ORIGINAL RESEARCH

The Features of *BRCA1* and *BRCA2* Germline Mutations in Hakka Ovarian Cancer Patients: *BRCA1* C.536 A>T Maybe a Founder Mutation in This Population

Yu Luo[1,2,*], Heming Wu [2,3,*], Qingyan Huang[2,3], Hui Rao[2,3], Zhikang Yu[2,3], Zhixiong Zhong [2,3]

[1]Department of Gynaecology, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China; [2]Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translational Research of Hakka Population, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China; [3]Center for Precision Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhikang Yu; Zhixiong Zhong, Center for Precision Medicine, Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translational Research of Hakka Population, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, No. 63 Huangtang Road, Meijiang District, Meizhou, 514031, People's Republic of China, Tel +753-2131-591, Email yuzhikang01@126.com; zhongzhixiong01@126.com

Objective: To investigate the frequencies of *BRCA1* and *BRCA2* mutations in Chinese Hakka patients with ovarian cancer.
Methods: The protein coding regions and exon intron boundary regions of the *BRCA* gene were sequenced using genomic DNA isolated from the lymphocytes of patients with next-generation sequencing. The patients' family history and clinical records were collected.
Results: A total of 195 patients with ovarian cancer were included in the study, and 52 distinct variants of the *BRCA* gene were identified. It was found that 64 patients (64/195, 32.8%) had *BRCA* gene mutations, including 32 patients (50.0%) with *BRCA1* mutation, 27 patients (42.2%) with *BRCA2* mutation, and 5 patients (7.8%) with both mutations. Furthermore, 22 pathogenic mutations were detected in 26 patients, 2 likely pathogenic variants in 2 patients, 12 variants of uncertain significance in 20 patients, and 16 likely benign variants in 24 patients. The mutations were mainly found to occur in exons 8, 14, and 17 of *BRCA1* and exons 10, 11, 14, and 15 of *BRCA2*. The results showed that the *BRCA* genes possess different mutation hotspots in different ethnic groups. In addition, recurrent mutations were noted in many patients. *BRCA1* c.536 A>T, considered a founder mutation, was identified in 10 patients (15.63%, 10/64), followed by *BRCA1* c.2635 G>T (6.25%, 4/64) and *BRCA2* c.2566 T>C (6.25%, 4/64).
Conclusion: The *BRCA1* c.536 A>T could be considered to be a founder mutation in this ovarian cancer population. This recurrent *BRCA1* mutation has rarely been observed in other ethnic groups. Our findings are expected to provide valuable data for clinical consultation and for designing individualized treatment for ovarian cancer.
Keywords: *BRCA* gene, ovarian cancer, variants, Hakka population

Introduction

Ovarian cancer is one of the most common cancers in women and a leading cause of death in women. Increased risk factors for cancer have led to an upward trend in the incidence of the disease globally.[1,2] There are several risk factors for ovarian cancer, such as genetic predisposition, ovulation, endometriosis, dietary factors, and ethnicity/race. Ovarian cancer can occur sporadically in any woman, including those without any notable risk factors.[3]

Ovarian cancer is thought to be divided into two main subtypes: type I and type II. Ovarian carcinoma type I includes patients with endometrioid carcinoma, low-grade serous carcinoma, low grade adenocarcinoma carcinoma, mucinous carcinoma, clear cell carcinoma, and transitional cell carcinoma, which are mostly confined to one ovary and are

associated with a good prognosis. Ovarian carcinoma type II includes patients with high-grade serous carcinoma, undifferentiated carcinoma, carcinosarcoma, and high grade adenocarcinoma. Unlike ovarian cancer type I, it is highly aggressive and progresses rapidly. Ovarian carcinoma type II is poorly differentiated, and is generally absent in early ovarian lesions.[4,5] The diagnosis of ovarian cancer involves pelvic examination, detection of serum tumor markers detection, blood cell analysis, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and other imaging techniques as well as the joint application of these detection methods. However, these tests used in the early diagnosis and condition monitoring of ovarian cancer have certain limitations; hence, the early diagnostic markers and disease characteristics of ovarian cancer need to be studied and clarified.

Studies have identified that family history and genetic factors are important risk factors for ovarian cancer. Ovarian cancer susceptibility genes include breast cancer susceptibility gene 1 (*BRCA1*), breast cancer susceptibility gene 2 (*BRCA2*), RAD51 recombinase (*RAD51*) gene family,[6] BRCA1-associated RING Domain protein 1 (*BARD1*) gene,[7] and murine double minute (MDM) gene family.[8,9] The two major ovarian cancer susceptibility genes, *BRCA1* (MIM #113705) and *BRCA2* (MIM #600185), have been explored the most.[10] *BRCA1* is located on chromosome 17q21 (containing 22 exons) and encodes and expresses a multi-domain protein containing 1863 amino acids.[11] *BRCA2* is located on chromosome 13q12-q13 (containing 27 exons) and encodes and expresses a multidomain protein containing 3418 amino acids. *BRCA1* is similar to *BRCA2*, but there is no significant homology in the exon region. BRCA1 plays a crucial role in important cell activities and in maintaining gene stability.[12–14] BRCA2 is thought to play a key role in the repair of double-strand breaks and in the partial regulation of RAD51 response via chromosome recombination mechanism.[15–17] Corresponding studies have also confirmed the correlation between the genetic marker on the chromosome 17q and the susceptibility to breast and ovarian cancer in some patients with a family history of these diseases.[18] The results of a genome-wide association analysis involving 15 high-risk breast cancer families revealed that *BRCA2*, localized on the chromosome 13q12-13, is associated with breast cancer.[19] Both *BRCA1* and *BRCA2* are tumor-suppressor genes, and mutations in these genes are seen in some patients with cancer. Mutations in the human *BRCA* gene may be race-specific in a given region and region-specific in a given ethnic group.[20,21]

