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NGS Analysis of the *BRCA*1/*BRCA*2 Variants in the Ovarian Cancer Patients at High versus Low Risk of Mutation

Dinh-Toi Chu, PhD*,**,
Nhat-Le Bui, BPharm, MSc*,**,
Hue Vu Thi, MSc*,**,
Thuy-Duong Vu, BPharm*,
Van-Thai Than, PhD*,**,
Yen Vy Nguyen Thi, BA*,**,
Duy Ha Nguyen, M.D., PhD***,
Quang Ngoc Nguyen, PhD****,
Thanh Hoa Nguyen Thi, M.D., MSc****,
Truong Dang Tien, M.D., PhD***,
Binh Pham Van, M.D., PhD****

** Center for Biomedicine and Community Health, International School, Vietnam National University, Hanoi, Vietnam*
*** Faculty of Applied Sciences, International School, Vietnam National University, Hanoi, Vietnam*
**** Vietnam Military Medical University, Hanoi, Vietnam*
***** Vietnam National Cancer Hospital, Hanoi, Vietnam*

ABSTRACT

Objectives: The pathogenic variant in breast cancer susceptibility gene 1/2 (*BRCA1/2*) is related to the risk of ovarian cancer, which is one of the most common cancers among women worldwide. Here, we aimed to understand the variants of these genes among Vietnamese population to improve early detection and diagnosis as well as treatment decisions.

Materials and Methods: *BRCA1/2* genes of tumor tissue from 33 ovarian cancer patients in high-risk and low-risk of mutation groups were sequenced by next-generation sequencing (NGS) using the *BRCA*accuTest™ PLUS kit.

Results: Among a total of 33 distinguished variants detected, we found 5 pathogenic and likely pathogenic (P/LP) variants, including c.1801_1808delCACAATTC, c.1016delA, c.1673_1674delAA, c.928C>T in *BRCA*1 and c.2865delC in *BRCA*2 with a frequency of 3.03%, except for c.1801_1808delCACAATTC (6.06%). All variants led to defects in coding protein. c.1801_1808delCACAATTC variant in *BRCA*1 and c.2865delC in *BRCA*2 was firstly introduced. The prevalence of *BRCA1/2* P/LP variants in the high-risk group was 22.73%, while this figure for the low-risk group was 9.09%. No statistically significant difference in other clinical features was found between P/LP variant-carried and non-P/LP variant-carried patients apart from age and comorbidities.

Conclusions: This study provided precious data for *BRCA1/2* variant mapping among Vietnamese ovarian cancer patients.

Keywords: ovarian cancer, NGS analysis, *BRCA*1/*BRCA*2 variants, mutation.

Correspondence to: *Dinh-Toi Chu, PhD., Center for Biomedicine and Community Health, International School, Vietnam National University, Hanoi, Vietnam. E-mail: chudinhtoi.hnue@mail.com, toicd@vnu.edu.vn, and Binh Pham Van, M.D., PhD., Vietnam National Cancer Hospital, Hanoi, Vietnam. Email: binhva@yahoo.fr*

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Introduction

According to GLOBOCAN 2020, more than 300,000 new cases and more than 200,000 death were recorded to be related to ovarian cancer per year. That makes ovarian cancer become one of the most common cancers in women in both incidence and mortality rate[1]. In Vietnam, the GLOBOCAN 2020 report showed that the incidence rate is about 2.9 per 100,000 women in 2020[1]. Ovarian cancer includes 4 main stages: stage I - cancer cells limited to one or two ovaries, stage II - cancer cells spread to the uterus or some surrounding organs, stage III - metastatic cancer spreads to lymph nodes or abdominal mucosal areas, stage IV – the cancer has spread to more distant organs in the body[2]. Regarding the classification of ovarian cancer, there are 3 main types: germ cell, sex-cord-stromal and epithelial. The most common currently is epithelial (about 95%), while the other two are about 5%[2].

One difficulty in ovarian cancer treatment is that patients are ussually at the advanced stages at the first time of diagnosis. Due to its complex nature, the detection and treatment of ovarian cancer have been a major challenge for global health. In particular, the source of all challenges has been attributed to be the vague early symptoms of ovarian cancer. Women with ovarian cancers have often been detected postmenopausal; thus, the development of ovarian tumors usually does not cause pronounced symptoms until they reach a large size and spread[3]. That has significantly hindered the development of ovarian cancer screening and early detection measures. Therefore, most patients were detected at a late stage and had low therapeutic efficacy. Up to 90% of cases were diagnosed at a late stage when tumors have spread to the pelvic or abdominal area[3]. The 5-year survival rate of ovarian cancer patients who have been detected at an early stage is 70%; however, it is disturbing that it is only 35% at a late stage[4]. That is the urgent need to develop comprehensive ovarian cancer early detection measures and treatment.

