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Original Article

Somatic mutation profiling in *BRCA*-negative breast and ovarian cancer patients by multigene panel sequencing

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Abstract: Targeted therapeutic agents such as poly (ADP-ribose) polymerases (PARP) inhibitors have emerged in treating cancers associated with germline *BRCA* mutations. Recently studies demonstrated the effectiveness of PARP inhibitors in treating patients with somatic *BRCA* mutations. Somatic mutations in 122 Chinese breast or ovarian cancer patients without *BRCA, PTEN and TP53* mutations were screened using multigene sequencing panel. The five most frequent pathogenic or likely pathogenic mutated genes identified in breast cancer patients were *PIK3CA* (28.6%), *TP53* (16.9%), *MAP3K1* (14.3%), *GATA3* (14.3%) and *PTEN* (5.2%). The five most frequently mutated genes identified in ovarian patients were *TP53* (52.9%), *KRAS* (23.5%) and *PIK3CA* (11.8%), *BRCA1* (5.9%) and *RB1* (5.9%). Somatic *PIK3CA and TP53* mutations were common events in both germline *BRCA*-negative breast and ovarian cancer patients. In contrast, somatic screening of *BRCA* mutations in *BRCA*-negative breast cancer patients has limited value. The results highlight the benefit of somatic testing to guide future research directions on other targeted therapies for breast and ovarian malignancies.

Keywords: Breast cancer, ovarian cancer, *BRCA*-negative, somatic mutations, multigene panel

Introduction

The link between genetic mutations and cancer pathogenesis has long been extensively studied, and genetic testing is taking on an increasingly important role to reduce the disease burden of breast cancer. The discoveries of *BRCA* germline mutations, which are associated with an estimated 20% of hereditary breast cancers, fueled the excitement for potential breast cancer treatment target [1-4]. Targeted therapeutic agents such as poly(ADP-ribose) polymerases (PARP) inhibitors have emerged with better outcomes in treating cancers with *BRCA* mutations and those with other homologous recombination (HR) deficiencies [5, 6].

The first PARP inhibitor was FDA-approved initially for ovarian cancer patients with germline *BRCA* mutations. Subsequent studies demonstrated their effective in those with somatic *BRCA* mutations and other HR deficiencies, which are present in up to 20% of serous ovarian cancers [7]. Olaparib and talazoparib are FDA-approved PARP inhibitors for metastatic breast cancer patients with germline *BRCA* mutations [8, 9] and metastatic triple negative breast cancer patient with somatic *BRCA1* mutation [10]. Therefore, it is postulated that somatic *BRCA*-mutated tumors might also respond to this new class of therapeutics similar to that in ovarian cancer setting. A phase II clinical trial is currently investigating the effectiveness of olaparib in both germline and somatic *BRCA*-positive breast cancer patients [11]. Other than PARP inhibitors, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) inhibitor, alpelisib, has been used to treat patients with *PIK3CA*-

mutated, hormonal receptor-positive, HER2-negative advanced breast cancer [12]. Several phase I and II clinical trials are conducted to investigate the efficacy of PIK3CA and AKT inhibitors on breast cancer patients with somatic mutations in the PI3K/AKT/mTOR pathway.

Somatic mutation analysis of 173 genes using in 2,433 breast tumors identified 13,084 somatic mutations which were predicted to affect protein sequence [13]. Another study identified mutations in 93 protein-coding cancer genes in 560 paired breast tumors and normal tissues by whole genome sequencing [14]. *PIK3CA* and *TP53* were the most frequently mutated genes found in breast tumors among these studies [13, 14]. Notably, the rate of somatic *BRCA* mutations in breast malignancy is rather low, implying that other mechanisms, such as epigenetics, might be a more important event in somatic breast cancers with BRCAness [14-18]. On the other hand, large-scale genetic mutation analysis from The Cancer Genome Atlas (TCGA) revealed *TP53* mutation was also found in 96% of high-grade serous ovarian cancer tissue samples [19] and commonly seen in different populations [20, 21]. Therefore, the presence of somatic *BRCA* mutations or other mutated genes must be ascertained so as to offer appropriate targeted therapies to improve treatment outcome in breast or ovarian cancers.

The objective of our study is to uncover the landscape of somatic mutations in germline (*BRCA, PTEN and TP53)* mutation-negative breast and ovarian cancer patients and identity the common somatic mutations which can potentially be targeted for therapeutic purposes.

Methods

Ethics statement

All human tissue samples in this study were used according to the Declaration of Helsinki. Written informed consent was obtained from all participants recruited in this study. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority West Cluster and other contributing hospitals in Hong Kong.

Samples and selection criteria

122 tumor tissues of breast or ovarian cancer tested negative for germline *BRCA1, BRCA2, TP53*, and *PTEN* mutations were retrieved for this study. They were recruited by the Hong Kong Hereditary and High Risk Breast Cancer Program from March 2007 to October 2017. The selection criteria were described previously with modification [4]. High-risk patients who were germline *BRCA/TP53/PTEN* negative and had not received any neoadjuvant chemotherapy were included.

DNA extraction & multigene panel sequencing

Genomic DNA was extracted from tissues with DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Extracted tumor DNA was subjected to Human Breast Cancer Panel from QIAseq Targeted DNA Panel (DHS-001Z, Qiagen) with 93 breast cancer predisposition genes. Sequencing libraries were prepared according the manufacturer's instructions. The quality and quantity of the re-purified DNA were assessed by Qubit Fluorometer (Thermo Fisher Scientific, Massachusetts, US). DNA was enzymatically fragmented and ligated with molecular barcodes and sample indexing adaptor. Ligated DNA was further enriched by PCR with a universal forward primer and the specific targeting primers from above panel which allows the targets and barcodes to enrich sufficiently. Another round of universal PCR amplification was carried out with platform specific adaptor sequences added for final completion of the library construct. The amplicon products of each sample were subjected to quality check with the use of Agilent DNA 1000 Kit on a 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA). The libraries were sequenced on MiSeq or NextSeq (Illumina, San Diego, CA) with QIAseq A Read 1 Primer I and the minimum sequencing depth was 50-fold and average depth of 500.

