Hereditary Cancer in Clinical Practice

RESEARCH Open Access

Somatic variants of potential clinical significance in the tumors of *BRCA* phenocopies

Lela Buckingham[1*], Rachel Mitchell[3], Mark Maienschein-Cline[4], Stefan Green[4], Vincent Hong Hu[4], Melody Cobleigh[2], Jacob Rotmensch[2], Kelly Burgess[2] and Lydia Usha[2]

Abstract

Background: *BRCA* phenocopies are individuals with the same phenotype (i.e. cancer consistent with Hereditary Breast and Ovarian Cancer syndrome = HBOC) as their affected relatives, but not the same genotype as assessed by blood germline testing (i.e. they do not carry a germline *BRCA1* or *BRCA2* mutation). There is some evidence of increased risk for HBOC-related cancers in relatives of germline variant carriers even though they themselves test negative for the familial variant (*BRCA* non-carriers). At this time, *BRCA* phenocopies are recommended to undergo the same cancer surveillance as individuals in the general population. This raises the question of whether the increased cancer risk in *BRCA* non-carriers is due to alterations (germline, somatic or epigenetic) in other cancer-associated genes which were not analyzed during *BRCA* analysis.

Methods: To assess the nature and potential clinical significance of somatic variants in *BRCA* phenocopy tumors, DNA from *BRCA* non-carrier tumor tissue was analyzed using next generation sequencing of 572 cancer genes. Tumor diagnoses of the 11 subjects included breast, ovarian, endometrial and primary peritoneal carcinoma. Variants were called using FreeBayes genetic variant detector. Variants were annotated for effect on protein sequence, predicted function, and frequency in different populations from the 1000 genomes project, and presence in variant databases COSMIC and ClinVar using Annovar.

Results: None of the familial *BRCA1/2* mutations were found in the tumor samples tested. The most frequently occurring somatic gene variants were *ROS1*(6/11 cases) and *NUP98* (5/11 cases). *BRCA2* somatic variants were found in 2/6 *BRCA1* phenocopies, but 0/5 *BRCA2* phenocopies. Variants of uncertain significance were found in other DNA repair genes (*ERCC1, ERCC3, ERCC4, FANCD2, PALB2*), one mismatch repair gene (*PMS2*), a DNA demethylation enzyme (*TET2*), and two histone modifiers (*EZH2, SUZ12*).

Conclusions: Although limited by a small sample size, these results support a role of selected somatic variants and epigenetic mechanisms in the development of tumors in *BRCA* phenocopies.

Keywords: Phenocopy, HBOC, NGS, Somatic mutations

* Correspondence: LelaBArnell@gmail.com
[1]Department of Pathology, Rush University Medical Center, Chicago, IL, USA
Full list of author information is available at the end of the article

Introduction

Cancer predisposition in hereditary breast and ovarian cancer (HBOC) syndrome is caused by pathogenic germline variants in the *BRCA1/2* genes (*germline BRCA* variants) and is inherited in an autosomal dominant pattern. The lifetime risk of breast cancer in female *germline BRCA* variant carriers is up to 85%; the lifetime risk of ovarian cancer is up to 50% [2, 6, 8, 11, 33, 34, 36, 38] Genetic alterations in the *BRCA1/2* genes cause over 90% of cases of HBOC [6, 33, 34, 36]. The genetic test recommended for patients suspected of carrying a *germline BRCA* variant involves sequencing of their *BRCA1* and *BRCA2* genes with deletion/duplication analyses, most commonly in blood and less frequently in saliva. A relative of a *germline BRCA* variant carrier who has tested negative for the known familial alteration is deemed to have normal (wild-type) germline *BRCA1* and *BRCA2* genes and is sometimes called a *BRCA* non-carrier.

There are conflicting reports on the relative risk ratio (RR) of breast and ovarian cancers in *BRCA* non-carriers when there is a known familial *BRCA* genetic alteration. Some authors argue that their cancer risk is the same as in the general population [24, 42], some conclude that their risk is the same as high risk families without identified germline *BRCA* pathogenic variants [20], but, overall, most authors agree that the risk is increased with a breast cancer RR of up to 5.1 [14, 27, 32, 35]. The studies are difficult to compare due to differences in methodology and patient populations [17] as well as different prognoses of breast cancers with *BRCA1* vs. *BRCA2* alterations [12, 25]. Germline *BRCA* non-carriers that develop breast or ovarian cancers are referred to as "*BRCA* phenocopies," meaning that they have the same phenotype (affected by cancer) as germline *BRCA* carriers, but do not have the same genotype (the known *BRCA* alteration as shown by germline genetic testing).

Explanations offered for HBOC malignancies in *BRCA* phenocopies include sporadic cancer related to familial lifestyle and/or environmental factors or pathogenic variants in other, possibly not yet identified, genes that cause HBOC. All of these explanations assume that cancers in *BRCA* non-carriers are not related to the familial *BRCA* variant. The risk of *BRCA* non-carriers developing an HBOC cancer is clinically important because it determines their cancer surveillance and prevention recommendations [16].

Undetectable germline variants in blood might also be due to revertant mosaicism where a rare spontaneous correction of a pathogenic variant might occur in *BRCA* pedigrees and result in false-negative testing for the familial variant. However, a study by Azzollini et al. [4] did not find the familial variants in tumor samples, blood leukocytes, buccal mucosa nor urine of *BRCA* phenocopies. We previously explored natural chimerism as an alternative explanation for *BRCA* phenocopies [28]. We hypothesized that breast and ovarian cancer can still be caused by familial *BRCA* variants, but transmitted in an alternative, non-mendelian fashion (e.g., through maternal-fetal or tetragametic chimerism) so that the altered genes are present in chimeric tissues rather than in blood. Since *BRCA* mutant cells are much more likely to give rise to cancer than non-mutants cells, in a chimeric organism, the tumor would be *BRCA*-mutant. We analyzed tumor tissue in *BRCA* phenocopies for the known familial variant using targeted PCR and qPCR methods [28]. In our cohort of 11 cases, we did not find the familial alteration in the tumor tissues. In the current study, we analyzed the tumor samples from the same cohort of patients. We used next generation sequencing (NGS) to investigate the possibility of other (somatic and/or germline) gene variants driving the cancer phenotype and the possibility of *BRCA1/2* epigenetic silencing in the context of familial cancer predisposition.