There are many causes leading to ovarian cancer, including genetic variants, and one of the most well-known genetic causes was germline variants in the breast cancer susceptibility gene 1/2 (*BRCA*1/2) genes[5, 6]. A study of Kast et al indicated that germline *BRCA*1/2 variants contribute to about 25% of the heredity ovarian cancer and breast cancer across the world[7]. Moreover, since *BRCA*1 and *BRCA*2 genes play roles in producing proteins for the purpose of repairing damaged deoxyribonucleic acid (DNA), pathogenic variants in the *BRCA*1/2 genes are considered as one of the strong predictors of patients' response to a drug class called poly adenosine diphosphate (ADP)-ribose polymerase inhibitor (PARPi). While previous studies provided low evidence about the relationship between genetic variants and the risk of non-epithelial ovarian cancer like germ cell or sex-cord-stromal[8], according to Candido-dos-Reis et al, mutation in

*BRCA*1/2 is responsible for about 5% to 15% of epithelial ovarian cancer cases, which is the most common type of ovarian cancers. However, epithelial ovarian cancer patients having *BRCA*1/2 mutations have more advantage than non-carrier patients in short-term (5 years) survival[9]. Another study reported that mutation in *BRCA*1/2 gene increase the lifetime risk of epithelial ovarian cancer into more than 10 times compared to general population[10].

Nowadays, the potential of personalized therapies has increasingly unraveled, ushering in a new era of precision medicine. However, the researchers could not approach this therapy if the ovarian cancer genetic information had not been elucidated. Next-generation sequencing (NGS) is a new method with outstanding advancement in the molecular profiling of tumors, including ovarian tumors. Through the ability to analyze multiple genes in one assay, NGS can identify the variant quickly and guide treatment decisions[11]. The technology has many advantages over traditional gene sequencing, such as faster reading times, extensive output data, and cost savings[11]. Studies in ovarian have often used NGS to identify *BRCA*1/2 gene variants. For example, NGS combined with MyChoice CDx testing is able to detect *BRCA*1/2 pathogenic large arrangements in ovarian tumors, which is commonly challenging[12]. A study in Italy by Paola Concolino et al was conducted using NGS in 132 ovarian cancer patients[13]. The results detected 37 *BRCA*1/2 variants, including 7 new ones that had never been reported. Another study in ovarian cancer patients from the North Caucasus also used NGS to provide founder *BRCA*1/2 alleles in all ethnic groups in this area[14].

For countries with limited resources, ovarian cancer management has been seen as a significant challenge. In particular, the genetic characteristics of the disease can differ significantly between ethnic groups and races[15]. Therefore, it is essential to have a comprehensive view of the genetic characteristics of ovarian cancer in Vietnamese people. Moreover, identifying high-risk groups based on genetic history should also be emphasized to make screening more convenient. This study was conducted to study *BRCA*1 and *BRCA*2 gene variants characteristics in ovarian cancer patients in Vietnam using NGS. The evidence can be valuable documents to build a standard ovarian genetic panel for Vietnamese people to develop effective screening and treatment strategies, which aligns with the current precision medicine era.

Materials and Methods

Selection of study patients

Among 141 patients diagnosed with ovarian cancer and treated at the Vietnam National Cancer Hospital (Hanoi, Vietnam), we selected a total of 60 patients that their tissue specimens after surgery have still been stored at the hospital. We devided 60 patients into high-risk and low-risk group of having genetic variants according to the following criteria: i) High-risk group included 40 patients with a confirmed diagnosis of ovarian cancer by histopathological examination results, and had one or both criteria of cancer syndrome breast – hereditary ovarian cancer: 1) age under 40; 2) patients with a history of breast cancer; 3) there was at least one family member with ovarian cancer and/or breast cancer; 4) higher stage of the disease. ii) The low-risk group included the remaining. There were 22 patients over 40 patients in the high-risk group and 11 patients over 20 patients in the low-risk group whose specimens were qualified for DNA extraction and sequencing (Fig. 1). Their medical information were collected from their medical records.