Data analysis

The bioinformatics analysis was performed on a Cray XC30 supercomputer (Cray, Seattle, WA). Paired sequencing reads were mapped to human reference genome sequence GRCh37/hg19 using BWA-MEM v0.7.7 [22] and default parameters. Post-alignment primer clipping and unique molecular identifier (UMI) extrac-

Table 1A. Clinicopathologic data of the breast cohort patients (N=108)

Sex	
Female	108 (100%)
Age (Mean/Range)	54.05 (25-86)
Diagnosis Age	
<35	5 (4.63%)
35-44	35 (32.41%)
45-54	25 (23.15%)
55-64	14 (12.96%)
≥65	29 (26.85%)
Bilateral Breast	18 (16.67%)
Personal cancers	
Breast Cancer only	90 (83.33%)
Both Breast & Ovarian or GYN cancers	3 (2.78%)
Breast cancer with multiple other cancers	15 (13.89%)
Menopause	
Yes	54 (50.00%)
Family History (1st or 2nd degree)	
Breast Cancer	37 (34.26%)
Ovarian Cancer	4 (3.70%)
BRCA Related Cancer (other than Breast & Ovarian)	43 (39.81%)
Histology of Breast tumors	
Ductal	82 (75.93%)
Ductal + Lobular/Medullary	3 (2.78%)
In situ carcinoma	7 (6.48%)
Lobular	6 (5.56%)
Medullary	1 (0.93%)
Others/Unclassified	9 (8.33%)
Molecular Subtypes of Breast tumors	
Hormonal +	75 (69.44%)
TNBC	19 (17.59%)
Her2	14 (12.96%)
Stage	
Stage 0	7 (6.48%)
Stage I	34 (31.48%)
Stage II	51 (47.22%)
Stage III	16 (14.81%)
Invasive Grade	
Grade 1	15 (15.46%)
Grade 2	49 (50.52%)
Grade 3	33 (34.02%)
Not stated	4
DCIS only	7

tion were performed using BAMClipper [23] adapted for single primer extension dataset. Samples having at least 75% of gene-specific primers with at least 100 detected UMI per primer were considered to pass quality control and subjected to variant calling by FreeBayes v1.0.2-15 [24]. Called variants with variant allelic fraction (VAF) at least 10% and sequencing depth at least 500× were annotated by Ensembl Variant Effect Predictor v75 [25]. A high stringency is adopted in order to eliminate the heterogeneity within tumors and also noise parameters.

Variant interpretation

With reference to the public databases including gnomAD (https://gnomad.broadinstitute.org/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), variants were interpreted and assigned classifications according to the four-tier terminology system of the joint guidelines from Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [26]: Tier I (variants with strong clinical significance); Tier II (variants with potential clinical significance); Tier III (variants of unknown significance) and Tier IV (benign or likely benign). Mutation variants that present in 1,000 Genomes resource or causing in-frame consequences were not analyzed. In addition, SNPs were excluded if the allele frequency ≥ 0.01% in East Asian population in the gnomAD database. Passenger mutations or mutations with high mutation background (e.g. *MUC16* and *KMT2C*) were omitted as they were seen in almost 99% of our samples.

Statistical analysis

Clinicopathological variables from pathogenic/likely pathogenic mutation carriers and non-carriers were tabulated in contingency tables. Statistical tests suitable for categorical data

Table 1B. Clinicopathologic data of the ovarian cohort patients (N=14)

Sex	
Female	14 (100%)
Age (Mean/Range)	45.29 (16-78)
Diagnosis Age	
<35	3 (21.43%)
35-44	2 (14.29%)
45-54	6 (42.86%)
55-64	2 (14.29%)
≥65	1 (7.14%)
Personal cancers	
Ovarian Cancer	14 (100%)
Menopause	
Yes	9 (64.29%)
Family History (1st or 2nd degree)	
Breast Cancer	2 (14.29%)
Ovarian Cancer	0 (0%)
BRCA Related Cancer (other than Breast & Ovarian)	2 (14.29%)
Histology of Ovarian tumors	
Serous	5 (35.71%)
Mucinous	2 (14.29%)
Endometrioid	6 (42.86%)
Metastatic adenocarcinoma	1 (7.14%)
FIGO Stage	
I	4 (30.77%)
II	1 (7.69%)
III	7 (53.85%)
IV	1 (7.69%)
Not stated	1
Grade	
1	1 (7.69%)
2	4 (30.77%)
3	8 (61.54%)
Not stated	1

were then considered. Since some variables had expected values less than 5 in some cells, and most of the variable did not have natural ordering, Fisher's exact test was finally adopted. Significance level was set at 5%. A *p*-value less than 0.05 would then indicate the rejection of null hypothesis of independence of variables. The computation was performed using R (version 3.4.2, Foundation for Statistical Computing, Vienna, Austria).

Results

Study population

Of 108 breast cancer patients, the mean age of diagnosis was 54.1 years (range, 25-86 years). Most of the patients were diagnosed at stage I (31.5%) and II (47.2%), and 14.8% of the patients had stage III. Majority of the tumors were invasive ductal carcinoma (82/108, 75.9%) and were grade 2 tumors (49/108, 50.5%). In particular, 43 (39.8%) had at least one first- or second-degree relative with *BRCA*-related cancer (other than breast or ovarian cancer) and 37 (34.3%) had family history of breast cancer. Clinicopathological characteristics of the breast cancer patients were listed in **Table 1A**.

Among 14 patients with ovarian tumors, the mean age of diagnosis was 45.3 years (range, 16-78 years). Most of the tumors are at high grade (8/14, 61.5%), 6 (42.9%) had endometrioid and 5 (35.7%) had serous cancer. Majority of the patients were diagnosed with stage I (4/14, 30.8%) and III (7/14, 53.9%). The patients' characteristics are summarized in **Table 1B**.

Mutation landscapes in breast tumors

With our customized bioinformatics pipeline for variant calling, 369 somatic mutations in 67 cancer predisposition genes were predicted to affect protein sequences in Tier I-IV. Among these mutations, there were 9 small insertions or deletions, 38 caused frameshift termination or early termination, and 19 were splice site variants. Each tumor had an average of 3.4 coding mutations and 25 of them harbored at least 5 coding mutations. Six of them were devoid of any mutation. *PIK3CA* (29.6%) and *TP53* (25.9%) mutations dominated the somatic mutation landscape of breast tumors. Five other common genes were *SYNE1* (20.4%), *BRCA2* (16.7%), *MSH2* (13.0%), *BRCA1* (11.1%) and *MLH1* (11.1%) (**Figure 1**).

Altogether 77 Tier I/II variants corresponding to 15 cancer predisposition genes were identified (**Table 2**), in which 49.1% of the breast tumors had at least one Tier I or Tier II variants.

