RESEARCH ARTICLE

Editorial Process: Submission:10/11/2022 Acceptance:04/14/2023

Genetic Characterization of Ovarian Tumor Tissues from Patients with Epithelial Ovarian Cancer in a Philippine Tertiary Hospital: A Descriptive Study

Ryan C V Lintao[1,2], Ana Joy P Padua[1,2], Yukiko Nakura[2], Erlidia F Llamas-Clark[3,4], Itaru Yanagihara[2]*

Abstract

Objective: This study identified genetic variations in ovarian tumor specimens from Filipino epithelial ovarian cancer (EOC) patients using next-generation sequencing. **Methods:** Genomic DNA was isolated from formalin-fixed paraffin-embedded ovarian specimens from 8 chemosensitive and 8 chemoresistant EOC patients. Targeted next-generation sequencing was done to identify mutations in hotspot regions of common oncogenes and tumor-suppressor genes. The mutations were cross-referenced with dbSNP and ClinVar databases to identify previously reported alterations, and potentially damaging variants were predicted using PolyPhen-2. **Results:** Our study has identified 85 unique variants, 35 in chemosensitive EOC, 22 in chemoresistant EOC, and 28 in both. Chemosensitive EOC specimens had more exonic single nucleotide variants than chemoresistant EOC specimens. Of the 50 oncogenes and tumor suppressor genes, KDR gene had the most frequent variations in EOC patients. Two of the unique KDR variants identified were novel mutations. Thirty-nine unique protein-modifying genetic variants were identified in all specimens, the majority of which have been previously reported in dbSNP and ClinVar. **Conclusion:** This study was the first non-BRCA genetic analysis done on ovarian cancer in Filipino patients. Next-generation sequencing was able to identify previously reported alterations with known therapeutic implications which may benefit from targeted therapy instead of standard chemotherapy regimen.

Keywords: Epithelial ovarian cancer- Philippines- targeted next-generation sequencing- chemotherapy resistance

Asian Pac J Cancer Prev, **24 (4)**, 1187-1197

Introduction

Cancer of the ovaries is the fifth most diagnosed cancer in women from the Philippines, following breast, cervix, colorectal, and lung cancers, and the second most common gynecologic malignancy (Laudico et al., 2015). Most ovarian cancer diagnosed worldwide are of epithelial type, comprising 90% of the cases, occurring primarily in postmenopausal women (Jelovac & Armstrong, 2011). Globally, quarter of ovarian cases are associated with germline mutations, with the rest arising from sporadic mutations. Of the hereditary ovarian cancers, 20% of the cases in the Philippines and worldwide are attributed to mutations in BRCA 1/2 (De Leon Matsuda et al., 2002; Konstantinopoulos et al., 2020).

Early-stage ovarian cancer usually does not present with any symptoms; if any, it presents as vague symptoms usually attributed to gastrointestinal pathology (Lheureux et al., 2019). Pelvic examination may detect ovarian masses, with serum tumor markers such as CA-125 and transvaginal ultrasound aiding in diagnosis. These modalities, however, are not ideal as screening tools because usage in average-risk women did not decrease the risk of mortality, and was associated with increased harm ranging from minor procedure-related adverse events (e.g., nausea, fainting) to more severe complications from cancer diagnosis or false-positive results (e.g., infection, bowel injury) (Buys et al., 2011). Non-specific symptomatology combined with a lack of effective screening tools contributes to late diagnosis of ovarian cancer, making it the most lethal gynecologic malignancy in terms of case-fatality rate (Doubeni et al., 2016).

Epithelial ovarian cancer (EOC) is treated with debulking surgery to remove the primary tumor and other

*[1]College of Medicine, University of the Philippines Manila, Ermita, Manila 1000 Philippines. [2]Department of Developmental Medicine, Research Institute, Osaka Women's and Children's Hospital, Izumi, Osaka, Japan. [3]Department of Obstetrics and Gynecology, College of Medicine and Philippine General Hospital, University of the Philippines Manila, Manila, Philippines. [4]Institute of Child Health and Human Development, National Institutes of Health, University of the Philippines Manila, Manila, Philippines. *For Correspondence: itaruy@wch.opho.jp. Ryan C. V. Lintao and Ana Joy P. Padua have equal contribution in this study.*

masses detected in the fallopian tube and the peritoneum during exploratory laparotomy, with a standard regimen of platinum-based chemotherapeutic drug and taxane (e.g. carboplatin-paclitaxel) as adjuvant therapy (Berek et al., 2018). The response to chemotherapy is classified as either resistant or sensitive, with chemoresistant ovarian cancer recurring within 6 months from the end of chemotherapy. In contrast, chemosensitive ovarian cancer recurs beyond 1 year (Stuart et al., 2011). Due to heterogeneity in chemotherapeutic response, there is a need to custom-fit treatment with the genetic profile of patients as part of precision medicine. In the Philippines, however, only a few genetic studies have been done on ovarian cancer, focusing on BRCA 1/2 mutations associated with hereditary breast-ovarian cancer (De Leon Matsuda et al., 2002; Nato, 2003; Que et al., 2018). This pioneering study described genetic variations in chemosensitive and chemoresistant ovarian specimens from Filipino patients that may be associated with pathogenesis or chemotherapeutic response via targeted next-generation sequencing of hotspot regions of common tumor suppressor genes and oncogenes.

Materials and Methods

Patient selection and sample preparation

Deidentified formalin-fixed paraffin-embedded (FFPE) ovarian tissues composed of 8 chemosensitive (CS) and 8 chemoresistant (CR) from randomly selected patients were retrieved from University of the Philippines - Philippine General Hospital. The inclusion criteria were (1) pathologic diagnosis of epithelial ovarian based on routine hematoxylin and eosin-stained histopathology, and (2) no family history of ovarian cancer. The response to chemotherapy was defined as (a) Resistant, progression-free interval since the last line of platinum-based chemotherapy of less than 6 months; and (b) Sensitive, progression-free interval since the last line of platinum-based chemotherapy of more than 12 months (Stuart et al., 2011). Clinical follow-up until at least 1 year should be completed to be included. This study was conducted upon approval of University of the Philippines Manila Research Ethics Board.

