original report

Endometrial Cancers in *BRCA1* or *BRCA2* Germline Mutation Carriers: Assessment of Homologous Recombination DNA Repair Defects

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abstract

PURPOSE Endometrial cancer (EC) is not considered a component of the hereditary breast and ovarian cancer syndrome but can arise in patients with germline *BRCA1/2* (g*BRCA1/2*) mutations. Biallelic *BRCA1/2* alterations are associated with genomic features of homologous recombination DNA repair deficiency (HRD) in cancer. We sought to determine if ECs in g*BRCA1/2* mutation carriers harbor biallelic alterations and/or features of HRD.

METHODS Of 769 patients with EC who underwent germline panel testing, 10 pathogenic g*BRCA1/2* mutation carriers were identified, and their tumor- and normal-derived DNA was subjected to massively parallel sequencing targeting at least 410 cancer-related genes. Three g*BRCA1/2*-associated ECs were identified in 232 ECs subjected to whole-exome sequencing by The Cancer Genome Atlas. Somatic mutations, copy number alterations, loss of heterozygosity, microsatellite instability (MSI), and genomic HRD features were assessed.

RESULTS Of the 13 patients included who had EC, eight harbored pathogenic g*BRCA1* mutations and five harbored g*BRCA2* mutations. Eight (100%) and two (40%) ECs harbored biallelic *BRCA1* and *BRCA2* alterations through loss of heterozygosity of the wild-type allele. All ECs harbored somatic *TP53* mutations. One monoallelic/sporadic g*BRCA2*-associated EC had *MLH1* promoter methylation and was MSI high. High large-scale state transition scores, a genomic feature of HRD, were found only in ECs with bi- but not monoallelic *BRCA1/2* alterations. The Signature Multivariate Analysis HRD signature Sig3 was enriched in biallelic g*BRCA1/2* ECs, and the three ECs from The Cancer Genome Atlas with *BRCA1* biallelic alterations subjected to whole-exome sequencing displayed a dominant HRD-related mutational signature 3.

CONCLUSION A subset of g*BRCA1/2*-associated ECs harbor biallelic *BRCA1/2* alterations and genomic features of HRD, which may benefit from homologous recombination–directed treatment regimens. ECs in *BRCA2* mutation carriers might be sporadic and even MSI high, and may potentially benefit from immune-checkpoint inhibition.

INTRODUCTION

The tumor suppressor genes *BRCA1* and *BRCA2* (*BRCA1/2*) play central roles in DNA repair via the homologous recombination (HR) pathway.[1] *BRCA1/2* pathogenic germline mutations account for a large proportion of cases of hereditary breast and ovarian cancer (HBOC) syndrome, increasing the lifetime risk of developing breast, ovarian, prostate, and/or pancreatic cancers.[2,3] Endometrial cancers (ECs) are currently not formally associated with HBOC but have been reported in patients with germline *BRCA1/2* (g*BRCA1/2*) mutations.[4,5]

Independent studies have suggested that ECs may be a component of HBOC[5] and specifically highlighted a possible increased risk of serous carcinomas in women with germline *BRCA1* (g*BRCA1*) mutations.[5,6] Conversely, in a recent pan-cancer analysis of pathogenic germline variants by The Cancer Genome Atlas (TCGA), enrichment of g*BRCA1/2* pathogenic variants in EC was not identified.[7]

Even if not formally accepted as part of the HBOC syndrome, the identification of ECs with defective HR DNA repair could have important treatment implications in the era of precision medicine. Biallelic inactivation of *BRCA1/2*, either through locus-specific loss of heterozygosity (LOH) of the *BRCA1/2* wild-type allele or through a somatic *BRCA1/2* pathogenic mutation, has been associated with genomic features of HR deficiency[8,9] and increased survival after DNA damage–inducing platinum-based chemotherapy.[9] Importantly, the frequency of LOH of the wild-type allele or somatic mutations of *BRCA1/2* in the context of g*BRCA1/2* mutation carriers varies according to tumor type.[9]

In this study, we sought to determine whether ECs arising in g*BRCA1/2* mutation carriers harbor biallelic

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To determine if endometrial cancers (ECs) that arise in patients with pathogenic *BRCA1* or *BRCA2* germline mutations are sporadic cancers or display genomic features of homologous recombination DNA repair deficiency (HRD), which may guide treatment strategies.

Knowledge Generated

ECs in patients with *BRCA1* germline mutations showed biallelic *BRCA1* alterations coupled with genomic features of HRD. In contrast, only a subset of ECs in *BRCA2* germline mutation carriers harbored biallelic *BRCA2* alterations; the remaining cases were likely sporadic cancers.

Relevance

Knowledge about the *BRCA1/2* allele status and/or HRD features of ECs in patients with germline *BRCA1/2* alterations may guide therapeutic decision making, given that tumors with biallelic alterations in *BRCA1/2* have been associated with increased response to HR-targeted therapies such as platinum salts and PARP inhibitors.

BRCA1/2 alterations and show features of HR deficiency, including the Catalog of Somatic Mutations in Cancer mutational signature 3, Signature Multivariate Analysis (SigMA) HR deficiency signature Sig3, or large-scale chromosomal breaks in the form of large-scale state transitions (LSTs). In addition, we assessed the clinicopathologic data of ECs in g*BRCA1/2* mutation carriers and explored the repertoire of somatic mutations present in these tumors.