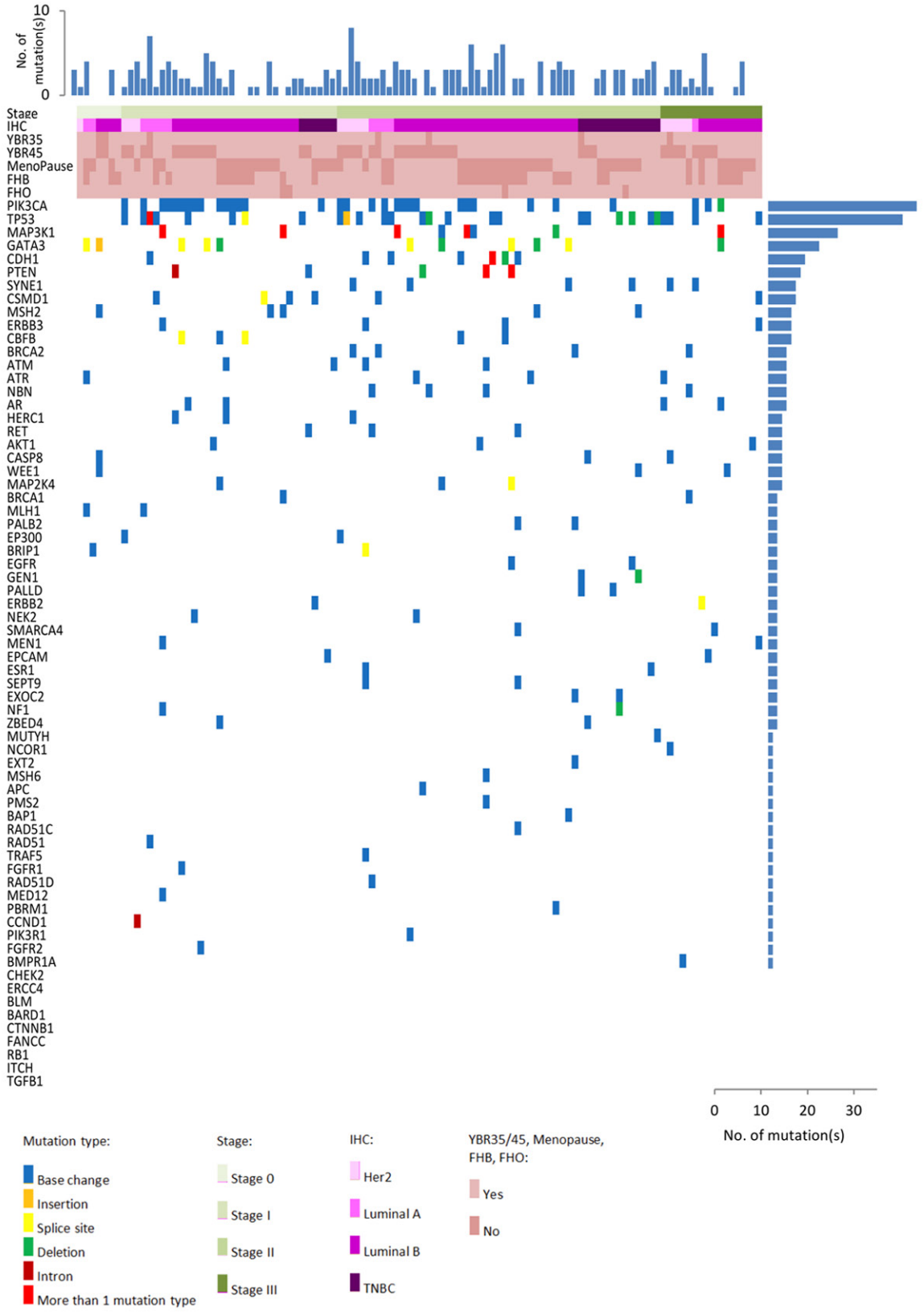

Figure 1. Heatmap of mutation identified in breast tumor tissues. Heatmap of most frequent somatic mutations identified in this study according to subtype. Mutation types are color-coded according to the legend. Top panel: the number of mutations found in each tumor is shown. Right Panel: The number of mutations identified in each

gene is shown. Left Panel: Different types of gene mutations carried by each tumors indicated. Abbreviation: IHC: Immunohistochemistry; YBR: Young Breast Cancer; FHB: Family history of breast cancer; FHO: Family history of ovarian cancer.

Table 2. Tier I and Tier II mutation variants called in Breast cohort

Gene	HVGS	Frequency
AKT1	c.49G>A; p.Glu17Lys	3
BRIP1	c.1936-1G>C	1
CBFB	c.133C>T; p.Gln45Ter	1
CBFB	c.165+2_165+3insT	1
CBFB	c.79-1G>T	1
CDH1	c.1345C>T; p.Gln449Ter	1
CDH1	c.2173delC; p.Leu725CysfsTer45	1
CSMD1	c.10039+1G>C	1
GATA3	c.1085delT; p.Ile362ThrfsTer43	1
GATA3	c.1103_1104delAA; p.Lys368AsnfsTer3	1
GATA3	c.1223_1224insT; p.Pro409AlafsTer99	1
GATA3	c.1278delA; p.Ser427ProfsTer49	1
GATA3	c.1299_1318delACACCACCCCTCCAGCATGG; p.His435ArgfsTer66	1
GATA3	c.925-3_925-2delCA	6
GEN1	c.562_563delAA; p.Lys188GlufsTer2	1
MAP2K4	c.219-2A>G	1
MAP2K4	c.274G>T; p.Glu92Ter	1
MAP3K1	c.1080_1081insC; p.Val361ArgfsTer24	1
MAP3K1	c.1152+1G>A	1
MAP3K1	c.1594C>T; p.Arg532Ter	1
MAP3K1	c.1624_1625insA; p.Thr542AsnfsTer17	1
MAP3K1	c.2262delT; p.Gly756AlafsTer6	1
MAP3K1	c.2479_2488delGTTACTACAG; p.Val827TyrfsTer9	1
MAP3K1	c.2867_2870delTTCA; p.Val956GlufsTer11	1
MAP3K1	c.3315_3316insCA; p.Ile1106GlnfsTer12	1
MAP3K1	c.3387_3396delCTCCAGTATT; p.Asn1129LysfsTer16	1
MAP3K1	c.3814_3817delAAAC; p.Lys1272ArgfsTer2	1
MAP3K1	c.3982+1_3982+2insG	1
MSH6	c.2194C>T; p.Arg732Ter	1
NEK2	c.952C>T; p.Arg318Ter	1
PALB2	c.3113G>A; p.Trp1038Ter	1
PIK3CA	c.1624G>A; p.Glu542Lys	1
PIK3CA	c.1633G>A; p.Glu545Lys	5
PIK3CA	c.3140A>G; p.His1047Arg	12
PIK3CA	c.3140A>T; p.His1047Leu	3
PIK3CA	c.353G>A; p.Gly118Asp	1
PTEN	c.127_149delGAAGGCGTATACAGGAACAATAT; p.Glu43Ter	1
PTEN	c.238A>T; p.Lys80Ter	1
PTEN	c.396delT; p.Val133Ter	1
PTEN	c.405_406insA; p.Cys136MetfsTer44	1
TP53	c.216_217insC; p.Val73ArgfsTer76	1
TP53	c.321C>G; p.Tyr107Ter	1
TP53	c.488A>G; p.Tyr163Cys	1

