease to chromosome 10q22-23. *Nat Genet*. 1996;13(1):114-116.
10. Pilarski R, Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. *J Med Genet*. 2004;41(5):323-326.
11. Bellido F, Pineda M, Aiza G, et al. POLE and POLD1 mutations in 529 kindred with familial colorectal cancer and/or polyposis: review of reported cases and recommendations for genetic testing and surveillance. *Genet Med*. 2016;18(4):325-332.
12. Mur P, García-Mulero S, Del Valle J, et al. Role of POLE and POLD1 in familial cancer. *Genet Med*. 2020;22(12):2089-2100.
13. Win AK, Reece JC, Dowty JG, et al. Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. *Int J Cancer*. 2016;139(7):1557-1563.
14. Pennington KP, Walsh T, Lee M, et al. BRCA1, TP53, and CHEK2 germline mutations in uterine serous carcinoma. *Cancer*. 2013;119(2):332-338.
15. Ring KL, Bruegl AS, Allen BA, et al. Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort. *Mod Pathol*. 2016;29(11):1381-1389.
16. de Jonge MM, de Kroon CD, Jenner DJ, et al.; for the Hebon Group. Endometrial cancer risk in women with germline BRCA1

or BRCA2 mutations: multicenter cohort study. *J Natl Cancer Inst*. 2021;113(9):1203-1211.
17. Shu CA, Pike MC, Jotwani AR, et al. Uterine cancer after risk-reducing salpingo-oophorectomy without hysterectomy in women with BRCA mutations. *JAMA Oncol*. 2016;2(11):1434-1440.
18. Heald B, Mokhtary S, Nielsen SM, et al. Unexpected actionable genetic variants revealed by multigene panel testing of patients with uterine cancer. *Gynecol Oncol*. 2022;166(2):344-350. doi:10.1016/j.ygyno.2022.05.023.
19. Levine MD, Pearlman R, Hampel H, et al.; for the Ohio Prevention and Treatment of Endometrial Cancer Initiative Study Group. Up-front multigene panel testing for cancer susceptibility in patients with newly diagnosed endometrial cancer: a multicenter prospective study. *J Clin Oncol Precis Oncol*. 2021;5:1588-1602.
20. Karpel HC, Chern JY, Smith JM, et al. Utility of germline multigene panel testing in patients with endometrial cancer. *Gynecol Oncol*. 2022;165(3):546-551.
21. Liu YL, Cadoo KA, Mukherjee S, et al. Multiple primary cancers in patients undergoing tumor-normal sequencing define novel associations. *Cancer Epidemiol Biomarkers Prev*. 2022;31(2):362-371.
22. Riaz N, Blecua P, Lim RS, et al. Pan-cancer analysis of bi-allelic alterations in homologous recombination DNA repair genes. *Nat Commun*. 2017;8(1):857.
23. Smith ES, Da Cruz Paula A, Cadoo KA, et al. Endometrial cancers in BRCA1 or BRCA2 germline mutation carriers: assessment of homologous recombination DNA repair defects. *J Clin Oncol Precis Oncol*. 2019;3(3):1.
24. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(6):703-713.
25. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT). *J Mol Diagn*. 2015;17(3):251-264.
26. Cheng DT, Prasad M, Chekaluk Y, et al. Comprehensive detection of germline variants by MSK-IMPACT, a clinical diagnostic platform for solid tumor molecular oncology and concurrent cancer predisposition testing. *BMC Med Genomics*. 2017;10(1):33.
27. Richards S, Aziz N, Bale S, et al.; for the ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
28. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *N Engl J Med*. 2015;372(23):2243-2257.
29. Mandelker D, Zhang L, Kemel Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA*. 2017;318(9):825-835.
30. Tung N, Domchek SM, Stadler Z, et al. Counselling framework for moderate-penetrance cancer-susceptibility mutations. *Nat Rev Clin Oncol*. 2016;13(9):581-588.
31. Momeni-Boroujeni A, Dahoud W, Vanderbilt CM, et al. Clinicopathologic and genomic analysis of TP53-mutated endometrial carcinomas. *Clin Cancer Res*. 2021;27(9):2613-2623.
32. Momeni-Boroujeni A, Nguyen B, Vanderbilt CM, et al. Genomic landscape of endometrial carcinomas of no specific molecular profile. *Mod Pathol*. 2022;35(9):1269-1278. doi:10.1038/s41379-022-01066-y.
33. Niu B, Ye K, Zhang Q, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics*. 2014;30(7):1015-1016.
34. Rios-Doria E. Molecular classification of endometrial carcinomas: a single-institution review. In: *SGO Annual Meeting on Women's Cancer*. Phoenix, AZ; 2022.
35. León-Castillo A, Britton H, McConechy MK, et al. Interpretation of somatic POLE mutations in endometrial carcinoma. *J Pathol*. 2020;250(3):323-335.
36. Middha S, Zhang L, Nafa K, et al. Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. *J Clin Oncol Precis Oncol*. 2017;2017(1):1.
37. Shen R, Seshan VE. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res*. 2016;44(16):e131.
38. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res*. 2006;66(15):7810-7817.
39. Stadler ZK, Maio A, Chakravarty D, et al. Therapeutic implications of germline testing in patients with advanced cancers. *J Clin Oncol*. 2021;39(24):2698-2709. doi:10.1200/jco.20.03661:Jco2003661.
40. National Comprehensive Cancer Network. *Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf. Published January 2022. Accessed November 2022.
41. National Comprehensive Cancer Network. *Genetics/Familial High-Risk Assessment: Colorectal*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf. Published February 2022. Accessed November 2022.
42. Offit K, Tkachuk KA, Stadler ZK, et al. Cascading after peridiagnostic cancer genetic testing: an alternative to population-based screening. *J Clin Oncol*. 2020;38(13):1398-1408.
43. Liu YL, Cadoo KA, Maio A, et al. Early age of onset and broad cancer spectrum persist in MSH6- and PMS2-associated Lynch syndrome. *Genet Med*. 2022;24(6):1187-1195.
44. Dedeurwaerdere F, Claes KB, Van Dorpe J, et al. Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer. *Sci Rep*. 2021;11(1):12880.
45. Manning-Geist BL, Liu YL, Devereaux KA, et al. Microsatellite instability-high endometrial cancers with MLH1 promoter hypermethylation have distinct molecular and clinical profiles. *Clin Cancer Res*. 2022;28(19):4302-4311.
46. de Bono J, Mateo J, Fizazi K, et al. Olaparib for metastatic castration-resistant prostate cancer. *N Engl J Med*. 2020;382(22):2091-2102.
47. Clarke MA, Devesa SS, Hammer A, et al. Racial and ethnic differences in hysterectomy-corrected uterine corpus cancer mortality by stage and histologic subtype. *JAMA Oncol*. 2022;8(6):895-903.
48. Whetstone S, Burke W, Sheth SS, et al. Health disparities in uterine cancer: report from the uterine cancer evidence review conference. *Obstetr Gynecol*. 2022;139(4):645-659. doi:10.1097/aog.00000000000047.
49. Manning-Geist BL, Liu YL, Devereaux KA, et al. Microsatellite instability-high endometrial cancers with MLH1 promoter hypermethylation have distinct molecular and clinical profiles. *Clin Cancer Res*. 2022;28(19):4302-4311.