Methods

Patients, clinical assessment, and germline genetic testing

Patients for this study were selected based on an HBOC cancer phenotype in the absence of a known familial *BRCA* mutation found in a first-degree relative. With approval by the Rush University Medical Center Institutional Review Board, each subject signed an informed consent form and tumor specimens were obtained from the Department of Pathology, Rush University Medical Center (Chicago, IL) and Pathology Departments of other institutions where participants had their cancer surgery. Cancer diagnoses were obtained from pathology reports and histologic evaluation. Clinical data were established from chart review and self-reported history forms. Patients were eligible if they were affected by cancer but had previously tested negative for a known familial pathogenic variant. Breast cancer patients under 45 (invasive or non-invasive), women with ovarian, fallopian tube, or primary peritoneal cancer at any age, endometrial cancer, patients with male breast cancer or pancreatic cancer were considered eligible for this study. Eleven cases that met these criteria were found. Familial mutations included *BRCA1* c.186_187delAG (p.L22_E23LVfs; 2 patients), c.1793delA (p.G559Vfs), c.17 + 3A > G, c.2841A > T (p.K947 N), c.3109_3110insAA (p.K1037 fs), c.5215G > A (p.D1739N), c.8107G > A, and *BRCA2* c.6794_6975 insA, c.5645C > A (p.S882*) and c.6174delT (p.F2058Lfs).

Four patients underwent expanded commercial germline genetic testing in addition to the *BRCA1/2* testing. Patients 1 and 9 underwent gene panel testing that included 23 genes: *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NFN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11,* and *TP53.* Patient 5 underwent a more comprehensive gene panel test due to her

significant personal and family history of cancer that included 49 genes: *APC, ATM, BAP1, BARD1, BRCA1, BRCA2, BRIP1, BMPR1A, CDH1, CDK4, CDKN2A, CHEK2, EPCAM FH, FLCN, GREM1, MAX, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TMEM127, TP53, TSC1, TSC2,* and *VHL.* Patient 3 underwent a gene panel specific to breast cancer risk that included 14 genes: *ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11,* and *TP53*. Patient 10 underwent Lynch syndrome testing (*MLH1, MSH2,* and *MSH6*) in addition to *BRCA1/2* due to a personal history of early onset endometrial cancer. No pathogenic variants were identified in the other genes tested.

DNA isolation

Hematoxylin and eosin-stained tissue 4 μm sections cut adjacent to unstained sections were examined by a pathologist. Using the stained slide as a guide, approximately $2mm^2$ of tumor tissue was manually scraped from the unstained slides. The tissue was digested in a solution of 1.0 mg/mL proteinase K (Sigma) in10mM Tris, pH 8.3, and 50 mM KCl. Digestions proceeded overnight at 56 °C. After fluorometric confirmation of adequate DNA concentration, the lysate was used directly for sequencing analysis.

Library preparation

The Nimblegen Cancer Gene panel was used in this study. Sequences were selected using biotinylated capture probes (Nimblegen, Hoffmann-La Roche, Ltd., Basel, Switzerland). The captured DNA was fragmented to an average DNA fragment size is 180–220 bp and end-repaired for ligation of adaptor sequences carrying primer binding sites. The DNA was then amplified with primers tailed with sequencing primer binding sites and patient-specific indexes.

Tumor DNA sequencing

That captured library (576 target genes) was sequenced on the MiSeq™ system (Illumina). The products of the indexing amplification were denatured and introduced to the flow cell for in situ amplification by bridge PCR on the MiSeq. The resulting clusters of immobilized templates were subjected to reversible dye terminator sequencing.

Variant calling

Raw reads were aligned to human reference genome hg19 using BWA MEM [23]. Apparent PCR duplicates were removed using Picard Mark Duplicates [40]. Variants were called using FreeBayes variant detection [21] annotated for effect on protein sequence, predicted function, and frequency in different populations from the 1000 genomes project, and presence in variant databases COSMIC and ClinVar using Annovar [39].

Results

Variants

The eleven patients studied carried diagnoses of infiltrating ductal carcinoma, ductal carcinoma in situ, invasive lobular carcinoma, ovarian adenocarcinoma and primary peritoneal carcinoma. One patient with endometrial cancer (non-HBOC) was included. Patient ages at diagnosis ranged from 26 to 66 years. None of the 11 patient tumors tested displayed the familial *BRCA* variant (Table 1). All tumors underwent expanded gene panel testing and were confirmed negative for the familial variants.

Between 2000 and 12,000 variants were detected per sample using the Nimblegen cancer gene panel. This panel is directed at high coverage of 572 genes with a reported role in carcinogenesis. Table 1 shows the analysis data for variants with potential clinical significance.

Variant data was annotated and screened for coverage (read depth or number of times sequenced). Samples 3, 6, 8, 10 and 11 had limiting DNA, resulting in lower coverage. The effect of the variant change on the protein product was calculated using PolyPhen for variants located in coding regions. The impact of the amino acid variants on protein structure (and function) was predicted from analysis of multiple sequence alignments and protein 3D-structures, predicting the effect of the DNA change on the cell (tumor) phenotype. All samples had at least one variant previously reported in the COSMIC or ClinVar databases. There are, however, caveats to using databases where submitted variants are at most classified into levels of clinical relevance as a source of information. Defined evidence categories provided by contributors may not sufficiently describe the medical significance of a variant [3].

The most frequently observed mutated gene was *ROS1* (6/11 cases), displaying variants p.S1109 L and p.I537M, neither of which predict a deleterious effect on protein function, and p.S1109 L, which may affect protein function (Polyphen score 0.908). *ROS1* encodes a receptor tyrosine kinase related to anaplastic lymphoma kinase (*ALK*), along with members of the insulin-receptor family. *ROS1* gene rearrangements lead to fusion of the entire tyrosine kinase domain of *ROS1* with 1 of 12 different partners, including fusions with: *TPM3, SDC4, SLC34A2, CD74* and *EZR*. *ROS1*gene rearrangements are present in about 1% of lung cancers where they are therapeutic targets for an FDA-approved agent Crizotinib. The same deleterious *RET* mutation p.S649 L (Polyphen score 1) was present in cases 8 and 4. *RET* is another receptor tyrosine kinase [10].