METHODS

Case Selection

All patients with EC and g*BRCA1/2* mutations who consented to germline analysis under an institutional review board–approved protocol at Memorial Sloan Kettering Cancer Center and whose tumors were subjected to targeted massively parallel sequencing via Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)[10,11] from July 2015 to May 2019 were identified (n = 769 sequenced; n = 10 with pathogenic g*BRCA1/2* mutation). All g*BRCA1/2* variants were reviewed by a board-certified molecular pathologist (D.M.) and classified according to the American College of Medical Genetics and Genomics criteria[12] as pathogenic. Clinicopathologic data, including age at diagnosis, cancer stage, and past cancer history, were obtained from medical records. All cases were centrally rereviewed by pathologists (A.P.M.S., R.A.S.) with experience and expertise in gynecologic pathology. In addition, ECs with pathogenic g*BRCA1/2* alterations (n = 3) subjected to whole-exome sequencing (WES) as part of The Cancer Genome Atlas (TCGA; n = 232)[13] were identified in a curated data set from Riaz et al,[8] as previously described.[14] WES-derived somatic mutation Multi-Center Mutation Calling in Multiple Cancers data of the cases were retrieved from TCGA Genomic Data Commons (https://gdc.cancer.gov/about-data/publications/mc3-2017),[15] and clinicopathologic data were obtained from the TCGA data portal.

Massively Parallel Sequencing Analysis

Tumor and matched normal DNA samples were subjected to targeted massively parallel sequencing using MSK-IMPACT, which targets all exons and selected introns of 410 (n = 6) or 468 (n = 4) cancer genes, as previously described.[10,11] The median depth of coverage was 613× (range, 406× to 992×) for tumor and 509× (range, 401× to 685×) for normal samples (Data Supplement). Analysis of sequencing data for the identification of single nucleotide variants (SNVs) and small insertions and deletions (indels) was performed as previously described.[16,17] FACETS[18] (fraction- and allele-specific copy number estimates from tumor sequencing) was used to determine copy number alterations (CNAs) and whether genes harboring somatic or germline mutations were targeted by loss of heterozygosity (LOH), as previously described.[16,17] The cancer cell fractions of all somatic mutations were computed using ABSOLUTE, version 1.0.6,[19] as previously described.[16,17] A combination of mutation function predictors was used to define the potential functional impact of each missense SNV.[20] Mutational hotspots were annotated according to Chang et al.[21]

Microsatellite Instability Score

The algorithm MSIsensor[22] was used to assess microsatellite instability (MSI), as previously described.[14] ECs subjected to MSK-IMPACT and WES (TCGA) with MSIsensor scores of greater than or equal to 10[23] and greater than or equal to 3.5[22], respectively, were deemed MSI high.

Mutational Signatures

Mutational signatures were inferred from all synonymous and nonsynonymous SNVs using the algorithm deconstructSigs[24] at default parameters, as previously described.[14] deconstructSigs was only applied to samples with at least 20 somatic SNVs.[14] In addition, Signature Multivariate Analysis (SigMA) was used for samples with at least five somatic SNVs; SigMA is a tool to detect the HR

deficiency mutational signature Sig3 and other signatures from targeted gene panels.[25]

Large-Scale State Transitions

For the assessment of genomic features of HR deficiency, large-scale state transitions (LSTs) were defined, and a cutoff of greater than or equal to 15 was used for LST-high cases, as previously described.[14,26]

DNA Mismatch Repair Immunohistochemistry and *MLH1* Promoter Methylation

Immunohistochemistry for the DNA mismatch repair (MMR) proteins MLH1, MSH2, MSH6, and PMS2 was performed in the clinical setting as previously described.[27] Loss of DNA MMR protein expression was defined as the complete absence from all tumor cell nuclei. The presence of a positive internal control (ie, blood vessels, stromal cells, lymphocytes) was required for interpretation. *MLH1* promoter methylation was assessed in the clinical setting on DNA obtained from formalin-fixed paraffin-embedded tumor samples, as previously described.[28]

Statistical Analyses

Fisher's exact and Mann-Whitney *U* tests were used for comparison of categorical and continuous variables, respectively. Statistical analyses were performed using SPSS Statistics, version.25 (IBM, Armonk, NY). Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathologic Features of ECs Arising in g*BRCA1/2* Carriers

Ten ECs from patients with pathogenic g*BRCA1/2* mutations subjected to MSK-IMPACT sequencing (n = 769 ECs; 1.3%) and three subjected to WES by TCGA (n = 232 ECs; 1.3%)[13] were included in this study. Of these 13 g*BRCA1/2*-associated ECs, eight and five patients with EC harbored g*BRCA1* and germline *BRCA2* (g*BRCA2*) mutations, respectively (Table 1). None of these g*BRCA1/2*-associated ECs displayed germline alterations in other genes associated with hereditary cancer susceptibility, including the DNA MMR genes.

The median age at EC diagnosis was 62 (range, 44 to 78) years, with g*BRCA1* patients (n = 8) being younger at EC diagnosis (median [range] age, 56 [44 to 64] years) than patients with EC harboring g*BRCA2* (n = 5; median [range] age, 68 [61 to 78] years; Mann-Whitney *U* test $P = .01$; Tables 1 and 2).

