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HEBON Grp; de Jonge, Marthe M.; Ritterhouse, Lauren L.; de Kroon, Cornelis D.; Vreeswijk, Maaike P. G.; Segal, Jeremy P.; Puranik, Rutika

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Germline *BRCA*-Associated Endometrial Carcinoma Is a Distinct Clinicopathologic Entity

Marthe M. de Jonge[1], Lauren L. Ritterhouse[2], Cornelis D. de Kroon[3], Maaike P.G. Vreeswijk[4], Jeremy P. Segal[2], Rutika Puranik[2]; for the HEBON Group[5], Harry Hollema[6], Matti A. Rookus[7], Christi J. van Asperen[8], Flora E. van Leeuwen[7], Vincent T.H.B.M. Smit[1], Brooke E. Howitt[9], and Tjalling Bosse[1]

Abstract

Purpose: Whether endometrial carcinoma (EC) should be considered part of the *gBRCA1/2*-associated hereditary breast and ovarian cancer (HBOC) syndrome is topic of debate. We sought to assess whether ECs occurring in *gBRCA* carriers are enriched for clinicopathologic and molecular characteristics, thereby supporting a causal relationship.

Experimental Design: Thirty-eight *gBRCA* carriers that developed EC were selected from the nationwide cohort study on hereditary breast and ovarian cancer in the Netherlands (HEBON), and these were supplemented with four institutional cases. Tumor tissue was retrieved via PALGA (Dutch Pathology Registry). Nineteen morphologic features were scored and histotype was determined by three expert gynecologic pathologists, blinded for molecular analyses (UCM-OncoPlus Assay including 1213 genes). ECs with LOH of the *gBRCA*-wild-type allele (*gBRCA*/LOHpos) were defined "*gBRCA*-associated," those without LOH (*gBRCA*/LOHneg) were defined "sporadic."

Results: LOH could be assessed for 40 ECs (30 *gBRCA1*, 10 *gBRCA2*), of which 60% were *gBRCA*/LOHpos. *gBRCA*/LOH-pos ECs were more frequently of nonendometrioid (58%, $P = 0.001$) and grade 3 histology (79%, $P < 0.001$). All but two were in the *TP53*-mutated TCGA-subgroup (91.7%, $P < 0.001$). In contrast, *gBRCA*/LOHneg ECs were mainly grade 1 endometrioid EC (94%) and showed a more heterogeneous distribution of TCGA-molecular subgroups: *POLE*-mutated (6.3%), MSI-high (25%), NSMP (62.5%), and *TP53*-mutated (6.3%).

Conclusions: We provide novel evidence in favor of EC being part of the *gBRCA*-associated HBOC-syndrome. *gBRCA*-associated ECs are enriched for EC subtypes associated with unfavorable clinical outcome. These findings have profound therapeutic consequences as these patients may benefit from treatment strategies such as PARP inhibitors. In addition, it should influence counseling and surveillance of *gBRCA* carriers.

Introduction

Inheritance of a pathogenic mutation in one allele of the breast cancer susceptibility genes, *BRCA1* or *BRCA2*, results in the hereditary breast and ovarian cancer (HBOC) syndrome, characterized by severely increased lifetime risk to develop breast cancer and tubo-ovarian cancer (OC; refs. 1, 2). Other cancer types reported to be increased in patients with germline *BRCA2* mutations (*gBRCA*) are pancreatic and prostate cancer (3, 4). Whether endometrial carcinoma (EC) should be considered part of *gBRCA*-associated HBOC syndrome is still under debate due to conflicting data (5–9). A number of studies have shown an increased risk to develop EC especially for *gBRCA1* carriers, with highest risks observed for an aggressive subtype of EC—the serous-like ECs (5–7, 9–11). However, others did not observe this increased risk or attributed it to previous tamoxifen treatment rather than to the *gBRCA* mutation (8, 9, 11).

LOH of the wild-type *BRCA1* or *BRCA2* allele (g*BRCA*/LOH-pos) is an important step in the carcinogenesis of *gBRCA*-associated tumors. This is supported by the observation that *gBRCA*/LOHpos breast cancers and OCs show significantly higher homologous recombination deficiency (HRD) scores compared with their *gBRCA*/LOHneg counterparts (12). The HRD score is based on the presence and quantification of "genomic scars" associated with *BRCA* deficiency, including the number of regions with LOH (13), large-scale state transitions (14), and telomeric allelic imbalances (15). Breast cancers and OCs arising in *gBRCA* carriers show variable LOH frequencies, with reported rates of 93% (*gBRCA1*) and 84% (*gBRCA2*)

[1]Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands. [2]Division of Genomic and Molecular Pathology, Department of Pathology, The University of Chicago, Chicago, Illinois. [3]Department of Gynaecology, Leiden University Medical Center, Leiden, the Netherlands. [4]Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands. [5]The Hereditary Breast and Ovarian Cancer Research Group Netherlands (HEBON), Coordinating Center: Netherlands Cancer Institute, Amsterdam, the Netherlands. [6]Department of Pathology, University Medical Center Groningen, Groningen, the Netherlands. [7]Department of Epidemiology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. [8]Department of Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands. [9]Department of Pathology, Stanford University School of Medicine, Stanford, California.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

M.A. Rookus, C.J. van Asperen, and F.E. van Leeuwen are writing on behalf of the HEBON Group; other authors are unaffiliated with the HEBON Group.

Corresponding Author: Tjalling Bosse, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands. Phone: 0031-7152-66639; Fax: 0031-7152-66952; E-mail: T.Bosse@lumc.nl

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Translational Relevance

We provide novel evidence in favor of endometrial carcinoma (EC) being part of the *gBRCA*-associated HBOC syndrome. By stratifying ECs that occurred in *gBRCA* mutation carriers by LOH of the *gBRCA* wild-type allele (LOH), we were able to identify ECs associated with the *gBRCA* mutation (*gBRCA*/LOHpos) and those that occurred sporadically (*gBRCA*/LOHneg). *gBRCA*-associated ECs are distinctly different from sporadic ECs by histology (high grade) and by molecular subtype (*TP53* mutant), both of which are associated with worst clinical outcome. These findings support the concept that EC is part of HBOC syndrome, which impacts genetic counseling and surveillance programs of *gBRCA* carriers. In addition, our work shows that LOH status should be considered when assessing PARP inhibitor sensitivity.

for OCs, and 90% (*gBRCA1*) and 54% (*gBRCA2*) for breast cancers (12). This signifies the relevance of LOH as a marker of causality and implies that *gBRCA*/LOHneg cancers are in fact sporadic tumors that develop independently of the *gBRCA* mutation and are not HRD.

