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# The Role and Significance of *BRCA1* c.4712delT Mutation in Ovarian Cancer

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KEYWORDS

*Ovarian Cancer;*
*BRCA1 Gene;*
*Gene Mutation;*
*Clinical Diagnosis*

ABSTRACT

**Objective:** To investigate the mutation status of BRCA1 c.4712delT in patients with sporadic ovarian cancer and to analyze its clinical significance in relation to clinical indicators such as HE4 and CA125.

**Methods: 1)**The BRCA1 c.4712delT mutation status in patients with sporadic ovarian cancer and healthy individuals was detected using Restriction Fragment Length Polymorphism (RFLP) and Sanger sequencing. **2)**Statistical analyses were conducted to assess differences in age, tumor markers, and coagulation function between ovarian cancer patients and healthy individuals, as well as between BRCA1 c.4712delT-mutated and non-mutated ovarian cancer patients.

**Results:** This study enrolled 209 ovarian cancer patients and 213 healthy individuals undergoing routine health checkups. In sporadic ovarian cancer patients, the BRCA1 c.4712delT mutation frequency was 1.67%, slightly higher than the 0.47% frequency in the healthy population; however, the difference was not statistically significant (P = 0.103). Levels of age, HE4, and CA125 showed statistically significant differences between ovarian cancer patients and healthy individuals. No statistically significant differences were observed in age, HE4, CA125, PT, APTT, TT, FIB, D-D, personal cancer history, family history, lymph node metastasis, pathological grade, or clinical stage between the BRCA1 c.4712delT-mutated and non-mutated groups. Bioinformatics analysis predicted that the BRCA1 c.4712delT mutation is deleterious. An ovarian cancer risk model established based on age, HE4, CA125 levels, and BRCA1 c.4712delT mutation status demonstrated that the combined model incorporating all factors yielded the best diagnostic efficacy for predicting ovarian cancer risk.

**Conclusions: 1)**The mutation frequency of BRCA1 c.4712delT in ovarian cancer patients is 1.67%. **2)**Changes in serum levels of the tumor markers HE4 and CA125 serve as effective indicators for the early diagnosis of ovarian cancer. **3)**BRCA1 c.4712delT mutation detection is a potential indicator for the early diagnosis of ovarian cancer.

## Introduction

Ovarian cancer is one of the most lethal malignancies of the female reproductive system and is characterized by high heterogeneity. Its biological behavior, molecular characteristics, and clinical outcomes differ markedly across histological types. According to the 2020 fifth edition of the World Health

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Organization (WHO) classification, ovarian cancer is clearly divided into five major subtypes: high-grade serous carcinoma (HGSC, 70%), endometrioid carcinoma (EC, 10%), clear cell carcinoma (CCC, 10%), mucinous carcinoma (MC, 3%), and low-grade serous carcinoma (LGSC, <5%). Each subtype possesses distinct epidemiological profiles, molecular alterations, and prognostic features. Globally, both the incidence and mortality of ovarian cancer rank among the highest in female cancers. In 2020, there were approximately 314,000 new cases and 207,000 deaths worldwide, with China accounting for 17.6% of global cases (approximately 57,000 new cases). Current diagnostic approaches primarily include imaging methods (transvaginal ultrasound, CT, and MRI), tumor markers (CA125 and HE4), and pathological diagnosis as the gold standard. Due to the insidious nature of early symptoms and the lack of effective screening methods, 75% of patients are diagnosed at advanced stages (stage III/IV), resulting in a 5-year survival rate of only 30%–40%. Studies have established that germline *BRCA1/2* mutations are closely associated with the occurrence and metastasis of ovarian cancer, suggesting that *BRCA1/2* mutation testing may serve as an effective approach for early clinical diagnosis.

Breast cancer susceptibility genes (*BRCA*) are critical tumor suppressor and cancer predisposition genes, primarily including *BRCA1* and *BRCA2* . *BRCA1* is located on chromosome 17 (17q12–21) and encodes 1,863 amino acids, while *BRCA2* is located on chromosome 13 (13q12–13) and encodes 3,418 amino acids. Both proteins maintain genomic stability by interacting with proteins such as RAD51 and PALB2 to repair DNA double-strand breaks via homologous recombination (HR), and they also regulate the cell cycle and transcription. When *BRCA1/2* are inactivated by mutation, HR repair is compromised, leading to increased genomic instability and promoting carcinogenesis. Furthermore, cells with *BRCA1/2* mutations exhibit significantly increased sensitivity to platinum-based drugs and PARP inhibitors, which informs clinical treatment strategies and prognosis. Women carrying pathogenic *BRCA1* mutations have a lifetime breast cancer risk exceeding 60% and an ovarian cancer risk ranging from 39.0% to 58.0%. Among the various *BRCA* mutations, *BRCA1* c.4712delT is a high-frequency deleterious germline mutation site.

The c.4712delT mutation is a frameshift mutation caused by the deletion of a thymine (T), resulting in a shift of the reading frame. This mutation typically leads to a premature termination codon, generating a truncated protein. Consequently, the BRCT domain of the BRCA1 protein may be lost, impairing its ability to bind DNA repair proteins and resulting in defective DNA repair. As a loss-of-function mutation, c.4712delT is closely associated with early-onset breast cancer (<45 years) and familial ovarian cancer.

Genome-wide association studies (GWAS) are statistical approaches used to identify genetic variants associated with specific phenotypes or diseases by scanning genome-wide single-nucleotide polymorphisms (SNPs) in large cohorts. To date, GWAS has identified approximately 27 genomic regions associated with epithelial ovarian cancer (EOC) risk, including key loci repeatedly validated across studies, such as 8q21 (rs11782652), 17q12 (rs757210), and 9p22 (rs3814113). Insertions and deletions refer to the addition or removal of one or more nucleotides in a DNA sequence; *BRCA1* c.4712delT belongs to this category. Studies indicate that the frequency of the *BRCA1* T-deletion in the Chinese population is 6.8%, significantly higher than the 3.5% reported in the BIC database for other populations. Therefore, in-depth research on this specific mutation is of substantial clinical significance.