Table 1 Variants with potential clinical significance. Putative somatic variants are identified as those with < 1% allele frequency in the 1000 genomes population and alternate allele frequency > 70% or < 30%, or those annotated in COSMIC

Case # Variant in First Degree Relative	Gene[a,b]	Loc	Variant	Read Depth	Variant Frequency[c]	Effect on Protein[d]	Population Frequency[e]
1 *BRCA2* c.C5645A	*PDE4DIP*	chr1	c.4993_4995CG	1084	0.39	NA	NA
	FOXP1	chr3	c.C343G:p.P115A	487	0.26	0.996	0.0005
	PIK3CA[a]	chr3	c.A3140G:p.H1047R	654	0.28	0.639	NA
	APC	chr5	c.G7450A:p.G2484S	274	0.43	0.234	0.01
	SMO	chr7	c.C1939T:p.P647S	65	0.72	1	0.0032
	CNTRL	chr9	c.G5809A:p.E1937K	349	0.44	0.841	0.0018
	TET1	chr10	c.C767T:p.A256V	414	0.58	0.022	0.1
	ZMYM2	chr13	c.G454C:p.D152H	441	0.28	0.997	0.0023
	ZMYM5	chr13	c.A691G:p.T231A	105	0.21	0.014	0.09
	MTUS2	chr13	c.C2870T:p.T957 M	461	0.27	0.062	0.02
2 *BRCA1*c.IVS17 + 3 A > G	*EPHA10*	chr1	c.C205T:p.R69C	521	0.68	1	0.0009
	ETV5	chr3	c.C287T:p.S96F	3463	0.52	0.999	NA
	CSF1R[a]	chr5	c.G1237A:p.G413S	2295	0.28	0.972	0.01
	ROS1	chr6	c.C3326T:p.S1109 L	3660	0.07	0.014	0.05
	NACA	chr12	c.A445G:p.K149E	2927	0.38	0.999	0.07
	ZNF668	chr16	c.G31A:p.D11N	3067	0.38	.	NA
	BRCA1[a]	chr17	c.A926G:p.Q309R	2105	0.06	0.999	0.03
	TP53[a]	chr17	c.A241G:p.K81E	1475	0.11	1	NA
3 *BRCA2* c.G8107A	*ERCC3*	chr2	c.C2111T:p.S704 L	59	0.29	0.642	0.0018
	TET2	chr4	c.C86G:p.P29R	62	0.25	0.993	0.09
	PALLD	chr4	c.A127T:p.T43S	142	0.62	0.992	0.0005
	PDGFRB	chr5	c.C1223G:p.S408C	51	0.33	1	0.0005
	MLLT4	chr6	c.A1264G:p.I422V	29	0.24	0.963	0.01
	ROS1	chr6	c.A1611G:p.I537M	61	0.44	0.275	0.07
	KIAA1549	chr7	c.G2800A:p.D934N	30	0.47	0.89	0.03
	PTPRD[a]	chr9	c.C2983T:p.R995C	44	0.44	0.321	0.05
	NUP98	chr11	c.C3424G:p.Q1142E	38	0.54	0.359	0.06
	TRIP11	chr14	c.G2134A:p.E712K	37	0.27	0.979	0.01
	MC1R[b]	chr16	c.G178 T:p.V60 L	34	0.32	0.988	0.05
4 *BRCA1* c.G5215A	*MYOC*	chr1	c.G227A:p.R76K	1834	0.37	0.984	0.08
	ROS1	chr6	c.G500A:p.R167Q	2239	0.46	0.908	0.06
	FGFR1OP[a]	chr6	c.T760C:p.S254P	1276	0.66	0.007	0.03
	FGFR1OP[a]	chr6	c.C470T:p.T157I	2039	0.68	0.868	0.09
	FGFR1OP[a]	chr6	c.G672 T:p.K224 N	1821	0.70	0.001	0.09
	KIAA1549	chr7	c.G2800A:p.D934N	1214	0.47	0.89	0.03
	TRIM24	chr7	c.C2514G:p.D838E	3339	0.46	0.743	0.0027
	RET[b]	chr10	c.C1946T:p.S649 L	1054	0.44	1	0.0009
	NUP98	chr11	c.C3424G:p.Q1142E	2139	0.54	0.359	0.06
	HNF1A	chr12	c.C293T:p.A98V	280	0.32	0.752	0.02
	MC1R[b]	chr16	c.G178 T:p.V60 L	700	0.32	0.988	0.05
5 *BRCA1* c.A2841T	*MUC1*	chr1	c.G586A:p.G196S	2460	0.45	0.669	0.01
	PAX3[b]	chr2	c.C941A:p.T314K	3586	0.42	0.837	0.0041
	PAX8	chr2	c.T985C:p.F329 L	2362	0.47	0.924	0.01

Table 1 Variants with potential clinical significance. Putative somatic variants are identified as those with < 1% allele frequency in the 1000 genomes population and alternate allele frequency > 70% or < 30%, or those annotated in COSMIC *(Continued)*

