Article

Glutathione S-transferase (GSTT1 and GSTP1) Polymorphisms Improves the Short- and Long-Term Efficacy of Concurrent Chemoradiotherapy in Advanced Ovarian Cancer

Xiao Xu 1, Zhuo Zhong 2, Dong-Mei Dou 3*, Fenfen Li 4*

1 Taizhou Hospital of Traditional Chinese Medicine, Taizhou 225300, China
2 Department of Oncology, Guangzhou Hospital of Integrated Traditional Chinese and Western Medicine, Guangzhou 510800, China
3 Oncology Department, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, China
4 Institute of Chronic Disease Risks Assessment, Henan University, Kaifeng 475001, China;

* Correspondence to: mydream9797@163.com (Dou D-M); lifenfenjxszyy@163.com (Li F)

Received: 13 August 2020; Accepted: 29 September 2020; Published: 12 October 2020

Abstract: Over the past four decades, patients with advanced ovarian cancer (OC) has enjoyed greatly improved survival rate considering advances in combined chemotherapy with platinum and paclitaxel. We investigated the association between polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1) and short- and long-term efficacy of concurrent chemoradiotherapy (CCRT) in advanced OC. Patients with advanced OC and healthy females were selected in our study. Multiplex polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP) were applied to detect homozygous deletions in GSTM1 and GSTT1, as well as codon 105 genetic polymorphisms in GSTP1 exons 5. All the patients with advanced OC were treated with CCRT. Toxicity of chemotherapy, short-term efficacy, recurrence and metastasis, respectively. Long-term survival outcomes were checked with a further examination and all patients were followed up for three years after CCRT. Compared with healthy females, patients with advanced OC showed higher frequencies of GSTT1 non-null genotype and homozygous mutation type GG of GSTP1. GSTP1 genetic polymorphisms were relevant to neutropenia resulted from CCRT in patients with advanced OC. Compared with patients with GSTT1 non-null genotype, those with GSTT1 null genotype appeared to enjoy a better short-term efficacy, lower recurrence and metastasis rate, higher survival rate and longer mean survival time. Compared with patients with GG genotype, those with GSTP1 AA + AG genotype also enjoyed more positive results. Our results reveal that GSTT1 null genotype and GSTP1 AA + AG genotype are associated with short- and long-term efficacy of CCRT in advanced OC.

Keywords: advanced ovarian cancer; GSTT1; GSTP1; GSTM1; Genetic polymorphisms; Concurrent chemoradiotherapy

1. Introduction

Ovarian cancer (OC) ranks the second among the lethal gynecologic malignancy and the 5th leading cause of cancer-related deaths worldwide [1]. The origin and pathogenesis of OC have long been investigated but still poorly understood [2]. Many epidemiologic studies have evaluated multiple risk factors for OC, including diet, obesity, smoking, pregnancy, chronic inflammation and non-steroidal anti-inflammatory drug use, hormone replacement therapy, hysterectomy, and infertility drug use [3]. Vague symptoms in early stage have caused difficulties in timely diagnosis and effective treatments, leading to a dismal 5-year survival rate lower than 30% [4,5]. Recently, OC treatments mainly include surgery, chemotherapy and radiotherapy [6]. It is found in studies that chemoradiotherapy exerts a better efficacy than surgery, chemotherapy or radiotherapy alone [7–9]. Concurrent chemoradiotherapy (CCRT), a combination of chemotherapy and radiotherapy, is the standard organ-preservation treatment for resectable diseases for its function in ensuring optimal locoregional control, and therefore has become a cornerstone of treatment [10]. Multiple studies have revealed that efficacy of chemotherapy and radiotherapy could be affected by a variety of factors, including bacteria, immune parameters and gene variations [11,12]. Accumulating studies reported the association of genetic polymorphisms with CCRT, for example, DNA repair gene polymorphisms with cisplatin-based CCRT in patients with cervical carcinoma, miRNA-related genetic polymorphisms and patients with esophageal squamous cell carcinoma after receiving CCRT, and single nucleotide polymorphisms (SNP) in predicting CCRT response in esophageal cancer [13–15].

Glutathione S-transferase (GST) is a phase II metabolic enzyme that plays an important role in cellular defense against numerous harmful chemicals produced both exogenously and endogenously [16]. Three functional polymorphisms of GST have been defined in the human genome: M1, T1 and P1, among which, GSTM1 is located in the short arm of the chromosome one (1p13.3), GSTT1 is located in 22q11.2, and GSTP1 is located in 11q13 [17]. There have been quite a few researches about associations between GST genes and efficacy of chemotherapy in patients with tumor in the past few years. For example, a recent study showed that GSTT1 genotype can be a useful prognostic marker for muscle invasive bladder cancer patients [18]. Another study revealed that null genotype of GSTM1 and GSTT1
polymorphisms can increase risks in breast cancer for Asians and GSTP1 Val105Ile (rs1695) polymorphism can increase breast cancer risks for Caucasians [19]. A meta-analysis demonstrated that advanced non-small cell lung cancer patients with GSTM1 null genotype tend to have better response to chemotherapy and a lower risk of death [20]. Presently, a study in 2016 found that GSTM1 polymorphism seemed to have an impact on OC prognosis as it predicted better responses to platinum-based chemotherapy, and hence an improved survival [21]. For further examination and verification, this study sought to explore the correlations of GST polymorphisms (GSTM1, GSTT1, and GSTP1) with short- and long-term efficacy of CCRT in advanced OC in a Chinese population.