Targeted next-generation sequencing platform

DNA was isolated from FFPE blocks with high tumor percentage using Maxwell® RSC DNA FFPE Kit (Promega) as described in the product manual. DNA samples were quantified using Qubit® 2.0 fluorometer with dsDNA BR assay kit (ThermoFisher Scientific) and stored at -20°C. About 10 ng of DNA for each sample was used for library preparation using Ion AmpliseqTM Kit for Chef DL8 and Ion ChefTM Instrument (ThermoFisher Scientific). Ion AmpliSeqTM Cancer Hotspot Panel v2 (ThermoFisher Scientific) was used for amplification of hotspot regions, including approximately 2,800 COSMIC mutations of 50 oncogenes and tumor suppressor genes such as APC, KDR, KIT, KRAS, PIK3CA, PTEN, and TP53 composed of 207 indexed, adaptor ligated, hybridization-captured primer pairs with average amplicon length 154 bp. Sequencing was performed using the Ion PGMTM Sequencer (ThermoFisher Scientific) and Ion PGMTM Hi-QTM View Chef Kit with the Ion 316TM Chip Kit described by the manufacturer.

Bioinformatics analysis

Data analysis of BAM files was carried Ion Torrent Sequencing platform (Life Technologies). Generated reads were aligned to the GRCh37 (hg19) human reference genome. Torrent Suite Software V.5.12 (Life Technologies) was used to call variants such as somatic single-nucleotide polymorphisms (SNPs), multi-nucleotide polymorphisms (MNPs), insertions, deletions, and block substitutions. The same software package was used to filter and annotate variants. Annotation for each variant included the type of variant, gene location, type of transcript, and amino acid change due to the variant. Annotation was cross-referenced with UCSC Genome Browser (Kent et al., 2002) (https://genome.ucsc.edu/). PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) was used to predict functional effect of an amino acid substitution in a certain protein (Adzhubei et al., 2010). dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) were used to identify previously reported genetic variants (Sherry et al., 2001) and their relationship to human diseases (Landrum et al., 2018), respectively.

Statistical analysis

Descriptive analysis using tables, frequency (%), and median values were used to summarize and analyze the data collected. Mann-Whitney test was done to compare the median age of the two groups. For other baseline characteristics, chi-square test for independence was done to determine the association between the two groups. P-values of 0.05 or less were considered statistically significant. Proportions and frequencies were reported for genetic variants due to limitations in the analysis due to small sample size. Data were analyzed using GraphPad Prism 9.3.1 (https://www.graphpad.com) (San Diego, CA, USA).

Results

Patient demographics and tumor characteristics

The study participants had a median age of 50.0 at the time of cytoreductive surgery, with age ranging from 23 to 57 years in chemosensitive group, and from 24 to 62 in chemoresistant group (Table 1). This was consistent with median age at diagnosis of 50-79 years reported by Momenimovahed et al (2019). The difference in median age between the two groups was not statistically significant (p=0.4564). Both groups had comparable gravidity (p=0.3149), number of abortions (p=0.5218) and number of preterm deliveries (p=0.5218). Majority of the patients (11 of 16) had advanced-stage disease at the time of cytoreductive surgery. The ovarian tumors surgically removed from patients were mostly serous, the most common histologic subtype in epithelial ovarian cancer (Torre et al., 2018). There was no statistically significant difference between chemosensitive and chemoresistant EOC groups in the cancer stage (p=0.3666) and histologic

Table 1. Summary of Patient Demographics and Tumor Characteristics in EOC.

	Total EOC group (n=16)	Chemosensitive EOC group (n=8)	Chemoresistant EOC group (n=8)	P-value
Median age (range)	50.0 (23-62)	49.5 (23-57)	55.5 (24-62)	0.4564
Gravidity				0.3149
Nulligravid	5	2	3	
Primigravid	2	2	0	
Multigravid	9	4	5	
Abortion	3	1	2	0.5218
Preterm delivery	3	1	2	0.5218
FIGO Stage				0.3666
I	3	2	1	
II	2	2	0	
III	8	3	5	
IV	3	1	2	
Histologic type				0.2276
Serous	12	5	7	
Endometrioid	2	2	0	
Clear cell	1	0	1	
Mucinous	1	1	0	

subtype (p=0.2276).

Chemosensitive EOC specimens have more exonic SNVs

A total of 305 genetic variants were detected in the EOC specimens, as shown in Table 2, of which 85 were unique. Supplementary Table A shows a list of all genetic variants identified via targeted next-generation sequencing. Of the 168 genetic variants detected in chemosensitive EOC specimens, 63 were unique. Similarly, 50 of the 137 genetic variants detected in chemoresistant EOC specimens were unique. Of the unique variants, 35/63 in chemosensitive EOC specimens and 22/50 in chemoresistant EOC specimens were exclusive to their corresponding groups. Twenty-eight