The finding of recurrent clinicopathologic and molecular features in *gBRCA*-associated breast cancers and OCs has supported the concept that these cancers are distinct entities belonging to the *gBRCA*-associated HBOC syndrome. These features can also help identify tumors more likely to harbor *BRCA1/2* mutations. For example, breast cancers arising in *gBRCA1* carriers prototypically are of high grade and of the basal-like subtype with more frequent necrosis and lymphocytic infiltration (16, 17). *BRCA1*-associated high-grade serous tubo-ovarian carcinoma (HGSOC) shows more frequent (partial) Solid, pseudoEndometrioid, and/or Transitional morphology (SET morphology), which is distinctly different from the prototypical papillary and infiltrative growth generally encountered in sporadic HGSOC. Other features more frequently observed in *BRCA1*-associated HGSOC are necrosis, a higher mitotic index, and an increased number of tumor-infiltrating lymphocytes (TILs; refs. 18–20). On a molecular level, *gBRCA*-associated breast cancers and OCs share similar somatic copy-number profiles [somatic copy-number alteration (SCNA)-high] and frequent *TP53* mutations (16–18, 20–22).

The Cancer Genome Atlas (TCGA) Research Network previously defined four distinct molecular subclasses with prognostic relevance in ECs (22). The "serous-like/SCNA-high" molecular subclass has poorest clinical outcome and interestingly displays molecular similarities to both basal-like breast cancer and HGSOC, including a high number of SCNAs and frequent *TP53* mutations. Moreover, recent studies demonstrated that serous-like/SCNA-high ECs also frequently are HRD (22–24). This raises the question whether ECs occurring in *gBRCA1/2* carriers might be enriched for certain features, but studies comprehensively evaluating this have not been performed to date.

We aimed to, for the first time, comprehensively describe the clinicopathologic and molecular features, stratified by LOH-status, of a large series of ECs that occurred in *gBRCA* carriers.

Materials and Methods

Patient selection

Patients with a history of EC and a pathogenic *gBRCA1/2* mutation were identified from the "*He*reditary *B*reast and *O*varian cancer study, the *N*etherlands (HEBON cohort study)" (25). The HEBON study is an ongoing nationwide study on families with HBOC for which all patients who undergo genetic testing for *BRCA1/2* and *CHEK2* mutations in one of the participating centers are eligible for inclusion [all eight university medical hospitals in the Netherlands and the Netherlands Cancer Institute (NKI)]. For participants, data on, among others, personal cancer history and therapeutic treatments are collected both retrospectively and prospectively through regular linkages with the Netherlands Cancer Registry. Data on prophylactic surgery are collected via the Dutch Pathology Registry (PALGA; ref. 26). All data are centrally collected and managed by trained data managers only. Women were eligible for inclusion when they had (i) a proven pathogenic germline *BRCA1/2* (*gBRCA1/2*) mutation (PLON class 4 or 5; ref. 27), (ii) provided written informed consent for the HEBON study, and (iii) had a history of epithelial EC or developed an EC during follow-up, defined as a tumor with an International Classification of Diseases Oncology, Third Edition, First Revision (ICD-O-3.1; http://codes.iarc.fr/) topographical code of either C54 (Corpus Uteri) or C55 (Uterus, NOS).

In total, 3,726 *gBRCA* carriers provided informed consent between 1999 and 2014, of which the majority was provided in 2012 and 2013 (62.5%). Of these women, 41 (1.1%) developed an EC. We were able to retrieve 39 of 41 tumors from pathology laboratories across the Netherlands. One tumor was a sarcoma and was therefore excluded. Of these 38 HEBON-ECs, 21 ECs occurred preceding to study enrollment (mean: 4.7 years, SD: 2.79) and 16 ECs occurred after study enrollment (mean: 4.5 years, SD 3.52). For one case, the date of study enrollment was not available. The HEBON-ECs were supplemented with four ECs from known *gBRCA1/2* carriers previously diagnosed in the Leiden University Medical Center (LUMC).

For all ECs, hematoxylin and eosin (H&E)-stained slides, anonymized pathology reports, and at least one representative formalin-fixed, paraffin embedded (FFPE)-tumor block were collected through the Dutch Pathology registry (PALGA; ref. 26) from pathology laboratories across the Netherlands. If applicable, material from the (salpingo-)oophorectomy or OC specimen was also requested. The HEBON study is approved by the medical ethical committees of all participating centers, and all participants gave written informed consent to participate in the study. The HEBON study is performed in accordance with the Declaration of Helsinki. Our study was performed after the study protocol was approved by the HEBON steering committee (date: November 30, 2017) and by the Institutional Review Board of the Netherlands Cancer Institute; IRBd18086. All specimens were handled in compliance with the Code of Conduct for dealing responsibly with human tissue in the context of health research (2011) drawn up by the Federation of Dutch Medical Scientific Societies.

Clinicopathologic characterization

All cases were independently reviewed by three expert gynecologic pathologists (V.T.H.B.M. Smit, T. Bosse, and B.E. Howitt). They were aware that the ECs occurred in *gBRCA* carriers; however,

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they were blinded for LOH status. The World Health Organization (2014) criteria were used for histologic subtype diagnosis. Reviewers were not allowed to use immunostains to aid classification and diagnoses were solely based on H&E stains. Cases were classified ambiguous when overlapping features of both high-grade endometrioid and serous carcinomas were present in the tumor and when tumors failed to show prototypic features of a certain subtype. Discordant cases were discussed during a consensus meeting to assign final histologic subtype. ECs with ambiguous morphology were considered nonendometrioid for statistical analyses. After final histologic subtype was assigned, histologic subgroups were made. For ambiguous cases, *TP53* mutation status was used to assign histologic subgroup. Cases were categorized as follows: "Endometrioid" for Endometrioid, mucinous and *TP53*-wild-type ambiguous carcinomas, "serous-like" for uterine serous carcinomas (USCs), uterine carcinosarcomas (UCSs) and *TP53*-mutant ambiguous carcinomas, or "clear cell" for clear cell carcinomas. Review of adnexa, depth of myometrial invasion, cervical involvement, lymph node status, and presence of lymphovascular space invasion was performed by one expert gynecologic pathologist (T. Bosse) on which FIGO-2009 stage was based upon. When slides were not available, these data were retrieved from the original pathology reports.

Nineteen morphologic characteristics were assessed by one expert gynecologic pathologist (B.E. Howitt) on all available tumor slides, blinded for LOH status. For additional details on this, see Supplementary Materials and Methods.

IHC

Cases were stained for p53 (clone DO-7, 1:2,000, DAKO), Wilms tumor 1 (WT-1, clone 6F-H1, 1:3,200, Invitrogen), estrogen receptor (ER, Clone EP1, 1:200, DAKO), progesterone receptor (PR, Clone Pgr636, 1:400, DAKO), and CD8 (Clone 4B11, 1:2,000, Novocastra). Procedures and scoring methods are described in the Supplementary Materials and Methods.