In addition to genetic testing, early clinical diagnosis of ovarian cancer relies heavily on tumor markers. CA125 (carbohydrate antigen 125), encoded by the *MUC16* gene, is a high-molecular-weight mucin-type glycoprotein. Serum CA125 levels exceed 35 U/mL in 83% of ovarian cancer patients, and its fluctuations correlate with disease progression or remission. HE4 (human epididymis protein 4), encoded by the *WFDC2* gene, is another crucial biomarker. HE4 is significantly overexpressed in ovarian cancer (especially serous and endometrioid carcinomas). A meta-analysis showed that HE4 has a sensitivity of 73% and specificity of 89% for diagnosing ovarian cancer, which is superior to CA125 (sensitivity 74%, specificity 82%). The combined detection of CA125 and HE4 further improves diagnostic sensitivity and specificity.

Beyond detection methods, exploring the relationship between clinical indicators such as coagulation function and ovarian cancer provides insight into disease progression. Commonly used coagulation indicators include prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), and D-dimer (D-D). This study uses these indicators to explore the association between coagulation function and the *BRCA1* c.4712delT mutation in ovarian cancer patients.

The present study utilizes experimental methods to analyze differences in the *BRCA1* c.4712delT mutation frequency between ovarian cancer patients and healthy individuals. By statistically analyzing clinical data—including age, tumor markers, coagulation function, and pathology—this study aims to elucidate the role of this mutation in ovarian cancer development and identify new avenues for early diagnosis.

## Materials and Methods

### Clinical Specimens

This study collected 2 mL of EDTA-anticoagulated whole blood and associated clinical data from 209 ovarian cancer patients (excluding 17 cases) treated at Hunan Cancer Hospital between December 23, 2024, and April 22, 2025. Addi-

**Table 1 | Primers for detection of BRCA1 c.4712delT site mutation**

| Primer Name | Direction | Sequence |
|---|---|---|
| 4712F | Forward | 5'-ACGTGTTAAGGTGTTTGCTACA-3' |
| 4712R | Reverse | 5'-GACTCTGGGGCTCTGTCTTC-3' |

**Table 2 | PCR amplification reaction system**

| Component | Volume |
|---|---|
| DNA Template | 2 μL |
| Forward Primer (10 μM) | 0.5 μL |
| Reverse Primer (10 μM) | 0.5 μL |
| PCR SuperMix | 10 μL |
| Nuclease-free Water | 7 μL |
| Total Volume | 20 μL |

**Table 3 | PCR amplification program**

| Temperature | Time | |
|---|---|---|
| 94℃ | 5 min | |
| 94℃ | 30 sec | 35 cycles |
| 58℃ | 30 sec | |
| 72℃ | 30 sec | |
| 72℃ | 5 min | |

**Table 4 | DNA enzyme digestion reaction system**

| Component | Volume |
|---|---|
| $ddH_2O$ | 16.5 μL |
| 10×FuniCut® Color Buffer | 3 μL |
| Substrate DNA | 10 μL |
| FuniCut® Earl | 0.5 μL |
| Total | 30 μL |

tionally, samples and data were collected from 213 healthy individuals (excluding 29 cases) undergoing physical examinations between February 5, 2025, and April 11, 2025, totaling 422 participants.

- **Inclusion criteria for the experimental group: 1)** Clinically and pathologically diagnosed with malignant ovarian tumors; **2)** Complete medical records.
- **Inclusion criteria for the control group: 1)** No history of malignant ovarian tumors; **2)** Age between 20 and 60 years; **3)** Valid clinical data. All pathological diagnoses were confirmed by experienced pathologists. This study was approved by the Medical Ethics Committee of Hunan Cancer Hospital, and informed consent was obtained from all participants.

## Reagents

Blood DNA extraction kits (spin column type) were purchased from Tiangen Biotech. PCR amplification reagents (2×EasyTaq® PCR SuperMix +dye) were purchased from TransGen Biotech. PCR primers were synthesized by Tsingke Biotechnology. The restriction enzyme FuniCut® Earl was purchased from Yeasen Biotechnology. Agarose (BIOWEST) and Tris-acetate electrophoresis buffer (50×TAE) were purchased from Aibowei Biotechnology.

## Experimental Methods

### *PCR Amplification*

Primers were designed based on the upstream and downstream sequences of the *BRCA1* c.4712delT mutation site (**Table 1**). PCR amplification was performed using the system and program outlined in **Tables 2 and 3**.

### *Enzyme Digestion*

Prepare the system reaction mixture according to the sample addition order in Table 4 (perform on ice). The reaction mixture was prepared on ice according to **Table 4**. The mixture was incubated at 37℃ for 30 minutes, followed by incubation at 80℃ for 20 minutes to inactivate the enzyme.

### *Electrophoresis*

Electrophoresis was conducted at a constant voltage of 120 V for 30 minutes. Wild-type samples displayed two clear bands (approximately 50 bp and 250 bp). Samples showing a single band (~300 bp) or three bands of different lengths were flagged as potential mutations.

### *Sanger Sequencing & Analysis*

Samples suspected of mutation based on electrophoresis were subjected to Sanger sequencing. The resulting sequences were aligned with the NCBI reference sequence for *BRCA1* (NC_000017.11) to determine the genotype (wild-type, heterozygous, or homozygous).