The Hakka is a Han ethnic group with a unique genetic background and originate from the Hakka ancestors of the Han nationality in Central China. They migrated southward for many times and fused with the ancient Yue residents in Guangdong, Fujian and Jiangxi.[22] Meizhou City is located in the northeast of Guangdong Province and has a large Hakka population. The *BRCA1/2* mutations and the characteristics of *BRCA1/2*-associated ovarian cancer in the Hakka population remain unclear. This study retrospectively analyzed the results of the *BRCA* gene in patients with ovarian cancer among the Hakka population using next-generation sequencing.

Materials and Methods
Participants
Enrollment for the ovarian cancer *BRCA* gene mutation screening trial was conducted between January 2018 and May 2021 of subjects visiting the Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences. Signed informed consent forms were obtained from all participants. The baseline data, including the general information, related medical history, hematological parameters, and staging (according to the AJCC 8th edition staging) of the enrolled subjects were collected. The ovarian cancer patients were categorized into three groups according to the pathological type: (1) type 1, patients with endometrioid, mucinous, clear cell, low-grade serous, and low grade adenocarcinoma, benign serous cystadenoma or Mullerian cystadenoma; (2) type 2, high grade serous, undifferentiated, carcinosarcoma, and high grade adenocarcinoma; and (3) others, including granulosa cell tumors, borderline tumors, and tumors with unavailable grade or histology data. The study was conducted on the basis of the Declaration of Helsinki, and was supported by the Ethics Committee of the Meizhou People's Hospital (Huangtang Hospital).

Detection of Serum Tumor Markers and Inflammatory Markers in the Sampled Blood
The subjects' blood samples (3 mL) were collected at the time of admission and at 2–3 days before treatment and the serum was immediately separated. Serum tumor markers analysis was performed on the Luminex 200 system (Luminex

Corporation, Austin, USA) to measure the concentration of serum carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), and alpha-fetoprotein (AFP) using relevant quantitative detection kits (TELLGEN Life Science and Technology Co., Ltd., China). After adding the samples and the labeled antibodies into each well, the reaction was performed in the dark at 37°C for 5 min, after which the fluorescence-encoded microspheres cross-linked with antibodies were added. After the reaction, the plate was incubated for 60 min in a darkroom at 37°C, the termination solution was added to terminate the reaction, and the microsphere signals of each well were read on the Luminex 200 system.

The blood sample (2 mL) was collected via venipuncture of an antecubital vein from each subject and collected in a tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The erythrocyte correlative indices were detected by using the Sysmex XE-2100 Blood Analyzer (Sysmex Corporation, Japan) according to the standard operating procedures (SOP). The blood routine results were collected before treatment and the inflammation index was calculated according to the following formulas: SII = platelet × neutrophil/lymphocyte,

SIRI = monocyte × neutrophil/lymphocyte,

NLR = neutrophil/lymphocyte,

PLR = platelet/lymphocyte,

LMR = lymphocyte/monocyte.

BRCA1 and *BRCA2* Mutations Were Detected by Next-Generation Sequencing (NGS)

The peripheral blood sample (2 mL) was collected from each participant and collected in a tube containing EDTA as an anticoagulant. Genomic DNA was extracted by using the QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA concentration and purity were quantified using the Nanodrop 2000^{TM} Spectrophotometer (ThermoFisher Scientific, Waltham, MA). The DNA samples were sequenced after library construction, template preparation and template enrichment according to standard operating procedures of the Life Technology Company. Then, 200–1000 ng DNA was sheared prior to library construction for 150 bp fragments. The NGS libraries were constructed using the IonPlus Fragment Library Kit (Life Technologies, Carlsbad, CA). Next, ligation was performed with barcode adapters, followed by ligated fragments amplification, library purification and concentration. Next-generation sequencing was performed on the Ion Proton instrument (Life Technologies) and tested by the CapitalBio Corporation (Beijing, China). Data were analyzed by the Torrent Suite 4.4.3 and 5.0.4 (Life Technologies) using optimized parameters: minimal depth 300×, detection threshold of 2% and 1% for hotspots. Variant call files from the variant caller were loaded on a galaxy platform and annotated using the Safir2report tool. The sequencing results were then compared with the standard human genome data to obtain the mutation sequence information of the samples to be tested. According to the Human Genome Variation Society (HGVS) guidelines, the genetic variations in this study, were named using the following reference sequences: NM_007294.4 (*BRCA1*) and NM_000059.4 (*BRCA2*).

Statistical Analyses

SPSS statistical software version 21.0 was used for data analyses. Continuous variable data are represented by mean ±SD, and analyzed using Student's *t*-test or the Mann–Whitney *U*-test. The Chi-square test was applied for analyzing the categorical variables, which were then presented as percentages. $P<0.05$ was considered to indicate statistical significance.