The histologic types of the g*BRCA1/2*-associated ECs varied. The majority of g*BRCA1/2*-associated ECs (n = 6 of 8; 75%) displayed an endometrioid histology (n = 2 and 4 International Federation of Gynecology and Obstetrics [FIGO] grades II and III, respectively) but lacked a solid/pseudoendometrioid/transitional cell-like pattern of morphology, which has been associated with g*BRCA1/2* mutations in high-grade serous ovarian cancers.[29,30] In contrast, ECs from patients with pathogenic g*BRCA2* mutations were more heterogeneous at the phenotypic level, and included endometrioid (n = 1; FIGO grade II), carcinosarcoma (n = 2), high-grade EC not otherwise specified (NOS; n = 1) and serous (n = 1) cancers (Tables 1 and 2). Of note, none of the g*BRCA1/2*-associated ECs included in this study were of serous histology.

g*BRCA1/2*-associated ECs presented at different clinical FIGO stages (2009 staging system[31]). Although a subset of the ECs occurring in pathogenic g*BRCA1* mutation carriers were uterus- or cervix-confined cancers (stage I, n = 3; stage II, n = 1; stage III, n = 4), all EC patients with pathogenic g*BRCA2* mutations presented at an advanced stage (stage III, n = 4; stage IV, n = 1; Tables 1 and 2).

Four of the patients with g*BRCA1/2*-associated EC had a breast cancer diagnosis prior to their diagnosis of EC (EC identifiers BEC-3, BEC-6, BEC-8, and BEC-12: BEC-3, g*BRCA1/2*, carcinosarcoma; BEC-6, g*BRCA1/2*, serous; BEC-8, g*BRCA1/2*, endometrioid; BEC-12, g*BRCA1/2*, high-grade EC NOS; Table 1). None of the patients received tamoxifen as part of their breast cancer treatment regimens. In addition, patient BEC-2 (g*BRCA1/2*, carcinosarcoma) had a rectal cancer 12 years before the EC diagnosis and had received pelvic radiation. Taken together, our data suggest the clinicopathologic features associated with ECs occurring in patients with pathogenic g*BRCA1* or g*BRCA2* mutations may be heterogeneous.

Biallelic *BRCA1/2* Alterations

Allele-specific copy number analysis revealed that 77% (n = 10 of 13) of the ECs in patients with pathogenic g*BRCA1/2* mutations displayed biallelic inactivation of *BRCA1/2* uniformly through LOH of the wild-type allele (Table 2; Fig 1). No somatic *BRCA1/2* mutations were identified in the ECs studied (Fig 1; Data Supplement). Not all ECs occurring in pathogenic g*BRCA1/2* mutation carriers harbored biallelic *BRCA1/2* alterations, which have previously been associated with HR deficiency.[8] Although all ECs in pathogenic g*BRCA1* mutation carriers (100%) harbored biallelic *BRCA1* inactivation, only two of the five ECs (40%) from patients with pathogenic g*BRCA2* mutations displayed biallelic *BRCA2* inactivation (Table 2; Fig 1). Biallelic *BRCA1/2* inactivation was found across all histologic subtypes and clinical stages of EC. ECs in *BRCA2* mutation carriers lacking LOH of the wild-type allele are likely sporadic tumors; all three were stage III at diagnosis and were endometrioid grade II, serous, or high-grade ECs (Table 2; Fig 1). These data suggest that the vast majority of g*BRCA1/2*-associated ECs, but only a subset of g*BRCA1/2*-associated ECs, harbor biallelic inactivation of the respective wild-type allele, and that some g*BRCA1/2*-associated ECs may, in fact, be sporadic.

Repertoire of Somatic Mutations and CNAs

The median number of somatic mutations detected in the 410 MSK-IMPACT genes (smallest gene panel) in the

TABLE 1. Demographics and Baseline Characteristics of g*BRCA1/2*-Associated Endometrial Cancers

Demographic or Characteristic	All *BRCA1/2* (n = 13)	*BRCA1* (n = 8)	*BRCA2* (n = 5)	*P**
Sequencing analysis				.23
MSK-IMPACT	10 (77)	5 (63)	5 (100)	
TCGA WES	3 (23)	3 (38)	—	
Age, median (range), years	62 (44-78)	56 (44-64)	68 (61-78)	.01
Histology				.16
Endometrioid (grade II/III)	7 (54)	6 (75)	1 (20)	
Carcinosarcoma	3 (23)	1 (13)	2 (40)	
Serous	1 (8)	—	1 (20)	
Mixed endometrioid/serous	1 (8)	1 (13)	—	
High-grade endometrial NOS	1 (8)	—	1 (20)	
Stage†				.74
I	3 (23)	3 (38)	—	
II	1 (8)	1 (13)	—	
III	8 (62)	4 (50)	4 (80)	
IV	1 (8)	—	1 (20)	
Past cancers‡				1.00
Breast	4 (40)	2 (40)	2 (40)	
Rectal	1 (10)	—	1 (20)	
Squamous cell carcinoma (face)	1 (10)	—	1 (20)	
No past cancers	4 (40)	3 (60)	1 (20)	
Prior radiation exposure‡				1.00
Breast (uterine serous, n = 1; carcinosarcoma, n = 1)	2 (20)	1 (50)	1 (20)	
Rectal (carcinosarcoma, n = 1)	1 (10)	—	1 (20)	
No prior radiation exposure	7 (70)	40 (80)	3 (60)	

NOTE. Data reported as No. (%) unless otherwise indicated.

Abbreviations: MSK-IMPACT, Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets; NOS, not otherwise specified; TCGA, The Cancer Genome Atlas; WES, whole-exome sequencing.

*Fisher's exact and Mann-Whitney *U* tests were used for comparison of categorical and continuous variables, respectively.

†Staging information was performed according to the International Federation of Gynecology and Obstetrics system.[31]

‡Past cancer histories and information regarding radiation exposure were available for the 10 MSK-IMPACT cases and reflect cancers diagnosed and treated prior to the diagnosis of EC.