Molecular analysis

DNA isolation. Tumor DNA was isolated from FFPE-tissue blocks either by using three 0.6-mm tumor cores ($n = 16$) or by using microdissected tissue from 5 to 10 tissue sections (10 μm; $n = 26$). DNA isolation was performed fully automated using the Tissue Preparation System (Siemens Healthcare Diagnostics) as described previously (28). The median tumor cell percentage of the isolated areas was 80% (range, 25%–90%).

Next-generation sequencing

Following extraction, DNA was quantified using the Qubit fluorometric assay (Thermo Fisher Scientific) and further assessed for quantity and quality using a quantitative PCR assay (hgDNA Quantitation and QC kit, KAPA Biosystems). Library preparation and sequencing were performed as previously described for the UCM-OncoPlus Assay (29). Briefly, approximately 100-ng DNA was fragmented using the Covaris S2 (Covaris). The fragmented DNA was amplified using the KAPA HTP Library Preparation Kit (Kapa Biosystems) along with a set of patient-specific indexes (Roche). The pooled library was captured using a custom SeqCap EZ capture panel (Roche) featuring a collection xGen LockdownProbes (IDT) for 1,213 genes. The pooled captured library was sequenced on the Illumina HiSeq 2500 system (Illumina) in rapid run mode (2×101 bp paired end sequencing). Somatic mutation and copy number calling were performed across all 1,213 genes using a custom in-house bioinformatics pipeline previously described (29). The five-tier pathogenicity classification described by Plon and colleagues, 2008, was used to categorize variants (27). Only class 4 (likely pathogenic) and 5 (pathogenic) mutations are reported in the manuscript.

LOH of *gBRCA1/2* mutations

Known *gBRCA1/2* mutations were assessed for LOH of the wild-type allele by evaluating the following parameters: estimated tumor cell purity, *BRCA1/2* mutation variant allele frequency (VAF), local copy number status, and adjacent SNP VAF, using a similar approach to what has been described by *Khiabanian and colleagues, 2018* (30). For LOH analyses, we applied the following model, taking into account the chromosomal copy number at the *BRCA* locus; $\text{VAF} = [(1-p) + \text{cmut} \times \text{p}]/[2 \times (1-p) + Y \times p]$, with p being the tumor purity, cMut being the mutation's chromosomal copy number, and Y being the ploidy of the tumor cells. LOH events occur when $\text{cMut} = 1$ and $Y = 1$ or $\text{cMut} > 1$ and $Y > 1$. Because all *BRCA1/2* mutations were germline mutations, the expected VAF in the absence of LOH was 1/2 (50%) for all cases. LOH of the *gBRCA1/2* wild-type allele was considered to be present if (i) $\text{cMut} = 1$ and $Y = 1$ or $\text{cMut} > 1$ and $Y > 1$, (ii) the observed *gBRCA1/2* mutation VAF was similar to the expected VAF according to the formula, (iii) adjacent observed SNP VAF (if present) supported the findings, and (iv) sequencing quality was sufficient. Mutations that were considered to have an LOH event were classified as either copy-neutral (no evidence of local copy-number change) or copy-number loss. *gBRCA*/LOHpos ECs were defined as *gBRCA*-associated, *gBRCA*/LOHneg ECs as "sporadic."

Copy-number calling

For the copy-number calling, we used a clinically validated bioinformatic tool that has previously been detailed and published (29). Briefly, copy-number analysis involved evaluation of average exon interval depths recorded via the Genome Analysis Toolkit DepthofCoverage module. A historical normalized baseline for each interval in the panel was generated using 24 non-malignant clinical samples. Test sample data were subjected to a normalization algorithm to control for individual gene profile run-specific variability. To detect the potential copy-number regions, fold change and Z-scores were calculated for each interval, and thresholds were set at >200% (gain) or <66% (loss) with Z-score >3 or ≤2, respectively. Genes with more than half the intervals showing copy-number changes in the same direction were then identified. Overall copy-number status was assessed manually by assessing the copy-number plots across the entire territory and determining how many large-scale (arm or subarm-level changes) copy-number alterations were present in each case. Cases considered to be "low" copy number had 0 large-scale copy-number alterations, "borderline" had 1 to 2 large-scale copy number alterations, and those considered "high" had >2 large-scale copy-number changes.

Microsatellite instability status

For MSI testing, a metric similar to that proposed by Kautto and colleagues 2017 (31) was employed to quantify the stability of a homopolymer locus. For each locus, distribution over different homopolymer lengths (normalized to a fraction of total depth at the locus) was generated. Then, absolute value of the stepwise difference between that sample distribution and normal distribution was calculated as a distance score (d). The baseline

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distribution was generated using average values across 23 non-malignant spleen samples. Thresholds for assignment of "stable" or "instable" status for a locus involved using training sets of MSI-stable and MSI-high samples, tested previously by PCR assay or IHC staining. Samples with unstable loci <9% were classified as microsatellite stable, 9% to 15% were classified as indeterminate, and >15% were classified as microsatellite instable (MSI).

Tumor mutational burden

Tumor mutational burden (TMB) was quantified as mutations/Mb using a 1,132-gene territory from the UCM-OncoPlus assay. Variants that met any of the following criteria were excluded from the calculation: <10% VAF, synonymous, variants present in either 1,000 genomes or ExAC population databases. In addition, variants were rescued if there were >10 entries in COSMIC database with an ExAC frequency of <0.001.

Molecular subgroups

The following surrogate markers were used to classify ECs in the four molecular subgroups defined by the TCGA (22, 32, 33); *POLE* exonuclease domain mutations for the *POLE*/ultramutated group, MSI-high profile for MSI-high/hypermutated group, *TP53* mutations for SCNA-high/serous-like group, and the absence of surrogate markers for no surrogate marker profile (NSMP)/SCNA-low group (22, 32, 33). When two molecular classifiers were present, subgroups were assigned in line with what has previously been published by the TCGA (22); *POLE*&MSI-high or *POLE*&*TP53* as *POLE* and MSI-high&*TP53* as MSI-high.

Statistical analysis

Associations between categorical variables were tested using a two-sided Fisher exact test or χ^2 statistics when more than two variables were compared. Associations between continuous variables were tested using the Mann–Whitney *U* test. Overall survival was calculated using the Kaplan–Meier Method with log-rank test and was calculated from the date of EC diagnosis to the date of death while patients who were alive were censored at the date of last follow-up. For HEBON cases, the date of last linkage with the Dutch Municipal Personal Record Database was used as last date of follow-up (April 11, 2019, for all except for case 2; December 23, 2016). *P* values <0.05 were considered significant. Statistical analysis was performed using IBM SPSS version 23.0 (SPSS, Inc.) and GraphPad Prism (GraphPad Software Inc.).

Results

In total, 42 ECs that occurred in *gBRCA1/2* carriers were analyzed (32 *gBRCA1*, 10 *gBRCA2*). Clinicopathologic characteristics of the complete cohort are described in Supplementary Table S1. The cohort comprised 26 endometrioid ECs (61.9%), of which 17 (40.5%) were grade 1, three (7.1%) were grade 2, five (11.9%) were grade 3, and one (2.4%) was a mucinous carcinoma. Sixteen ECs were classified as nonendometrioid (38.1%), of which seven (16.7%) were USC, four (9.5%) were UCS, and five (11.9%) were classified as high-grade ambiguous.