TP53	c.493delC; p.Gln165SerfsTer5	1
TP53	c.559+2T>C	1
TP53	c.635_636delTT; p.Phe212SerfsTer3	1
TP53	c.723delC; p.Cys242AlafsTer5	2
TP53	c.734G>A; p.Gly245Asp	2
TP53	c.817C>T; p.Arg273Cys	1
TP53	c.916C>T; p.Arg306Ter	2

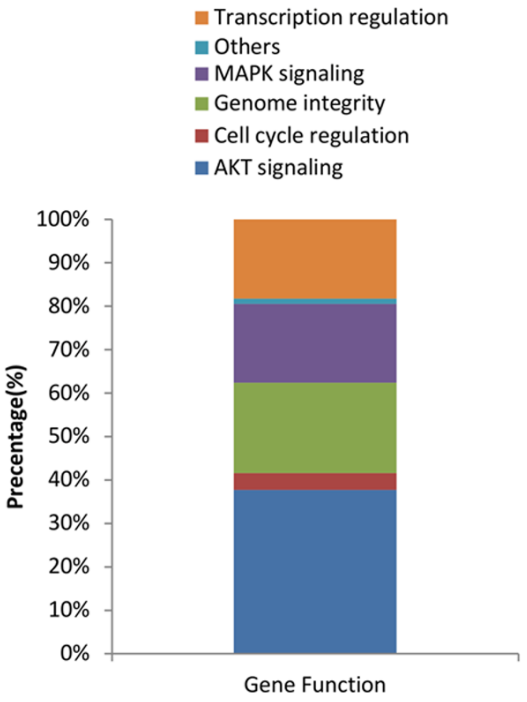

Figure 2. Identification of 15 mutated gene function. Genes carried mutations were classified according to functions: transcription regulation; MAPK signaling; genome instability; cell cycle regulation and AKT signaling.

The most frequent mutated genes were *PIK3CA* (28.6%), *TP53* (16.9%), *MAP3K1* (14.3%), *GATA3* (14.3%) and *PTEN* (5.2%). *PIK3CA* c.3140A>G (11.1%), *GATA3* c.925-3_925-2delCA (5.6%) and *PIK3CA* c.1633G>A (4.6%) were common in breast tumors. Also, the major mutations were the cancer driver genes involved in AKT signaling (37.7%), genome integrity (20.8%), transcription regulation (18.2%) and MAPK signaling pathways (18.2%) (**Figure 2**). Apart from Tier I/II mutations, a total of 144 variants of Tier III in 55 cancer susceptibility genes from 66 breast tumors were identified (Table S1).

A total of 221 mutation variants (Tier I/II: 77; Tier III: 144) from 108 tumors were identified (**Table 3**). Surprisingly, 6 of the tumors had no somatic mutation found and the rest carried an average of 2.2 mutations. Among these 221 mutation variants, 13 dominant recurrence mutation variants were seen in 46.2% of the tumors (50/108) and none of them have been reported in gnomAD within the East Asian population (Table S2). Of note, there were 6 *BRCA* mutations in Tier III (*BRCA1*:2; *BRCA2*:4) but none was in Tier I/II, in which 4 of the *BRCA* mutations were confirmed to be germline. Interestingly, there was only one case with both Tier III somatic *BRCA1* and *BRCA2* mutations.

Clinical and pathological association

Association of clinical and pathological parameters and dominant gene mutations from 77 Tier I/II mutation variants were analyzed by Fisher Exact test. *PIK3CA* mutations were common in Luminal A tumors (P=0.0294). Mutations in *GATA3* (P=0.0172) were significantly associated with ER positivity. For other mutations, there were no significant association between the mutation and young onset age, patient status, staging and menopausal status.

Somatic single nucleotide variants (SNVs)

566 SNVs were detected, of which 340 of them were unique variants. The median SNV was 5.29 and ranged from 1-18 SNVs per tumor, where five patients got >10 SNVs and three patients got one SNV only. Early-onset and HER2+ tumors often acquired a G to T transversion while tumors from menopause patients yielded mostly a T to G transversion (**Figure 3** and **Table 4**). The identified SNVs include 21 nonsense mutation variants in 15 genes. Recurrent mutations were seen in *BMPR1A*, *MAP3K1*, *PALB2* and *TP53* genes. For missense mutations, 322 mutations were fo-