Case # Variant in First Degree Relative	Gene[a,b]	Loc	Variant	Read Depth	Variant Frequency[c]	Effect on Protein[d]	Population Frequency[e]
	HECW1	chr7	c.A3692G:p.N1231S	4508	0.46	0.998	0.0014
	WRN	chr8	c.G340A:p.V114I	3761	0.54	0.002	0.06
	PTPRD[a]	chr9	c.C2983T:p.R995C	3365	0.44	0.321	0.07
	NUP98	chr11	c.C3424G:p.Q1142E	3553	0.54	0.359	0.06
	CASC5	chr15	c.A4339G:p.T1447A	4457	0.40	0.336	0.05
	IGF1R[b]	chr15	c.G1532A:p.R511Q	3795	0.43	0.476	0.0009
	CBFA2T3	chr16	c.G308C:p.R103P	2797	0.43	0.811	0.04
	SPECC1	chr17	c.C579G:p.S193R	2353	0.60	1	0.1
	SSX1	chrX	c.A149G:p.Y50C	7574	0.46	0.97	0.0006
6 *BRCA1* c.187delAG	*FCRL4*	chr1	c.C34T:p.P12S	49	0.28	0.941	0.0046
	MYOC	chr1	c.G227A:p.R76K	24	0.37	0.984	0.01
	KIAA1549	chr7	c.G2800A:p.D934N	35	0.47	0.89	0.03
	LTBP3	chr11	c.C1313T:p.A438V	24	0.25	0.997	0.05
	NUP98	chr11	c.C3424G:p.Q1142E	27	0.54	0.359	0.06
	BRCA2[b]	chr13	c.A865C:p.N289H	103	0.20	0.991	0.06
	BRCA2[b]	chr13	c.A2971G:p.N991D	50	0.18	0	0.06
	ERCC4	chr16	c.G1244A:p.R415Q	52	0.48	1	0.03
	MC1R[b]	chr16	c.G178 T:p.V60 L	17	0.32	0.988	0.05
	CHD6	chr20	c.C7165T:p.R2389C	29	0.53	1	0.006
7 *BRCA1* c.187delAG	*PDE4DIP*	chr1	c.A6002G:p.E2001G	927	0.58	0.999	0.1
	PPARG[b]	chr3	c.C34G:p.P12A	923	0.46	0	0.07
	ROS1	chr6	c.G500A:p.R167Q	968	0.46	0.908	0.06
	ROS1	chr6	c.A1611G:p.I537M	1094	0.44	0.275	0.07
	PMS2[b]	chr7	c.G59A:p.R20Q	877	0.45	0.924	0.07
	HNF1A	chr12	c.C293T:p.A98V	182	0.32	0.752	0.02
	CBFA2T3	chr16	c.G308C:p.R103P	676	0.43	0.811	0.04
8 *BRCA1* c.3109insAA	*PDE4DIP*	chr1	c.A6002G:p.E2001G	42	0.58	0.999	0.1
	FGFR1OP[a]	chr6	c.C470T:p.T157I	29	0.69	0.868	0.03
	FGFR1OP[a]	chr6	c.T760C:p.S254P	22	0.66	0.007	0.03
	ROS1	chr6	c.G500A:p.R167Q	29	0.46	0.908	0.06
	RET[b]	chr10	c.C1946T:p.S649 L	13	0.44	1	0.0009
	NUP98	chr11	c.G4759A:p.E1587K	25	0.79	1	0.01
	NUP98	chr11	c.C3424G:p.Q1142E	23	0.54	0.359	0.06
	BLM	chr15	c.C2603T:p.P868L	64	0.2	0.81	0.05
9 *BRCA1* c.1793 delA	*FANCD2*	chr3	c.A1634G:p.N545S	1102	0.27	0.06	0.0046
	FGFR1OP	chr6	c.G688C:p.A230P	1640	0.38	0.934	0.02
	ROS1	chr6	c.A1611G:p.I537M	1497	0.44	0.275	0.07
	AKAP9	chr7	c.G4519C:p.D1507H	1623	0.45	0.874	0.0005
	MYC	chr8	c.A77G:p.N26S	838	0.56	0.977	0.02
	WRN	chr8	c.C3236T:p.S1079 L	1327	0.29	0.405	0.02
	CARS	chr11	c.G38A:p.R13H	626	0.79	1	0.0032
	NUP98	chr11	c.G4759A:p.E1587K	952	0.79	1	0.01
	BRCA2[b]	chr13	c.A865C:p.N289H	1907	0.20	0.991	0.06

Table 1 Variants with potential clinical significance. Putative somatic variants are identified as those with < 1% allele frequency in the 1000 genomes population and alternate allele frequency > 70% or < 30%, or those annotated in COSMIC *(Continued)*

Case # Variant in First Degree Relative	Gene[a,b]	Loc	Variant	Read Depth	Variant Frequency[c]	Effect on Protein[d]	Population Frequency[e]
	BRCA2[b]	chr13	c.A2971G:p.N991D	1331	0.18	0	0.06
	TSC2[b]	chr16	c.G1100A:p.R367Q	557	0.58	0.999	0.02
	ASXL1	chr20	c.C3692T:p.S1231F	1399	0.55	0.03	0.02
	CHD6	chr20	c.C7165T:p.R2389C	1628	0.53	1	0.01
10 *BRCA2* c.6794 insA	*ROS1*	chr6	c.G500A:p.R167Q	22	0.46	0.908	0.06
	NUP98	chr11	c.C3424G:p.Q1142E	10	0.54	0.359	0.06
	OMD	chr9	c.G662A:p.S221 N	41	0.46	0	0.03
	ARNT	chr1	c.T1506G:p.D502E	18	0.44	0.018	0.01
	PAX3	chr2	c.C941A:p.T314K	14	0.42	0.837	0.02
	ARHGEF12	chr11	c.A2861T:p.Y954F	68	0.33	0.01	0.05
	PALB2	chr16	c.G2993A:p.G998E	71	0.27	1	0.01
	EZH2	chr7	c.G436C:p.D146H	76	0.66	0.898	0.07
11 *BRCA2* c.6174 del T	*PAX3*[b]	chr2	c.C941A:p.T314K	109	0.42	0.837	0.02
	PPARG[b]	chr3	c.C34G:p.P12A	49	0.46	0	0.07
	PMS2[b]	chr7	c.G59A:p.R20Q	76	0.45	0.924	0.07
	MC1R	chr16	c.G178 T:p.V60 L	34	0.32	0.988	0.05
	MYOC	chr1	c.G227A:p.R76K	84	0.37	0.984	0.08
	PTPRD[a]	chr9	c.C2983T:p.R995C	44	0.44	0.321	0.07
	SSX1	chrX	c.A149G:p.Y50C	192	0.46	0.97	0.0006
	MUC1	chr1	c.G586A:p.G196S	110	0.45	0.669	0.01
	FANCD2	chr3	c.G521A:p.R174Q	34	0.21	0.96	0
	CNTRL	chr9	c.C6221A:p.A2074D	34	0.83	0.8	0.0041
	PDE4DIP	chr1	c.G2838A:p.M946I	25	0.59	0.028	0.09
	PDE4DIP	chr1	c.G4111A:p.V1371I	78	0.24	0.012	0.07

[a]Variant annotated in the Catalogue of Somatic Mutations in Cancer (COSMIC)
[b]Variant annotated ClinVar (NCBI Clinical Variant Database)
[c]Variant frequencies of 0.5 ± 0.05 or 1.0 ± 0.05 may be germline
[d]Polyphen algorithm score for predicting damaging mutations (non-Mendelian) (1 = most severe; http://genetics.bwh.harvard.edu/pph2/)
[e]Frequency reported in the combined 1000 genomes database

The next most frequent variant found was *NUP98*, found in 5/11 cases. This gene encodes a 186 kDa precursor protein that undergoes auto-proteolytic cleavage to generate a 98 kDa nucleoporin and 96 kDa nucleoporin, the latter portion is a scaffold component of the nuclear core complex that regulates transport of macromolecules between the nucleus and cytoplasm. Translocations between this gene and many other partner genes have been observed in myeloid leukemia and myelodysplastic syndrome [41].