## Statistical Analysis

Data analysis was performed using SPSS 27.0. Normality was assessed using the Shapiro-Wilk test. Quantitative data (e.g., age, HE4, CA125, coagulation markers) did not follow a normal distribution and are presented as mean ± standard deviation (X ± SD); comparisons were made using the Mann-Whitney U test. Ordinal data (pathological grade, clinical stage) were analyzed using the Mann-Whitney U test. Categorical data (cancer history, family history, metastasis) are expressed as frequencies (n, %) and were compared using the Chi-square test or Fisher's exact test. Logistic regression was used to construct risk assessment models. A $P$ -value < 0.05

**Table 5 | Summary of clinical data for ovarian cancer patients (experimental group) (n = 209)**

| Indicator | Category | N(%) |
|---|---|---|
| Age | ≤ 40 | 17 (8.13) |
| | 41–50 | 55 (26.32) |
| | 51–60 | 88 (42.11) |
| | ≥ 61 | 49 (23.44) |
| | Mean | 54.06 ± 10.70 |
| Histological subtype | Serous carcinoma | 169 (80.86) |
| | Mucinous carcinoma | 5 (2.39) |
| | Endometrioid carcinoma | 5 (2.39) |
| | Clear cell carcinoma | 18 (8.61) |
| | Other | 11 (5.26) |
| | Unknown | 1 (0.48) |
| Clinical stage | I | 21 (10.05) |
| | II | 27 (12.92) |
| | III | 93 (44.50) |
| | IV | 55 (26.32) |
| | Unknown | 13 (6.22) |
| Histologic grade | Low grade | 6 (2.87) |
| | Intermediate grade | 2 (0.96) |
| | High grade | 163 (77.99) |
| | Unknown | 38 (18.18) |
| Lymph node metastasis | Yes | 99 (47.37) |
| | No | 79 (37.80) |
| | Unknown | 31 (14.83) |

**Table 6 | Comparative analysis between the experimental group and the control group.**

| Indicator | experimental group (n=209) | control group (n=213) | Z | P |
|---|---|---|---|---|
| Age | 54.06 ± 10.70 | 34.41 ± 12.05 | -13.142 | <0.001 |
| HE4 | 532.52 ± 505.01 | 47.42 ± 8.96 | -8.543 | <0.001 |
| CA125 | 1492.42 ± 4156.48 | 13.86 ± 6.44 | -12.748 | <0.001 |

**Table 7 | Comparative analysis of genotypes between the experimental group and the control group**

| Genotype | Experimental group | Control group | P |
|---|---|---|---|
| AA | 202 | 211 | |
| A- | 7 | 2 | |
| Total | 209 | 213 | 0.103 |

**Table 8 | Bioinformatics prediction results for the BRCA1 c.4712delT variant**

| Items | Results |
|---|---|
| Gene | *BRCA1* |
| Nucleotide change | *BRCA1* c.4712delT |
| Exon | exon16 |
| Effect on protein | p.E1571fs |
| variant_function | frameshift substitution |
| dbSNP ID | rs886037790 |
| Mutation type | Frameshift del |
| Clinically Importance | Yes |
| Previously reported | BIC/ClinVar/UMD/LOVD |
| Ethnicity | Eastern-European, Chinese, Han, None Specified, None-Specified, Not Specified, Western |
| MetaSVM/MetaLR/SIFT/Poly-Phen | deleterious |

**Table 10 | Summary of baseline clinical data in the mutant group (n = 7)**

| Indicator | Category | N (%) |
|---|---|---|
| Age | ≤ 40 | 1 (14.29) |
| | 41–50 | 2 (28.57) |
| | 51–60 | 4 (57.14) |
| | ≥ 61 | 0 (0.00) |
| | Mean (years) | 50.29 ± 8.08 |
| Histological subtype | Serous carcinoma | 5 (71.43) |
| | Mucinous carcinoma | 0 (0.00) |
| | Endometrioid carcinoma | 0 (0.00) |
| | Clear cell carcinoma | 2 (28.57) |
| Histological subtype | Other | 0 (0.00) |
| | Unknown | 0 (0.00) |
| Clinical stage | I | 1 (14.29) |
| | II | 1 (14.29) |
| | III | 5 (71.43) |
| | IV | 0 (0.00) |
| | Unknown | 0 (0.00) |
| Histologic grade | Low grade | 0 (0.00) |
| | Intermediate grade | 0 (0.00) |
| | High grade | 5 (71.43) |
| | Unknown | 2 (28.57) |
| Lymph node metastasis | Yes | 3 (42.86) |
| | No | 4 (57.14) |
| Personal history of cancer | Yes | 0 (0.00) |
| | No | 7 (100.00) |
| HBOC-associated tumors | Yes | 1 (14.29) |
| | No | 6 (85.71) |
| Family history | Yes | 3 (42.86) |

**Table 9 | Clinical data of seven patients with the mutant genotype**

| ID | H46 | H74 | H87 | H165 | H168 | H172 | H173 |
|---|---|---|---|---|---|---|---|
| Age (years) | 60 | 54 | 38 | 45 | 51 | 59 | 45 |
| Histological type | Serous adenocarcinoma | Clear cell carcinoma | Serous adenocarcinoma | Serous adenocarcinoma | Clear cell carcinoma | Serous adenocarcinoma | Serous adenocarcinoma |
| Histologic grade | High grade | Not reported | High grade | High grade | Not reported | High grade | High grade |
| Clinical stage | III | I | III | II | III | III | III |
| Family history of cancer | No | Yes | No | Yes | No | No | Yes |
| HBOC syndrome | No | No | No | No | No | No | Yes |
| Lymph node metastasis | No | No | Yes | No | No | Yes | Yes |