Table 3. Mutation frequency (N=221) of different genes in breast cohort

Gene (s)	Tier I/II (N=77)	Tier III (N=144)	Total (N=221)
AKT1	3 (3.9%)	-	3 (1.36%)
APC	-	1 (0.69%)	1 (0.45%)
AR	-	4 (2.78%)	4 (1.81%)
ATM	-	4 (2.78%)	4 (1.81%)
ATR	-	4 (2.78%)	4 (1.81%)
BAP1	-	1 (0.69%)	1 (0.45%)
BMPR1A	-	1 (0.69%)	1 (0.45%)
BRCA1	-	2 (1.39%)	2 (0.9%)
BRCA2	-	4 (2.78%)	4 (1.81%)
BRIP1	1 (1.3%)	1 (0.69%)	2 (0.9%)
CASP8	-	3 (2.08%)	3 (1.36%)
CBFB	3 (3.9%)	2 (1.39%)	5 (2.26%)
CCND1	-	1 (0.69%)	1 (0.45%)
CDH1	2 (2.6%)	6 (4.17%)	8 (3.62%)
CSMD1	1 (1.3%)	5 (3.47%)	6 (2.71%)
EGFR	-	2 (1.39%)	2 (0.9%)
EP300	-	2 (1.39%)	2 (0.9%)
EPCAM	-	2 (1.39%)	2 (0.9%)
ERBB2	-	2 (1.39%)	2 (0.9%)
ERBB3	-	5 (3.47%)	5 (2.26%)
ESR1	-	2 (1.39%)	2 (0.9%)
EXOC2	-	2 (1.39%)	2 (0.9%)
EXT2	-	1 (0.69%)	1 (0.45%)
FGFR1	-	1 (0.69%)	1 (0.45%)
FGFR2	-	1 (0.69%)	1 (0.45%)
GATA3	11 (14.29%)	-	11 (4.98%)
GEN1	1 (1.3%)	1 (0.69%)	2 (0.9%)
HERC1	-	3 (2.08%)	3 (1.36%)
MAP2K4	2 (2.6%)	1 (0.69%)	3 (1.36%)
MAP3K1	11 (14.29%)	4 (2.78%)	15 (6.79%)
MED12	-	1 (0.69%)	1 (0.45%)
MEN1	-	2 (1.39%)	2 (0.9%)
MLH1	-	2 (1.39%)	2 (0.9%)
MSH2	-	5 (3.47%)	5 (2.26%)
MSH6	1 (1.3%)	-	1 (0.45%)
MUTYH	-	1 (0.69%)	1 (0.45%)
NBN	-	4 (2.78%)	4 (1.81%)
NCOR1	-	1 (0.69%)	1 (0.45%)
NEK2	1 (1.3%)	1 (0.69%)	2 (0.9%)
NF1	-	2 (1.39%)	2 (0.9%)
PALB2	1 (1.3%)	1 (0.69%)	2 (0.9%)
PALLD	-	2 (1.39%)	2 (0.9%)
PBRM1	-	1 (0.69%)	1 (0.45%)
PIK3CA	22 (28.57%)	10 (6.94%)	32 (14.48%)
PIK3R1	-	1 (0.69%)	1 (0.45%)
PMS2	-	1 (0.69%)	1 (0.45%)

und in 69 different genes, in which *SYNE1* (n=24) and *TP53* (n=24) were common. Among them, 72 of these SNVs were listed in the "Candidate Cancer Gene Database" category A potential cancer drivers [27] and 471 of the SNVs from 49 genes which were actively relevant to cancer according to "Cancer Gene Census" database [28].

Mutation spectrum of breast tumors versus ovarian tumors

Analysis revealed 17 Tier I/II mutation variants in all 14 tumors, and each tumor carried an average of 1.21 mutations. The mutation signature of ovarian tumors was different from breast tumors (**Table 5**), the most frequently mutated genes were *TP53* (52.9%), *KRAS* (23.5%), *PIK3CA* (11.8%), *BRCA1* (5.9%) and *RB1* (5.9%). Of note, Tier I/II *BRCA1* somatic mutation was seen in ovarian tumor (1/14, 7.1%) but not in breast tumor. The complete list of Tier I-III variants of ovarian cancers was shown in Table S3.

Discussion

In Hong Kong, approximately 10% of the breast cancer cases are inherited with *BRCA* mutations [4] and these patients are offered risk reduction surgery or targeted therapy such as PARP inhibitors. However, limited study has investigated the prevalence of somatic mutation in germline *BRCA* mutation-negative breast and ovarian cancer patients, who may potentially benefit from other targeted therapies. Interpretation of somatic variants are likely to impact clinical managements including estimation of the sensitivity and resistance of specific drug treatments. With the increase demand of genetic information, screening of germline and somatic mutations have been incorporated into the routine clinical practice for treatment decision making in different cancer types including breast cancer [29].

PTEN	4 (5.19%)	3 (2.08%)	7 (3.17%)
RAD51	-	1 (0.69%)	1 (0.45%)
RAD51C	-	1 (0.69%)	1 (0.45%)
RAD51D	-	1 (0.69%)	1 (0.45%)
RET	-	3 (2.08%)	3 (1.36%)
SEPT9	-	2 (1.39%)	2 (0.9%)
SMARCA4	-	2 (1.39%)	2 (0.9%)
SYNE1	-	6 (4.17%)	6 (2.71%)
TP53	13 (16.88%)	16 (11.11%)	29 (13.12%)
TRAF5	-	1 (0.69%)	1 (0.45%)
WEE1	-	3 (2.08%)	3 (1.36%)
ZBED4	-	2 (1.39%)	2 (0.9%)

Somatic *PIK3CA* and *TP53* mutations were found in 27-40% and 23-34% of breast cancer respectively [30-32]. In a large cohort study (n=1,794), somatic *TP53* mutation rate was lowered in older age groups (>59 years) [33], similar as in our cohort, *TP53* mutations were seen in 40% young breast patient's (≤ 45 years) tumor, while 18% were age >45 years. On the contrary, there was one study reported that 20% of the tumors had no association with early-onset breast cancer [31]. Patients with somatic *TP53* mutations had a poor overall survival in ER-positive than in ER-negative patients [33]. Docetaxel has been suggested as a better therapeutic option than anthracyclines in treating *TP53*-mutated breast cancer patients than the wild-type patients.

Somatic *PIK3CA* mutations, another common mutated gene, was identified in 20.4% of our breast tumors. Studies showed that 25-46.5% of breast cancer patients had *PI3K* mutations were significantly associated with ER-positive tumors [34-39]. Similarly, mutation hotspots E542K, E545K and H1047R mutations were also observed in our cohort [38]. Furthermore, several retrospective and prospective studies had contradictory conclusions on prognostic and predictive values of *PIK3CA* mutations in breast cancer tumors [40-43]. *PIK3CA* mutations can co-exist with other PI3K-enhancing mechanisms, such as HER2 amplification. HER2-targeted therapy is suggested in *PIK3CA*-mutated patients [44]. However, some studies indicated that *PIK3CA* mutations may predict resistance to trastuzumab [45, 46]. The antitumor activity of combining BKM120 and trastuzumab were promising in patients with HER2-positive advanced or metastatic breast cancer developed resistant to trastuzumab [47]. To develop PI3K inhibitors as novel therapeutics for HER2-positive advanced or metastatic breast cancer, we still need to overcome challenge of maximizing efficacy of these agents with minimum side effects.