Somatic *BRCA2* variants were observed in three tumors, two of which were from *BRCA1* phenocopies, and each had two *BRCA2* variants: N289H and N991D, only one of which is deleterious (Polyphen scores 0.991 and 0, respectively). A third tumor from a *BRCA1* phenocopy had a *BRCA1* Q309R deleterious variant (Polyphen score .999). All *BRCA* variants have been reported in COSMIC or ClinVar. None of familial *BRCA* pathogenic variants were found among the variants.

A *PALB2* p.E672Q variant (Polyphen score 0.275) was present in one tumor and a deleterious *PALB2* p.G998E variant found in another (Polyphen score 1) . Both tumors were from *BRCA2* phenocopies. *PALB2* serves as the molecular scaffold in the formation of the homologous recombination *BRCA1-PALB2-BRCA2* complex [37].

Fanconi anemia complementation group D2, *FANCD2* variants p.N545S and p.R174Q were present in separate samples, the latter variant having predicted effects on protein function (Polyphen score .96), but also having low sequence depth. *PALB2* and *BRCA2* are members of this complementation group.

Other DNA repair complexes are represented amongst the variants including *PMS2* with p.V738F and p.R20Q,

ERCC3 p.S704 L and *ERCC4* p.R415Q. These variants, however, had low sequence coverage (< 50).

A somatic p.S1079 L variant in *WRN* was found in one case. Germline *WRN* variants are associated with premature aging. The *WRN* gene also functions in DNA repair and may have implications in tumorigenesis [9, 31].

Variants in genes that regulate histone and DNA methylation were present in three cases. *TET1* p.A256V has unlikely protein effect, while *TET2* p.P29R has a high Polyphen score .993. The ten-eleven translocation (*TET*) genes encode oxidases that demethylate methylated cytosine in DNA. A histone methylase gene variant, *EZH2* p.D146H (Polyphen score .898) was found in a different specimen. *EZH2* encodes a member of the Polycomb-group (PcG) protein family. PcG family members form multimeric protein complexes which maintain the transcriptional repressive state of genes.

Variants in genes that regulate histone and DNA methylation were present in three cases. *TET1* p.A256V is unlikely to affect the protein function, while *TET2* p.P29R has a high Polyphen score 0.993. The ten-eleven translocation (*TET*) genes encode oxidases that demethylate methylated cytosine in DNA. A histone methylase gene variant, *EZH2* p.D146H (Polyphen score .898) was found in a different specimen. *EZH2* encodes a member of the Polycomb-group (PcG) protein family. PcG family members form multimeric protein complexes which maintain the transcriptional repressive state of genes.

Discussion

The current study addresses the origin of a disease phenocopy, an HBOC syndrome, in the absence of a familial (germline) gene pathogenic variant. We previously tested DNA from 11 tumors from women who come from families carrying *BRCA1* or *BRCA2* pathogenic variants, but who do not carry the variant themselves as defined by blood testing [28]. Although genetic alterations in any of the 11 tumor samples have not been demonstrated, several potential driver mutations were observed through testing with the Agilent 572 oncogene panel. There were no HBOC-related gene variants shared in all cases, although other somatic variants were present including *BRCA* variants in three cases and a *PALB2* variant in one case. The familial pathogenic variants in 6/11 cases are frameshift insertions and deletions and one intronic variant, while most of the detected pathogenic variants were exonic single nucleotide variant (SNV). This may be due to the gene panel used which is designed to detect SNV in exonic regions.

In the absence of available normal tissue from most of these cases, the identification of germline variants was limited. Based on allele frequencies highly divergent from 50% and low frequency in population studies of germline variation, some of the reported variants might be somatic. Some apparently benign variants such as *BRCA2* p.N991D and *PPARG* p.P12A are reported in ClinVar. ClinVar hosts germline and somatic variants. It contains all categories of germline variants including pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, and benign. ClinVar has 18 reports of *BRCA2* p.N991D, including familial breast and breast-ovarian cancer, Fanconi anemia, and HBOC syndrome. A germline mutation *PPARG* p.P12A was reported as a risk factor for obesity, noninsulin-dependent diabetes mellitus, and familial partial lipodystrophy was reported in a single study. Both of these variants were included in Table 1 because of their presence in the annotated database.

There was limited DNA for four of the 11 specimens, which is reflected in the coverage levels in Table 1. As a result of low coverage, the variants may not be included in a clinical report. For variants with strong clinical significance based on clinical data (called Tier 1 variants) [22], repeat sequencing or confirmation by other methods would be recommended. The rarity of *BRCA* phenocopies also limits this study. Additional samples would further confirm the lack of familial pathogenic variants as well as any commonalities in family variants or in the somatic variants of the phenocopies. Interestingly, the one patient with non-HBOC phenotype (endometrial carcinoma) had a complex family history of maternal HBOC history (breast and ovarian cancers) with pathogenic 6794 insA variant in *BRCA2* and paternal Lynch syndrome history (colon, lung and bone cancers) without any familial variant reported [28]. The possibility exists, therefore, that this patient is a phenocopy of a paternally inherited variant.

In addition to genetic drivers, epigenetic changes may also contribute to the disruption of cell phenotype and tumorigenesis. DNA hypermethylation in gene promoters is a mechanism for loss of function of genes. Epigenetic mechanisms independent of DNA sequence can affect phenotype in a heritable manner. DNA methylation patterns are reprogrammed during gamete production by erasure of epigenetic tags (DNA methylation and histone alterations). This should reset the DNA imprinting but rare survival of imprinted genes may persist [18, 29]. Furthermore, loss of function epigenetic regulators such as *TET* and *EZH2* through DNA mutation, may result in aberrant methylation in offspring.