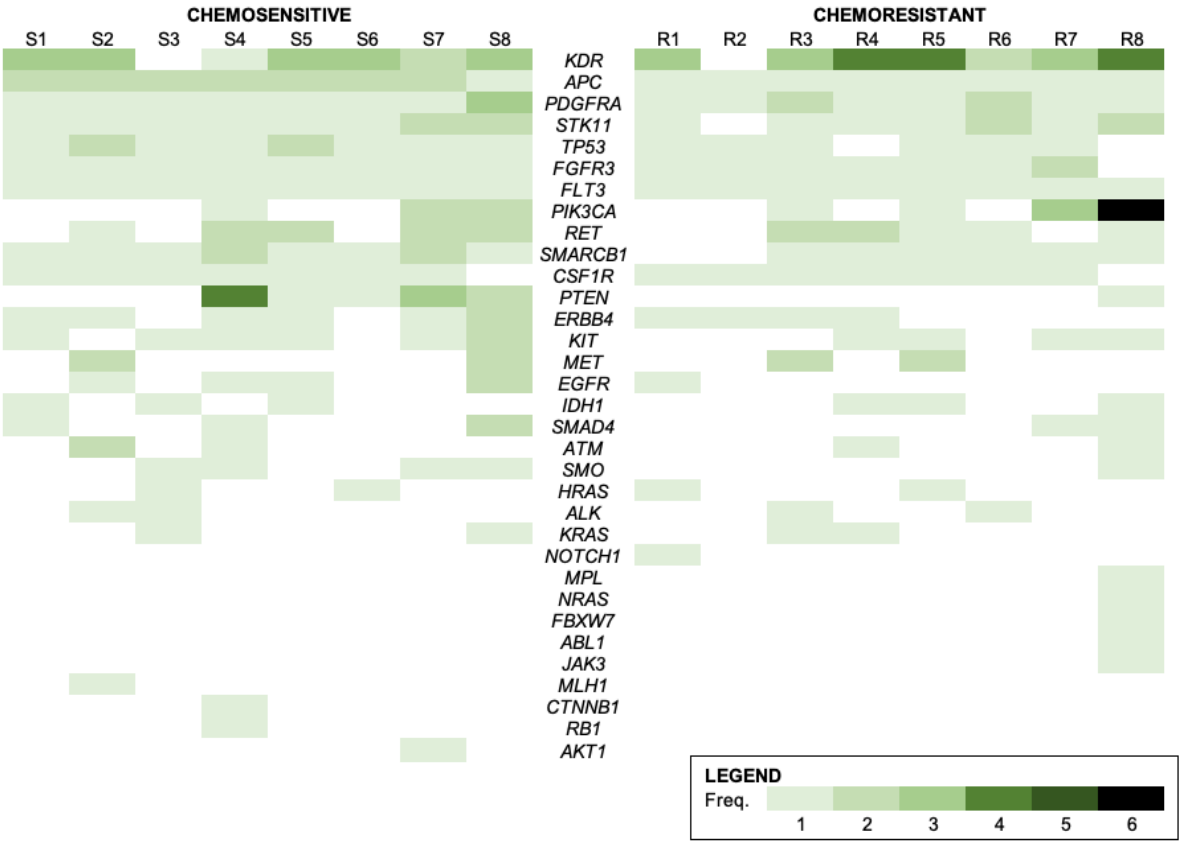

Figure 1. Frequency Heatmap of Genetic Variations Across Chemosensitive and Chemoresistant EOC Specimens.

Table 2. Summary of Genetic Variants in both Chemosensitive and Chemoresistant EOC Groups.

	Chemosensitive EOC group (n=8)	Chemoresistant EOC group (n=8)
Total genetic variants	168	137
Unique variants	63	50
Variants exclusive to the group	35	22
Type of alteration		
Insertion-deletion (Indel)	2	2
Single nucleotide variant (SNV)	33	20
Multi-nucleotide variant (MNV)	0	0
SNVs by location		
Exonic	28	12
Non-exonic	5	8
Exonic SNVs by effect		
Synonymous	7	5
Missense	18	7
Nonsense	3	0

unique variants were shared between chemosensitive and chemoresistant EOC specimens. These 28 common variants were mostly single nucleotide variants or SNVs (26, 92.86%), with 1 insertion-deletion and 1 multi-

Table 3. Frequency of Genetic Variants Common to Both Chemosensitive and Chemoresistant EOC Specimens.

Position	Type	Geno-type	Gene	Location	Functional Consequence	Frequency	
						CS	CR
chr2:209113192	SNV	G/A	*IDH1*	exonic	synonymous	3	3
chr2:212812097	SNV	T/C	*ERBB4*	intronic	-	5	3
chr2:29432625	SNV	C/A	*ALK*	intronic	-	1	2
chr4:1807894	SNV	G/A	*FGFR3*	exonic	synonymous	8	7
chr4:55141055	SNV	A/G	*PDGFRA*	exonic	synonymous	8	8
chr4:55152040	SNV	C/T	*PDGFRA*	exonic	synonymous	1	2
chr4:55593464	SNV	A/C	*KIT*	exonic	missense	1	2
chr4:55593481	SNV	A/G	*KIT*	exonic	synonymous	5	2
chr4:55946354	SNV	G/T	*KDR*	intronic	-	3	4
chr4:55962545	INDEL	-/G	*KDR*	intronic	-	3	4
chr4:55972974	SNV	T/A	*KDR*	exonic	missense	4	7
chr4:55980239	SNV	C/T	*KDR*	intronic	-	6	6
chr5:112175770	SNV	G/A	*APC*	exonic	synonymous	8	8
chr5:149433596	MNV	TG/GA	*CSF1R*	3' UTR	-	7	7
chr7:55249063	SNV	G/A	*EGFR*	exonic	synonymous	3	1
chr7:116339672	SNV	C/T	*MET*	exonic	synonymous	1	2
chr7:116340262	SNV	A/G	*MET*	exonic	missense	1	2
chr10:43613843	SNV	G/T	*RET*	exonic	synonymous	5	5
chr10:43615633	SNV	C/G	*RET*	exonic	synonymous	3	2
chr11:534242	SNV	A/G	*HRAS*	exonic	synonymous	2	2
chr12:25398284	SNV	C/T	*KRAS*	exonic	missense	1	2
chr13:28610183	SNV	A/G	*FLT3*	intronic	-	8	8
chr17:7579472	SNV	G/C	*TP53*	exonic	missense	8	5
chr18:48586344	SNV	C/T	*SMAD4*	intronic	-	2	2
chr19:1220321	SNV	T/C	*STK11*	intronic	-	8	7
chr19:1223125	SNV	C/G	*STK11*	exonic	missense	2	2
chr22:24134064	SNV	C/A	*SMARCB1*	exonic	missense	6	3
chr22:24176287	SNV	G/A	*SMARCB1*	intronic	-	4	3

Table 4. Frequency of Exclusive Genetic variants in Chemosensitive and Chemoresistant EOC Specimens.