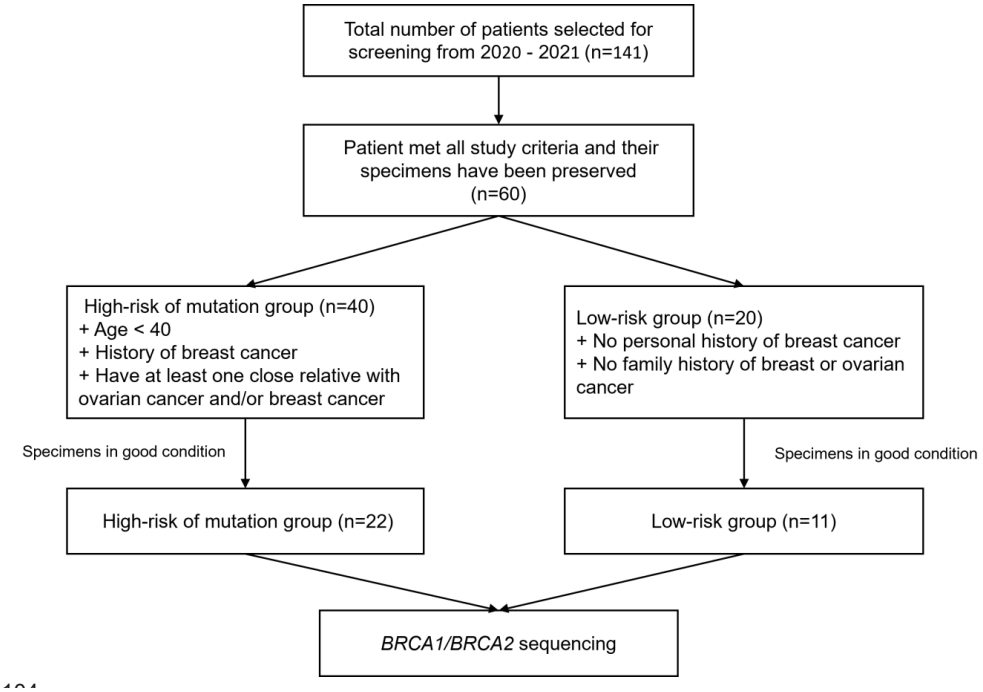

Fig. 1. Selection of study objects.

BRCA: breast cancer susceptibility gene

Collection of tumor tissue samples

In this study, the 33 clinical samples of tumor tissue were obtained from 33 ovarian cancer patients after their surgery and were stored under formalin-fixed paraffin-embedded (FFPE) tumor tissue conditions at the Vietnam National Cancer Hospital (Hanoi, Vietnam). All FFPE tissue specimens in the research were collected under institutional review board approval by certified medical pathologists. Sample tissues were fixed in 10% neutral-buffered formalin (NBF) within 30 to 60 minutes of surgery excision. The samples were fixed for 18 – 24 hours at room temperature, subsequent embedded in Immunohistochemistry (IHC) - grade paraffin. Standard tissue sample size was 1x1x0.5cm, but can vary based on the nature of the disease or tissue type. FFPE tissues were stored at room temperature. FFPE blocks were cut to slices (5µm thick) and stained with hematoxylin & eosin (H&E) using the Dako Coverstainer system (Agilent, CA 95051, USA) for histopathological evaluation. Diagnostic pathology was made by more than two pathologists based on the American Joint Committee on Cancer (AJCC, 2009) guidelines.

DNA extraction and next generation sequencing

Genomic DNA was extracted from 5 to 10 sections of 10 µm thickness of macro-dissected ovarian tumor tissues using QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). The extracted samples contain at least 30% tumor cells. Quality and quantity of extracted DNA was measured using BioDrop UV-Visible spectrophotometer (Biochrom, United Kingdom). Furthermore, the double strand DNA was assessed by Qubit 4 Flourometer (Invitrogen, Thermo Fisher Scientific Inc, MA, USA). Sample met the inclusion criteria was involved in this study.

BRCAaccuTest™ PLUS kit (NGeneBio Co., Ltd, South Korea) was utilized for library preparation of *BRCA*1 and *BRCA*2 (transcript accession of NM_007294.3 and NM_000059.3, respectively). A total of 100ng of high-quality genomic DNA was used for two separate library preparation reactions

following the manufacturer's protocol. DNA library was validated by Agilent 4200 TapeStation D1000 ScreenTape Assay. The size of the prepared DNA library was checked by size marker (Fig. 2).

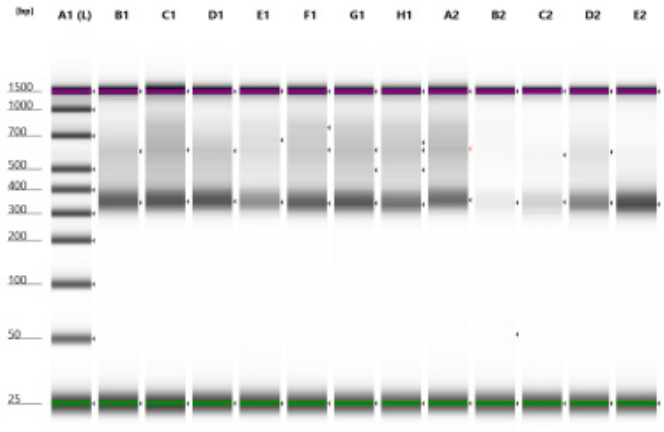

Fig. 2. TapeStation gel image of DNA library preparation for sequencing.

DNA: damaged deoxyribonucleic acid

The length DNA library segments must be between 300 to 500 bp. The size of the prepared DNA library was confirmed through the horizontal axis of the graph. If there was an abnormal peak of small size (under 200 bp, dimer of primer), the library had been re-prepared since it may affect the analysis result even though the size of the main peak appeared within the normal range (300 ~ 500 bp).

MiSeqDx instrument (Illumina, Inc. USA) and MiSeq Reagent Micro v2 (300 cycles) (Illumina, San Diego, CA, USA) were used for NGS. 3 sequencing runs were performed, each run included 1 control DNA and 11 somatic samples. More detail was provided in our previous work[16].