TP53 and *KRAS* are the most dominated somatic mutations in our ovarian cancer cohort. *TP53* mutations were seen in 90-96% of high-grade ovarian tumors [19, 48]. Several on-going phase I/II clinical trials are conducted to assess the efficacy of *TP53* activators (APR-246 and MK-1775-004) in treating platinum-sensitive ovarian cancer and platinum-resistant high-grade serous ovarian cancer [49, 50]. Also, the use of AMG 510 in treating *KRAS* c.34G>T mutation, a common mutation in solid tumors, showed promising results in patients with non-small-cell lung carcinoma [51, 52]. Also, a mRNA-derived *KRAS*-targeted vaccine, mRNA-5671, targeting both *KRAS* c.35G>A and c.34G>T mutations is undergoing Phase I trial [51]. Herein, somatic mutation screening is likely beneficial for ovarian patients without germline mutations to provide better treatment strategies in the future.

Several clinical trials of olaparib revealed promising results on high-grade serous ovarian cancer patients with germline or somatic *BRCA* mutation, the median PFS was improved in olaparib group than placebo (11.2 months vs 4.3 months) [53]. Besides, clinical trials of other PARP inhibitors (rucaparib and niraparib) have extended the study to platinum-based therapy in high-grade ovarian cancer patient regardless of *BRCA* status and yield positive results. On the other hand, the prevalence of somatic *BRCA* mutations in sporadic breast cancer was around 3.5% [54], yet very little information in hereditary breast cancer is available. In this testing cohort, no somatic *BRCA* mutation was identified, hence, we believed that *BRCA*-negative breast cancer patients are unlikely to carry somatic *BRCA* mutation. In light of a relatively low reported frequency in hereditary breast cancer, somatic *BRCA* mutation testing for non-*BRCA* breast cancer patients are of limited value, unlike as in ovarian cancer.

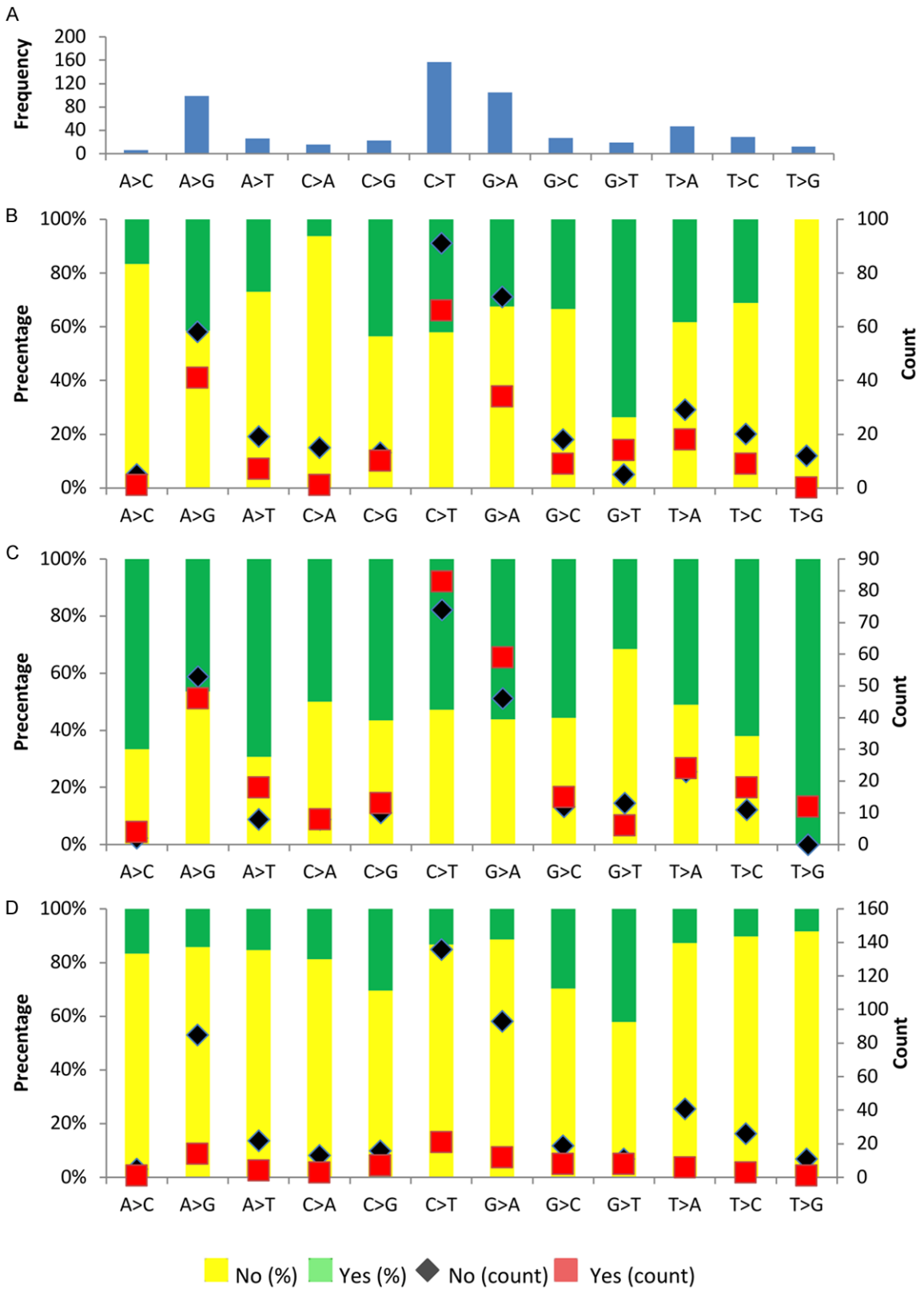

Figure 3. Nucleotide mutational profile. A. Frequency of nucleotide change. B. Distribution of YBR45. C. Distribution of Menopause. D. Distribution of Her2.