Position	Type	Genotype	*Gene*	Location	Functional Consequence	Freq.
Chemosensitive EOC specimens only						
chr2:212576848	SNV	T/C	*ERBB4*	exonic	missense	1
chr2:212578395	SNV	G/A	*ERBB4*	intronic		1
chr2:29443617	SNV	C/G	*ALK*	exonic	synonymous	1
chr3:178916876	SNV	G/A	*PIK3CA*	exonic	missense	2
chr3:178921547	SNV	C/T	*PIK3CA*	exonic	synonymous	2
chr3:178927980	SNV	T/C	*PIK3CA*	exonic	missense	1
chr3:37067240	SNV	T/A	*MLH1*	exonic	missense	1
chr3:41266113	SNV	C/A	*CTNNB1*	exonic	missense	1
chr4:55144628	SNV	C/T	*PDGFRA*	exonic	missense	1
chr4:55597497	SNV	C/T	*KIT*	5' splice site		1
chr4:55972955	SNV	G/A	*KDR*	exonic	missense	1
chr4:55979624	SNV	G/A	*KDR*	exonic	nonsense	1
chr5:112175952	INDEL	A/-	*APC*	exonic	frameshift deletion	7
chr7:116339662	SNV	G/A	*MET*	exonic	missense	1
chr7:116340176	SNV	C/T	*MET*	exonic	synonymous	1
chr7:128845088	SNV	A/G	*SMO*	exonic	synonymous	4
chr7:55211110	SNV	C/T	*EGFR*	exonic	missense	1
chr7:55241701	SNV	G/A	*EGFR*	exonic	missense	1
chr10:43617372	SNV	C/T	*RET*	intronic		1
chr10:89692905	SNV	G/A	*PTEN*	exonic	missense	2
chr10:89711899	SNV	C/T	*PTEN*	exonic	missense	2
chr10:89711910	SNV	T/G	*PTEN*	exonic	nonsense	1
chr10:89720725	SNV	T/C	*PTEN*	exonic	synonymous	1
chr10:89720812	INDEL	A/-	*PTEN*	exonic	frameshift deletion	4
chr10:89720870	SNV	T/G	*PTEN*	exonic	missense	1
chr11:108137941	SNV	C/A	*ATM*	exonic	nonsense	1
chr11:108180917	SNV	T/C	*ATM*	exonic	synonymous	1
chr11:108236264	SNV	C/G	*ATM*	3' UTR		1
chr12:25378656	SNV	T/C	*KRAS*	exonic	synonymous	1
chr13:48942677	SNV	G/T	*RB1*	exonic	missense	1
chr14:105246565	SNV	C/T	*AKT1*	intronic		1
chr17:7577108	SNV	C/A	*TP53*	exonic	missense	1
chr17:7577570	SNV	C/A	*TP53*	exonic	missense	1
chr18:48591907	SNV	C/T	*SMAD4*	exonic	missense	1
chr18:48603030	SNV	A/T	*SMAD4*	exonic	missense	1
Chemoresistant EOC specimens only						
chr1:115256542	SNV	C/T	*NRAS*	exonic	missense	1
chr1:43815034	SNV	C/T	*MPL*	intronic		1
chr2:212578389	SNV	A/G	*ERBB4*	intronic		1
chr3:178917005	SNV	A/G	*PIK3CA*	intronic		3
chr3:178936091	SNV	G/A	*PIK3CA*	exonic	missense	2
chr3:178952020	SNV	C/T	*PIK3CA*	exonic	synonymous	2
chr3:178952085	SNV	A/G	*PIK3CA*	exonic	missense	1
chr3:178952151	SNV	G/A	*PIK3CA*	exonic	synonymous	1
chr3:178952202	SNV	T/C	*PIK3CA*	3' UTR		1
chr3:178952228	SNV	G/A	*PIK3CA*	3' UTR		1
chr4:153250867	SNV	G/A	*FBXW7*	exonic	missense	1
chr4:1807864	SNV	C/T	*FGFR3*	exonic	synonymous	1

Table 4. Continued

Position	Type	Genotype	Gene	Location	Functional Consequence	Freq.
Chemoresistant EOC specimens only						
chr4:55953852	SNV	G/A	*KDR*	exonic	missense	1
chr4:55973048	SNV	G/A	*KDR*	intronic		1
chr7:128845966	SNV	A/G	*SMO*	intronic		1
chr9:133738374	SNV	G/A	*ABL*	exonic	synonymous	1
chr9:139399411	INDEL	CCA/-	*NOTCH1*	exonic	non-frameshift deletion	1
chr10:89692902	SNV	G/A	*PTEN*	exonic	missense	1
chr11:108225611	SNV	T/C	*ATM*	intronic		1
chr11:108236232	SNV	G/A	*ATM*	exonic	synonymous	1
chr17:7579437	INDEL	C/-	*TP53*	exonic	frameshift deletion	1
chr19:17954206	SNV	C/T	*JAK3*	exonic	missense	1

nucleotide variant. Eighteen SNVs were exonic, of which 11 were silent mutations, and 7 were missense mutations.

In the chemosensitive EOC group, 33 of the 35 exclusive variants were SNVs, while 2 were insertion-deletion. Of the SNVs, 28 (84.85%) were exonic, of which 18 were missense mutations, 3 were nonsense mutations, and 7 were silent mutations. On the other hand, 20 of the 22 variants exclusive to the chemoresistant EOC group were SNVs, of which 12 (60.00%) were exonic. Five exonic SNVs were silent mutations, while seven were missense mutations.

Genetic variants common to all EOC specimens

Of the 305 non-unique genetic variants across 16 EOC specimens, KDR genetic variants had the highest frequency with 41 (13.44%), followed by APC (23, 7.54%), PDGFRA (20, 6.56%), and STK11 (19, 6.23%). FGFR3, FLT3, PIK3CA, RET, and TP53 each had 16 variants, accounting for 5.25% each. The frequency of the variants per gene in each specimen is shown in Figure 1. Table 3 shows the genetic variants common to chemosensitive and chemoresistant EOC specimens. SNVs in PDGFRA, APC, and FLT3 were present in all 16 specimens, followed by STK11 and FGFR3 (n=15 each). The most common SNVs which resulted in alteration in protein sequence were missense mutations in TP53 (n=13), KDR (n=11), and SMARCB1 (n=9). The gene with the most variants was KDR, with 1 insertion-deletion and 3 SNVs, of which 1 was a missense mutation. KDR was followed by PDGFRA, KIT, MET, RET, STK11 and SMARCB1 with 2 genetic variants each. Of the five genes, only MET and SMARCB1 had a genetic variant that resulted in a missense mutation. There were 7 variants that resulted in protein modification due to missense mutation in KIT, KDR, MET, KRAS, TP53, STK11 or SMARCB1.