13 g*BRCA1/2*-associated ECs was six (range, 2 to 38), with a median of five (range, 2 to 32) nonsynonymous mutations (Data Supplement). We observed that all ECs analyzed, irrespective of the presence of mono- or biallelic *BRCA1/2* alterations, harbored somatic *TP53* mutations, of which 12 (92%) were hotspot mutations (Fig 1; Data Supplement). Alterations affecting the PI3K pathway were common, with *PIK3CA* mutations present in five ECs (38%), *PIK3R1* mutations in two ECs (15%), and *PTEN* mutations/homozygous deletions in three ECs (23%; Figs 1 and 2). Other recurrently altered genes included *FAT1*, *PTCH1, KRAS*, and *MAP3K1* (each n = 2; Fig 1, Data Supplement).

We noted that BEC-1, a likely sporadic EC from a g*BRCA2* mutation carrier with a monoallelic *BRCA2* alteration, had a high mutational burden with 32 nonsynonymous somatic mutations. In addition, this case was MSI high with a high MSIsensor score (≥ 10; Fig 1, Table 2). None of the DNA MMR genes in BEC-1 harbored any somatic or germline mutations; however, consistent with the protein loss of MLH1 and PMS2 as assessed by immunohistochemistry (Fig 3), this case displayed *MLH1* promoter methylation.

The levels of copy number alterations (CNAs) varied across the g*BRCA1/2*-associated ECs studied, with some having very few CNAs and others displaying high levels of genomic instability (Fig 2). The ECs with very low levels of gene CNAs (ie, BEC-1, BEC-6, and BEC-12) had monoallelic *BRCA2* alterations and were likely sporadic, with one (BEC-1) being MSI high, as mentioned. On the other hand, ECs with the highest levels of CNAs had biallelic *BRCA1/2* alterations (ie, BEC-2, BEC-3, BEC-5, BEC-7, and TCGA-3; Fig 2). When assessing amplifications and deletions, *PTEN* was the only recurrent homozygous deletion identified in two biallelic *BRCA1* ECs (BEC-3 and TCGA-2). In addition, we found an *NF1* homozygous deletion in the biallelic *BRCA1* grade II endometrioid TCGA-2 and a *SMARCA4* homozygous deletion in the biallelic *BRCA2* carcinosarcoma BEC-2. Of note, SMARCA4 (BRG1) loss is commonly found in undifferentiated carcinoma of the endometrium,[32,33] and BEC-2 was a carcinosarcoma with heterologous elements, which, in addition to the carcinoma and sarcomatous components, also displayed an undifferentiated component. Finally, amplification of *BCL6*, *TP63*, and *EED* was found in the biallelic *BRCA1* grade III endometrioid TCGA-1 EC (Fig 2).

Taken together, we found that ECs in patients with a g*BRCA1/2* mutation are heterogeneous at the mutational and gene copy number levels. In addition, in germline carriers of pathogenic *BRCA2* mutations, the ECs may be sporadic and even have a high mutational burden and be MSI high.

HR DNA Repair Deficiency in g*BRCA1/2* ECs

We first assessed the presence of LSTs, a genomic feature of HR deficiency, in all cases. We observed that ECs with biallelic *BRCA1/2* alterations (n = 10) had high LST scores, whereas ECs with monoallelic *BRCA1/2* alterations did not (Table 2; Fig 3A). In addition, mutational signature analysis was performed using deconstructSigs[24] for ECs with at least 20 SNVs (ie, n = 1 MSK-IMPACT EC; n = 3 TCGA ECs), as previously described.[14] All biallelic, g*BRCA1/2*-associated, TCGA ECs had a dominant mutational signature 3 associated

TABLE 2. *BRCA1/2* Alterations, Clinicopathologic Information and Sequencing Results of g*BRCA1/2*-Associated Endometrial Cancers