Results

Population Characteristics

A total of 195 patients with ovarian cancer (all women) were included in the present study. There were 14 women (7.2%) <35 years of age, 54 cases (27.7%) between 35 and 50 years of age, and 127 women (65.1%) >50 years of age. None of the 195 patients had a family history of breast or ovarian cancer. The findings show that ovarian cancer mainly occurs in people >50 years of age. Some studies have observed that the risk of ovarian cancer decreases with the number of pregnancies.[23,24] We intended to analyze the relationship between the number of pregnancies and *BRCA* mutations in the

patients with ovarian cancer. Accordingly, 8 women (4.1%) never had a pregnancy, 94 women (48.2%) had 1–3 pregnancies, 34 women (17.4%) had 4–5 pregnancies, 14 women (7.2%) had >5 pregnancies, and the remaining 45 (23.1%) were unknown. These results show that the risk of ovarian cancer decreases with the number of pregnancies. The CEA, CA199, AFP, CA125, NLR, LMR, and PLR levels of these patients were 11.56 ± 114.89 ng/mL, 144.34 ± 769.04 U/mL, 225.98 ± 1779.13 ng/mL, 733.15 ± 1553.06 U/mL, 4.22 ± 4.17, 3.61 ± 2.24, and 245.88 ± 169.87, respectively (Table 1).

Clinical Features of Patients with Different Types of Ovarian Cancer

There were 29 (14.9%) patients with type 1 ovarian cancer, 119 (61.0%) cases with type 2 ovarian cancer and 47 (24.1%) cases with other ovarian cancer types. The majority of the patients had type 2 ovarian cancer. It was found that 1 patient (3.4%) was <35 years of age, 6 patients (20.7%) were between 35 and 50 years of age, and 22 patients (75.9%) were >50 years of age in the type 1 group. On the other hand, in the type 2 group, 2 patients (1.7%) were <35 years of age, 28 patients (23.5%) were between 35 and 50 years of age, and 89 patients (74.8%) were >50 years of age. In the other types, there were 11 patients (23.4%) <35 years of age, 20 patients (42.6%) between 35 and 50 years of age, and 16 patients (34.0%) >50 years of age. There were significant differences in age distribution among the three groups of patients with ovarian cancer ($P < 0.001$). The results show that type 1 and type 2 ovarian cancers mainly occur in people >50 years of

Table 1 Clinical Characteristics of Ovarian Cancer Patients

	Number (Mean±SD)	Percentage (%)
Gender		
Female	195	100.0
Male	0	0
Age (year)		
<35	14 (25.14±6.83)	7.2
35–50	54 (45.15±4.28)	27.7
>50	127 (60.40±6.88)	65.1
Family history of breast cancer		
No	195	100.0
Yes	0	0
Family history of ovarian cancer		
No	195	100.0
Yes	0	0
Number of pregnancy/pregnancies		
Not pregnant	8	4.1
1–3 times	94	48.2
4–5 times	34	17.4
>5 times	14	7.2
Unknown	45	23.1
Type of ovarian cancer		
Type 1	29	14.9
Type 2	119	61.0
Other	47	24.1
CEA, ng/mL	11.56±114.89	
CA199, U/mL	144.34±769.04	
AFP, ng/mL	225.98±1779.13	
CA125, U/mL	733.15±1553.06	
NLR	4.22±4.17	
LMR	3.61±2.24	
PLR	245.88±169.87	

Note: Number of pregnancy/pregnancies, the number of time/times a female has gone through the process/processes from conception to abortion or delivery of fetus/fetuses or embryo/embryos.
Abbreviations: NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio.

age, whereas the other types of cancer mainly occur in people in the age group of 35–50 years. Regarding the number of pregnancies, in the group of type 1 ovarian cancer, 17 women (58.6%) had never been pregnant or had 1–3 pregnancies, and 8 women (27.6%) had ≥4 pregnancies. In the type 2 ovarian cancer group, 55 women (46.2%) had never been pregnant or had 1–3 pregnancies, and 32 (26.9%) had ≥4 pregnancies. Although there was no significant difference in the number of pregnancies among the groups, these types of ovarian cancer were predominated by women with ≤3 pregnancies (Table 2). There were significant differences in CEA ($P = 0.035$) and AFP ($P = 0.012$) levels among the groups. The CEA level was the highest in patients with type 1 ovarian cancer (62.24 ± 296.74 ng/mL vs 2.87 ± 7.89 and 2.28 ± 2.26 ng/mL), whereas the AFP level was the lowest in patients with type 1 ovarian cancer (2.92 ± 1.50 ng/mL vs 16.83 ± 120.70 and 893.17 ± 3565.47 ng/mL) (Table 2).

The Frequencies and Distributions of the *BRCA* Gene Mutations

The protein coding region and exon-intron boundary region of the *BRCA1* and *BRCA2* genes of the patients were sequenced using next-generation sequencing. There were 64 patients (64/195, 32.8%) with *BRCA* gene mutations, among whom 32 patients (32/64, 50.0%) had *BRCA1* gene mutation/mutations, 27 patients (27/64, 42.2%) had *BRCA2* gene mutation/mutations, and 5 patients (5/64, 7.8%) had both mutations. The numbers of patients with type 1, type 2, and other types of ovarian cancer who had *BRCA* mutations were 7, 42, and 15, respectively. The numbers of patients with type 1 ovarian cancer who had *BRCA1, BRCA2*, and both mutations were 3 (42.9%), 4 (57.1%), and 0(0), respectively. The corresponding numbers of patients with type 2 ovarian cancer and other types of cancer were 23 (54.8%), 15 (35.7%), 4 (9.5%) and 6 (40.0%), 8 (53.3%), 1 (6.7%), respectively. These results show that type 1 ovarian cancer with *BRCA* mutation mainly involves the *BRCA2* gene, while type 2 ovarian cancer with *BRCA* mutation mainly involves the *BRCA1* gene. The frequencies and distributions of *BRCA1* and *BRCA2* gene mutations are presented in Table 3.