Molecular analysis was conducted to stratify for LOH of the *gBRCA1/2*-wild-type allele, which succeeded for all but two cases ($n = 40$, 95.2%), which were excluded from final analyses (one USC and one EEC grade 1, Supplementary Table S2). The known *gBRCA1/2* mutation was confirmed in all 40 cases included in final analyses. Overall, 60% (24/40) of ECs were *gBRCA*/LOHpos. When stratified for *gBRCA1* and *gBRCA2* mutations, 66.7% ($n = 20/30$) and 40% ($n = 4/10$) showed LOH, respectively ($P = 0.159$; Fig. 1; Supplementary Table S2). Plotting the position of the *gBRCA* mutations across the coding DNA sequence for *BRCA1* and *BRCA2* did not show enrichment of mutations in a specific region of the gene [www.cbioportal.org/visualize (34, 35); Supplementary Fig. S1].

Clinicopathologic, morphologic, and molecular characteristics of *gBRCA* ECs stratified by LOH status

Clinicopathologic characteristics stratified by LOH status are summarized in Table 1 and Fig. 1. Compared with *gBRCA*/LOHneg ECs, *gBRCA*/LOHpos ECs were significantly more often FIGO grade 3 (6.3% vs. 79.2%, $P < 0.001$) with nonendometrioid and serous-like histology (both 6.3% vs. 58.3%, $P = 0.001$) and more often presented with lymphovascular space invasion (41.7% vs. 0%, $P = 0.003$). The 5-year overall

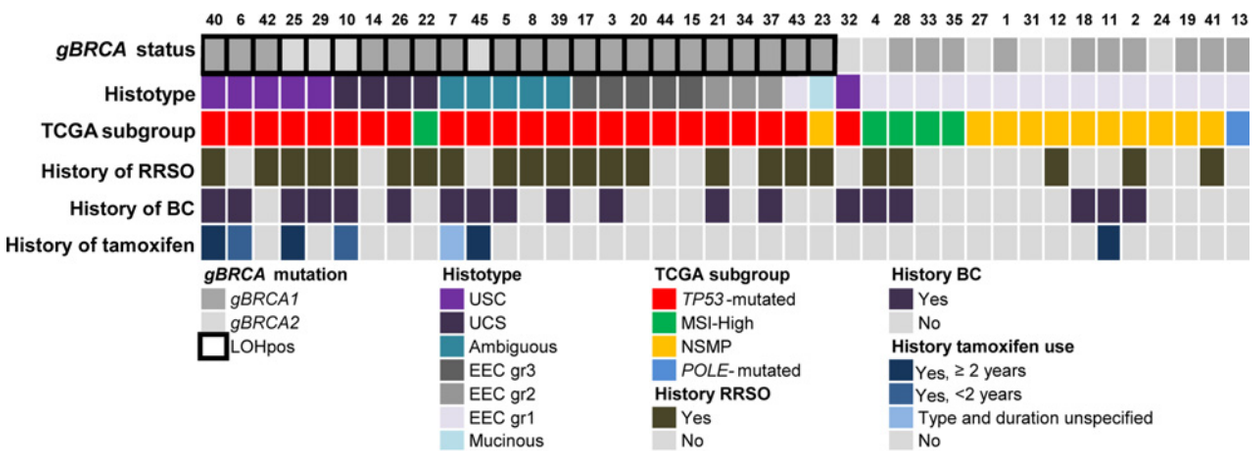

Figure 1.

Clinicopathologic and molecular characteristics stratified by LOH status. Case 22 and case 4 were MSI-high and had a *TP53* mutation; they were classified in the MSI-high subgroup in accordance to what is described in the Supplementary Material and Methods. EEC, endometrioid endometrial carcinoma grade; gr, grade; LOH, loss of heterozygosity of the *gBRCA1/2* wild-type allele.

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Table 1. Clinicopathologic characteristics stratified by LOH status

	LOHpos ($n = 24$)	LOHneg ($n = 16$)	*P*
Germline *BRCA1/2* mutation, *n* (%)			
g*BRCA1*	20 (83.3)	10 (62.5)	0.159
g*BRCA2*	4 (16.7)	6 (37.5)	
Age at diagnosis, median (range), y	60.5 (33–74)	57 (44–67)	0.267
FIGO 2009, *n* (%)			
I, II	19 (79.2)	14 (87.5)	0.681
III, IV	5 (20.8)	2 (12.5)	
Salpingo-oophorectomy, *n* (%)[a]			
History of RRSO	18 (75)[b]	5 (31.3)	**0.009**[c]
RRSO at the time of EC diagnoses	0 (0)	2 (12.5)	
At the time of hysterectomy	5 (20.8)	8 (50)	
Therapeutic	0 (0)	1 (6.3)	
History of, *n* (%)			
OC	0 (0)	0 (0)	
BC	13 (54.2)	6 (37.5)	0.349
Tamoxifen use	6[d] (25)	1 (6.3)	0.21
STIC or adnexal involvement, *n* (%)	0 (0)	0 (0)	
LVSI present, *n* (%)[e]	10 (41.7)	0 (0)	**0.003**
Not assessable	*1 (2.4)*	*1 (6.3)*	
Histologic subtype, *n* (%)			
Endometrioid	10 (41.7)	15 (93.8)	**0.001**[f]
Mucinous	*1 (4.2)*	*0 (0)*	1.00
Nonendometrioid	14 (58.3)	1 (6.3)	
Serous	*5 (20.8)*	*1* (6.3)	0.373
Carcinosarcoma, serous	*2 (8.3)*	*0 (0)*	0.136[g]
Carcinosarcoma, ambiguous	*2 (8.3)*	*0 (0)*	
Ambiguous	*5 (20.8)*	*0 (0)*	0.071
Histologic subgroups, *n* (%)			
Endometrioid	10 (41.7)	15 (93.8)	**0.001**
Serous-like	14 (58.3)	1 (6.3)	
Histologic grade, *n* (%)			
1 & 2	5 (20.8)	15	**<0.001**
3	19 (79.2)	1 (6.3)	

NOTE: *P* values in boldface are considered significant ($P < 0.05$).
Abbreviations: CN, copy number; LOH, loss of heterozygosity of the *gBRCA1/2* wild-type allele; LVSI, lymphovascular space invasion.
[a]For one case (case 15), no history of salpingo-oophorectomy was reported and they were not removed during hysterectomy.
[b]For one case, only an ovariectomy (without salpingectomy) was performed; this was not considered as RRSO.
[c]*P* value was calculated over history of RRSO or not.
[d]Includes one patient for which the specific hormone treatment was unknown.
[e]Not evaluable for two cases, which were left out from statistical analyses.
[f]*P* value was calculated over endometrioid and nonendometrioid ECs.
[g]*P* value was calculated over carcinosarcoma versus other histotypes (independent of epithelial component).

survival rate of *gBRCA*/LOHpos ECs was lower (81.3%) compared with *gBRCA*/LOHneg ECs (93.3%, $P = 0.084$; Supplementary Fig. S2).