Sample ID	Germline Mutation	Amino Acid Change†	Histology	FIGO Grade at Diagnosis	FIGO Stage at Diagnosis	Age at Diagnosis (years)	Somatic *BRCA1/2* LOH	Nonsynonymous Mutations (n = 410 MSK-IMPACT genes), No.	MSI Sensor‡	Dominant/ Secondary Mutational Signatures§	SigMa Dominant/ Secondary Mutational Signatures (%)‖	LST Score¶	Sequencing Type
BEC-1	*BRCA2*	p.S1982Rfs*22/c.5946delT	Endometrioid	II	III	68	No	32	High (14.8)	6 (MSI)/1 (aging)	MSI (97)/Sig8 (3)	1	MSK-IMPACT
BEC-2	*BRCA2*	p.R2520*/c.7558C>T	Carcinosarcoma	N/A	III	68	Yes	4	Low (3.3)	NP	NP	15	MSK-IMPACT
BEC-3	*BRCA1*	p.Q1756Pfs*74/c.5266dupC	Carcinosarcoma	N/A	III	53	Yes	7	Low (1.2)	NP	HRD Sig3 (87)/ HRD Sig8 (6)	37	MSK-IMPACT
BEC-5	*BRCA1*	p.S1655Yfs*16/c.4964_ 4982delCTGGCCTGACCCCAGAAGA	Endometrioid	II	II	47	Yes	4	Low (3.8)	NP	Clock (68)/ HRD Sig3 (21)	27	MSK-IMPACT
BEC-6	*BRCA2*	p.S1982Rfs*22/c.5946delT	Serous	N/A	III	78	No	5	Low (0)	NP	APOBEC (87)/ HRD Sig3 (11)	0	MSK-IMPACT
BEC-7	*BRCA2*	p.N1706Lfs*5/c.5116_5119delAATA	Carcinosarcoma	N/A	IV	61	Yes	7	Low (2.0)	NP	Clock (50), HRD (26 Sig 3, 22 Sig8)	16	MSK-IMPACT
BEC-8	*BRCA1*	p.E23Vfs*17/c.68_69delAG	Endometrioid	III	III	62	Yes	4	Low (0)	NP	HRD Sig3 (73)/ Clock (6)	15	MSK-IMPACT
BEC-9	*BRCA1*	p.C61G/c.181T>G	Endometrioid	III	III	62	Yes	9	Low (3.96)	NP	Clock (55), HRD (26 Sig3, 13 Sig8)	22	MSK-IMPACT
BEC-12	*BRCA2*	p.E3309*/c.9925G>T	High-grade endometrial carcinoma NOS (focal serous component)	III	III	69	No	5	Low (0)	NP	NP	0	MSK-IMPACT
BEC-13	*BRCA1*	p.C61G/c.181T>G	Mixed endometrioid and serous	III	III	59	Yes	5	Low (2.2)	NP	Clock (80), HRD (7 Sig3, 11 Sig8)	43	MSK-IMPACT
TCGA-1	*BRCA1*	p.E1368fs/c.4102delG	Endometrioid	III	I	50	Yes	6	Low (0.3)	3 (HRD)/1 (aging)	HRD Sig3 (100)	37	WES (TCGA)
TCGA-2	*BRCA1*	p.E1203*/c.3607G>T	Endometrioid	II	I	44	Yes	2	Low (0.3)	3 (HRD)/1 (aging)	HRD Sig3 (97)/ Clock (3)	23	WES (TCGA)
TCGA-3	*BRCA1*	p.E23Vfs*17/c.68_69delAG	Endometrioid	III	I	64	Yes	7	Low (0.9)	3 (HRD)/25 (unknown)	HRD Sig3 (100)	36	WES (TCGA)

Abbreviations: FIGO, International Federation of Gynecology and Obstetrics; HRD, homologous recombination DNA repair deficiency; ID, identifier; LOH, loss of heterozygosity; LST, large-scale state transition; MSI, microsatellite instability; MSK-IMPACT, Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets; N/A, not applicable; NOS, not otherwise specified; NP, not performed; SigMA, Signature Multivariate Analysis; TCGA, The Cancer Genome Atlas.

†All germline mutations are pathogenic according to the American College of Medical Genetics and Genomics criteria.[12]

‡Cutoffs for MSIsensor are ≥ 10 for MSK-IMPACT[23] and ≥ 3.5 for WES.[22]

§Mutational signatures were defined using descontructSigs[24] for samples with ≥ 20 single nucleotide variants, as previously described.[14]

‖SigMA mutational signatures were defined for samples with at least five single nucleotide variants.[25]

¶Cutoff LST high ≥ 15.[26]

FIG 1. Landscape of somatic mutations in endometrial cancers (ECs) in patients with pathogenic *BRCA1/2* germline mutations. Nonsynonymous somatic mutations identified in 410 cancer-related cancer genes in ECs from germline *BRCA1* (n = 8) and germline *BRCA2* (n = 5) mutation carriers (left) are shown. The mutation types are color coded according to the legend. Selected amplifications and homozygous deletions are shown at the bottom of the figure. Information on the germline mutation status, somatic loss of heterozygosity of *BRCA1/2*, histologic type, microsatellite instability (MSI) status, and sequencing type is provided in the phenobar below the sample names. indel, insertions and deletions; MSK-IMPACT, Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets; NOS, not otherwise specified; SNV, single nucleotide variant; TCGA, The Cancer Genome Atlas; WES, whole-exome sequencing.