The presence of variants that can alter methylation state led to the investigation of *BRCA 1/2* gene promoter methylation in the phenocopy DNA as measured by cytosine methylation. Aberrant DNA methylation patterns in gene promoters are strong regulators of gene expression and phenotype. In a preliminary analysis, *BRCA* promoter methylation status in tumor tissue DNA from phenocopies was compared to the DNA

methylation in tumor tissue of *BRCA* carriers. Results so far in a small number of samples show a threefold increase in *BRCA*2 promoter methylation in phenocopy tumor tissue compared to tumor tissue from *BRCA* PV carriers suggesting that *BRCA2* promoter methylation in the phenocopy tumor tissues (from familial *BRCA*2 backgrounds) is consistently higher in phenocopy tumor DNA than in non-malignant and tumor tissue from the control germline *BRCA* PV carriers. A more thorough analysis of the *BRCA* promoter regions in tumor and matched non-malignant tissue will confirm the presence of the increased methylation and studies of directed methylation in cell lines would demonstrate the actual effect.

A complex cancer phenotype is probably not driven solely by a single genetic or epigenetic variant, even in the presence of a highly penetrant familial pathogenic variant [5, 30, 38]. The standard polygenic model of carcinogenesis proposes phenotypes produced by multiple loci acting independently and additively [13, 26]. The contribution of multiple loci could explain observed genetic inheritance characteristics, such as phenotypic variability, penetrance and anticipation. A combination of single nucleotide polymorphisms (SNPs), which singularly may not produce a malignant phenotype, may establish a genetic context within which PVs in high-penetrance cancer genes (or other variants not classified as such) can produce it [7, 15]. In the current study, *ROS*1 PVs were repeatedly observed. Either the genetic background promotes *ROS1* to a driver mutation, or *ROS1* is a passenger to a yet undiscovered driver mutation in another gene. Theoretically, then, a somatic or germline *BRCA* variant may be a driver only in the context of particular combinations of other variants. This idea is consistent with results of an exploratory study by Agarwal et al. which identified cancer gene germline-somatic mutation pairs that co-occurred more frequently than would be expected by chance. The authors concluded that germline polymorphisms might function as pre-existing driver "hits", which together with acquired complementary somatic mutations would act to dysregulate key pathways in malignant transformation [1].

A familial genetic context would provide both conditions, with inheritance of a familial *BRCA* PV being manifested when present. In the absence of germline *BRCA* PVs, a combination of variants in other genes (or *BRCA* promoter methylation) may take this role. The polygenic inheritance might also affect the penetrance of the driver mutation through generations, as observed with anticipation phenomenon where cancer develops at a younger age in subsequent generations in some *BRCA*-positive families [19]. Once established, the polygenic background may select for additional variants or variant losses which increase the "malignant context," i.e. establish an environment with greater cancer risk.

Conclusions

An initial hypothesis that *BRCA* phenocopies were secondary to chimerism was not confirmed in our previous study. This prompted further analysis by extensive sequencing of DNA derived from tumor cells, to look for further insight into the pathogenesis of these tumors. The sequencing results confirmed that none of the familial pathogenic variants were present in the tumors of *BRCA* phenocopies. It also revealed several presumably somatic variants with potential oncologic significance. At least one variant in each case was previously reported in annotated databases. Somatic mutations in *ROS1* were the most frequently represented in this small case group, but their significance in breast and ovarian cancer is unknown at this time. Several presumably somatic variants were found in DNA repair genes which share homologous DNA repair function with *BRCA1* and *BRCA2* and are in the same Fanconi anemia pathway. Epigenetic silencing through increased DNA methylation and/or polygenic background context can be other underlying mechanisms explaining *BRCA* phenocopies.

Acknowledgements

We would like to acknowledge the generous contributions of Mr. and Mrs. Eugene and Shirley Deutsch, Mr. and Mrs. Mark and Maha Halabi Ditsch, Mr. George Ruwe, and Mr. and Mrs. Douglas and Sarah Criner, which made this work possible.

Authors' contributions

Authors LU, RM, MC, JR provided medical services to patients, obtained consent and participated in study design. Genetic background and inheritance data were collected and provided by KB. Nucleic acid isolation and molecular testing was performed by LB. MM-C, VH and SG performed NGS and bioinformatic analysis. All authors contributed to writing and editing of the article. All authors read and approved the final manuscript.

Funding

This study is funded by the philanthropic Metastatic Breast Cancer Research Fund at Rush (Dr. Lydia Usha).

Availability of data and materials

Deidentified complete sequence data analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Rush University Medical Center Institutional Review Board, Office of Research Affairs. Patients have provided informed consent for use of their archival tissue. Patients were made aware that the data collected is available on an individual basis. The IRB human subjects' protection division designated this study as low risk to subjects with deidentification of data, once recorded so that subjects cannot be linked directly or by an identifier to the data reported.

Consent for publication

The authors warrant that the manuscript does not infringe upon any copyright or other right(s), and that we are the sole and exclusive owners of the rights conveyed to Hereditary Cancer in Clinical Practice. The authors guarantee that the contribution to the work has not been previously published elsewhere.

Competing interests

The authors have disclosed that they have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors.

Author details
[1]Department of Pathology, Rush University Medical Center, Chicago, IL, USA. [2]Rush Cancer Institute, Rush University Medical Center, Chicago, IL, USA. [3]Tennessee Oncology, Shelbyville, TN, USA. [4]University of Illinois at Chicago Research Resources Center, Chicago, IL, USA.