Table 2 Clinical Characteristics of Participants with Type 1, Type 2, and Other Ovarian Cancer

	Type 1	Type 2	Others	*P* value
Gender				
Female	29	119	47	–
Male	0	0	0	
Age (year)				
<35	1(3.4%)	2(1.7%)	11(23.4%)	<0.001
35–50	6(20.7%)	28(23.5%)	20(42.6%)	
>50	22(75.9%)	89(74.8%)	16(34.0%)	
Family history of breast cancer				
No	29	119	47	–
Yes	0	0	0	
Family history of ovarian cancer				
No	29	119	47	–
Yes	0	0	0	
Number of pregnancy/pregnancies				
≤3 times	17(58.6%)	55(46.2%)	30(63.8%)	0.210
≥4 times	8(27.6%)	32(26.9%)	8(17.0%)	
Unknown	4(13.8%)	32(26.9%)	9(19.1%)	
CEA, ng/mL	62.24±296.74	2.87±7.89	2.28±2.26	0.035
CA199, U/mL	332.58±1062.33	117.51±792.59	96.11±411.42	0.358
AFP, ng/mL	2.92±1.50	16.83±120.70	893.17±3565.47	0.012
CA125, U/mL	305.91±902.34	867.58±1642.59	656.41±1608.63	0.202
NLR	2.94±1.93	4.35±4.21	4.67±4.93	0.186
LMR	4.23±1.91	3.36±2.18	3.89±2.49	0.108
PLR	205.44±111.35	252.78±158.08	253.35±221.00	0.383

Note: Number of pregnancy/pregnancies, the number of time/times a female has gone through the process/processes from conception to abortion or delivery of fetus/fetuses or embryo/embryos.
Abbreviations: NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio.

Table 3 The Frequencies and Distributions of *BRCA1* and *BRCA2* Gene Mutations

	Patients with *BRCA* Mutation	Type 1	Type 2	Others	*P* value
Total	64	7	42	15	
BRCA1	32(50.0)	3(42.9)	23(54.8)	6(40.0)	0.736(χ^2=2.207)
BRCA2	27(42.2)	4(57.1)	15(35.7)	8(53.3)	
BRCA1 and *BRCA2*	5(7.8)	0(0)	4(9.5)	1(6.7)	
BRCA1					
Exon 8	1(3.1)	0(0)	1(4.3)	0(0)	
Exon 12	1(3.1)	0(0)	1(4.3)	0(0)	
Exon 14	15(46.9)	0(0)	14(60.9)	1(16.7)	
Exon 16	1(3.1)	1(33.3)	0(0)	0(0)	
Exon 17	9(28.1)	2(66.7)	3(13.0)	4(66.7)	
Exon 20	1(3.1)	0(0)	0(0)	1(16.7)	
Exon 14 and 14	1(3.1)		1(4.3)		
Exon 14 and 17	1(3.1)	0(0)	1(4.3)	0(0)	
Exon 17 and 22	1(3.1)	0(0)	1(4.3)	0(0)	
Intron 7	1(3.1)	0(0)	1(4.3)	0(0)	
BRCA2					
Exon 3	1(3.7)	0(0)	1(6.7)	0(0)	
Exon 5	1(3.7)	0(0)	1(6.7)	0(0)	
Exon 10	3(11.1)	1(25.0)	1(6.7)	1(12.5)	
Exon 11	7(25.9)	0(0)	5(33.3)	2(25.0)	
Exon 14	5(18.5)	0(0)	2(13.3)	3(37.5)	
Exon 15	2(7.4)	0(0)	1(6.7)	1(12.5)	
Exon 16	1(3.7)	0(0)	1(6.7)	0(0)	
Exon 18	2(7.4)	1(25.0)	1(6.7)	0(0)	
Exon 21	1(3.7)	0(0)	1(6.7)	0(0)	
Exon 23	1(3.7)	0(0)	0(0)	1(12.5)	
Exon 27	1(3.7)	1(25.0)	0(0)	0(0)	
Exon 6 and 11	1(3.7)	0(0)	1(6.7)	0(0)	
Exon 11 and 11	1(3.7)	1(25.0)	0(0)	0(0)	
BRCA1 and *BRCA2*					
BRCA1 exon 13 and *BRCA2* exon 11	1(20.0)	0(-)	1(25.0)	0(0)	
BRCA1 exon 14 and *BRCA2* exon 10	1(20.0)	0(-)	1(25.0)	0(0)	
BRCA1 exon 14 and *BRCA2* exon 11	1(20.0)	0(-)	0(0)	1(100.0)	
BRCA1 exon 14 and *BRCA2* exon 18	1(20.0)	0(-)	1(25.0)	0(0)	
BRCA1 exon 17 and *BRCA2* exon 11	1(20.0)	0(-)	1(25.0)	0(0)	

In this study, a total of 52 variants of the *BRCA* gene were detected. The sequence analysis revealed 22 distinct pathogenic mutations detected in 26 patients, 2 likely pathogenic variants in 2 patients, 12 variants of uncertain significance (VOUS) in 20 patients, and 16 likely benign variants in 24 patients. The mutations were predominantly seen in exons 8, 14, and 17 of the *BRCA1* gene and exons 10, 11, 14 and 15 of the *BRCA2* gene (Figure 1A and B). Among the *BRCA1* gene variants, 15 (62.5%) were pathogenic variants, 2 (8.3%) were likely pathogenic variants, 5 (20.8%) were likely benign variants, and 2 (8.3%) were VOUSs. On the other hand, among the *BRCA2* mutations, 7 (25.0%) were pathogenic variants, 11 (39.3%) were likely benign variants, and 10 (35.7%) were VOUSs (Figure 1C).