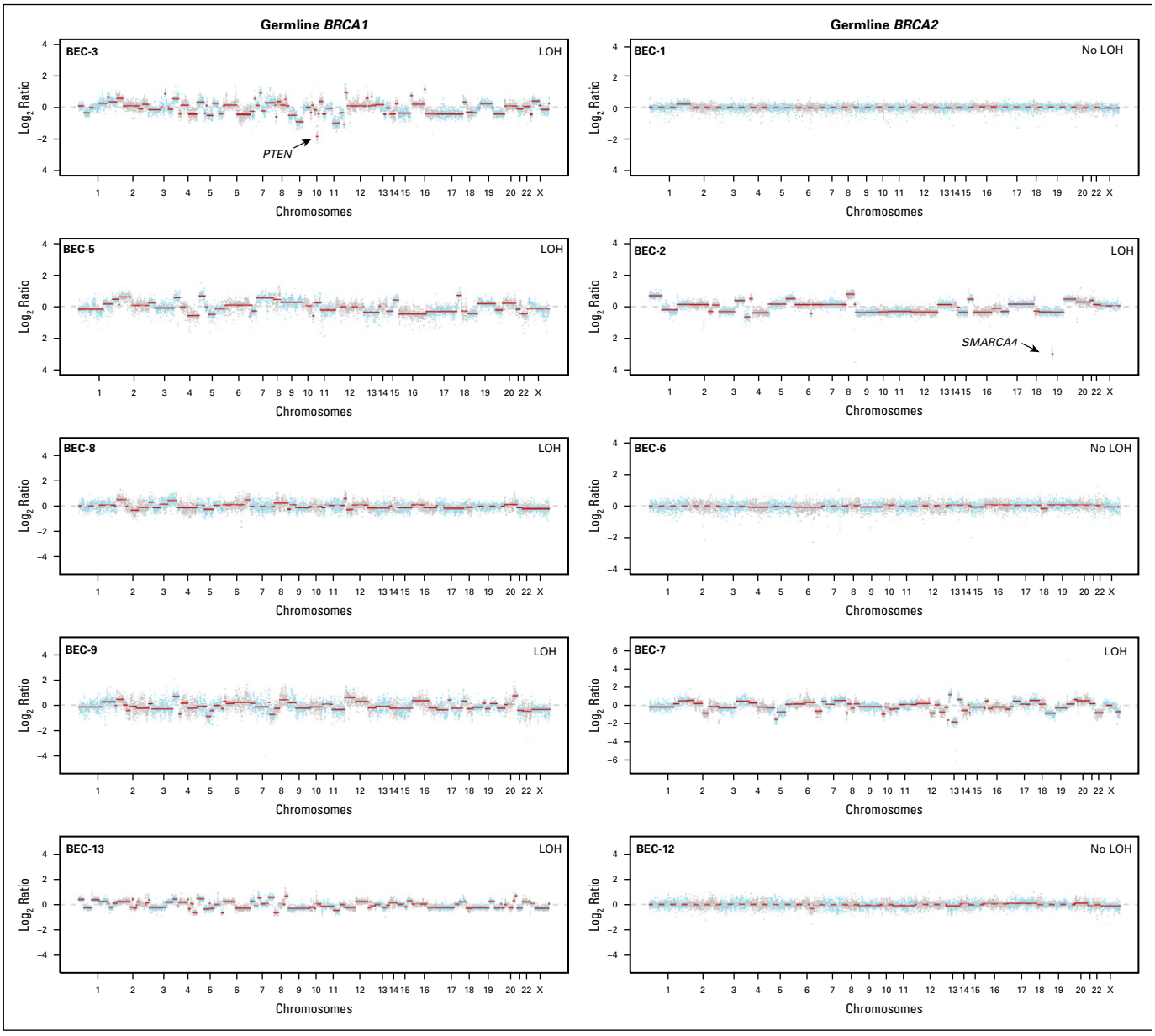

FIG 2. Copy number alterations in germline (g*BRCA1/2*)-associated endometrial cancers. Chromosome plots of the g*BRCA1/2*- and g*BRCA1/2*-associated endometrial cancers included in this study are presented. Copy number $\log_2$-ratios are depicted on the *y*-axis with the chromosome location on the *x*-axis. The presence of somatic loss of heterozygosity (LOH) of *BRCA1/2* is displayed in the top right corner within each plot. Black arrows point to selected cancer gene amplifications and homozygous deletions. TCGA, The Cancer Genome Atlas.

with HR deficiency (Fig 3B).[14] Given the limited number of SNVs and indels in the ECs subjected to MSK-IMPACT sequencing, other genomic HR-deficiency features such as indel length, microhomology, or mutational signatures using decomposition algorithms could not be used. Therefore, we used SigMA, a recently described, likelihood-based measure signature analysis combined with machine-learning techniques, which can be used to detect the mutational signature Sig3 associated with HR deficiency from targeted gene panels including MSK-IMPACT (sensitivity range, 48% to 62% for uterine cancers).[25] Consistent with the LST analysis, all six biallelic, *BRCA1/2*-associated ECs subjected to MSK-IMPACT and with at least five SNVs displayed either a dominant HR deficiency-related Sig3 (n = 2) or a dominant clock signature with secondary HR-deficiency signatures Sig3 (n = 3) or Sig8[34] (n = 1), in contrast to the two sporadic ECs with monoallelic *BRCA2* alterations, which displayed MSI (SigMA)/signature 6 (deconstructSigs) and APOBEC signatures (Table 2; Fig 3C). The three biallelic g*BRCA1/2*-associated ECs from TCGA subjected to WES displayed a strong dominant SigMA Sig3 (Table 2). These data provide evidence to suggest that in some patients with g*BRCA1/2* mutations, ECs may have biallelic *BRCA1/2* alterations and be HR deficient.

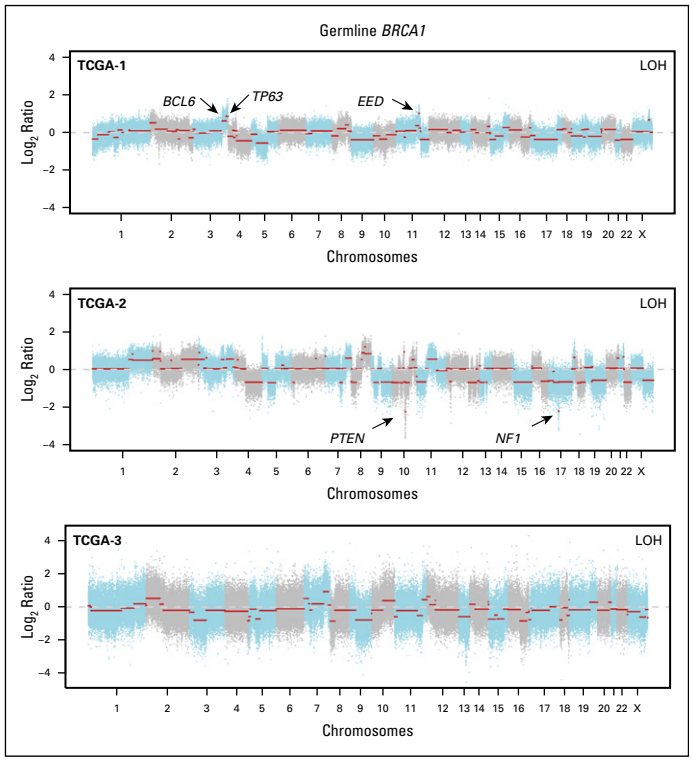

FIG 2. (Continued).