In total, morphologic characteristics were informative for 39 cases (one case was excluded because of neoadjuvant therapy). A higher frequency of "SET features" in *gBRCA*/LOHpos ECs was observed compared with *gBRCA*/LOHneg ECs (52.2% vs. 0%, $P < 0.001$; Fig. 2). Other histologic features that were significantly more often observed in *gBRCA*/LOHpos ECs were destructive type of invasion, desmoplastic stromal reaction, nonglandular dominant growth pattern, geographic necrosis, trabecular growth pattern, slit-like spaces, high nuclear grade, tumor giant cells, and a higher median mitotic index (Table 2; Supplementary Fig. S3). We did not find a significant difference for intraepithelial TILs or peritumoral lymphocytes assessed on H&E, nor for CD8-positive T cells (Supplementary Fig. S4). *gBRCA*/LOHpos ECs were more often estrogen receptor negative (45.5% vs. 6.8%, $P = 0.012$) and progesterone receptor negative (79.2% vs. 12.5%, $P < 0.001$) compared with *gBRCA*/LOHneg ECs.

All ECs were classified into one of the four molecular subgroups previously defined by the TCGA (Fig. 1). All but two *gBRCA*/LOHpos ECs were classified in the *TP53*-mutated subgroup, compared with only one of the *gBRCA*/LOHneg ECs (91.7% vs. 6.3%, $P < 0.001$). In line with this, *gBRCA*/LOHpos ECs more often had a CN-high profile compared with *gBRCA*/LOHneg ECs (95.5% vs. 0%, $P < 0.001$; Fig. 3). Compared with *gBRCA*/LOHneg ECs, *gBRCA*/LOHpos ECs had significantly more mutations in *TP53* (95.8% vs. 12.5%, $P < 0.001$) and fewer mutations in *PTEN* (16.7% vs. 93.8%, $P < 0.001$), *PIK3CA* (16.7% vs. 56.3%, $P = 0.015$), *PIK3R1* (4.2% vs. 43.8%, $P = 0.004$), *ARID1A* (4.2% vs. 43.8%, $P = 0.004$), and *CTNNB1* (0% vs. 37.5%, $P = 0.002$; Fig. 3). In total, *gBRCA*/LOHpos ECs harbored significantly fewer class 4 or 5 mutations (other than the *gBRCA* mutation) compared with *gBRCA*/LOHneg ECs; no statistically significant difference was observed for TMB (Supplementary Fig. S5A and S5B).

gBRCA/LOHpos ECs are not misclassified ovarian cancers

To ensure that the ECs did not represent misclassified OCs, salpingo-oophorectomy specimens were rereviewed to detect (pre)malignant lesions. Of the 40 cases included in our final cohort, 39 (97.5%) cases underwent salpingo-oophorectomy either prior to or at the time of hysterectomy. Women who developed *gBRCA*/LOHpos ECs more often previously underwent a risk-reducing salpingo-oophorectomy (RRSO) compared with women with *gBRCA*/LOHneg ECs (75% vs. 31.3%, $P = 0.009$), and the time interval between the RRSO and EC diagnosis was significantly longer; 73.2 months (range, 35.7–187) versus 12.2 months (range, 4.9–82.9, $P = 0.037$). Because this is a historical cohort, sectioning and extensively examining the fimbriated end was not routinely performed. In total, 36 of 39 (92%) adnexal specimens were available for rereview, of which the fimbriae could be (partially) examined for 16 of 22 (72.7%) of *gBRCA*/LOHpos ECs and seven of 14 (50%) of *gBRCA*/LOHneg ECs. None of the ECs showed adnexal involvement and none of the RRSO specimens showed a serous tubal intraepithelial carcinoma (STIC). In two cases, tubal lesions were detected at the time of hysterectomy; one *TP53* signature (case 6, USC) and one serous tubal intraepithelial lesion (STIL, case 35, EEC grade 1). In addition, according the pathology report of case 31 (EEC grade 1, adnexa not available for review), the tubal lining showed focal epithelial "atypia and p53 positivity," which could indicate the presence of a p53 signature, STIL, or STIC. Case 31 presented with a simultaneous EEC and endometrioid ovarian cancer, which were considered to be synchronous primary tumors and not to be secondary adnexal involvement of the EC.

A minority of cases displayed WT-1 positivity ($n = 7$, 17.5%), of which three (7.5%) displayed heterogenous staining; two USCs, one UCS, and four (10%) displayed diffuse staining; one USC, one UCS, and two ambiguous cases (Table 2). Six of seven women with a WT-1-positive EC had a history of RRSO, none of which showed a (pre)malignant lesion upon rereview. For all but one (case 5), slides available for rereview included sections through the fimbriae. For case 5 (EC diagnosis 2015), the fimbriae could not be examined because of scarring of the fimbriae as a result of a previous bilateral oophorectomy (1995) performed prior to the salpingectomy (2005), as the complete tubes were submitted for histology review. For the one WT-1-positive EC that did not have a history of RRSO (case 6), both adnexa were removed during

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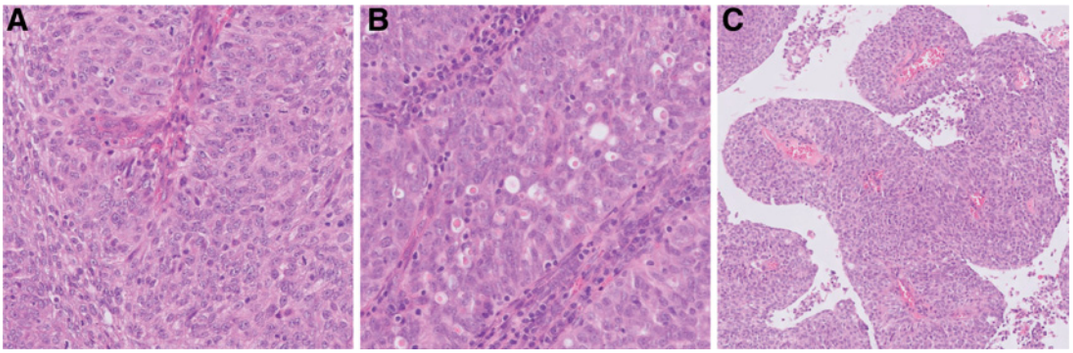

Figure 2.