Table 4 provides detailed information on each patient with *BRCA* mutation/mutations, including ClinVar information (pathogenic, likely pathogenic, likely benign, and VOUS) pertaining to the *BRCA1* and *BRCA2* genes. Data on mutations, mutation types, family history of cancer, and number of pregnancies are presented. In this study, 10 patients (15.63%, 10/64) carried the *BRCA1* gene c.536 A>T variant, 4 patients (6.25%, 4/64) carried the *BRCA1* gene c.2635 G>T variant, 4 patients (6.25%, 4/64) carried the *BRCA1* gene c.2566 T>C variant, and 3 patients (4.69%, 3/64) carried the *BRCA2* gene
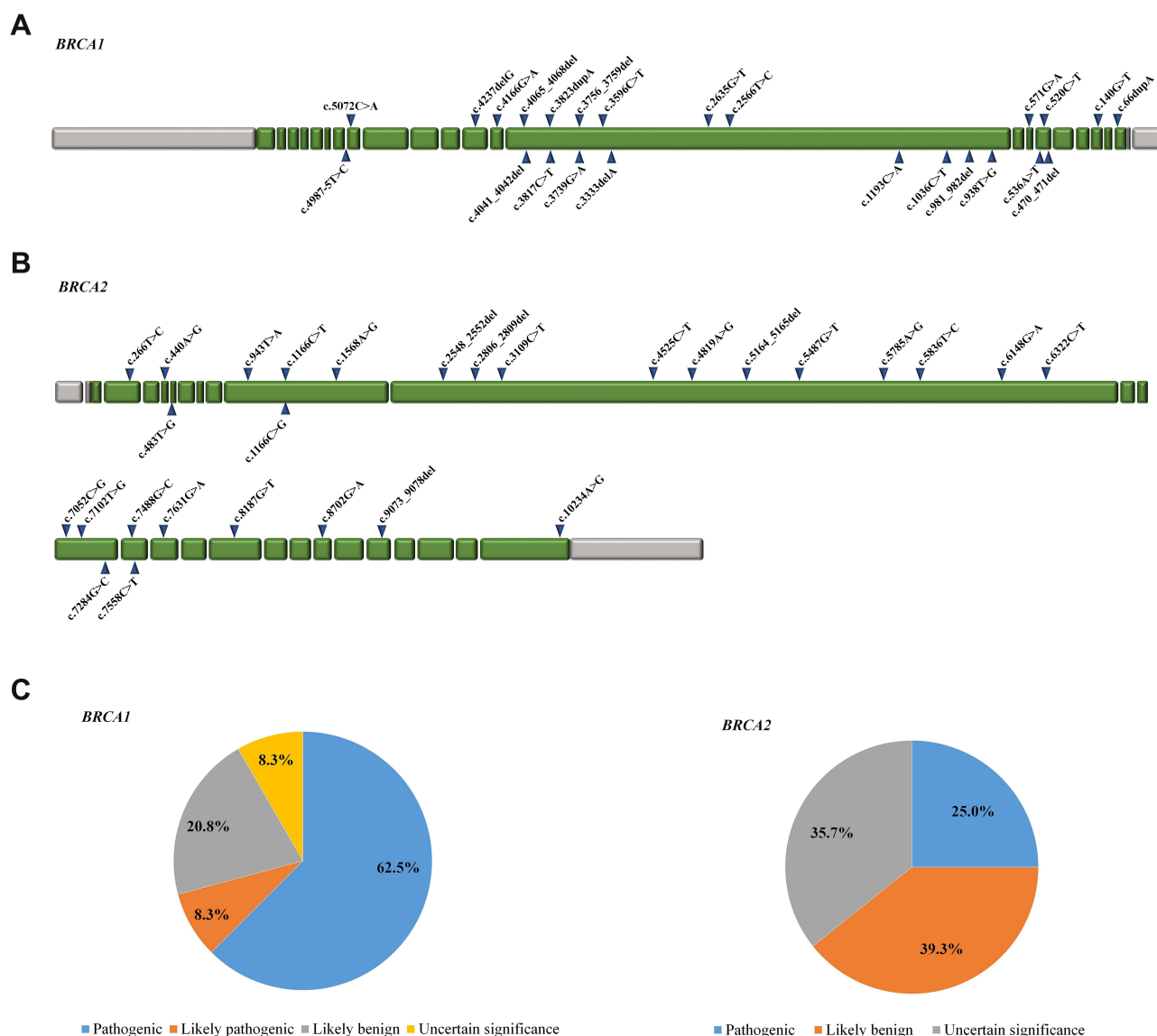
Figure I All the *BRCA1* (**A**) and *BRCA2* (**B**) variants and their locations on the protein coding region and exon intron boundary region identified in ovarian cancer patients, and the overall frequencies of the ClinVar grades of variants in ovarian cancer patients (**C**). Gray boxes represent untranslated regions and green boxes represent coding exons.

c.5785 A>G variant, 3 patients (4.69%, 3/64) carried the *BRCA2* gene c.8187 G>T variant. Based on these observations, *BRCA1* c.536 A>T might be a founder mutation in this population.