**Table 11 | Comparative analysis of clinical data between the mutant group and the wild-type group**

| Indicator | Mutant group (n = 7) | Wild-type group (n = 202) | Z | P |
|---|---|---|---|---|
| Age (years) | 50.29 ± 8.08 | 54.19 ± 10.77 | -1.059 | 0.290 |
| HE4 | 316.93 ± 425.50 | 533.72 ± 487.53 | -1.538 | 0.124 |
| CA125 | 1882.82 ± 1937.76 | 1212.96 ± 1868.83 | -0.297 | 0.766 |
| PT | 11.31 ± 0.81 | 11.62 ± 0.84 | -1.212 | 0.225 |
| APTT | 26.10 ± 2.89 | 27.14 ± 3.20 | -0.601 | 0.548 |
| TT | 20.10 ± 7.90 | 16.96 ± 1.71 | -1.224 | 0.221 |
| FIB | 4.65 ± 1.96 | 4.84 ± 6.72 | -0.057 | 0.954 |
| D-dimer (D-D) | 4.30 ± 4.21 | 3.69 ± 3.83 | -0.235 | 0.814 |
| Personal history of cancer | 0 (0) | 11 (5.4%) | — | 1.000 |
| Family history | 3 (42.9%) | 56 (27.7%) | — | 0.406 |
| Lymph node metastasis | 3 (42.9%) | 95 (55.6%) | — | 0.636 |
| Histologic differentiation | — | — | -0.536 | 0.592 |
| Well differentiated | 0 (0) | 6 (3%) | — | — |
| Moderately differentiated | 0 (0) | 2 (1%) | — | — |
| Poorly differentiated | 7 (100%) | 194 (96%) | — | — |
| Clinical stage | — | — | -1.255 | 0.210 |
| Stage I | 1 (14.3%) | 19 (10.1%) | — | — |
| Stage II | 1 (14.3%) | 25 (13.2%) | — | — |
| Stage III | 5 (71.4%) | 91 (48.1%) | — | — |
| Stage IV | 0 (0) | 54 (28.6%) | — | — |

was considered statistically significant.

**Table 12 | Statistical analysis and correlation between serological biomarkers and clinical characteristics in the experimental group**

| Indicator | Category | N (%) | HE4 (mean ± SD) | CA125 (mean ± SD) |
|---|---|---|---|---|
| Age | ≤ 40 | 17 (8.13) | 390.66 ± 539.39 | 751.44 ± 1145.35 |
| | 41–50 | 55 (26.32) | 421.96 ± 503.52 | 1102.33 ± 1632.96 |
| | 51–60 | 88 (42.11) | 556.80 ± 501.85 | 1514.13 ± 2335.46 |
| | ≥ 61 | 49 (23.44) | 642.52 ± 487.35 | 2148.34 ± 7793.06 |
| | P | | 0.009 | 0.367 |
| Histological subtype | Serous carcinoma | 169 (80.86) | 599.02 ± 514.28 | 1716.39 ± 4555.03 |
| | Mucinous carcinoma | 5 (2.39) | 133.27 ± 171.89 | 80.10 ± 61.89 |
| | Endometrioid carcinoma | 5 (2.39) | 51.82 ± 13.96 | 224.48 ± 413.37 |
| | Clear cell carcinoma | 18 (8.61) | 164.70 ± 197.37 | 531.66 ± 1387.91 |
| | Other | 11 (5.26) | 309.05 ± 367.22 | 603.79 ± 1251.82 |
| | Unknown | 1 (0.48) | 1312.7 ± 0 | 4111 ± 0 |
| | P | | < 0.001 | < 0.001 |
| Clinical stage | I | 21 (10.05) | 129.26 ± 155.25 | 124.80 ± 172.13 |
| | II | 27 (12.92) | 202.83 ± 289.97 | 622.48 ± 1434.86 |
| | III | 93 (44.50) | 611.08 ± 487.23 | 2150.08 ± 5958.27 |
| | IV | 55 (26.32) | 660.86 ± 527.37 | 1480.73 ± 1688.11 |
| | Unknown | 13 (6.22) | 679.58 ± 647.87 | 853.05 ± 1084.73 |
| | P | | < 0.001 | < 0.001 |
| Histologic grade | Low grade | 6 (2.87) | 227.62 ± 228.31 | 36.01 ± 17.65 |
| | Intermediate grade | 2 (0.96) | 194.90 ± 0 | 2061.09 ± 2860.83 |
| | High grade | 163 (77.99) | 615.32 ± 509.38 | 1748.15 ± 4630.47 |
| | Unknown | 38 (18.18) | 236.58 ± 381.98 | 595.46 ± 1243.34 |
| | P | | < 0.001 | < 0.001 |
| Lymph node metastasis | Yes | 99 (47.37) | 590.17 ± 487.35 | 2148.41 ± 5718.41 |
| | No | 79 (37.80) | 353.23 ± 418.76 | 558.94 ± 1230.92 |
| | P | | < 0.001 | < 0.001 |
| Personal history of cancer | Yes | 11 (5.26) | 645.91 ± 618.65 | 722.52 ± 856.14 |
| | No | 196 (93.78) | 525.31 ± 498.21 | 1550.33 ± 4281.63 |
| | P | | 0.672 | 0.630 |
| HBOC syndrome | Yes | 15 (7.18) | 636.75 ± 580.42 | 1845.85 ± 2792.01 |
| | No | 191 (91.39) | 523.93 ± 499.27 | 1463.71 ± 4272.79 |
| | P | | 0.462 | 0.305 |
| Family history | Yes | 59 (28.23) | 482.94 ± 481.41 | 1199.81 ± 1882.68 |
| | No | 147 (70.33) | 554.77 ± 515.55 | 1608.63 ± 4802.48 |
| | P | | 0.345 | 0.990 |