Table 4. Frequency of transition and transversion mutations

		To			
		A	C	G	T
From	A		6 (4.58%)	99 (75.57%)	26 (19.85%)
	C	16 (8.16%)		23 (11.73%)	157 (80.1%)
	G	105 (83.33%)	2 (1.59%)		19 (15.08%)
	T	47 (53.41%)	29 (32.95%)	12 (13.64%)	

Table 5. Mutation spectrum of breast and ovarian tumors

	Breast (N=108)			Ovarian (N=14)		
	Tier I/II	Tier III	Total	Tier I/II	Tier III	Total
APC		1	1			
AR		4	4			
ATM		4	4		1	1
ATR		4	4		1	1
BRCA1		2	2	1	1	2
BRCA2		4	4			
BRIP1	1	1	2			
CBFB	3	2	5			
CDH1	2	6	8			
CSMD1	1	5	6			
ERBB3		5	5		1	1
EXOC2		2	2		1	1
EXT2		1	1			
GATA3	11		11			
HERC1		3	3		1	1
KRAS				4		4
MAP2K4	2	1	3		1	1
MAP3K1	11	4	15		2	2
MSH2		5	5			
NBN		4	4			
NF1		2	2		1	1
PALB2	1	1	2		2	2
PIK3CA	22	10	32	2		2
PTEN	4	3	7		1	1
RAD51C		1	1			
RB1				1		1
SYNE1		6	6			
TP53	13	16	29	9	2	11

In conclusion, characterization of somatic mutations in breast and ovarian tumors could provide insights into tumorigenesis and reveal candidates for targeted therapeutics. We found that somatic *PIK3CA* and *TP53* mutations were common events in germline mutation-negative breast cancer patients and had distinct spectrum than in ovarian cancer. Results from this study exemplify the necessity of somatic testing in breast and ovarian cancer patients, besides germline mutation screening, and to guide future research directions on other targeted therapies.

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Disclosure of conflict of interest

None.

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Table S1. Tier III mutation variants identified from 108 breast tumors

Tier III					
Mutation variants	Freq	Mutation variants	Freq	Mutation variants	Freq
APC: c.8412G>T; p.Gln2804His	1	ERBB3: c.985A>G; p.Lys329Glu	1	RET: c.2342A>G; p.Gln781Arg	1
AR: c.2659A>G; p.Met887Val	1	ESR1: c.1370G>A; p.Gly457Glu	1	RET: c.2980A>G; p.Lys994Glu	1
AR: c.302G>A; p.Arg101His	1	ESR1: c.620C>A; p.Ala207Asp	1	RET: c.565C>T; p.Arg189Cys	1
AR: c.528C>A; p.Ser176Arg	2	EXOC2: c.350G>A; p.Arg117His	1	SEPT9: c.1204C>T; p.Arg402Cys	1
ATM: c.2803A>G; p.Thr935Ala	1	EXOC2: c.557C>T; p.Ala186Val	1	SEPT9: c.38G>C; p.Ser13Thr	1
ATM: c.3078G>T; p.Trp1026Cys	1	EXT2: c.962A>G; p.Asn321Ser	1	SMARCA4: c.1532C>T; p.Thr511Met	1
ATM: c.7115A>T; p.Asp2372Val	1	FGFR1: c.2284A>G; p.Met762Val	1	SMARCA4: c.923C>T; p.Thr308Met	1
ATM: c.8173G>C; p.Asp2725His	1	FGFR2: c.1535C>T; p.Ala512Val	1	SYNE1: c.10288G>A; p.Gly3430Arg	1
ATR: c.1453C>A; p.Pro485Thr	1	GEN1: c.2606A>G; p.Asp869Gly	1	SYNE1: c.12553A>C; p.Lys4185Gln	1
ATR: c.1909G>A; p.Val637Met	1	HERC1: c.14525T>C; p.Met4842Thr	1	SYNE1: c.15497C>G; p.Ala5166Gly	1
ATR: c.637C>G; p.Leu213Val	1	HERC1: c.4274G>A; p.Arg1425Gln	1	SYNE1: c.2374C>T; p.Leu792Phe	1
ATR: c.6640G>C; p.Asp2214His	1	HERC1: c.8626G>T; p.Val2876Leu	1	SYNE1: c.26372C>T; p.Thr8791Met	1
BAP1: c.1031A>G; p.Asn344Ser	1	MAP2K4: c.563_565delTTTinsGTA; p.PheTyr188CysAsn	1	SYNE1: c.911C>G; p.Ser304Cys	1
BMPR1A: c.1318A>G; p.Met440Val	1	MAP3K1: c.1694G>A; p.Gly565Glu	1	TP53: c.1073A>T; p.Glu358Val	1
BRCA1: c.5072C>A; p.Thr1691Lys	1	MAP3K1: c.1739G>A; p.Arg580Lys	1	TP53: c.394A>G; p.Lys132Glu	1
BRCA1: c.5096G>A; p.Arg1699Gln	1	MAP3K1: c.4298G>C; p.Trp1433Ser	1	TP53: c.533A>C; p.His178Pro	1
BRCA2: c.2401A>G; p.Asn801Asp	1	MAP3K1: c.65C>T; p.Pro22Leu	1	TP53: c.646G>A; p.Val216Met	1
BRCA2: c.3040A>G; p.Asn1014Asp	1	MED12: c.1883C>T; p.Ala628Val	1	TP53: c.659A>G; p.Tyr220Cys	1
BRCA2: c.5942C>T; p.Ala1981Val	1	MEN1: c.1496C>T; p.Pro499Leu	1	TP53: c.700_702delTAC; p.Tyr234del	1
BRCA2: c.8419T>G; p.Ser2807Ala	1	MEN1: c.1546G>T; p.Val516Leu	1	TP53: c.711G>A; p.Met237Ile	1
BRIP1: c.2158G>A; p.Val720Met	1	MLH1: c.1154G>A; p.Arg385His	1	TP53: c.713G>C; p.Cys238Ser	1
CASP8: c.38G>C; p.Ser13Thr	1	MLH1: c.1780G>C; p.Glu594Gln	1	TP53: c.722C>T; p.Ser241Phe	1
CASP8: c.458G>T; p.Gly153Val	1	MSH2: c.1204C>G; p.Gln402Glu	1	TP53: c.747G>T; p.Arg249Ser	1
CASP8: c.932C>T; p.Pro311Leu	1	MSH2: c.1886A>G; p.Gln629Arg	3	TP53: c.775G>T; p.Asp259Tyr	1
CBFB: c.189T>G; p.Asn63Lys	1	MSH2: c.260C>A; p.Ser87Tyr	1	TP53: c.814G>A; p.Val272Met	1
CBFB: c.80T>G; p.Ile27Ser	1	MUTYH: c.1165C>A; p.Leu389Met	1	TP53: c.814G>T; p.Val272Leu	1
Mutation variants	Freq	Mutation variants	Freq	Mutation variants	Freq
CCND1: c.724-8_724-4delCTCT	1	NBN: c.1616C>T; p.Ser539Phe	1	TP53: c.824G>A; p.Cys275Tyr	1
CDH1: c.1022A>C; p.Tyr341Ser	1	NBN: c.2176G>C; p.Glu726Gln	1	TP53: c.833C>T; p.Pro278Leu	1
CDH1: c.1031_1033delTGG; p.Val345del	1	NBN: c.235A>G; p.Asn79Asp	1	TP53: c.838A>G; p.Arg280Gly	1
CDH1: c.1103C>T; p.Thr368Ile	1	NBN: c.800G>C; p.Gly267Ala	1	TRAF5: c.1591G>C; p.Glu531Gln	1
CDH1: c.1213A>G; p.Asn405Asp	1	NCOR1: c.5504A>G; p.Asp1835Gly	1	WEE1: c.241C>T; p.Pro81Ser	1
CDH1: c.61C>G; p.Leu21Val	1	NEK2: c.289G>T; p.Val97Leu	1	WEE1: c.269A>G; p.Glu90Gly	2
CDH1: c.875A>G; p.Asp292Gly	1	NF1: c.3510C>G; p.His1170Gln	1	ZBED4: c.1429T>G; p.Cys477Gly	1