Exclusive genetic variants in chemosensitive and chemoresistant EOC

There were 35 genetic variants found only in chemosensitive EOC specimens, as shown in Table 4. Twenty-three exclusive variants resulted in protein modification, 18 of which were due to missense mutations, 3 to nonsense mutations, and 2 to frameshift deletion. A frameshift deletion in APC was the most common genetic variant in this group (n=7), followed by a frameshift deletion in PTEN and an SNV in SMO (both n=4 each). The gene with the most variants was PTEN with 1 frameshift deletion as mentioned and 5 SNVs, of which 3 were missense mutations and 1 was a nonsense mutation. PTEN was followed by PIK3CA and ATM with 3 genetic variants each. In terms of variants that resulted in protein modifications, PTEN was followed by KDR at 1 missense and 2 nonsense mutations, followed by PIK3CA, EGFR, SMAD4 and TP53 with 2 missense mutations each. MLH1, CTNNB1, RB1, and AKT1 variants were found only in chemosensitive EOC group.

On the other hand, of the 22 chemoresistant EOC-exclusive genetic variants, 9 resulted in protein modification. All genetic variants were present in 3 specimens or less. The gene with the most variants was PIK3CA with 6 SNVs. Interestingly, all 6 SNVs were found in one specimen. PIK3CA (2 missense) and PTEN (1 non-frameshift deletion, 1 missense) were the genes with most variants resulting in protein modification. MPL, NRAS, FBXW7, NOTCH1, ABL1 and JAK3 variants were exclusive to chemoresistant EOC group.

In silico functional prediction reveals potentially damaging gene variants

Table 5 shows the 39 unique protein-modifying genetic variants identified in all EOC specimens. Seven protein-modifying genetic variants were common to both EOC groups, all of which have been previously reported in dbSNP. Using ClinVar as reference database, 4 of these variants were deemed benign or likely benign, 1 was pathogenic, and 1 was not previously reported in ClinVar. The remaining variant had no interpretation provided.

Of the 23 protein-modifying variants in chemosensitive EOC group, 13 were previously deposited in dbSNP. Eight of these 13 variants were reported as pathogenic or likely pathogenic, 1 was reported to be benign, 3 were of uncertain significance, and 1 was not previously reported in ClinVar. In particular, PIK3CA, PTEN, and TP53 variants described in this study were found to be associated with pathogenic conditions. Using PolyPhen-2 to predict functional effect of amino acid substitution in 8 previously unreported SNVs, 6 variants were predicted to be deleterious while 2 were predicted to be benign.

Table 5. *In silico* Functional Prediction of Exonic Genetic Variants in Chemosensitive and Chemoresistant EOC Specimens.

Position	Genotype	Gene	Change	PolyPhen-2 HumDiv score	dbSNP	ClinVar Interpretation	ClinVar Var ID
Common to chemosensitive and chemoresistant EOC specimens							
chr4:55593464	A/C	KIT	M541L	Benign (0.009)	rs3822214	Benign/Likely benign	41599
chr4:55972974	T/A	KDR	Q472H	Benign (0.003)	rs1870377	Not provided	134603
chr7:116340262	A/G	MET	N375S	Benign (0.038)	rs33917957	Benign	41611
chr12:25398284	C/T	KRAS	G12D	Benign (0.380)	rs121913529	Pathogenic	12582
chr17:7579472	G/C	TP53	P72R	Benign (0.083)	rs1042522	Benign	12351
chr19:1223125	C/G	STK11	F354L	Benign (0.025)	rs59912467	Benign/Likely benign	7461
chr22:24134064	C/A	SMARCB1	T72K	Benign (0.008)	rs1568936152	Not Reported in ClinVar	n/a
Chemosensitive EOC specimens only							
chr2:212576848	T/C	ERBB4	S351G	Probably Damaging (0.985)	n/a	n/a	n/a
chr3:178916876	G/A	PIK3CA	R88Q	Probably Damaging (1.000)	rs121913287	Pathogenic	376049
chr3:178927980	T/C	PIK3CA	C420R	Probably Damaging (0.997)	rs121913272	Pathogenic	31945
chr3:37067240	T/A	MLH1	V384D	Probably Damaging (1.000)	rs63750447	Benign	41632
chr3:41266113	C/A	CTNNB1	S37Y	Probably Damaging (1.000)	rs121913403	Pathogenic/Likely pathogenic	375894
chr4:55144628	C/T	PDGFRA	P701L	Benign (0.027)	n/a	n/a	n/a
chr4:55972955	G/A	KDR	P479S	Probably Damaging (0.999)	n/a	n/a	n/a
chr4:55979624	G/A	KDR	R275*	n/a	rs1720648779	Not Reported in ClinVar	n/a
chr5:112175952	A/-	APC	E1554fs	n/a	n/a	n/a	n/a
chr7:116339662	G/A	MET	C175Y	Probably Damaging (1.000)	rs1584877055	Uncertain significance	948372
chr7:55211110	C/T	EGFR	A118V	Probably Damaging (1.000)	n/a	n/a	n/a
chr7:55241701	G/A	EGFR	V717M	Probably Damaging (0.998)	n/a	n/a	n/a
chr10:89692905	G/A	PTEN	R130Q	Probably Damaging (1.000)	rs121909229	Pathogenic	7829
chr10:89711899	C/T	PTEN	R173C	Probably Damaging (1.000)	rs121913293	Pathogenic	189500
chr10:89711910	T/G	PTEN	Y176*	n/a	rs1057522285	Likely pathogenic	492732
chr10:89720812	A/-	PTEN	N323fs	n/a	rs121913291	Likely pathogenic	928675
chr10:89720870	T/G	PTEN	F341V	Probably Damaging (1.000)	rs1554825652	Uncertain significance	495806
chr11:108137941	C/A	ATM	S837*	n/a	n/a	n/a	n/a
chr13:48942677	G/T	RB1	R355I	Probably Damaging (0.959)	n/a	n/a	n/a
chr17:7577108	C/A	TP53	C277F	Probably Damaging (1.000)	rs763098116	Uncertain significance	458567
chr17:7577570	C/A	TP53	M237I	Probably Damaging (1.000)	rs587782664	Likely pathogenic	634770