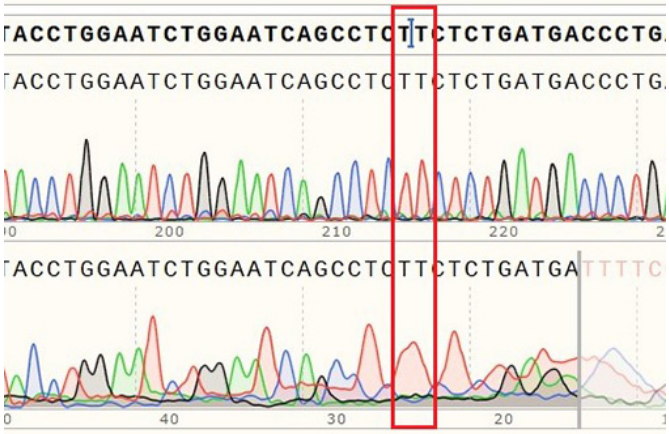


**Figure 1 | Wild-type Sanger sequencing chromatogram and sequence alignment**

*Note: (1) The bold text at the top indicates the reference sequence BRCA1 (NC_000017.11). (2) The two chromatograms, from top to bottom, correspond to the forward and reverse sequences, respectively. (3) The four colored peaks represent the four nucleotides. (4) The target site is indicated within the red box.*

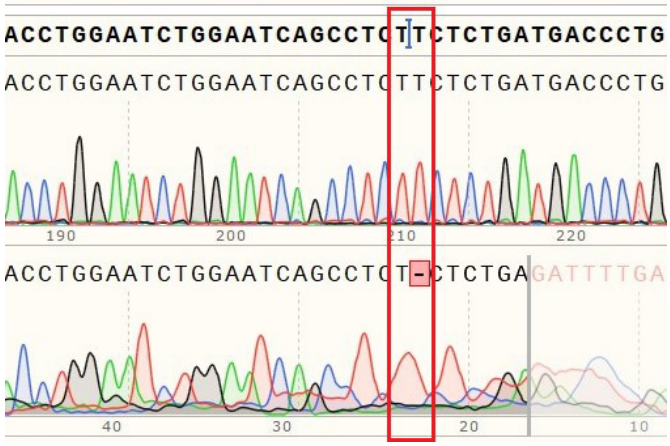


**Figure 2 | Heterozygous mutant Sanger sequencing chromatogram and sequence alignment**

*Note: (1) The bold text at the top indicates the reference sequence BRCA1 (NC_000017.11). (2) The two chromatograms, from top to bottom, correspond to the forward and reverse sequences, respectively. (3) The four colored peaks represent the four nucleotides. (4) The target site is indicated within the red box.*

## Results

### Comparative Analysis Between Experimental and Control Groups

The clinical characteristics of the 209 sporadic ovarian cancer patients are summarized in **Table 5**. The mean age was 54.06 ± 10.70 years. The predominant histological type was serous carcinoma (80.86%), and the majority of patients had high-grade tumors (77.99%). Most patients were diagnosed at advanced clinical stages (Stage III: 44.50%; Stage IV: 26.32%), and lymph node metastasis was common (47.37% confirmed yes).

Comparative analysis (**Table 6**) revealed that Age ($P < 0.001$), HE4 ($P < 0.001$), and CA125 ($P < 0.001$) were significantly higher in the experimental group compared to the healthy control group.

### *BRCA1* c.4712delT Mutation Status

The electrophoresis results indicated 18 samples in the experimental group and 10 samples in the control group with suspected mutations and/or failed restriction enzyme digestion, for a total of 28 samples. Sanger sequencing was performed on these 28 samples, and the resulting nucleotide sequences were compared with the BRCA1 reference sequence in the database (NC_000017.11). In the experimental group, 9 cases were wild type, 7 cases were heterozygous mutations, 0 cases were homozygous mutations, and 2 cases were indeterminate (excluded). In the control group, 7 cases were wild type, 2 cases were heterozygous mutations, 0 cases were homozygous mutations, and 1 case was indeterminate (excluded). The mutation frequency of BRCA1 c.4712delT was 1.67% (7/418) in the experimental group and 0.47% (2/426) in the control group. **Figure 1** shows the sequencing result for the wild-type genotype, and **Figure 2** shows the sequencing result for the heterozygous mutant genotype.

The experimental results were summarized and subjected to statistical analysis (**Table 7**), showing that the difference in genotype distribution between the experimental group and the control group was not statistically significant ($P > 0.05$).

In the preliminary phase of this project, the protein functional impact of the BRCA1 c.4712delT variant was evaluated using the bioinformatics prediction tools MetaSVM and MetaLR, as well as the online tools SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) (**Table 8**). The predictions from MetaSVM, MetaLR, SIFT, and PolyPhen-2 all indicated that this germline variant is deleterious, suggesting that it impairs the function of the encoded protein. Specifically, alterations were identified in amino acids downstream of the 1571st amino acid in exon 16 of BRCA1, resulting in truncation of the BRCA1 protein beyond this position and loss of normal BRCA1 function. This truncation is expected to hinder the interaction of BRCA1 with homologous recombination repair–related proteins such as RAD51 and PALB2, thereby disrupting key processes including homologous recombination repair of DNA double-strand breaks, cell-cycle regulation, and transcriptional regulation. It may further impair centrosome duplication control, leading to centrosome dysregulation, genomic instability, and malignant cellular transformation, ultimately contributing to carcinogenesis.