Data analysis

Raw sequencing data under FASTQ format were experienced the following pipeline workflow of bioinformatics: quality control of data, trimming adapter, alignment, variant calling and annotation. Raw data was firstly checked by FASTQC. The Sickle v1.33 tool was used to trim sequencing adapters and low Q-score base (the cut-off threshold of Q-score was Q-score ≥ 30), then the Burrows–Wheeler aligner-maximum exact matches (BWA-MEM) v0.7.10 aligned processed sequences to the Human hg19 genome as reference genome. The unmapped reads were extracted (mapping quality (MAPQ) < 60). Both single nucleotide variants and short indels (insertion/deletion) were identified by FreeBayes v9.9.2 and GATK v.2.3. Variant annotation was performed by snpEff v4.2, which all were included in NGeneAnalySys™ software (NGeneBio Co., Ltd, South Korea). MEGA v.11.0.13 was also utilized for visualization and alignment. Variants were categorized based on the five-tier criteria of the American College of Medical Genetics and Genomics (ACMG) standards and guidelines. In this study, pathogenic and likely pathogenic variants were mostly focused on. Copy number variant (CNV) detection was not recommended by the manufacturer.

Statistics were performed by SPSS v.22.0 (SPSS Inc., Chicago, Illinois). Quantititive data were presented in mean and standard deviation, while qualitative data were presented in frequency and percentage. Fisher's exact test and Wilcoxon test were performed to compare several features between two groups.

Results

Characteristics of selected subjects

Our study involved 33 patients divided into the high-risk group (n = 22) and the low-risk group (n = 11) (Table 1). The mean age of diagnosis among studied patients was 49.06 ± 14.79 years, and the mean value of body mass index (BMI) was 21.15 ± 2.45 kg/m^2. Only 2 patients whose families had cancer patients, and both of them were in the high-risk group (accounted for 6.06%). No statistically significant difference was found between the high-risk group and low-risk group in age, BMI, patient's medical history as well as their family history, and patient's comorbidities. Regarding pathological classification, our results showed that most of the studied patients had epithelial cancer, which accounted for at least 76.00% of all patients. The prevalence of non-epithelial ovarian cancer patients among the low-risk group was significantly higher than in the high-risk group. Statistics also indicated that patients in the low-risk group were diagnosed to be at an earlier stage than those in the high-risk group (p = 0.003). Most of the patients in the high-risk group were at advanced stages (stage III and stage IV) when diagnosed with a prevalence of 63.64%, while most of the patients in the low-risk group were at stage I of diagnosis with the same prevalence (63.64%).

BRCA1 and BRCA2 variants

The variant calling results showed that a total of 323 variants were recorded in 33 patients. After removing overlapped variants and several variants that are ubiquitous variants of the Asian population, including 4563A>G, c.6513G>C, and c.7397T>C in *BRCA*2[17], 33 distinguished variants were found in *BRCA*1/2 (Table 2).

Table 1. Characteristics of selected subjects.

Features		High risk group		Low risk group		Total		p value
	Age*	47.09	3.67	53.00	2.21	49.06	14.79	0.349
	BMI*	21.44	0.52	20.64	1.03	21.15	2.45	0.571
Tumor pathological classification**	Epithelial cancer	17	77.27%	8	72.73%			0.041
	Non-epithelial cancer	2	9.09%	3	27.27%			
	Not mentioned	3	13.64%	0	0.00%			
Stage**	I	3	13.64%	7	63.64%	10	30.30%	0.003
	II	1	4.55%	2	18.18%	3	9.09%	
	III	13	59.09%	1	9.09%	14	42.42%	
	IV	1	4.55%	0	0.00%	1	3.03%	
	Not mentioned	4	18.18%	1	9.09%	5	15.15%	
Family history**	Having cancer patient	2	9.09%	0	0.00%	2	6.06%	0.542
	Not having cancer patient	20	90.91%	11	100.00%	31	75.76%	
Comorbidities**	Diabetes	0	0.00%	1	9.09%	1	3.03%	0.261
	Hypertension	2	9.09%	2	18.18%	4	12.12%	
	None	20	90.91%	8	72.73%	28	84.85%	
Medical history**	Ovarian cyst	1	4.55%	1	9.09%	2	6.06%	0.592
	Cancer	1	4.55%	0	0.00%	2	6.06%	
	None	20	90.91%	10	90.91%	29	87.88%	
BRCA1/BRCA2 variants**	Benign	22	100.00%	11	100.00%	6	18.18%	0.637
	Uncertain significance	9	40.91%	6	54.55%	15	45.45%	
	Pathogenic	2	9.09%	1	9.09%	3	9.09%	
	Likely pathogenic	3	13.64%	0	0.00%	3	9.09%	

*Data are presented as mean ± standard deviation and %; and **Data are presented as n and %

BMI: body mass index, BRCA: breast cancer susceptibility gene

Table 2. Characteristics of *BRCA*1 and *BRCA*2 variants in ovarian cancer patients.