Table 5. Continued

Position	Genotype	Gene	Change	PolyPhen-2 HumDiv score	dbSNP	ClinVar Interpretation	Var ID
Chemosensitive EOC specimens only							
chr18:48591907	C/T	SMAD4	S357F	Probably Damaging (1.000)	n/a	n/a	n/a
chr18:48603030	A/T	SMAD4	H444L	Benign (0.109)	n/a	n/a	n/a
Chemoresistant EOC specimens only							
chr1:115256542	C/T	NRAS	D57N	Probably Damaging (0.996)	rs1465850103	Not Reported in ClinVar	n/a
chr3:178936091	G/A	PIK3CA	E545K	Probably Damaging (0.970)	rs104886003	Pathogenic/Likely pathogenic	13655
chr3:178952085	A/G	PIK3CA	H1047R	Benign (0.263)	rs121913279	Pathogenic	13652
chr4:153250867	G/A	FBXW7	S398F	Probably Damaging (1.000)	n/a	n/a	n/a
chr4:55953852	G/A	KDR	P1195L	Possibly Damaging (0.780)	rs748810441	Not Reported in ClinVar	n/a
chr9:139399411	CCA/-	NOTCH1	V1578del	n/a	rs761020817	Not Reported in ClinVar	n/a
chr10:89692902	G/A	PTEN	G129E	Probably Damaging (1.000)	rs121909218	Pathogenic	7812
chr17:7579437	C/-	TP53	A84fs	n/a	rs1597374343	Pathogenic	646068
chr19:17954206	C/T	JAK3	E135K	Benign (0.004)	n/a	n/a	n/a

On the other hand, of the 9 protein-modifying variants in chemoresistant EOC group, 7 were reported in dbSNP. Four variants were reported in ClinVar as pathogenic or likely pathogenic, while 3 were not previously reported in ClinVar. Of the 2 unreported SNVs, the FBXW7 variant was predicted to be damaging while the JAK3 variant was predicted to be benign.

Discussion

Based on a small follow-up prevalence study of protein-modifying FBXW7, KDR, NOTCH1 and PTEN variants seen exclusively in chemoresistant EOC (Supplementary Table B), no samples harbored the selected genetic variants, implying that these variants exist in low frequency i.e. sporadic. However, there were some interesting observations obtained from this study. KDR, which encodes for vascular epithelial growth factor receptor 2 or VEGFR-2, was found to be the gene with the most common alterations in all EOC specimens in this study. As KDR has the most significant prevalence of alterations in lung adenocarcinoma, colon adenocarcinoma, cutaneous melanoma (American Association for Cancer Research (AACR) Project GENIE Consortium, 2017), the role of KDR variants in epithelial ovarian cancer needs further scrutiny. Six of the KDR variants were already described in dbSNP, of which 4 did not have clinical significance yet as reported in ClinVar database. A missense mutation (genotype A>T) at chr4:55972974, which resulted in Q472H alteration of VEGFR-2, has been implicated in cancer susceptibility, but was not previously reported in ovarian cancer (Bodian et al., 2014). Two SNVs, one at chr4:55972955 and one at chr4:55973048 have not been deposited in dbSNP, thus this study is the first to report these mutations. In particular, the missense mutation at chr4:55972955 resulted in P479S alteration, which might increase cancer susceptibility.

Of the protein-modifying genetic variants in chemoresistant EOC, three have been described in Personalized Cancer Therapy Knowledge Base for Precision Oncology. This knowledge base provides information on the functional effects of these genetic variants and their therapeutic implications (Dumbrava and Meric-Bernstam, 2018). A missense mutation in PIK3CA resulted in E545K alteration, an activating alteration associated with increased cell proliferation, colony formation, and invasiveness (Bader et al., 2006; Dogruluk et al., 2015; Gymnopoulos et al., 2007; Ikenoue et al., 2005; Kang et al., 2005; Ng et al., 2018; Samuels et al., 2005; Zhang et al., 2008). Another missense mutation in PIK3CA resulted in H1047R alteration within the kinase domain. The most frequently encountered alteration in somatic cancer results in the activation of PI3K/AKT/mTOR pathway to induce cell proliferation and survival, colony formation, and anchorage-independent growth (Berenjeno et al., 2017; Chang et al., 2016; Hart et al., 2015; Zhang et al., 2008). On the other hand, a missense mutation in PTEN resulted in G129E alteration deficient in lipid phosphatase activity (Furnari et al., 1998; Han et al., 2000; Leslie et al., 2007; Myers et al., 1998). This alteration, which has been reported in gliomas, endometrial

cancers, melanomas as a somatic mutation, and Cowden syndrome as a germline mutation, is associated with colony formation and increased cell growth (Byron et al., 2008; Furnari et al., 1998; Hansen-Kiss et al., 2017; Steelman et al., 2008; Van Allen et al., 2014; Wang et al., 2000). The two PIK3CA alterations were responsive to PI3K/AKT/mTOR inhibitors, while the PTEN alteration was responsive to mTOR inhibitor rapamycin, implying an opportunity for targeted therapy given the resistance of cancerous lesions to platinum-based chemotherapy (Beaver et al., 2013; Elkabets et al., 2013; Garnett et al., 2012; Gonzalez-Angulo and Blumenschein, 2013; Janku et al., 2014; Liu et al., 2011; Mayer et al., 2014; Ng et al., 2018; Rashmi et al., 2014; Sangai et al., 2012; Steelman et al., 2008; Zhao et al., 2016).

Two deletions were found in chemoresistant EOC specimens, both at exonic locations. A non-frameshift deletion at NOTCH1 gene resulted to deletion of one valine residue in the LVVVL sequence between residue 1570 and 1580. On the other hand, a deletion at TP53 gene resulted in a frameshift starting at residue 84 of p53. While TP53 mutations were long implicated in platinum chemoresistance in ovarian cancer (Reles et al., 2001), the relationship of Notch signaling with platinum chemoresistance has only been described previously in non-small cell lung cancer and colon cancer (Kukcinaviciute et al., 2018; Zhang et al., 2017). Of the genes with variants exclusively ascribed to chemoresistant EOC in this study, JAK3 is notable as mutations in JAK could affect downstream response of tumor cells to various interleukins such as IL-6 and IL-7 leading to platinum chemoresistance (Meng et al., 2020; Sun et al., 2019).