Growth pattern associated with LOH. Hematoxylin and eosin (H&E) slide of a *gBRCA*/LOHpos endometrial carcinoma classified as ambiguous showing Solid (**A**), pseudoEndometrioid (**B**), and Transitional (**C**; SET) features.

therapeutic hysterectomy and a p53 signature was detected in one fallopian tube. When excluding all ECs that displayed WT-1 staining, nonendometrioid and serous-like histology remained significantly more common in *gBRCA*/LOHpos ECs than in *gBRCA*/LOHneg ECs (both $n = 7/17$, 41.2% vs. $n = 1/16$, 6.3%, $P = 0.039$).

gBRCA/LOHpos ECs are not exclusively the result of previous tamoxifen treatment

In total, 19 women had a history of breast cancer, which was not significantly different for women with *gBRCA*/LOHpos ECs compared with *gBRCA*/LOHneg ECs (54.2% vs. 37.5%, $P = 0.349$). Although women with *gBRCA*/LOHpos ECs more frequently had a history of tamoxifen use (including one case for which the type of hormone treatment was not specified), this difference was not significant ($n = 6$, 25% vs. $n = 1$, 6.3%, $P = 0.210$; Table 1; Fig. 1). When excluding all tamoxifen-treated individuals, nonendometrioid and serous-like histology remained significantly more common in *gBRCA*/LOHpos ECs than in *gBRCA*/LOHneg ECs (both $n = 8/18$, 44.4% vs. $n = 1/15$, 6.7%, $P = 0.021$). Across the entire cohort (both *gBRCA*/LOHpos and *gBRCA*/LOHneg), a history of tamoxifen use was significantly associated with serous-like histology ($n = 6/15$, 40% vs. $n = 1/25$, 4.0%, $P = 0.007$). When only including women who received tamoxifen for 2 or more years (excluding the patient for which hormone treatment duration was unknown), this association was not observed anymore ($n = 3/14$, 21.4% vs. $n = 1/25$, 4%, $P = 0.123$).

Discussion

This is the first study to describe *gBRCA*-associated EC as a distinct entity enriched for high-grade, nonendometrioid tumors with frequent *TP53* mutations and recurring morphologic features. LOH of the wild-type *gBRCA* allele was present in 60% of ECs diagnosed in *gBRCA* carriers, and therefore these should be regarded as "*gBRCA*-associated ECs." Importantly, the remaining 40% did not show LOH and therefore are "sporadic ECs" despite the presence of a *gBRCA* mutation. *gBRCA*-associated ECs were histologically high-grade in 79%, which is much more frequent than the 21% to 28% of ECs that would be expected on the basis of population frequencies (36, 37). We have shown that these tumors are not misclassified OCs, nor exclusively the result of previous tamoxifen treatment. In summary, our findings strongly support that EC is part of the *gBRCA*-associated HBOC syndrome.

There are no strict criteria to which a tumor type should adhere to be considered part of a hereditary cancer syndrome. It is generally accepted, however, that tumors that are part of a cancer syndrome should occur more frequently and develop at a younger age compared with what would be expected in the general population. A distinct phenotype of tumors in a cancer syndrome is considered to be in support of a causal relationship. Although previous studies show contradictory results about excess risk of EC (all histotypes) for *gBRCA* carriers (6–11, 38), most recent studies did find increased risks to develop serous-like ECs, with reported standardized incidence ratios (SIR) ranging from 14.29 to 32.2 (6, 7, 10). These SIRs are comparable with the reported relative risk increase for prostate cancer (up to 20-fold) and pancreatic cancer (up to 10-fold) for *gBRCA2* carriers (1). The *gBRCA*-associated ECs in our study were diagnosed at a median age of 60.5 years (range, 33–74 years). Because these tumors were enriched for EC histotypes that generally occur at an older age (e.g., USC, UCS, EEC grade 3; refs. 36, 37), our data are suggestive that *gBRCA*-associated ECs indeed occur at a younger age compared with their sporadic counterparts, although no definitive conclusions can be drawn without a proper control group. The combination of the excess risk reported in literature and the phenotype of *gBRCA*-associated EC described here strongly support adding (*TP53*-mutated/serous-like) EC to the HBOC syndrome.

Our observation that *gBRCA*-associated (*gBRCA*/LOHpos) EC and sporadic (*gBRCA*/LOHneg) EC show marked histologic and molecular differences supports previous findings that tumors arising in *gBRCA* carriers are not necessarily causally related to the *gBRCA1/2* mutation (12). ECs arising in g*BRCA* carriers showed LOH relatively infrequently (67.7% of *gBRCA1* and 40% *gBRCA2*) compared with OCs and breast cancers in *gBRCA1* carriers (93% and 90%) and OCs in *gBRCA2* carriers (84%) but with similar rates to what has been found for breast cancers in *gBRCA2* carriers (54%; ref. 12). This is an important finding, as it emphasizes that tumors that develop in *gBRCA* carriers are not HRD per default and thereby may not respond to treatments targeting this DNA repair defect. This concept impacts the interpretation of clinical trials assessing efficacy of PARP inhibitors in tumors with *BRCA1/2* mutations that show LOH relatively infrequently and suggests that LOH should be included in stratification algorithms for studies assessing therapy efficacy in tumors

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Table 2. Morphologic characteristics stratified by LOH status

	LOHpos ($n = 23$)	LOHneg ($n = 16$)	*P*
Tumor slides assessed/case, median (range)	7 (1–21)	4.5 (1–18)	0.074
Invasion type, *n* (%)			
Destructive	17 (73.9)	4 (25)	**0.004**[a]
Pushing/broad front	2 (8.7)	3 (18.8)	
MELF type	0 (0)	1 (6.3)	
Adenomyosis-like	0 (0)	3 (18.8)	
No invasion	2 (8.7)	3 (18.8)	
Not analyzable	2 (8.7)	2 (12.5)	
Desmoplastic stromal reaction, *n* (%)[b]	16 (69.6)	5 (31.3)	**0.042**
Predominant growth pattern, *n* (%)			
Glandular	7 (30.4)	16 (100)	**0.001**
"SET-like"	8 (34.8)	0 (0)	
Papillary	4 (17.4)	0 (0)	
Solid	3 (13)	0 (0)	
Mucinous	1 (4.3)	0 (0)	
SET features (any percentage), *n* (%)			
Solid	15 (65.2)	0 (0)	<**0.001**
Cribriform/pseudoEndometrioid	9 (39.1)	0 (0)	**0.005**
Transitional cell carcinoma-like	5 (21.7)	0 (0)	0.066
SET features present ≥25%, *n* (%)	12 (52.2)	0 (0)	<**0.001**
Comedo necrosis, *n* (%)	10 (43.5)	2 (12.5)	0.076
Geographic necrosis, *n* (%)[c]	6 (26.1)	0 (0)	**0.03**
Squamous differentiation, *n* (%)	4 (17.4)	6 (37.5)	0.264
Papillary growth, *n* (%)	15 (65.2)	13 (81.3)	0.471
Trabecular growth, *n* (%)[d]	8 (34.8)	0 (0)	**0.006**
Jagged lumina, *n* (%)	8 (34.8)	1 (6.3)	0.056
Slit-like spaces, *n* (%)[c]	10 (43.5)	2 (12.5)	**0.04**
Hobnailing, *n* (%)[c]	1 (4.3)	1 (6.3)	1
Nuclear atypia, *n* (%)			
Grade 1/2	4 (17.4)	15 (93.8)	<**0.001**
Grade 3	19 (82.6)	1 (6.3)	
Tumor giant cells, *n* (%)	11 (47.8)	1 (6.3)	**0.012**
Mitotic index/10 HPF, median (range)	48 (1–197)	12 (1–28)	<**0.001**
Intraepithelial TILs, *n* (%)	9 (39.1)	6 (37.5)	1
Peritumoral lymphocytes, *n* (%)[c]	16 (69.6)	9 (56.3)	0.323
<10% ER, *n* (%)	11 (45.8)	1 (6.3)	**0.012**
<10% PR, *n* (%)	19 (79.2)	2 (12.5)	<**0.001**
WT-1, *n* (%)			
Negative: ≤1%	17 (70.8)	16 (100)	**0.029**[e]
Heterogeneous: 2%–75%	3 (12.5)	0 (0)	
Diffuse positive >75%	4 (16.7)	0 (0)	