No.	NT Change	Frequency	Pathogenicity	Gene	Type	Consequence	Exon	dbSNP ID	Zygosity	AA Change
1	c.4837A>G	48.48%	B	BRCA1	snv	missense	15/23	rs1799966	heterozygote	p.Ser1613Gly
2	c.4308T>C	48.48%	B	BRCA1	snv	synonymous	12/23	rs1060915	heterozygote	p.Ser1436Ser
3	c.3548A>G	48.48%	B	BRCA1	snv	missense	10/23	rs16942	heterozygote	p.Lys1183Arg
4	c.3113A>G	48.48%	B	BRCA1	snv	missense	10/23	rs16941	heterozygote	p.Glu1038Gly
5	c.2612C>T	48.48%	B	BRCA1	snv	missense	10/23	rs799917	heterozygote	p.Pro871Leu
6	c.2311T>C	48.48%	B	BRCA1	snv	synonymous	10/23	rs16940	heterozygote	p.Leu771Leu
7	c.2082C>T	48.48%	B	BRCA1	snv	synonymous	10/23	rs1799949	heterozygote	p.Ser694Ser
8	c.114G>A	6.06%	B	BRCA1	snv	synonymous	3/23	rs1800062	heterozygote	p.Lys38Lys
9	c.1114A>C	42.42%	B	BRCA2	snv	missense	10/27	rs144848	heterozygote	p.Asn372His
10	c.3396A>G	60.61%	B	BRCA2	snv	synonymous	11/27	rs1801406	heterozygote	p.Lys1132Lys
11	c.7242A>G	60.61%	B	BRCA2	snv	synonymous	14/27	rs1799955	heterozygote	p.Ser2414Ser
12	c.865A>C	18.18%	B	BRCA2	snv	missense	10/27	rs766173	heterozygote	p.Asn289His
13	c.1365A>G	18.18%	B	BRCA2	snv	synonymous	10/27	rs1801439	heterozygote	p.Ser455Ser
14	c.2229T>C	18.18%	B	BRCA2	snv	synonymous	11/27	rs1801499	heterozygote	p.His743His
15	c.2971A>G	18.18%	B	BRCA2	snv	missense	11/27	rs1799944	heterozygote	p.Asn991Asp
16	c.3807T>C	27.27%	B	BRCA2	snv	synonymous	11/27	rs543304	heterozygote	p.Val1269Val
17	c.1801_ 1808delCACAATTC	6.06%	LP	BRCA1	del	frameshift	10/23	New	homozygote	p.His601fs
18	c.2865delC	3.03%	LP	BRCA2	del	frameshift	11/27	New	heterozygote	p.Asn955fs
19	c.1016delA	3.03%	P	BRCA1	del	frameshif	10/23	rs80357569	heterozygote	p.Lys339fs
20	c.1673_ 1674delAA	3.03%	P	BRCA1	del	frameshift	10/23	rs80357600	heterozygote	p.Lys558fs
21	c.928C>T	3.03%	P	BRCA1	snv	stop_gained	10/23	rs397509338	heterozygote	p.Gln310*
22	c.825C>T	3.03%	US	BRCA1	snv	synonymous	10/23	rs397509328	heterozygote	p.Gly275Gly
23	c.2566T>C	6.06%	US	BRCA1	snv	missense	10/23	rs80356892	heterozygote	p.Tyr856His
24	c.1362A>G	6.06%	US	BRCA2	snv	synonymous	10/27	rs55919657	heterozygote	p.Lys454Lys
25	c.6322C>T	3.03%	US	BRCA2	snv	missense	11/27	rs55794205	heterozygote	p.Arg2108Cys
26	c.8187G>T	6.06%	US	BRCA2	snv	missense	18/27	rs80359065	heterozygote	p.Lys2729Asn
27	c.5785A>G	3.03%	US	BRCA2	snv	missense	11/27	rs79538375	homozygote	p.Ile1929Val
28	c.943T>A	3.03%	US	BRCA2	snv	missense	10/27	rs79483201	heterozygote	p.Cys315Ser
29	c.3445A>G	3.03%	US	BRCA2	snv	missense	11/27	rs80358589	heterozygote	p.Met1149Val
30	c.2133C>T	3.03%	US	BRCA2	snv	synonymous	11/27	rs535547513	heterozygote	p.Cys711Cys
31	c.2920G>A	3.03%	US	BRCA2	snv	missense	11/27	rs539613324	heterozygote	p.Asp974Asn
32	c.3420T>C	3.03%	US	BRCA2	snv	synonymous	11/27	rs118093942	heterozygote	p.Ser1140Ser
33	c.7052C>G	3.03%	US	BRCA2	snv	missense	14/27	rs80358932	heterozygote	p.Ala2351Gly

*B: Benign, US: Uncertain significance, LP: Likely pathogenic, P: Pathogenic

Of the 33 above variants, 14 variants were detected in the *BRCA*1 gene (accounted for 38.8%), while this figure for *BRCA*2 was 61.1%. Only 4 variants were deletions, which accounted for 12.12%, while the remaining were single nucleotide variants. About 45.45% of variants were missense, followed by synonymous variants with 39.39%. Only 4 variants were frameshift, and 1 variant was a stop variant. Most of the *BRCA*1 variants were detected on exon 10 over 23 exons of *BRCA*1, while each of exons 3, 12, and 15 had 1 variant. Variants in *BRCA*2 were found in exons 10, 11, 14, and 18 over 27 exons of *BRCA*2. However, almost 60% of them were on exon 11. Except for c.5785A>G in *BRCA*2 and c.1801_1808delCACAATTC in *BRCA*1, all variants were heterozygote (Table 2). According to ACMG classification, the percentage of pathogenic and likely pathogenic was 15.15%, while this figure for benign was 48.48%, followed by uncertain significance with 36.36% (Fig. 3).