Discussion

Ovarian cancer is one of the most common malignant tumors that threaten women's health and quality of life and has the highest mortality rate among gynecological tumors.[25] *BRCA* is an important gene that determines the genetic susceptibility to cancer by participating in the regulation of DNA damage and repair, cell growth and apoptosis and by playing an indispensable role in maintaining the genetic stability of cells.[26,27] Mutations in the *BRCA* gene can lead to ovarian cancer. Screening for *BRCA* gene mutations can effectively assess and predict the risk for ovarian cancer, intervene to reduce the incidence of the disease, and guide precise treatment.

Worldwide, the incidence of *BRCA* mutations in patients with ovarian cancer is approximately 10%-15%. *BRCA* mutations are chiefly distributed in Europe and North America, among which the incidence of *BRCA1* mutation is significantly higher than that of *BRCA2*.[28] The incidence of *BRCA1/2* mutations among Mexican patients with ovarian

Table 4 All Mutations and the Characteristics of Ovarian Cancer Patients

Gene	Exon/ Intron	Mutation	Mutation Type	ClinVar	Clinical Staging of Cancer	Type of Ovarian Cancer	Age (Years)	Family History of Ovarian Cancer	Family History of Breast Cancer	Number of Pregnancy/ Pregnancies
BRCA1	Intron 7	c.4987–5T>C	SNV	Pathogenic	IV	Type 2	68	0	0	2
	Exon 8	c.5072C>A	SNV	Likely pathogenic	I	Type 2	54	0	0	5
	Exon 12	c.4237delG	DEL	Pathogenic	II	Type 2	54	0	0	4
	Exon 13	c.4166G>A	SNV	Likely benign	IV	Type 2	47	0	0	9
	Exon 14	c.4065_4068del	DEL	Pathogenic	IV	Type 2	55	0	0	9
	Exon 14	c.4041_4042del	DEL	Pathogenic	IV	Type 2	53	0	0	4
	Exon 14	c.3823dupA	DUP	Pathogenic	III	Type 2	50	0	0	6
	Exon 14	c.3817C>T	SNV	Pathogenic	IV	Type 2	68	0	0	4
	Exon 14	c.3756_3759del	DEL	Pathogenic	III	Type 2	45	0	0	3
	Exon 14	c.3756_3759del	DEL	Pathogenic	IV	Type 2	46	0	0	9
	Exon 14	c.3739G>A	SNV	Likely benign	III	Type 2	54	0	0	3
	Exon 14	c.3596C>T	SNV	Uncertain significance	I	Type 2	58	0	0	5
	Exon 14	c.3333delA	SNV	Pathogenic	IV	Type 2	54	0	0	9
	Exon 14	c.2635G>T	SNV	Pathogenic	III	Type 2	47	0	0	9
	Exon 14	c.2635G>T	SNV	Pathogenic	IV	Type 2	50	0	0	9
	Exon 14	c.2635G>T	SNV	Pathogenic	IV	Type 2	56	0	0	2
	Exon 14	c.2635G>T	SNV	Pathogenic	III	Type 2	53	0	0	3
	Exon 14	c.2566T>C	SNV	Likely benign	III	Other	28	0	0	2
	Exon 14	c.2566T>C	SNV	Likely benign	IV	Type 2	50	0	0	9
	Exon 14	c.2566T>C	SNV	Likely benign	III	Type 2	47	0	0	3
	Exon 14	c.2566T>C	SNV	Likely benign	I	Type 2	52	0	0	2
	Exon 14	c.1193C>A	SNV	Pathogenic	IV	Type 2	70	0	0	9
	Exon 14	c.1036C>T	SNV	Likely benign	III	Type 2	65	0	0	2
	Exon 14	c.981_982del	DEL	Pathogenic	Unknown	Other	72	0	0	9
	Exon 14	c.938T>G	SNV	Pathogenic	III	Type 2	67	0	0	9

	Exon 16	c.571G>A	SNV	Likely benign	I	Type 1	81	0	0	6
	Exon 17	c.536A>T	SNV	Uncertain significance	III	Type 1	68	0	0	3
	Exon 17	c.536A>T	SNV	Uncertain significance	III	Type 2	48	0	0	2
	Exon 17	c.536A>T	SNV	Uncertain significance	II	Type 2	49	0	0	2
	Exon 17	c.536A>T	SNV	Uncertain significance	IV	Type 2	49	0	0	1
	Exon 17	c.536A>T	SNV	Uncertain significance	Unknown	Other	46	0	0	3
	Exon 17	c.536A>T	SNV	Uncertain significance	Unknown	Type 1	38	0	0	0
	Exon 17	c.536A>T	SNV	Uncertain significance	IV	Type 2	56	0	0	2
	Exon 17	c.536A>T	SNV	Uncertain significance	I	Other	16	0	0	0
	Exon 17	c.536A>T	SNV	Uncertain significance	IV	Type 2	43	0	0	1
	Exon 17	c.536A>T	SNV	Uncertain significance	III	Other	50	0	0	6
	Exon 17	c.520C>T	SNV	Pathogenic	III	Type 2	63	0	0	2
	Exon 17	c.470_471del	DEL	Pathogenic	Unknown	Other	48	0	0	3
	Exon 20	c.140G>T	SNV	Likely pathogenic	III	Other	44	0	0	9
	Exon 22	c.66dupA	DUP	Pathogenic	III	Type 2	48	0	0	2
BRCA2	Exon 3	c.266T>C	SNV	Uncertain significance	II	Type 2	56	0	0	2
	Exon 5	c.440A>G	SNV	Likely benign	II	Type 2	64	0	0	9
	Exon 6	c.483T>G	SNV	Uncertain significance	IV	Type 2	73	0	0	9
	Exon 10	c.943T>A	SNV	Likely benign	III	Type 2	65	0	0	2
	Exon 10	c.1166C>G	SNV	Uncertain significance	III	Other	69	0	0	4
	Exon 10	c.1166C>T	SNV	Likely benign	I	Type 1	54	0	0	4
	Exon 10	c.1568A>G	SNV	Likely benign	I	Type 2	53	0	0	2
	Exon 11	c.2548_2552del	DEL	Pathogenic	III	Type 2	62	0	0	3
	Exon 11	c.2806_2809del	DEL	Pathogenic	IV	Other	49	0	0	3
	Exon 11	c.3109C>T	SNV	Pathogenic	IV	Type 2	47	0	0	9
	Exon 11	c.4525C>T	SNV	Pathogenic	III	Type 2	49	0	0	2
	Exon 11	c.4819A>G	SNV	Uncertain significance	IV	Type 2	73	0	0	9
	Exon 11	c.5164_5165del	DEL	Pathogenic	IV	Type 2	43	0	0	1
	Exon 11	c.5487G>T	SNV	Uncertain significance	III	Type 1	62	0	0	3
	Exon 11	c.5785A>G	SNV	Likely benign	III	Type 1	62	0	0	3
	Exon 11	c.5785A>G	SNV	Likely benign	Unknown	Other	72	0	0	9
	Exon 11	c.5785A>G	SNV	Likely benign	II	Type 2	76	0	0	8
	Exon 11	c.5836T>C	SNV	Uncertain significance	Unknown	Type 2	31	0	0	1
	Exon 11	c.6148G>A	SNV	Uncertain significance	I	Other	49	0	0	3
	Exon 11	c.6322C>T	SNV	Likely benign	III	Type 2	57	0	0	9