### Comparative Analysis Between Genotype Groups

The experimental group was stratified by genotype into a mutant subgroup and a wild-type subgroup. The basic clinical information of the mutant subgroup was summarized and

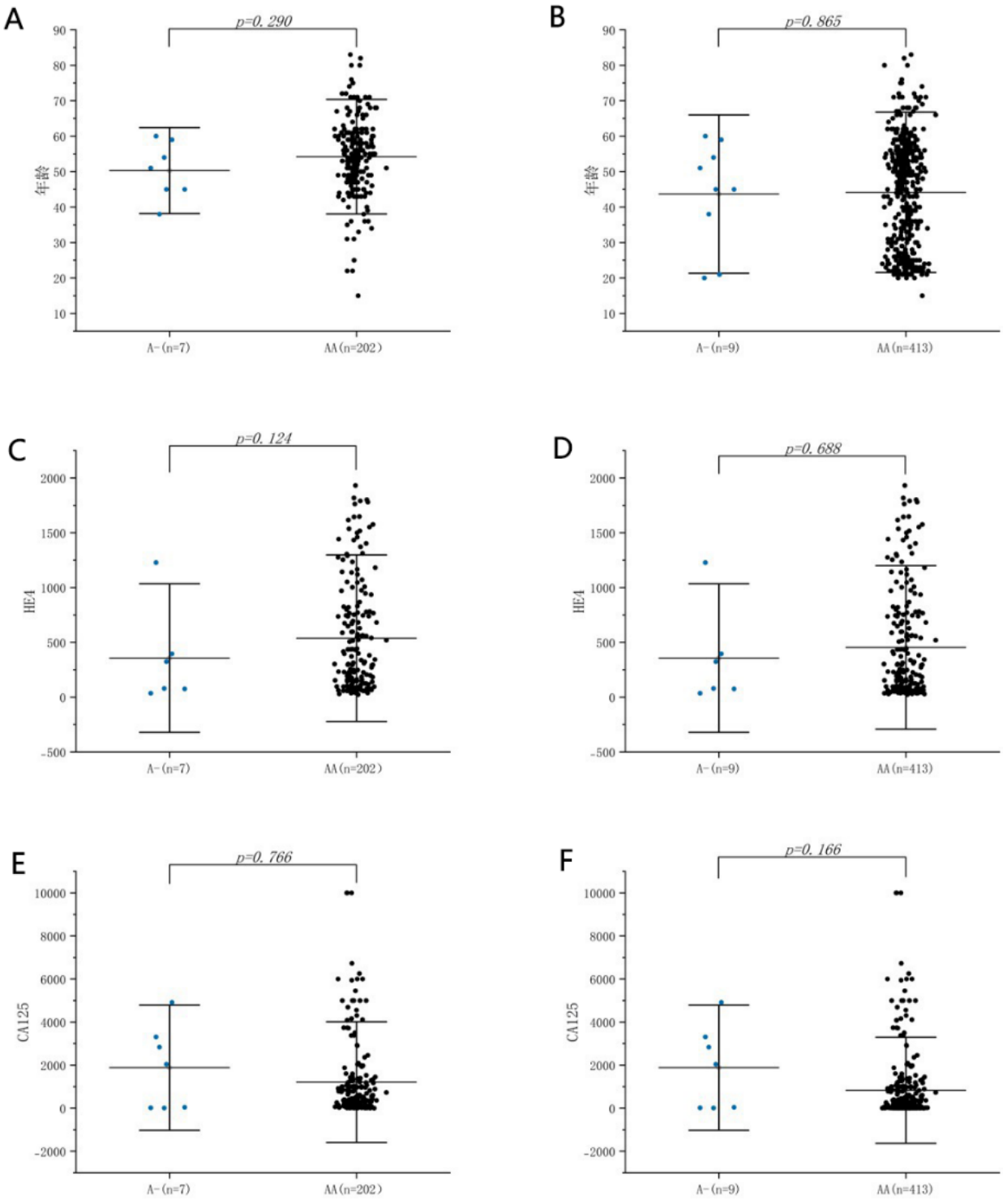


**Figure 3 | Comparative analysis of clinical parameters across genotype groups**

*Note: (1) Panels A, C, and E present comparisons between the mutant and wild-type subgroups within the experimental group, whereas Panels B, D, and F present comparisons between the mutant and wild-type subgroups in the pooled dataset combining the experimental and control groups. (2) The x-axis indicates genotype, where A− denotes the heterozygous mutant genotype and AA denotes the wild-type genotype. (3) The lines in each panel represent the mean and standard deviation for the corresponding group.*

**Table 13 | Predictive performance of logistic regression models for ovarian cancer**

| Model variables | Sensitivity | Specificity | AUC |
|---|---|---|---|
| HE4 | 0.891 | 0.973 | 0.945 |
| CA125 | 0.871 | 1.000 | 0.958 |
| Age | 0.919 | 0.723 | 0.870 |
| HE4, CA125 | 0.935 | 1.000 | 0.988 |
| HE4, Age | 0.870 | 1.000 | 0.950 |
| CA125, Age | 0.919 | 0.968 | 0.978 |
| HE4, CA125, Age | 0.935 | 1.000 | 0.989 |
| HE4, CA125, Age, Genotype | 0.946 | 1.000 | 0.991 |

analyzed (**Tables 9 and 10**). Differences between the two subgroups were then compared across 13 indicators, including age, HE4, CA125, PT, APTT, TT, FIB, D-dimer, personal history of cancer, family history, lymph node metastasis, histologic grade, and clinical stage. Among these, age, HE4, CA125, PT, APTT, TT, FIB, and D-dimer were continuous variables and are presented as mean ± SD; personal history of cancer, family history, and lymph node metastasis were categorical variables and are presented as n (%); histologic grade and clinical stage were ordinal variables and are presented by category as n (%). Statistical analyses showed that none of these indicators differed significantly between the mutant and wild-type subgroups ($P > 0.05$). The comparative results for all indicators are shown in **Table 11**.

According to the clinical characteristics of patients with the mutation (Table 9), the predominant pathological subtype was high-grade serous adenocarcinoma, and most cases were at stage III.