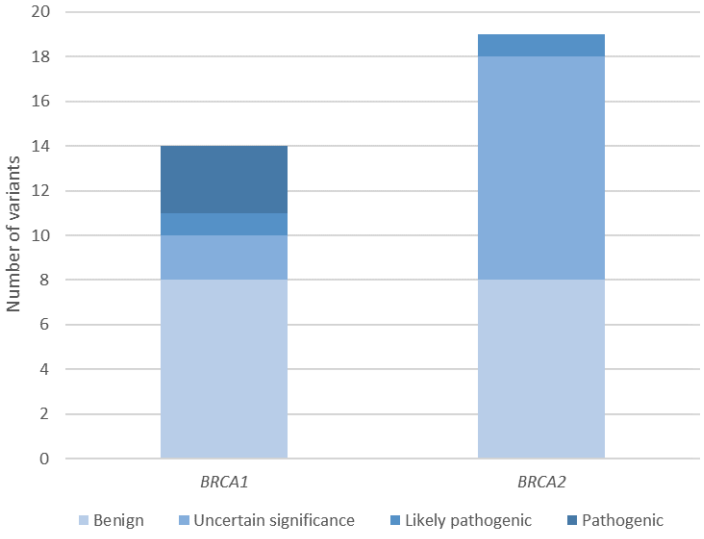

Fig. 3. The distribution of 4 variant types among BRCA1 and BRCA2 detected variants.
BRCA: breast cancer susceptibility gene

Regarding the high-risk and low-risk group, although we found *BRCA*1 and *BRCA*2 benign variants in all 33 patients (4563A>G, c.6513G>C, and c.7397T>C variants in *BRCA*2 were not taken into account), uncertain significance variants were detected in 9 patients of the high-risk group (40.91%) and 6 patients of the low-risk group (54.55%) (Table 1). Pathogenic and likely pathogenic (P/LP) variants were found in more than 20% of the high-risk group while this figure for the low-risk group was 9.09%. However, statistical analysis found no significant difference in the prevalence of variants between the high-risk and low-risk groups.

Pathogenic and likely pathogenic variants of *BRCA*1 and *BRCA*2 genes

Of 33 variants detected in *BRCA*1 and *BRCA*2 genes among 33 ovarian cancer patients involved in this study, 5 variants were classified as P/LP variants in the *BRCA*1/*BRCA*2 genes in 6 out of 33 patients (18.18%), including c.1801_1808delCACAATTC, c.1016delA, c.1673_1674delAA, c.928C>T in *BRCA*1 and c.2865delC in *BRCA*2 (Fig. 4 and Fig. 5).

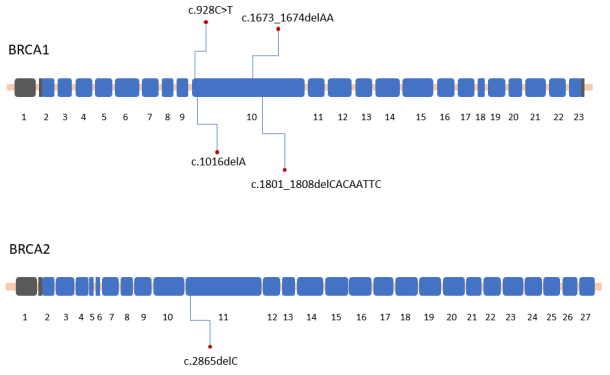

Fig. 4. Pathogenic and likely pathogenic variants of BRCA1 and BRCA2.

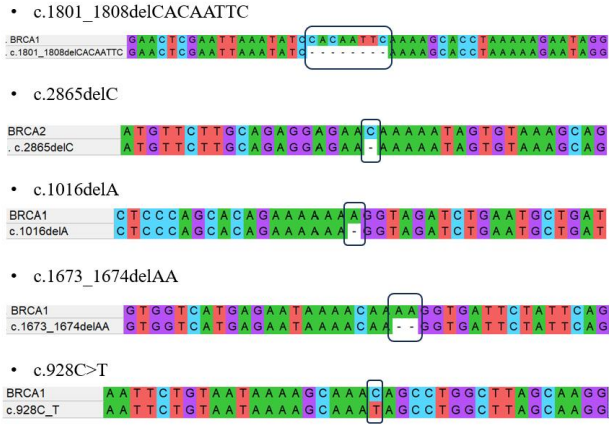

Fig. 5. Sequence data of pathogenic and likely pathogenic variants in BRCA1 and BRCA2 genes.

Among them, four were frameshift and 1 was stop-gained. All detected pathogenic variants were presented in the coding regions, particularly, in *BRCA*1 exons 10 and in *BRCA*2 exon 11. The c.1801_1808delCACAATTC was detected in 2 patients with a frequency of 6.06%, while others was detected in one patient with a frequency of 3.03%. c.1016delA, c.1673_1674delAA, and c.928C>T variants were reported in previous publication, while c.1801_1808delCACAATTC variant in *BRCA*1 and c.2865delC in *BRCA*2 were firstly recorded (Table 3). c.1801_1808delCACAATTC was also the unique homozygote variant among 33 detected variants.