Somatic mutations in BRCA-ve patients

CSMD1: c.4184G>C; p.Gly1395Ala	1	NF1: c.8509_8512delAGAT; p.Lys2837del	1	ZBED4: c.1585G>A; p.Ala529Thr	1
CSMD1: c.694G>A; p.Ala232Thr	1	PALB2: c.3428T>A; p.Leu1143His	1		
CSMD1: c.7504G>A; p.Gly2502Arg	1	PALLD: c.455T>C; p.Val152Ala	1		
CSMD1: c.8014G>A; p.Glu2672Lys	1	PALLD: c.790G>A; p.Ala264Thr	1		
CSMD1: c.850A>G; p.Ile284Val	1	PBRM1: c.2746G>A; p.Glu916Lys	1		
EGFR: c.2749G>A; p.Gly917Arg	1	PIK3CA: c.1035T>A; p.Asn345Lys	5		
EGFR: c.926G>C; p.Arg309Pro	1	PIK3CA: c.1258T>C; p.Cys420Arg	3		
EP300: c.2563C>T; p.Pro855Ser	1	PIK3CA: c.1637A>C; p.Gln546Pro	1		
EP300: c.4477G>A; p.Asp1493Asn	1	PIK3CA: c.314_325delTAGGCAACCGTG; p.Val105_Arg108del	1		
EPCAM: c.391A>G; p.Thr131Ala	1	PIK3R1: c.869T>C; p.Ile290Thr	1		
EPCAM: c.785A>G; p.Gln262Arg	1	PMS2: c.1251T>G; p.Ile417Met	1		
ERBB2: c.1738-3_1738-2insC	1	PTEN: c.210-7_210-3delCTTTT	1		
ERBB2: c.2018T>A; p.Ile673Asn	1	PTEN: c.389G>A; p.Arg130Gln	1		
ERBB3: c.1166C>T; p.Thr389Ile	1	PTEN: c.406T>C; p.Cys136Arg	1		
ERBB3: c.1300A>G; p.Lys434Glu	1	RAD51: c.290G>A; p.Arg97His	1		
ERBB3: c.1553T>C; p.Leu518Ser	1	RAD51C: c.246C>G; p.His82Gln	1		
ERBB3: c.2611G>T; p.Ala871Ser	1	RAD51D: c.179A>G; p.Gln60Arg	1		

Table S2. Recurrence mutation variants identified from 108 breast tumors

Tier	Gene	Mutation Variants	Frequency
Tier I/II	AKT1	c.49G>A; p.Glu17Lys	3
	GATA3	c.925-3_925-2delCA	6
	PIK3CA	c.1633G>A; p.Glu545Lys	5
	PIK3CA	c.3140A>G; p.His1047Arg	12
	PIK3CA	c.3140A>T; p.His1047Leu	3
	TP53	c.723delC; p.Cys242AlafsTer5	2
	TP53	c.734G>A; p.Gly245Asp	2
	TP53	c.916C>T; p.Arg306Ter	2
Tier III	AR	c.528C>A; p.Ser176Arg	2
	MSH2	c.1886A>G; p.Gln629Arg	3
	PIK3CA	c.1035T>A; p.Asn345Lys	5
	PIK3CA	c.1258T>C; p.Cys420Arg	3
	WEE1	c.269A>G; p.Glu90Gly	2

Table S3. Mutation variants identified from 14 ovarian tumors

Tier I/II		Tier III	
Mutation variants	Freq	Mutation variants	Freq
BRCA1: c.1674delA; p.Gly559Valfs*11	1	ATM: c.4724G>A; p.Arg1575His	1
KRAS: c.35G>A; p.Gly12Asp	2	ATR: c.7667C>G; p.Thr2556Ser	1
KRAS: c.35G>T; p.Gly12Val	2	AXIN2: c.863G>C; p.Gly288Ala	1
PIK3CA: c.1624G>A; p.Glu542Lys	1	BRCA1 LOH	1
PIK3CA: c.3145G>C; p.Gly1049Arg	1	ERBB3: c.2864G>A; p.Arg955His	1
RB1: c.1899_1902dupCTCA; p.Ala635Leufs*25	1	EXOC2: c.1706A>G; p.Asn569Ser	1
TP53: c.97-3_103delinsG	1	HERC1: c.1268C>T; p.Ser423Phe	1
TP53: c.250delG; p.Ala84Profs*39	1	MAP2K4: c.239G>A; p.Ser80Asn	1
TP53: c.524G>A; p.Arg175His	2	MAP3K1: c.233_234delTCinsCT; p.Leu78Pro	1
TP53: c.527G>T; p.Cys176Phe	1	MAP3K1: c.2617G>A; p.Val873Ile	1
TP53: c.659A>G; p.Tyr220Cys	1	NCOR1: c.2746G>A; p.Val916Ile	1
TP53: c.742C>T; p.Arg248Trp	1	NF1: c.4766C>A; p.Ala1589Glu	1
TP53: c.743G>A; p.Arg248Gln	1	PALB2: c.1492G>T; p.Asp498Tyr	1
TP53: c.818G>A; p.Arg273His	1	PALB2: c.2423G>A; p.Gly808Glu	1
		PTEN: c.729_730delGGinsCT; p.Met243_Gly244delinsIleCys	1
		TP53: c.376G>A; p.Ala26Thr	1
		TP53: c.785_786delinsTA; p.Gly262Val	1