In addition, this study further evaluated differences in age, HE4, and CA125 across genotypes within the experimental group, and in the pooled dataset combining the experimental and control groups (**Figure 3**).

### Statistical Analysis of Associations Between Serological Biomarkers and Clinical Characteristics in Ovarian Cancer

To evaluate the clinical utility of HE4 and CA125 as serological tumor biomarkers, this study examined the associations between these two markers and the clinical and pathological characteristics of patients with ovarian cancer. As shown in **Table 12**, HE4 differed significantly across age groups, histological subtypes, clinical stages, histologic grades, and lymph node metastasis status ($P < 0.05$), whereas no significant differences were observed according to personal history of cancer, HBOC syndrome, or family history ($P > 0.05$). CA125 differed significantly across histological subtypes, clinical stages, histologic grades, and lymph node metastasis status ($P < 0.05$), but showed no significant differences across age groups or according to personal history of cancer, HBOC syndrome, or family history ($P > 0.05$). These findings indicate that HE4 and CA125 effectively discriminate between pathological characteristics and may aid in the diagnosis of ovarian cancer.

### Disease Risk Assessment Model

To further investigate the effects of age, HE4 level, CA125 level, and the presence of the BRCA1 c.4712delT variant on ovarian cancer status, a logistic regression–based risk assessment model was developed (**Table 13**; **Figure 4**). The combined model using HE4 and CA125 demonstrated markedly better predictive performance (AUC = 0.988, sensitivity = 0.935, specificity = 1.000) than either biomarker alone, indicating that their combined use provides a more reliable clinical diagnostic indicator than HE4 or CA125 individually. In addition, compared with the joint model including HE4, CA125, and age, incorporating genotype as an additional predictor further improved performance (AUC = 0.991, sensitivity = 0.946, specificity = 1.000), yielding the best diagnostic performance among the evaluated ovarian cancer risk prediction models.

## Discussion

Among the various BRCA1 variants, BRCA1 c.4712delT is regarded as a high-frequency deleterious germline variant. Investigating differences in its mutation rate between ovarian cancer patients and healthy individuals is important for evaluating its potential utility as an early detection marker for ovarian cancer. A previous study reported a BRCA1 c.4712delT mutation rate of 5% (2/40) among ovarian cancer patients with hereditary breast and ovarian cancer (HBOC) syndrome in Hunan Province. In the present study, the mutation rate in the experimental group was 3.35% (7/209), with a mutation frequency of 1.67% (7/418); in the control group, the mutation rate was 0.94% (2/213), with a mutation frequency of 0.47% (2/426). The mutation rate observed in the experimental group was lower than that reported in the above study. This may be explained by two factors. First, the EDTA-anticoagulated whole-blood samples collected in this study were obtained from ovarian cancer patients with different histological subtypes and were not strictly limited to HBOC patients, resulting in fewer inclusion restrictions. Sec-

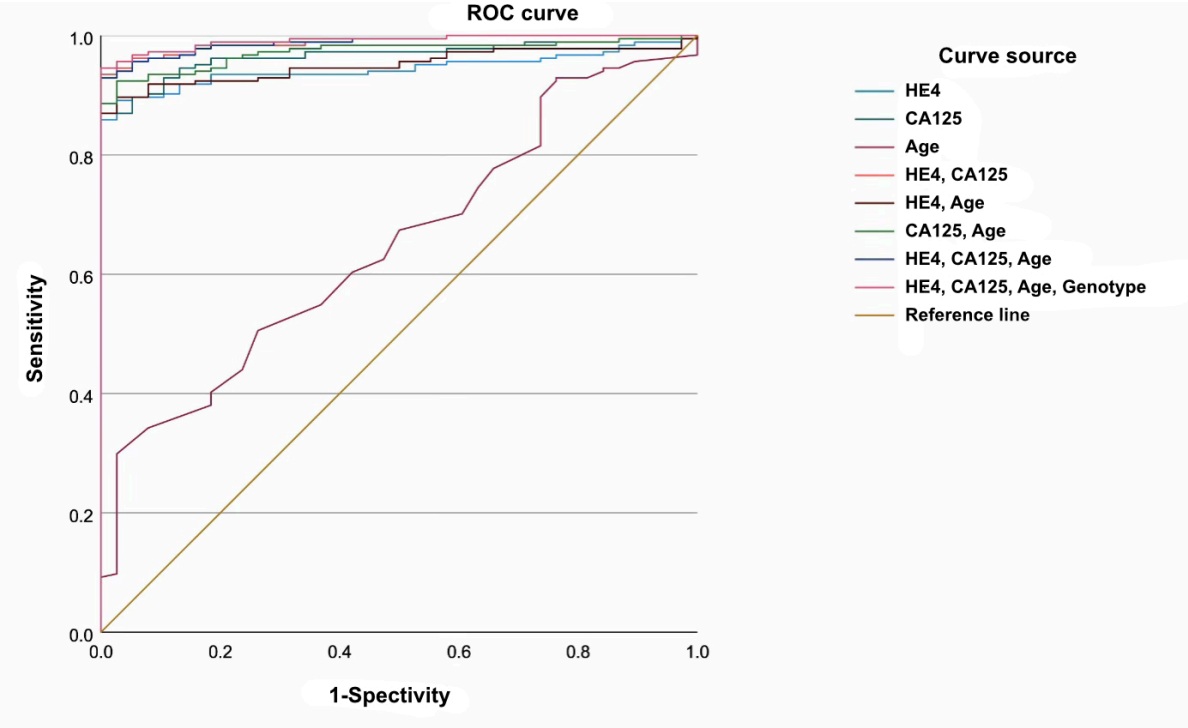


**Figure 4 | ROC curves of logistic regression models for ovarian cancer**

ond, the sample size in this study was limited, and the influence of random variation on the results cannot be excluded. Moreover, statistical analysis showed no significant difference in mutation rate between the experimental and control groups ($P > 0.05$), suggesting that BRCA1 c.4712delT is not significantly associated with ovarian cancer status; this finding is clearly inconsistent with the expert consensus on BRCA germline variant screening in Chinese populations [8]. Nevertheless, although the difference was not statistically significant, an apparent upward trend in the BRCA1 c.4712delT mutation rate was observed in the experimental group compared with the control group. The lack of statistical significance may still be attributable to the relatively small sample size.