Table 3. Characteristics of BRCA1 and BRCA2 pathogenic and likely pathogenic variants in ovarian cancer patients.

Patient ID	NT Change	AA Change	Frequency	Pathogenicity	Gene	Exon	Type	Consequence	Start Position	Fraction	Depth	Zygosity	Note
OC13, OC26	c.1801_1808delCACAATTC	p.His601fs	6.06%	LP	BRCA1	10/23	del	FS	41245739	2.18	3984	homozygote	New
OC27	c.2865delC	p.Asn955fs	3.03%	LP	BRCA2	11/27	del	FS	32911356	45.79	439	heterozygote	New
OC29	c.1016delA	p.Lys339fs	3.03%	P	BRCA1	10/23	del	FS	41246531	55.99	1704	heterozygote	rs80357569
OC36	c.1673_1674delAA	p.Lys558fs	3.03%	P	BRCA1	10/23	del	FS	41245873	49.21	1331	heterozygote	rs80357600
OC48	c.928C>T	p.Gln310*	3.03%	P	BRCA1	10/23	snv	SG	41246620	56.15	1560	heterozygote	rs397509338
31	c.2920G>A	3.03%	US	BRCA2	snv	missense	11/27	rs539613324	heterozygote	p.Asp974Asn			
32	c.3420T>C	3.03%	US	BRCA2	snv	synonymous	11/27	rs118093942	heterozygote	p.Ser1140Ser			
33	c.7052C>G	3.03%	US	BRCA2	snv	missense	14/27	rs80358932	heterozygote	p.Ala2351Gly			

*FS: Frameshift; SG: Stop-gained

Clinical features and BRCA1/BRCA2 variants

The mean age of patients who did not carry P/LP variants in *BRCA*1/2 was significantly lower than those having P/LP variants, which was 46.44 ± 2.72 versus 60.83 ± 5.17 years (Table 4). Although all patients having P/LP variants were classified as epithelial cancer patients, no statistical relationship was found between the tumor pathological features and the status of variant carrying. There are 5 over 6 P/LP variant-carried patients (accounting for 83.34%) who were diagnosed to be in the advanced stage and only 1 patient in stage II. No P/LP variants in *BRCA*1 and *BRCA*2 genes were found in stage I patients. Of 33 patients, we did not find any P/LP variants in the 2 patients whose families had cancer patients. No statistically significant difference was found between non-variant and variant patients in BMI, pathological classification, diagnosed stage, their families, and their medical history (Table 4).

Table 4. Clinical features between non-P/LP variant-carried patients and P/LP variant-carried patients.

Features		Non-P/LP variant carrying		P/LP variant carrying		p value
Age*		46.44	2.72	60.83	5.17	0.019
BMI*		21.38	0.56	19.94	0.8	0.289
Tumor pathological classification**	Epithelial cancer	19	70.37%	6	100.00%	
	Non-epithelial cancer	5	18.52%	0	0.00%	0.167
	Not mentioned	3	11.11%	0	0.00%	
Stage**	I	10	37.04%	0	0.00%	
	II	2	7.41%	1	16.67%	
	III	10	37.04%	4	66.67%	0.066
	IV	0	0.00%	1	16.67%	
	Not mentioned	5	18.52%	0	0.00%	
Family history**	Having cancer patient	2	7.41%	0	0.00%	1
	Not having cancer patient	25	92.59%	6	100.00%	
Comorbidities**	Diabetes	1	3.70%	0	0.00%	
	Hypertension	3	11.11%	1	16.67%	0.017
	None	23	85.19%	5	83.33%	
Medical history**	Ovarian cyst	2	7.41%	0	0.00%	
	Cancer	0	0.00%	2	33.33%	0.1
	None	25	92.59%	4	66.67%	

* Data are presented as mean± standard deviation and %, **Data are presented as n and %

BMI: body mass index, P/LP: pathogenic and likely pathogenic

Discussion

Among a total of 33 ovarian cancer recruited in our study, the *BRCA*1/2 variant frequency was 18.18%, which was within the range of variant frequency in ovarian patients of different ethnicities (5-30%)[18]. Although more variants were found in patients with high risk of variants compared to low risk group (5/22 versus 1/11), this difference was not statistically significant (p = 0.076). This result was corresponding to our classifications of patients into high-risk and low-risk of variants.

Using NGS, five *BRCA*1/2 deleterious variants have been identified, including two new variants. All four *BRCA*1 variants are located in coding regions of

exon 10. Mutations of the central portion of this exon are widely known for their association with an increased risk of ovarian cancer[19]. The remaining variant is in coding regions of exon 11 of *BRCA2* gene which is also associated with higher ovarian cancer risk than other regions of the gene[19]. Two variants found, c.1673_1674delAA and the novel variant c.1801_1808delCACAATTC are located in the ovarian cancer cluster region (OCCR) of *BRCA*1 gene, which is understandable since the incidence of ovarian cancer is significantly high in patients with a *BRCA* variant in the OCCR[20]. It also means that these variants have higher risk of ovarian cancer than those in other regions[21]. Among five variants, c.1016delA was also detected in another study on Vietnamese patients with ovarian cancer[21]. Additionally, such variant was presented in other populations including Canada, the USA, Norway and Czech Republic according to the database of NCBI (dbSNP). This database also showed that besides Vietnam, variant named c.928>T was described in Taiwanese people, another Asian population.