Although our study includes a small sample of patients, to our knowledge, this is the first study conducted to study the genetic profiling of Filipino ovarian cancer patients outside of BRCA1 and BRCA2. As this is a descriptive study, we recommend functional studies looking into the effect of these unreported mutations not previously ascribed to cancer. Future direction includes conducting a prospective cohort study with larger sample size to identify potential prognostic and predictive biomarkers in conjunction with sonographic findings to help improve clinical outcomes in women with EOC.

Author Contribution Statement

A.J.P.P., E.F.L.C., I.Y. conceptualized and designed the study, with additional input from R.C.V.L.; A.J.P.P., Y.N., I.Y. performed the experiments and collected the data; R.C.V.L., A.J.P.P., Y.N., I.Y. analyzed the data; R.C.V.L., A.J.P.P., Y.N., E.F.L.C., I.Y. interpreted the results; R.C.V.L. prepared the initial manuscript; R.C.V.L., A.J.P.P., Y.N., E.F.L.C., I.Y. reviewed and approved the final version of manuscript.

Acknowledgements

General

Two of the authors (R.C.V.L. and A.J.P.P.) are current and past trainees, respectively, of the MD-PhD in Molecular Medicine Program, supported by Department of Science and Technology - Philippine Council for Health Research and Development (DOST-PCHRD), and administered through University of the Philippines Manila.

Funding Statement

This study was supported by research grants from JSPS KAKENHI, Grant Number JP20H03564 (I.Y.).

Approval

This study was approved as an independent research work by the Technical Review Board of the Department of Obstetrics and Gynecology, College of Medicine and Philippine General Hospital, University of the Philippines Manila.

Ethical Declaration

This study underwent ethical review by the University of the Philippines Manila Research Ethics Board (UPMREB), and was performed in accordance with the principles stated in the Declaration of Helsinki.

Data Availability

Any information needed to reanalyze the data is available from the corresponding author upon reasonable request.

Conflict of Interest

All authors have no personal and/or financial conflicts of interest to declare.

References

Adzhubei IA, Schmidt S, Peshkin L, et al (2010). A method and server for predicting damaging missense mutations. *Nat Methods*, **7**, 248-9.

American Association for Cancer Research (AACR) Project GENIE Consortium (2017). AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discov*, **7**, 818-31.

Bader AG, Kang S, Vogt PK (2006). Cancer-specific mutations in PIK3CA are oncogenic in vivo. *Proc Natl Acad Sci U S A*, **103**, 1475-9.

Beaver JA, Gustin JP, Yi KH, et al (2013). PIK3CA and AKT1 mutations have distinct effects on sensitivity to targeted pathway inhibitors in an isogenic luminal breast cancer model system. *Clin Cancer Res*, **19**, 5413-22.

Berek JS, Kehoe ST, Kumar L, Friedlander M (2018). Cancer of the ovary, fallopian tube, and peritoneum. *Int J Gynaecol Obstet*, **143**, 59-78.

Berenjeno IM, Piñeiro R, Castillo SD, et al (2017). Oncogenic PIK3CA induces centrosome amplification and tolerance to genome doubling. *Nat Commun*, **8**, 1773.

Bodian DL, McCutcheon JN, Kothiyal P, et al (2014). Germline variation in cancer-susceptibility genes in a healthy, ancestrally diverse cohort: implications for individual genome sequencing. *PLoS One*, **9**, e94554.

Buys SS, Partridge E, Black A, et al (2011). Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. *JAMA*, **305**, 2295-303.

Byron SA, Gartside MG, Wellens CL, et al (2008). Inhibition of activated fibroblast growth factor receptor 2 in endometrial cancer cells induces cell death despite PTEN abrogation.