NOTE: *P* values in boldface are considered significant ($P < 0.05$).

Abbreviations: HPF, high-power field (0.2 mm^2); LOH, loss of heterozygosity of the g*BRCA1/2* wild-type allele; MELF, microcystic, elongated, and fragmented; SET, Solid, pseudoEndometrioid, Transitional.

[a]*P* value was calculated over destructive type of invasion versus other.

[b]Not applicable for nine cases that were left out from statistical analysis [five times absence of invasion, four times invasion not analyzable (curettage)].

[c]Not evaluable for one case, which was left out from statistical analysis.

[d]Not evaluable for two cases, which were left out from statistical analysis.

[e]*P* value was calculated over negative nuclear WT-1 expression or positive nuclear WT-1 expression (encompassing both heterogeneous and diffuse positive staining).

from *gBRCA* carriers (39–42). In fact, LOH status may explain the less pronounced efficacy of olaparib (PARP-inhibitor) for *gBRCA2* carriers with HER2-negative metastatic breast cancer compared with g*BRCA1* carriers as observed in the OlympiAD-trial (42).

Our observation should increase awareness of the association between *gBRCA* and high-grade EC and may have clinical implications in selecting patients with EC and their families for *gBRCA* testing. Previous studies testing *gBRCA* mutations in unselected EC cohorts resulted in relatively low incidences (0.5% and 0.6%), with only minor increase (1.1% and 3%) when limited to USC and UCS (43, 44). The morphologic clues described in our study, however, may serve to enrich for *gBRCA* carriers and therefore facilitate cost-effective *gBRCA* testing in patients with EC and their families, a concept that merits further study. Currently, one might consider *gBRCA* testing in patients with high-grade EC with a previous history of breast cancer or a positive family history for *gBRCA*-associated malignancies. Although our study was not aimed to determine the excess risk in women with *gBRCA1/2* mutations to develop EC compared with the general population, our study supports to at least inform *gBRCA* carriers about the association with EC, as the ECs arising in this background are of an unfavorable subtype.

In this study, it was relevant to ascertain that all included carcinomas were of endometrioid and not of tubo-ovarian origin. To exclude misclassification of secondary involvement of the endometrium by HGSOC as EC, we rereviewed all available salpingo-oophorectomy slides with emphasis on putative precursor lesions in the distal fallopian tube. None of the serous-like ECs showed adnexal involvement, supporting the endometrium as primary origin. In addition, we stained all ECs for WT-1, a marker that assists in distinguishing between USC and HGSOC, with reported nuclear positivity rates ranging from 0% to 44% for USCs and 95% to 100% for serous OCs (45–48). Although cutoff values for WT-1 positivity are unclear, "diffuse WT-1" is generally accepted to be uncommon in EC. WT-1 positivity was observed in seven of 40 ECs (17.5%), of which four (10%) showed diffuse WT-1 positivity. There was no macro- and microscopic indication for a tubo-ovarian carcinoma in the WT-1 positive ECs; nevertheless, we cannot completely rule out the theoretical possibility of a "drop metastasis" from the fallopian tube. The large time interval between the RRSO and EC diagnosis (median 5.7 years, range, 4.0–9.4 years) that was previously performed in six of seven cases, in combination with the absence of any tubal involvement upon rereview, favors primary endometrial origin. For the remainder WT-1-positive EC (case 6), both adnexa were removed during therapeutic hysterectomy, in which a p53 signature was detected unrelated to the EC. We therefore conclude that all cancers in this study, including those that showed WT-1 positivity, are most likely of primary endometrial origin.

Another relevant aspect is a history of tamoxifen treatment, as 2 or more years of tamoxifen treatment has been associated with a two- to sevenfold increased risk to develop ECs (49–52). ECs of tamoxifen-treated individuals are enriched for less favorable histologic subtypes compared with nontreated individuals, especially carcinosarcomas and sarcomas (10.6%–13.8% vs. 2.9%–8.7%, respectively), and for ECs with abnormal p53 expression (49, 53, 54). Tamoxifen is thought to have a stimulatory effect on the endometrium and uterine body while having an antiestrogenic effect in breast tissue (49, 55). This stimulatory effect on the endometrium is unlikely the responsible mechanism for the observed association with serous-like ECs as these ECs are mostly hormone independent (49). A more plausible, alternative hypothesis for this association may be the DNA-damaging effect of tamoxifen. It has been suggested that tamoxifen induces the generation of reactive oxygen species (ROS; ref. 56). ROS can cause DNA damage resulting in replicative stress and DNA double-stranded break formation (1, 57). Previous literature showing the association between tamoxifen use and EC risk did not take *gBRCA* status into account. In our study cohort of *gBRCA* carriers, we found an enrichment for serous-like histology in women previously treated with tamoxifen. We recently showed that