(*Continued*)

Table 4 (Continued).

Gene	Exon/ Intron	Mutation	Mutation Type	ClinVar	Clinical Staging of Cancer	Type of Ovarian Cancer	Age (Years)	Family History of Ovarian Cancer	Family History of Breast Cancer	Number of Pregnancy/ Pregnancies
	Exon 14	c.7052C>G	SNV	Likely benign	I	Other	17	0	0	0
	Exon 14	c.7052C>G	SNV	Likely benign	II	Type 2	54	0	0	2
	Exon 14	c.7102T>G	SNV	Likely benign	III	Type 2	54	0	0	2
	Exon 14	c.7102T>G	SNV	Likely benign	III	Other	37	0	0	2
	Exon 14	c.7284G>C	SNV	Uncertain significance	III	Other	34	0	0	3
	Exon 15	c.7488G>C	SNV	Uncertain significance	II	Other	60	0	0	6
	Exon 15	c.7558C>T	SNV	Pathogenic	III	Type 2	56	0	0	6
	Exon 16	c.7631G>A	SNV	Uncertain significance	IV	Type 2	61	0	0	2
	Exon 18	c.8187G>T	SNV	Likely benign	III	Type 1	41	0	0	3
	Exon 18	c.8187G>T	SNV	Likely benign	III	Type 2	67	0	0	9
	Exon 18	c.8187G>T	SNV	Likely benign	IV	Type 2	67	0	0	9
	Exon 21	c.8702G>A	SNV	Likely benign	IV	Type 2	48	0	0	9
	Exon 23	c.9073_9078T	DEL	Pathogenic	IV	Other	64	0	0	9
	Exon 27	c.10234A>G	SNV	Likely benign	IV	Type 1	45	0	0	2

Abbreviations: SNV, single-nucleotide variant; DEL, deletion; DUP, duplication.

cancer is approximately 28%, of which *BRCA1* constitutes 88% and *BRCA2* constitutes 12%.[29] Another study on patients with ovarian cancer in Mexico found that the incidence of *BRCA1/2* mutations was 33%, of which 66.1% was *BRCA1* and 33.9% was *BRCA2*.[30] These differences may be related to the geographical distribution of the selected population. The prevalence of *BRCA* mutations among Spanish patients with ovarian cancer was 16%, of which the prevalence of *BRCA2* mutations was 63%, which is contrary to most studies.[31] The prevalence of *BRCA* mutations in Israeli Arab patients with ovarian cancer was 32%.[32] The prevalence of *BRCA* mutations in Korean patients with ovarian cancer was 24.6%.[33] At present, there are several studies based on large samples in China. Shi et al[34] performed *BRCA1/2* gene detection in 916 patients with epithelial ovarian cancer. The results showed that the incidence of *BRCA1/2* gene mutation was 16.7%, of which *BRCA1* mutation accounted for 13.1%, *BRCA2* mutation accounted for 3.9%, and the simultaneous presence of both mutations accounted for 0.3%. Wu et al[35] conducted the *BRCA* test on 826 patients with ovarian cancer in a multiCenter nationwide study, and the incidence of *BRCA1/2* gene mutations was found to be 28.5%, of which the *BRCA1* mutation accounted for 20.8% and the *BRCA2* mutation accounted for 7.6%. Bu et al[36] attempted to detect *BRCA* mutation in 547 patients with ovarian cancer and found the incidence of *BRCA1/2* gene mutations to be 23.6%, of which *BRCA1* mutation accounted for 15.4% and *BRCA2* mutation accounted for 8.2%. The proportion of *BRCA* mutations was 5.41% in breast and ovarian cancer in a Hakka population.[37] The proportion of patients with *BRCA* mutation in Hong Kong was 15.3%.[38] In this study, the incidence of *BRCA1/2* gene mutations was 32.8% in patients with ovarian cancer, of which *BRCA1* mutation accounted for 50.0%, *BRCA2* mutation accounted for 42.2%, and both mutations accounted for 7.8%. In the future, multicenter *BRCA* gene mutation studies should be conducted in China with a larger sample size by adopting unified standards so as to create a *BRCA* gene mutation database consistent with the characteristics of the Chinese population.