Received: 2 May 2019 Accepted: 27 June 2019
Published online: 16 July 2019

References

1. Agarwal D, Nowak C, Zhang NR, Pusztai L, Hatzis C. Functional germline variants as potential co-oncogenes. NPJ Breast Cancer. 2017;3:46. https://doi.org/10.1038/s41523-017-0051-5.
2. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Ee. 2003Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet. 2003;72(5):1117–30.
3. Azzariti DR, Riggs ER, Niehaus A, Rodriguez LL, Ramos EM, Kattman B, Landrum MJ, Martin CL, Rehm HL. Points to consider for sharing variant-level information from clinical genetic testing with ClinVar. Cold Spring Harbor Mol Case Stud. 2018;4(1). https://doi.org/10.1101/mcs.a002345.
4. Azzollini J, Pesenti C, Ferrari L, Fontana L, Calvello M, Peissel B, et al. Revertant mosaicism for family mutations is not observed in BRCA1/2 phenocopies. PLoS One. 2017;12(2):e0171663.
5. Baquero JM, Benítez-Buelga C, Fernández V, Urioste M, García-Giménez JL, Perona R; CIMBA Consortium, Benítez J, Osorio A. 2019 "A common SNP in the UNG gene decreases ovarian cancer risk in BRCA2 mutation carriers." Mol Oncol. 13(5):1110–1120.
6. Brose MS, Rebbeck TR, Calzone KA, Stopfer JE, Nathanson KL, Weber BL. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst. 2002;94(18):1365–72.
7. Carter H, Marty R, Hofree M, Gross AM, Jensen J, Fisch KM, Wu X, DeBoever C, Van Nostrand EL, Song Y, Wheeler E, Kreisberg JF, Lippman SM, Yeo GW, Gutkind JS, Ideker T. Interaction landscape of inherited polymorphisms with somatic events in cancer. Cancer Discovery. 2017;7(4):410-23.
8. Chen S, Iversen ES, Friebel T, Finkelstein D, Weber BL, Eisen A, et al. Characterization of BRCA1 and BRCA2 mutations in a large United States sample. J Clin Oncol. 2006;24(6):863–71.
9. Comai L, Li B. The Werner syndrome protein at the crossroads of DNA repair and apoptosis. Mech Ageing Dev. 2004;125(8):521–8.
10. Drilon A, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. Nat Rev Clin Oncol. 2018;15(3):151–67.
11. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast cancer linkage consortium. Am J Hum Genet. 1995;56(1):265–71.
12. Engel C, Fischer C, Zachariae S, Bucksch K, Rhiem K, Giesecke J, Herold N, Wappenschmidt B, Hübbel V, Maringa M, Reichstein-Gnielinski S, Hahnen E, Bartram CR, Dikow N, Schott S, Speiser D, Horn D, Fallenberg EM, Kiechle M, Quante AS, Vesper AS, Fehm T, Mundhenke C, Arnold N, Leinert E, Just W, Siebers-Renelt U, Weigel S, Gehrig A, Wöckel A, Schlegelberger B, Pertschy S, Kast K, Wimberger P, Briest S, Loeffler M, Bick U, Schmutzler RK; German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC). 2019. "Breast cancer risk in BRCA1/2 mutation carriers and noncarriers under prospective intensified surveillance." Int J Cancer. doi: 10.1002/ijc.32396
13. Evans DG, Brentnall A, Byers H, Harkness E, Stavrinos P, Howell A, et al. The impact of a panel of 18 SNPs on breast cancer risk in women attending aUK familial clinic: a case-control study. J Med Genet. 2017;54(2):111–3.
14. Fischer C, Engel C, Sutter C, Zachariae S, Schmutzler R, Meindl A, et al. BRCA1/2 testing: uptake, phenocopies, and strategies to improve detection rates in initially negative families. Clin Genet. 2012;82(5):478–83.
15. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The breast cancer linkage consortium. Am J Hum Genet. 1998;62(3):676–89.
16. Goldgar D, Venne V, Conner T, Buys S. BRCA phenocopies or ascertainment bias? J Med Genet. 2007;44(8):e86 author reply e88.
17. Gronwald J, Cybulski C, Lubinski J, Narod SA. Phenocopies in breast cancer 1 (BRCA1) families: implications for genetic counselling. J Med Genet. 2007;44(4):e76.
18. Illum LRH, Bak ST, Lund S, Nielsen AL. DNA methylation in epigenetic inheritance of metabolic diseases through the male germ line. J Mol Endocrinol. 2018;60(2):R39–56.
19. Kuchenbaecker KB, McGuffog L, Barrowdale D, Lee A, Soucy P, Dennis J, et al. Evaluation of polygenic risk scores for breast and ovarian cancer risk preditions in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst. 2017;107(5). https://doi.org/10.1093/jnci/djw302.
20. Kurian AW, Gong GD, John EM, Johnston DA, Felberg A, West DW, et al. Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: findings from the breast cancer family registry. J Clin Oncol. 2011;29(34):4505–9.
21. Lee WP, Stromberg MP, Ward A, Stewart C, Garrison EP, Marth GT. MOSAIK: a hash-based algorithm for accurate next-generation sequencing short-read mapping. PLoS One. 2014;9(3):e90581.
22. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer. J Mol Diagn. 2017;19:4–20.
23. Li H. Toward better understanding of artifacts in variant calling from high-coverage samples. Bioinformatics. 2014;30(20):2843–51.
24. Mangold KA, Wang V, Weissman SM, Rubenstein WS, Kaul KL. Detection of BRCA1 and BRCA2 Ashkenazi Jewish founder mutations in formalin-fixed paraffin-embedded tissues using conventional PCR and heteroduplex/amplicon size differences. J Mol Diagn. 2010;12:20–6.
25. Nones K, Johnson J, Newell F, Patch AM, Thorne H, Kazakoff SH, de Luca XM, Parsons MT, Ferguson K, Reid L, McCart Reed AE, Srihari S, Lakis V, Davidson AL, Mukhopadhyay P, Holmes O, Xu Q, Wood S, Leonard C; Kathleen Cuningham Foundation Consortium for Research into Familial Aspects of Breast Cancer (kConFab); Australian Breast Cancer Tissue Bank (ABCTB); Brisbane Breast Bank (BBB), Beesley J, Harris J, Barnes D, Degasperi A, Ragan MA, Spurdle AB, Khanna KK, Lakhani SR, Pearson JV, Nik-Zainal S, Chenevix-Trench G, Waddell N, Simpson PT. 2019. "Whole-genome sequencing reveals clinically relevant insights into the aetiology of familial breast cancers." Ann Oncol. https://doi.org/10.1093/annonc/mdz132.
26. Mavaddat N, Pharoah PD, Michailidou K, Tyrer J, et al. Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst. 2015;107(5ii). https://doi.org/10.1093/jnci/djv036.
27. Metcalfe KA, Finch A, Poll A, Horsman D, Kim-Sing C, Scott J, et al. Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation. Br J Cancer. 2009;100(2):4.
28. Mitchell R, Buckingham L, Cobleigh M, Rotmensch J, Burgess K, Usha L. Can chimerism explain breast/ovarian cancers in BRCA non-carriers from BRCA-positive families? PLoS One. 2018;13(4):e0195497.
29. Northstone K, Golding J, Davey Smith G, Miller LL, Pembrey M. Prepubertal start of father's smoking and increased body fat in his sons: further characterisation of paternal transgenerational responses. Eur J Hum Genet. 2014;22:1382–6.
30. Osorio A, Milne RL, Kuchenbaecker K, Vaclová T, Pita G, Alonso R, Peterlongo P, Blanco I, de la Hoya M, Duran M, Díez O, Ramón Y Cajal T,Konstantopoulou I, Martínez-Bouzas C, Andrés Conejero R, Soucy P, McGuffog L, Barrowdale D, Lee A, Swe-Brca, Arver B, Rantala J, Loman N, Ehrencrona H, Olopade OI, Beattie MS, Domchek SM, Nathanson K, Rebbeck TR, Arun BK, Karlan BY, Walsh C, Lester J, John EM, Whittemore AS, Daly MB, Southey M, Hopper J, Terry MB, Buys SS, Janavicius R, Dorfling CM, van Rensburg EJ, Steele L, Neuhausen SL, Ding YC, Hansen TV, Jønson L, Ejlertsen B, Gerdes AM, Infante M, Herráez B, Moreno LT, Weitzel JN, Herzog J, Weeman K, Manoukian S, Peissel B, Zaffaroni D, Scuvera G, Bonanni B, Mariette F, Volorio S, Viel A, Varesco L, Papi L, Ottini L, Tibiletti MG, Radice P, Yannoukakos D, Garber J, Ellis S, Frost D, Platte R, Fineberg E, Evans G, Lalloo F, Izatt L, Eeles R, Adlard J, Davidson R, Cole T, Eccles D, Cook J, Hodgson S, Brewer C, Tischkowitz M, Douglas F, Porteous M, Side L, Walker L, Morrison P, Donaldson A, Kennedy J, Foo C, Godwin AK, Schmutzler RK, Wappenschmidt B, Rhiem K, Engel C, Meindl A, Ditsch N, Arnold N, Plendl HJ, Niederacher D, Sutter C, Wang-Gohrke S, Steinemann D, Preisler-Adams S, Kast K, Varon-Mateeva R, Gehrig A, Stoppa-Lyonnet D, Sinilnikova OM, Mazoyer S, Damiola F, Poppe B, Claes K, Piedmonte M, Tucker K, Backes F, Rodríguez G, Brewster W, Wakeley K, Rutherford T, Caldés T, Nevanlinna H, Aittomäki K, Rookus MA, van Os TA, van der Kolk L, de Lange JL, Meijers-Heijboer HE, van der Hout AH, van Asperen CJ, Gómez Garcia EB, Hoogerbrugge N, Collée JM, van Deurzen CH, van der Luijt RB, Devilee P, Hebon, Olah E, Lázaro C, Teulé A, Menéndez M, Jakubowska A, Cybulski C, Gronwald J, Lubinski J, Durda K, Jaworska-Bieniek K, Johannsson OT, Maugard C, Montagna M, Tognazzo S, Teixeira MR, Healey S, Investigators K, Olswold C, Guidugli L, Lindor N, Slager S, Szabo CI, Vijai J, Robson M, Kauff N, Zhang L, Rau-Murthy R, Fink-Retter A, Singer CF, Rappaport C, Geschwantler Kaulich D, Pfeiler G, Tea MK, Berger A, Phelan CM, Greene MH, Mai PL, Lejbkowicz F, Andrulis I, Mulligan AM, Glendon G, Toland AE,