DISCUSSION

The inclusion of EC as a component of HBOC syndrome could have substantial clinical impact on the screening, evaluation, and treatment of patients with g*BRCA1/2* mutations. Previous reports of ECs in this population have acknowledged that the prevalence of EC in g*BRCA1/2* mutation carriers is low.[5] Here, through an in-depth analysis of ECs from patients with pathogenic g*BRCA1/2* mutations, we demonstrate that the majority of these cases (77%) harbored biallelic *BRCA1/2* alterations and displayed genomic features of HRD, providing evidence to suggest an etiological link between the pathogenic g*BRCA1/2* mutations and the development of these ECs. In fact, this phenomenon was uniformly observed in patients with pathogenic g*BRCA1* mutations; conversely, only two of the five patients with pathogenic g*BRCA2* mutations had somatic inactivation of the *BRCA2* wild-type allele and genomics features of HR deficiency. In ovarian cancer, patients with HR-deficient tumors have improved overall survival with platinum-based therapy and have better responses to HR deficiency–directed treatments such as PARP inhibitors than their wild-type counterparts.[35,36]

Knowledge of the allele status of *BRCA1/2* alterations and/or HR-deficiency features could be beneficial in therapeutic decision making for patients with g*BRCA1/2* mutations and EC. Our analysis has demonstrated that not all ECs arising in the context of pathogenic g*BRCA2* mutations harbor LOH of the wild-type allele and genomics features of HR deficiency. In addition, it revealed that one of these cases was MSI high, likely representing a sporadic (ie, non-*BRCA1/2*) EC arising in a g*BRCA2* mutation carrier. In this context, this patient would potentially benefit from immune checkpoint inhibitors, which have been approved for the management of recurrent MSI-high/DNA MMR–deficient cancers.[37] In fact, a recent case report highlighted a complete remission after PD-1 blockade in a patient with MMR-deficient EC and a pathogenic monoallelic g*BRCA1* mutation.[38]

The majority (75%) of biallelic g*BRCA1/2*-associated ECs were of intermediate/high-grade endometrioid as opposed to serous histology in previous reports.[5,6] All six biallelic g*BRCA1/2*-associated endometrioid ECs harbored *TP53* mutations, had high LST scores, and relatively high levels of CNAs (Table 2; Fig 2), and would likely be of copy-number high (serous-like) molecular subtype, as described by TCGA.[13] It should be noted, however, that of these six endometrioid ECs with biallelic *BRCA1* alterations, four harbored somatic mutations characteristic of endometrioid ECs, including *PIK3CA* mutations, *PIK3R1* mutations, and *PTEN* mutations/homozygous deletions. Conversely, two of these ECs lacked alterations affecting genes recurrently altered in endometrioid ECs (Fig 1), suggesting that at the genetic level, these latter two cases resembled serous rather than endometrioid carcinomas, and that there is heterogeneity in g*BRCA1/2*-associated ECs. Carcinosarcomas of the uterus are rare, representing less than 5% of all uterine tumors.[39] We noted a high frequency (30%) of carcinosarcomas in the 10 g*BRCA1/2*-associated ECs subjected to MSK-IMPACT sequencing, whereas overall, only 10% of the 769 ECs subjected to germline MSK-IMPACT sequencing were carcinosarcomas (remaining cases: 55% endometrioid, 15% serous, 10% endometrial cancer NOS, 4% mixed endometrial, 3% clear cell, 1% de- or undifferentiated, and 1% other). Interestingly, recurrent somatic *BRCA1/2* mutations and *BRCA2* deletions also have been reported in uterine carcinosarcomas.[40-42] These findings suggest that ECs arising in g*BRCA1/2* mutation carriers are heterogeneous at the histologic level and are potentially enriched for endometrioid tumors and carcinosarcomas.

The data presented here do provide insight into the genomics of ECs in patients with pathogenic g*BRCA1/2* mutations, which may play a role in the tumorigenesis and/or progression of EC in some patients. This study has several limitations, however, primarily driven by the small sample size, and it does not resolve the controversy of EC as a feature of HBOC syndrome. The low incidence of these cases (1.3%; n = 10 of 769 ECs subjected to germline MSK-IMPACT testing) not only limits the sample size of this study but also, on its own, presents a challenge in defining EC as part of the HBOC syndrome spectrum of malignancies. In contrast, during the same time, 4.5% and 11.1% of breast and ovarian cancers tested by germline MSK-IMPACT were g*BRCA1/2* associated, respectively. The MSK cohort during the time studied is enriched for advanced-stage disease,[10,11] and neither the actual prevalence of pathogenic g*BRCA1/2*