To the best of our knowledge, this study is the largest on *BRCA1* and *BRCA2* gene sequencing in patients with ovarian cancer for mutation screening analysis in the Chinese Hakka population. According to researches, mutations in the *BRCA1* gene are concentrated in exons 8, 11, 22, and 24 and mutations in the *BRCA2* gene are concentrated in exons 10, 11, 14, and 21 in Mainland China populations.[39–43] Our results suggested that mutations in the *BRCA1* gene mainly occur in exons 8, 14, and 17, whereas mutations in the *BRCA2* gene chiefly occur in exons 10, 11, 14, and 15. The findings indicate that the *BRCA* genes have different mutation hotspots in different ethnic groups and regions.

In addition, some variants were present at higher frequencies when compared with the other variants. It was observed that 10 patients (15.63%, 10/64) carried the *BRCA1* gene c.536 A>T variant, 4 patients (6.25%, 4/64) carried the *BRCA1* gene c.2635 G>T variant, 4 patients (6.25%, 4/64) carried the *BRCA1* gene c.2566 T>C variant, and 3 patients (4.69%, 3/64) carried the *BRCA2* gene c.5785 A>G variant, 3 patients (4.69%, 3/64) carried the *BRCA2* gene c.8187 G>T variant. Whether these mutations are founder and hotspot mutations in the patients with ovarian cancer in the Chinese Hakka population remains to be confirmed. Founder mutations in the *BRCA* genes have been reported in many nations and ethnic groups worldwide. It has been documented that *BRCA1* c. 5470_5477del8 is a *BRCA1* founder mutation of ovarian cancer in the Chinese population;[34,44] this mutation may be the *BRCA1* founder mutation unique to Asians.[45] The mutation *BRCA2* c.3109 C>T is a founder mutation in the Southern Chinese population.[46] Another study showed that the most common mutation was *BRCA1* ex9-12del, a Mexican founder mutation.[30] Mutational data show that the most frequently recorded *BRCA1* c.5266dupC mutation is the founder mutation in Italian,[47] Northeastern Romanian,[48] and Turkish populations.[49] Slavic *BRCA1* and *BRCA2* founder mutations include *BRCA1* c.5266dupC, *BRCA1* c.4034delA, and *BRCA1* c.68_69delAG.[50] *BRCA1* c.4136_4137delCT and c.1140dupG represent the founder mutations in the Middle Eastern population.[51] *BRCA2* c.3922G>T is a founder mutation in the Puerto Rican population.[52] *BRCA1* c.5266dupC and c.181T>G are founder mutations in the Polish population.[53] *BRCA1* c.798_799delTT might be a founder mutation in the North African population.[54] *BRCA1* c.3319G>T might be a founder mutation in the Western Danish population.[55] In the present study, the *BRCA1* c.536 A>T mutation was observed which might be considered a founder mutation in this ovarian cancer population. The recurrent *BRCA1* mutation reported herein has rarely been observed in other ethnic groups.

In general, the mutation frequency of the *BRCA1/2* gene in the Hakka patients with ovarian cancer in Southern China is different from that in other ethnic groups. Moreover, differences exist in the exon regions where the mutations occur. This study provides a basis and serves as a reference for clinical counseling and for devising individualized prevention

and treatment strategies to combat ovarian cancer. Identifying founder and recurrent mutations is an important way to improve genetic counseling because molecular testing can target the founder and recurrent mutations, thereby enabling faster and less expensive testing. As the frequency of founder mutations increases, molecular testing can analyze a large number of cases and provide accurate information on the relationship between the patient's mutation status and disease risk, thereby improving disease management. Owing to the small sample size of this study, the distributions of the *BRCA* gene mutations among the patients with ovarian cancer in the Hakka population of Southern China have not been entirely revealed. The distributions of the *BRCA* gene mutations in different populations, and the relationship between mutation status and disease risk and pathological features need to be investigated further.

In this population, the significance of identifying the founder mutations mainly lies in reducing the cost of population screening. Genetic screening can be performed by first focusing on the founder mutation. In this manner, genetic screening can be easily implemented in the Hakka population. Although this study has shed light on the founder *BRCA1* mutation in the Hakka population, we cannot rule out the possibility that other founder *BRCA* mutations may exist in the larger patient population. However, given the economic advantages of genetic screening, we believe that this study would pave the way for future studies in the Hakka population.

Conclusions

In this study, the *BRCA* gene mutations were found to account for a certain proportion of the patients with ovarian cancer in the Hakka population of Southern China. The *BRCA1* c.536 A>T mutation was detected among in 10/64 (15.63%) of the individuals with *BRCA* mutation/mutations in the cohort and can, therefore, be considered a founder mutation in this ovarian cancer population. This recurrent *BRCA1* mutation has rarely been observed in other ethnic groups. Understanding the frequency of *BRCA1* and *BRCA2* gene mutations in the Hakka patients with ovarian cancer will provide valuable data for clinical consultation and for devising individualized therapeutic strategies for patients with ovarian cancer.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate

This study was conducted on the basis of the Declaration of Helsinki, and was supported by the Ethics Committee of the Meizhou People's Hospital.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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