Bojesen A, Pedersen IS, Sunde L, Thomassen M, Kruse TA, Jensen UB, Friedman E, Laitman Y, Shimon SP, Simard J, Easton DF, Offit K, Couch FJ, Chenevix-Trench G, Antoniou AC, Benitez J. 2014. "DNA glycosylases involved in base excision repair may be associated with cancer risk in BRCA1 and BRCA2 mutation carriers." PLoS Genet. 10(4):e1004256.
31. Opresko PL, Calvo JP, von Kobbe C. Role for the Werner syndrome protein in the promotion of tumor cell growth. Mech Ageing Dev. 2007;128(7–8):423–36.
32. Robson M. Do women remain at risk even if they do not inherit a familial BRCA1/2 mutation? J Clin Oncol. 2011;29(34):4477–8.
33. Satagopan JM, Offit K, Foulkes W, Robson ME, Wacholder S, Eng CM, et al. The lifetime risks of breast cancer in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. Cancer Epidemiol Biomark Prev. 2001;10(5):467–73.
34. Schorge JO, Modesitt SC, Coleman RL, Cohn DE, Kauff ND, Duska LR, et al. SGO white paper on ovarian cancer: etiology, screening and surveillance. Gynecol Oncol. 2010;199(1):7–17.
35. Smith A, Moran A, Boyd MC, Bulman M, Shenton A, Smith L, et al. Phenocopies in BRCA1 and BRCA2 families: evidence for modifier genes and implications for screening. J Med Genet. 2007;44(1):10–5.
36. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med. 1997;336(20):1401–8.
37. Sy SM, Huen MS, Chen J. PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proc Natl Acad Sci U S A. 2009;106(17):7155–60.
38. Thorlacius S, Struewing JP, Hartge P, Olafsdottir GH, Sigvaldason H, Tryggvadottir L, et al. Population-based study of risk of breast cancer in carriers of BRCA2 mutation. Lancet. 1998;352(9137):1337–9.
39. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.
40. Wysoker A, Tibbetts K, Fennell T. Picard tools version 1.107; 2013. Accessed 2013
41. Xu H, Valerio DG, Eisold ME, Sinha A, Koche RP, Hu W, et al. NUP98 fusion proteins interact with the NSL and MLL1 complexes to drive Leukemogenesis. Cancer Cell. 2016;30(6):863–87.
42. Yunis EJ, Zuniga J, Romero V, Yunis EJ. Chimerism and tetragametic chimerism in humans: implications in autoimmunity, allorecognition and tolerance. Immunol Res. 2007;38(1–3):213–36.

Publisher's Note