The interaction between germline and somatic variants in the *BRCA*1 gene was reported in a study by Kanchi et al (2014), the authors also suggested that analyzing both germline and somatic variants could identify notably altered pathways in ovarian cancer[22]. Although the sequencing was performed using FFPE samples instead of blood samples, we did not detect any somatic variants (data not shown). All detected variants were germline. This result was consistent with the findings in a study by Mafficini et al (2016), in which 11/13 pathogenic variants were germline[23]. According to Alsop et al (2012), having a *BRCA*1/*BRCA*2 germline mutation even without any family history of ovarian cancer was still alert for ovarian cancer risk[24].

Two novel variants, c.1801_1808delCACAATTC and c.2865delC variants, have not been described in public databases. The prior variant located in exon 10 of the OCCR causes a sequence frameshift involving amino acid number 601, histamine. Meanwhile, c.2865delC was the only identified *BRCA*2 variant in our study. The deletion of nucleotide cytosine leads to the alteration of asparagine at the position of 955 in the polypeptide chain. The pathogenesis of this variant is remaining unknown since it is not located in *BRCA*2 OCCR or any protein binding sites.

We observed that all likely pathogenic and pathogenic variants were found in patients diagnosed with epithelial cancer. Such finding is reasonable since germline variants in *BRCA*1/2 genes accounted for up to 18% of epithelial cancer cases and somatic alterations are observed in another 7%[20]. It has raised a new hope for patients with epithelial cancer because *BRCA*1/2 variants are related to higher sensitivity to platinum regimens and other chemotherapeutic agents and therefore, better prognosis. Patients with *BRCA*1/2 variants, especially *BRCA*1/2 somatic variants are particularly sensitive to PARPi and DNA damaging agents such as platinum-based compounds possibly due to the precedence of error-prone DNA-repair pathways of *BRCA*-altered cells and the presence of homologous recombination deficiency (HRD)[25]. Therefore, HRD testing is currently crucial for ovarian cancer patient management, especially when solid evidence has shown ovarian patients with HRD tumors gain greater benefit from PARPi treatment than patients with non-HRD tumors[26].

Our study results also revealed that *BRCA*-variant carriers were older than non-variant carriers at the time of diagnosis ($p < 0.05$). However, Hamdi et al stated that early age at onset was significantly associated with *BRCA* status in a study on breast and ovarian cancer patients in Tunisia[27]. Another study in China showed that no significant differences were found in onset age[28]. The discrepancy in the results of such papers might be because of differences in ethnicity and most of variants in our study being germline mutations. The mean age of cancer diagnosis for the variant carrier group in our study was 60.83 ± 5.17 years. It was in agreement with another study in the Denmark population in which the mean age of diagnosis for *BRCA*1 and *BRCA*2-

variant carriers were 61 ± 1.59 and 60 ± 0.54 years, respectively[29]. Besides, other clinical features including tumor pathology, FIGO stage, family history, BMI, comorbidities and medical history had no association with the status of variants.

There were several limitations in our study. Firstly, the sample size of our study was relatively small, so we could not find a large number of variants as well as founder variants for Vietnamese population. However, to date, there have been a few studies on *BRCA*1/2 variants in Vietnam and our study was among those with adequate sample size. We also detected variants in tumors of high-risk of mutation patients to maximize the number of possible variants detected. Our study met the same challenge as several previous studies in CNV analysis using NGS[30], moreover, the FFPE samples used in our study are not recommended by manufacturers in CNV detection. Besides, NGS results are commonly confirmed by using Sanger sequencing afterwards. Nevertheless, Kim et al proved that NGS applied to analyze data from *BRCA*accuTest™ (NGeneBio), which we used as an in vitro diagnostic reagent, provided 100% overall diagnostic consistency with Sanger sequencing[17].

Conclusion

Our study provides new insights into the genetic landscape of ovarian cancer patients in Vietnamese population. 18.18% of our Vietnamese cohort carried *BRCA*1/2 variants, which was comparable to global statistics. Five *BRCA*1/2 P/LP variants had been detected, including two novel variants that have not been described in public databases. All five variants are in coding regions and cause premature termination of translation. One of the newly detected variants is located in OCCR of *BRCA*1 gene, which produces more evidence to support the role of these regions in the pathogenesis of ovarian cancer. Age of onset was significantly associated with the status of *BRCA*1/2 variants, with *BRCA*-variant carriers being older than non-variant group, which was different from other previous work. All five deleterious *BRCA*1/2 variants were detected in patients with epithelial cancer, which benefits the results of chemotherapeutics in these patients. Research in *BRCA*1/2 mutational spectrum in Vietnamese population helps to not only support early detection of the disease, but only develop personalized treatments for *BRCA*-related diseases, especially for ovarian cancer.

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Ethics approval

The research was approved by the Institute of Genome Research Institutional Review Board according to the decision number: 02-2022/NCHG-HĐĐĐ on March 09, 2022.

Potential conflicts of interest

The authors declare no conflicts of interest.

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