Cancer Res, **68**, 6902-7.
Chang MT, Asthana S, Gao SP, et al (2016). Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat Biotechnol*, **34**, 155-63.
De Leon Matsuda ML, Liede A, Kwan E, et al (2002). BRCA1 and BRCA2 mutations among breast cancer patients from the Philippines. *Int J Cancer*, **98**, 596-603.
Dogruluk T, Tsang YH, Espitia M, et al (2015). Identification of Variant-Specific Functions of PIK3CA by Rapid Phenotyping of Rare Mutations. *Cancer Res*, **75**, 5341-54.
Doubeni CA, Doubeni AR, Myers AE (2016). Diagnosis and Management of Ovarian Cancer. *Am Fam Physician*, **93**, 937-44.
Dumbrava EI, Meric-Bernstam F (2018). Personalized cancer therapy-leveraging a knowledge base for clinical decision-making. *Cold Spring Harb Mol Case Stud*, **4**, a001578.
Elkabets M, Vora S, Juric D, et al (2013). mTORC1 inhibition is required for sensitivity to PI3K p110α inhibitors in PIK3CA-mutant breast cancer. *Sci Transl Med*, **5**, 196ra199.
Furnari FB, Huang HJ, Cavenee WK (1998). The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. *Cancer Res*, **58**, 5002-8.
Garnett MJ, Edelman EJ, Heidorn SJ, et al (2012). Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature*, **483**, 570-5.
Gonzalez-Angulo AM, Blumenschein GR (2013). Defining biomarkers to predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer. *Cancer Treat Rev*, **39**, 313-20.
Gymnopoulos M, Elsliger MA, Vogt PK (2007). Rare cancer-specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci U S A*, **104**, 5569-74.
Han SY, Kato H, Kato S, et al (2000). Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay. *Cancer Res*, **60**, 3147-51.
Hansen-Kiss E, Beinkampen S, Adler B, et al (2017). A retrospective chart review of the features of PTEN hamartoma tumour syndrome in children. *J Med Genet*, **54**, 471-8.
Hart JR, Zhang Y, Liao L, et al (2015). The butterfly effect in cancer: a single base mutation can remodel the cell. *Proc Natl Acad Sci U S A*, **112**, 1131-6.
Ikenoue T, Kanai F, Hikiba Y, et al (2005). Functional analysis of PIK3CA gene mutations in human colorectal cancer. *Cancer Res*, **65**, 4562-7.
Janku F, Hong DS, Fu S, et al (2014). Assessing PIK3CA and PTEN in early-phase trials with PI3K/AKT/mTOR inhibitors. *Cell Rep*, **6**, 377-87.
Jelovac D, Armstrong DK (2011). Recent progress in the diagnosis and treatment of ovarian cancer. *CA Cancer J Clin*, **61**, 183-203.
Kang S, Bader AG, Vogt PK (2005). Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci U S A*, **102**, 802-7.
Kent WJ, Sugnet CW, Furey TS, et al (2002). The human genome browser at UCSC. *Genome Res*, **12**, 996-1006.
Konstantinopoulos PA, Norquist B, Lacchetti C, et al (2020). Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *J Clin Oncol*, **38**, 1222-45.
Kukcinaviciute E, Jonusiene V, Sasnauskiene A, et al (2018). Significance of Notch and Wnt signaling for chemoresistance of colorectal cancer cells HCT116. *J Cell Biochem*, **119**, 5913-20.
Landrum MJ, Lee JM, Benson M, et al (2018). ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res*, **46**, D1062-7.
Laudico AV, Mirasol-Lumague MR, Medina V, et al (2015). Philippine Cancer Facts and Estimates. Philippine Cancer Society, Manila.
Leslie NR, Yang X, Downes CP, Weijer CJ (2007). PtdIns(3,4,5) P(3)-dependent and -independent roles for PTEN in the control of cell migration. *Curr Biol*, **17**, 115-25.
Lheureux S, Gourley C, Vergote I, Oza AM (2019). Epithelial ovarian cancer. *Lancet*, **393**, 1240-53.
Liu R, Liu D, Trink E, et al (2011). The Akt-specific inhibitor MK2206 selectively inhibits thyroid cancer cells harboring mutations that can activate the PI3K/Akt pathway. *J Clin Endocrinol Metab*, **96**, 577-85.
Mayer IA, Abramson VG, Isakoff SJ, et al (2014). Stand up to cancer phase Ib study of pan-phosphoinositide-3-kinase inhibitor buparlisib with letrozole in estrogen receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol*, **32**, 1202-9.
Meng J, Liu K, Shao Y, et al (2020). ID1 confers cancer cell chemoresistance through STAT3/ATF6-mediated induction of autophagy. *Cell Death Dis*, **11**, 137.
Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H (2019). Ovarian cancer in the world: epidemiology and risk factors. *Int J Womens Health*, **11**, 287-99.
Myers MP, Pass I, Batty IH, et al (1998). The lipid phosphatase activity of PTEN is critical for its tumor supressor function. *Proc Natl Acad Sci U S A*, **95**, 13513-8.
Nato AQ (2003). Risk modeling and screening for BRCAI mutations among Filipino breast cancer patients [Thesis]. University of the Philippines Diliman, Quezon City.
Ng PK, Li J, Jeong KJ, et al (2018). Systematic Functional Annotation of Somatic Mutations in Cancer. *Cancer Cell*, **33**, 450-62.
Que FVF, Ang D, Andal JJ, et al (2018). Incidence of germline BRCA1 and BRCA2 mutations among Filipinos. *J Clin Oncol*, **36**, e13628.
Rashmi R, DeSelm C, Helms C, et al (2014). AKT inhibitors promote cell death in cervical cancer through disruption of mTOR signaling and glucose uptake. *PLoS One*, **9**, e92948.
Reles A, Wen WH, Schmider A, et al (2001). Correlation of p53 mutations with resistance to platinum-based chemotherapy and shortened survival in ovarian cancer. *Clin Cancer Res*, **7**, 2984-97.
Samuels Y, Diaz LA, Schmidt-Kittler O, et al (2005). Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell*, **7**, 561-73.
Sangai T, Akcakanat A, Chen H, et al (2012). Biomarkers of response to Akt inhibitor MK-2206 in breast cancer. *Clin Cancer Res*, **18**, 5816-28.
Sherry ST, Ward MH, Kholodov M, et al (2001). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*, **29**, 308-11.
Steelman LS, Navolanic PM, Sokolosky ML, et al (2008). Suppression of PTEN function increases breast cancer chemotherapeutic drug resistance while conferring sensitivity to mTOR inhibitors. *Oncogene*, **27**, 4086-95.
Stuart GC, Kitchener H, Bacon M, et al (2011). 2010 Gynecologic Cancer InterGroup (GCIG) consensus statement on clinical trials in ovarian cancer: report from the Fourth Ovarian Cancer Consensus Conference. *Int J Gynecol Cancer*, **21**, 750-5.
Sun CY, Nie J, Huang JP, Zheng GJ, Feng B (2019). Targeting STAT3 inhibition to reverse cisplatin resistance. *Biomed Pharmacother*, **117**, 109135.
Torre LA, Trabert B, DeSantis CE, et al (2018). Ovarian cancer statistics, 2018. *CA Cancer J Clin*, **68**, 284-96.
Van Allen EM, Wagle N, Sucker A, et al (2014). The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov*, **4**, 94-109.

Wang X, Gjörloff-Wingren A, Saxena M, et al (2000). The tumor suppressor PTEN regulates T cell survival and antigen receptor signaling by acting as a phosphatidylinositol 3-phosphatase. *J Immunol*, **164**, 1934-9.

Zhang H, Liu G, Dziubinski M, et al (2008). Comprehensive analysis of oncogenic effects of PIK3CA mutations in human mammary epithelial cells. *Breast Cancer Res Treat*, **112**, 217-27.

Zhang Y, Xu W, Guo H, et al (2017). NOTCH1 Signaling Regulates Self-Renewal and Platinum Chemoresistance of Cancer Stem-like Cells in Human Non-Small Cell Lung Cancer. *Cancer Res*, **77**, 3082-91.

Zhao S, Cao Y, Liu SB, et al (2016). The E545K mutation of PIK3CA promotes gallbladder carcinoma progression through enhanced binding to EGFR. *J Exp Clin Cancer Res*, **35**, 97.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.