In this cohort of 209 ovarian cancer patients, analysis of BRCA variants in whole-blood samples in relation to clinical characteristics indicated that, because ovarian cancer lacks routine screening and effective early detection strategies, most patients are already at an advanced stage at initial diagnosis. Given the high proportions of serous adenocarcinoma (80.86%), advanced FIGO stage (III and IV, 70.82%), and lymph node metastasis among middle-aged and older patients in this study, these features may partly explain the relatively high BRCA mutation frequency observed. Bioinformatic predictions suggest that the BRCA1 c.4712delT variant alters amino acids downstream of the 1571st amino acid in exon 16 of BRCA1, leading to truncation of the BRCA1 protein beyond this position and loss of normal protein function. This truncation is expected to impede binding to homologous recombination repair–related proteins such as RAD51 and PALB2, thereby disrupting key processes including homologous recombination repair of DNA double-strand breaks, cell-cycle regulation, and transcriptional regulation. It may further impair centrosome duplication control, resulting in centrosome dysregulation, genomic instability, and malignant cellular transformation, ultimately contributing to tumorigenesis. Clinically, mutation carriers in this study were predominantly diagnosed with high-grade serous adenocarcinoma and were mainly classified as stage III, which to some extent suggests an association between this variant and pathological characteristics. In addition, logistic regression modeling showed that including genotype improved the predictive performance for ovarian cancer risk, supporting the possibility that BRCA1 c.4712delT may contribute to ovarian cancer development and may have potential as an early diagnostic biomarker. Increasing the sample size in future studies may help to further verify this association.

In the experiment to determine the genotype of each sample, this study first employed the restriction fragment length polymorphism method, and conducted a preliminary screening for whether a mutation occurred by visually observing the bands obtained from electrophoresis. The screened samples were then subjected to Sanger sequencing, and the final determination was made as to whether the BRCA1 c.4712delT

mutation occurred and whether the mutation type was a homozygous mutation or a heterozygous mutation. The advantage of performing electrophoresis first and sequencing afterward is that it can reduce experimental costs and is more suitable for the detection of large batches of samples; the disadvantage is that the electrophoresis results need to be judged by visual observation, which has strong subjective dependence, and the possibility of missing mutant samples is higher than performing Sanger sequencing for all samples, thereby possibly leading to a lower mutation rate obtained in the experiment, which may also be one of the reasons why the mutation rate in the experimental group in this study was relatively low.

Based on the clinical data collected in this study and the statistical analysis results, the differences in HE4 levels and CA125 levels between the experimental group and the control group were statistically significant ($P < 0.05$), and the differences between the two markers across pathological subtype, clinical stage, histologic grade, and lymph node metastasis status were all significant ($P < 0.05$), indicating that HE4 and CA125 can significantly reflect the differences in the occurrence and progression of ovarian cancer among different patients, which is consistent with various previous studies [18]. In addition, the predictive model performance of the combined use of HE4 and CA125 was superior (AUC = 0.988, sensitivity = 0.935, specificity = 1), indicating that their combined use can more effectively predict the occurrence and progression of ovarian cancer. Both HE4 and CA125 are relatively mature clinical biomarkers for ovarian malignant tumors, and elevated levels of these markers are of important significance for the diagnosis of ovarian cancer; this study further corroborates this point.

When this study compared the indicators between the mutant group and the wild-type group, the results showed that there were no statistically significant differences between the two groups in 13 indicators, including age, HE4, CA125, PT, APTT, TT, FIB, D-D, personal history of cancer, family history, lymph node metastasis status, histologic grade, and clinical stage ($P > 0.05$), indicating that the BRCA1 c.4712delT mutation has no significant effect on tumor marker levels, coagulation function, lymph node metastasis status, histologic grade, or clinical stage in ovarian cancer patients, and has no clear association with age, which is inconsistent with the expected results. This may be due to the too small number of mutant samples and the lack of effective clinical data, such that intergroup differences could not be well reflected. Even so, compared with the wild-type group, the CA125 level in the mutant group still showed a relatively obvious upward trend. In subsequent experiments, further increasing the sample size and the amount of clinical data is expected to convert this trend into a statistically significant difference.

In summary, there are significant differences in HE4 and CA125 levels between the ovarian cancer patient population and the healthy population, further corroborating the effectiveness of HE4 and CA125 as biomarkers for ovarian malignant tumors. In addition, detection of the BRCA1 c.4712delT mutation, as a potential early diagnostic method for ovarian cancer, has demonstrated certain potential, and further investigation of the effects of this type of mutation on ovarian cancer risk and various clinical indicators is of important significance. This study has limitations in the detection method and sample size; subsequent experiments should expand the sample size and improve the experimental methods to enhance the reliability of the experimental results.

## Conclusions

1) The mutation frequency of BRCA1 c.4712delT in ovarian cancer patients was 1.67% (7/418);
2) Changes in the serum levels of the tumor markers HE4 and CA125 can serve as effective indicators for the early diagnosis of ovarian cancer;
3) Detection of the BRCA1 c.4712delT variant is a potentially useful indicator for the early diagnosis of ovarian cancer.

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