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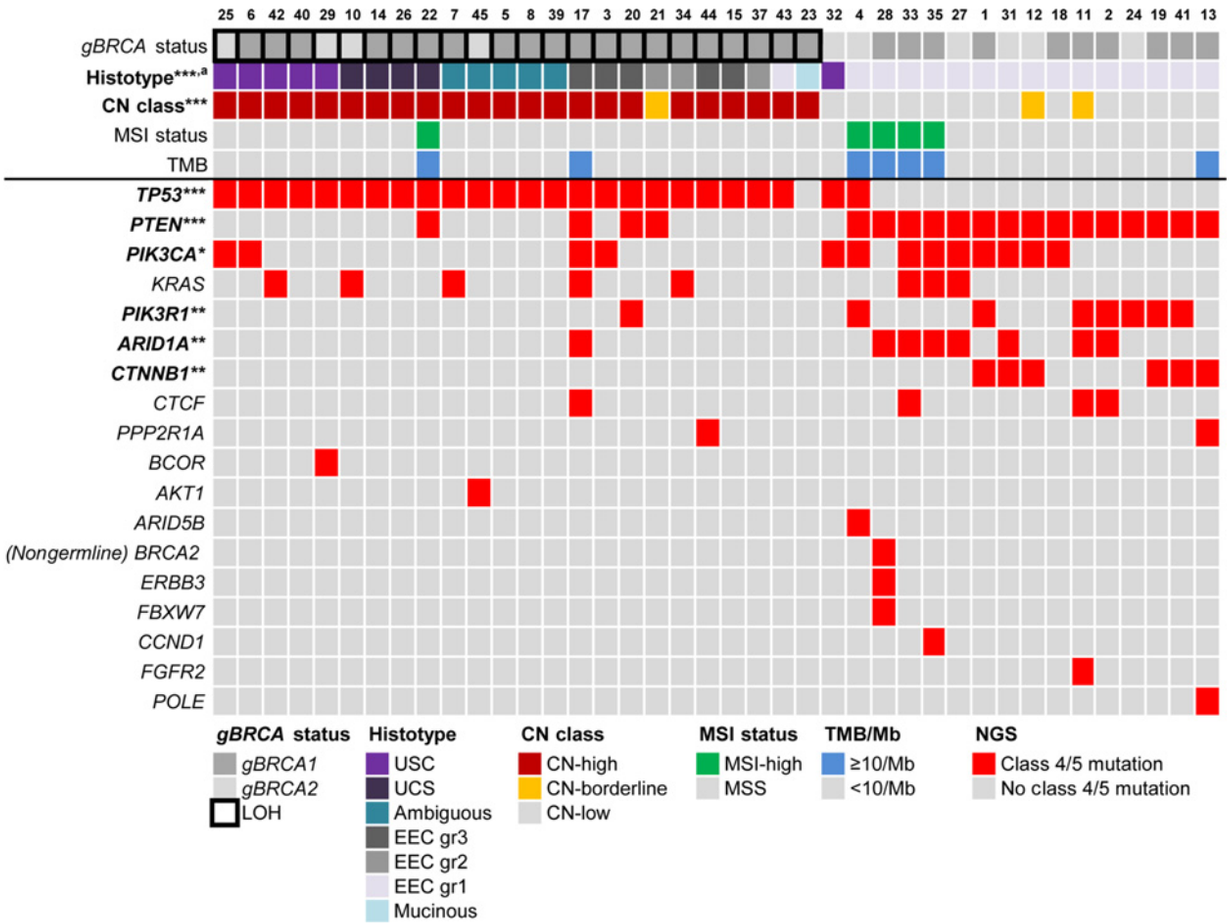

Figure 3.

Molecular characteristics of *gBRCA1/2* ECs grouped by LOH status. Case 29 contains a *TP53* mutation NM_000546.5:c.375+5G>T that was considered as likely pathogenic, given the predicted effect on splicing in combination with abnormal p53 expression ("null pattern") in IHC. Bolded cases were considered significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. [a]*P* value was calculated over endometrioid and nonendometrioid EC. CN, copy number; EEC, endometrioid endometrial carcinoma; gr, grade; LOH, loss of heterozygosity of the *gBRCA1/2* wild-type allele; MSI-high, microsatellite instability high; MSS, microsatellite stable; TMB/Mb, tumor mutational burden/megabase.

BRCA1/2-mediated HR is commonly abrogated in *TP53*-mutated serous-like ECs (24). Cells that are HRD are more prone to DNA damage due to the error-prone repair of the DNA double-strand breaks caused by ROS and estrogen metabolites (58). Thereby, we hypothesize that tamoxifen might facilitate (but not initiate) early carcinogenesis of serous-like precursors in *gBRCA1/2* carriers, as these women are already more prone to develop these tumors. This hypothesis should be further studied, as it may alter the balance between advantages and disadvantages of tamoxifen treatment in *gBRCA* carriers.

This study has some limitations. First, we did not include a matched control group of ECs from non-*gBRCA1/2* carriers. Therefore, we are unable to assign sensitivity and specificity of the morphologic features described. Second, we have defined *gBRCA*-associated EC based on LOH status alone and did not interrogate the presence of *BRCA*-related genomic scars to support our definition of *gBRCA*-associated EC. Third, the study design, in which women were included only after providing informed consent and in which ECs were collected both retrospectively (period before providing informed consent) and prospectively (period after providing informed consent), may have led our study cohort to be enriched for ECs with more favorable histotype and survival.

In conclusion, we provide novel evidence that EC is part of the *gBRCA*-related tumor spectrum, with enrichment for EC subtypes associated with unfavorable clinical outcome and distinct histopathologic and molecular features. We also show that tumors with and without LOH of the *gBRCA1/2* wild-type allele are clearly different, thereby providing evidence that establishing LOH status is critical when assessing treatment efficacy of drugs targeting HRD in *BRCA1/2*-mutated tumors.

Disclosure of Potential Conflicts of Interest

L.L. Ritterhouse reports receiving speakers bureau honoraria from Bristol-Myers Squibb, Abbvie, Loxo Oncology, and Personal Genome Diagnostics. J.P. Segal is an unpaid consultant/advisory board member for Novartis, Bristol-Myers Squibb, and AstraZeneca, and reports receiving commercial research support from Abbvie. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.M. de Jonge, C.D. de Kroon, M.P.G. Vreeswijk, C.J. van Asperen, V.T.H.B.M. Smit, T. Bosse

Development of methodology: M.M. de Jonge, J.P. Segal, M.A. Rookus, B.E. Howitt, T. Bosse

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.M. de Jonge, L.L. Ritterhouse, C.D. de Kroon, J.P. Segal, HEBON Group, H. Hollema, M.A. Rookus, C.J. van Asperen, F.E. van Leeuwen, V.T.H.B.M. Smit, B.E. Howitt, T. Bosse

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.M. de Jonge, L.L. Ritterhouse, C.D. de Kroon, M.P.G. Vreeswijk, J.P. Segal, M.A. Rookus, F.E. van Leeuwen, V.T.H.B.M. Smit, B.E. Howitt, T. Bosse

Writing, review, and/or revision of the manuscript: M.M. de Jonge, L.L. Ritterhouse, C.D. de Kroon, M.P.G. Vreeswijk, J.P. Segal, R. Puranik, H. Hollema, M.A. Rookus, C.J. van Asperen, F.E. van Leeuwen, V.T.H.B.M. Smit, B.E. Howitt, T. Bosse

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.M. de Jonge, L.L. Ritterhouse, R. Puranik, T. Bosse

Study supervision: C.J. van Asperen, V.T.H.B.M. Smit, T. Bosse

Other (responsible for quality evaluation, processing, and troubleshooting of samples on next-generation sequencing platform in Clinical Laboratory): R. Puranik

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