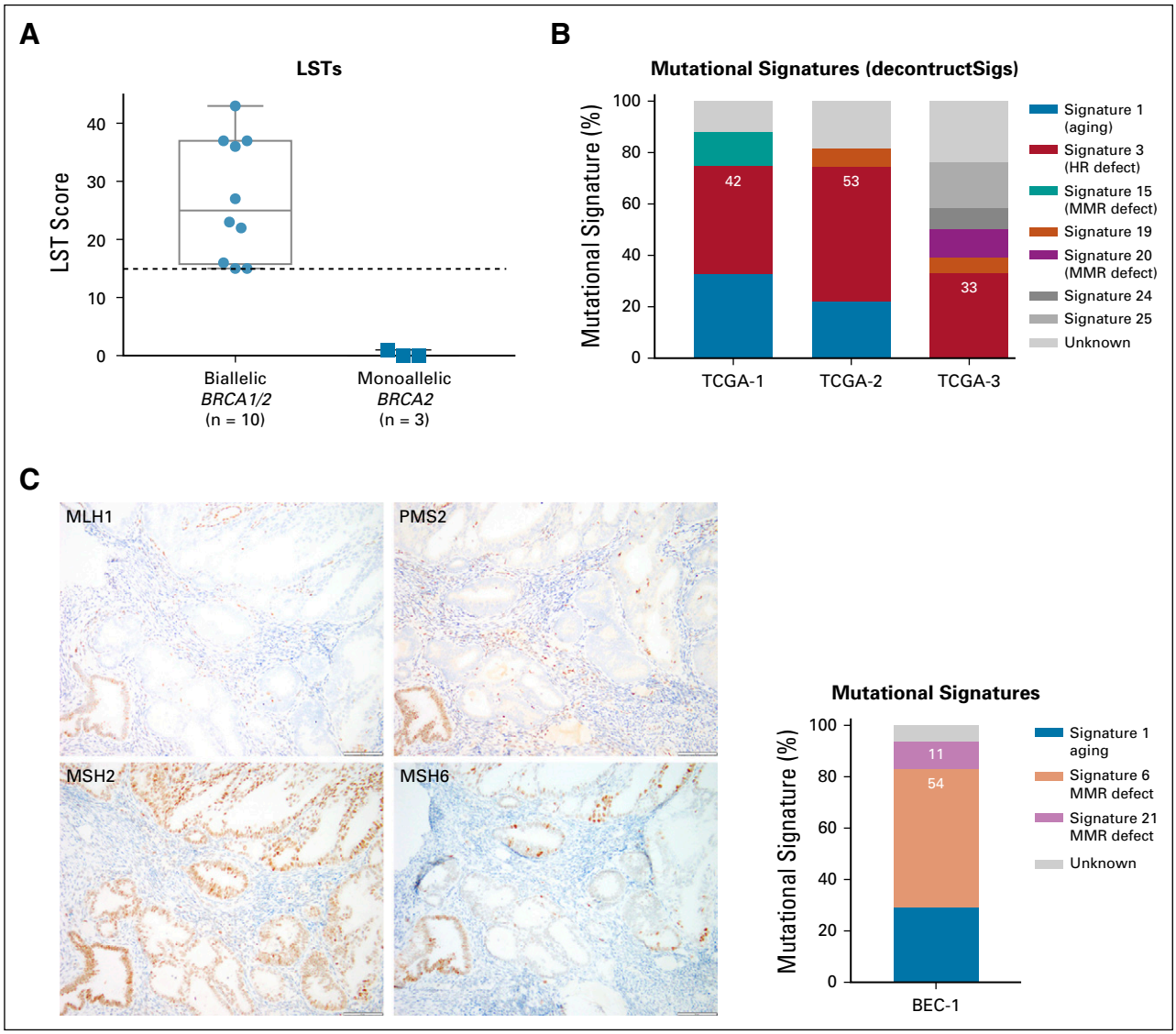

FIG 3. Genomic features of homologous recombination (HR) DNA repair deficiency. (A) Large-scale state transition (LST) scores in germline (g*BRCA1/2*)-associated endometrial cancers with biallelic (n = 10) and monoallelic (n = 3) *BRCA1/2* alterations. Dashed line indicates the cutoff (≥ 15) for high LST scores.[26] Biallelic *BRCA1/2* alterations are associated with high LST scores. (B) Mutational signatures defined by deconstructSigs[24] in three g*BRCA1/2*-associated endometrial cancers from The Cancer Genome Atlas (TCGA) subjected to whole-exome sequencing.[13,14] Mutational signatures are color coded according to the legend on the right. All three germline g*BRCA1/2*-associated endometrial cancers have a dominant homologous recombination DNA repair deficiency–related mutational signature 3. (C) Immunohistochemical analysis of the (left) DNA mismatch repair (MMR) proteins MLH1, PMS2, MSH2, and MSH6 and (right) mutational signatures of case BEC-1, a monoallelic/ sporadic g*BRCA1/2*-associated endometrial cancer. Mutational signatures defined by deconstructSigs[24] are color coded according to the legend on the right. BEC-1 shows loss of MLH1 and PMS2 expression, *MLH1* promoter hypermethylation (not shown), and dominant mutational signatures 6 and 21 associated with defective DNA MMR.

mutations in patients with EC nor the impact of these mutations on the outcome of patients with EC could be fully defined. Although we have assessed the genomics features of HR deficiency, including LSTs and SigMA HR deficiency signature Sig3 in all cases, HR deficiency is more accurately assessed with whole-genome sequencing.[43] Hence, additional studies testing ECs developing in the context of pathogenic g*BRCA1/2* mutations but lacking loss of the wild-type allele by whole-genome sequencing are warranted.

Despite these limitations, here we demonstrate the importance of germline and somatic genetic characterization of ECs. In fact, ECs developing in the context of pathogenic g*BRCA1/2* mutations may harbor genomics features of HR deficiency and be causally linked to the loss of function of these tumor suppressor genes. However, the presence of a pathogenic g*BRCA1/2* mutation does not rule out the possibility of an EC being sporadic and displaying DNA MMR deficiencies.

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