2. Materials and Methods

2.1. Ethical Statement

This study was approved by the Ethics Committee of our hospital, and all the study subjects provided signed informed consent.

2.2. Study Subjects

From January 2010 to June 2012, 161 patients with advanced OC were selected from our hospital as a case group, aged between 22 to 74 years (mean age 47.8 ± 9.3 years). According to their B-mode ultrasound and multi-detector computed tomography (CT) examinations, lumps of different sizes were found in their lower abdomen, the largest being 8.0 cm × 7.6 cm × 5.4 cm and the smallest 3.4 cm × 2.1 cm × 1.6 cm. Among them, 35 cases have much ascites, 50, moderate ascites, 47, little ascites, and 29 exhibit obvious symptoms. As for pathological types, serous carcinoma were found in 51 cases, mucinous carcinoma in 57, endometrioid carcinoma in 23, clear cell carcinoma in 9, sex cord-stromal carcinoma in 6, germ cell carcinoma in 4 and others in 11 cases. Their Federation of Gynecology and Obstetrics (FIGO) stages were stage III to IV [22].

The patients were enrolled into our study if they were pathologically confirmed with advanced OC; if they had a Kamofsky score > 60 points; if they were absent of previous radiotherapy or chemotherapy; if they had no malignant tumors in other body parts or contraindication to radiotherapy or chemotherapy; if they had normal hepatic and renal function, blood circulation and diet (more than 1500 calories per day); If they had an expected survival time > three months; and if they were mentally healthy, willing to participate and sign a written informed consent, and compliant. Correspondingly, these patients who met with the following criteria were excluded: OC patients were not accorded with the above-mentioned pathological types; patients had anemia, thrombocytopenia and leucopenia resulting from primary diseases of liver, kidney or splenic hyperfunction; patients had anemia, thrombocytopenia and leucopenia resulting from hematological system diseases; patients had a history of severe allergy or idiosyncrasy; patients were involved in other treatments; and patients had personality or mental disorders, or refuse or couldn’t sign the written informed consent of this research. At the same time, a control group was set consisting of 163 healthy females who came to our hospital for physical examination. Their age ranged from 32 to 78 years, with an average age of (50.7 ± 8.7 years).

2.3. Blood Sampling and DNA Extraction

Fasting venous blood samples (3 mL) from the patients in the case group prior to CCRT and from females in the control group were collected. Ethylenediaminetetraacetic acid (EDTA) was added to the samples as anticoagulant. Phenol-chloroform method was used for DNA extraction. The concentration and purity of the sample DNA was detected by Nanodrop2000 and set at 25 mg/L. Sample DNA ran for electrophoresis test and electrophoresis bands shall be larger than 10 kb and showed no obvious degradation. The extracts were kept at -80°C in a refrigerator for later use.

2.4. DNA Genotyping

Multiplex-polymerase chain reaction (PCR) was applied to detect homozygous deletions in GSTM1 and GSTT1. For genotyping of GSTM1 and GSTT1, albumin gene was amplified as an internal positive control. The primers for GSTT1 were 10 pmol, for albumin gene, 10 pmol and for GSTM1, 20 pmol. The PCR reaction (25 μL) was prepared containing 3 μL of 4×dNTP, 2.5 μL of 10× buffer, 3 mmol/L MgCl2, 1.5 U Taq DNA polymerase, 1 μL of DNA sample, 1 μL of forward primer and 1 μL of reverse primer (together three pairs, respectively for GSTM1, GSTP1 and GSTT1). PCR reaction conditions were: denaturation at 95°C for 5 min; 35 cycles (denaturation at 95°C for 45 s and extension for 45 s at 72°C), and extension at 72°C for 5 min. PCR primers were designed and synthesized by Takara Biotechnology Co., Ltd., (Dalian, China) (Table 1). The PCR products were separated by electrophoresis using 2% agarose gel and stained with ethidium bromide.
Table 1. PCR primer sequences used for polymerase chain reaction.

| Locus   | Primer sequence (5' - 3')                        | PCR amplicon size |
|---------|--------------------------------------------------|-------------------|
| GSTM1   | F: GAACTCCCTGAAAAGCTAAAGC                        | 215 bp            |
|         | R: GTTGGGCTCAAATATACGGTG                       |                   |
|         |                                                 |                   |
| GSTT1   | F: TTTCTTACTGTGTTCTCACATTC                     | 480 bp            |
|         | R: TCACCGGATCATGGCCAGCA                        |                   |
|         |                                                 |                   |
| GSTP1   | F: TCTTCCACGCACATCTCT-3                         | 268 bp            |
|         | R: AAGCCCTTTTCCTTGCTCAG-3                      |                   |
|         |                                                 |                   |
| β-globin | F: CAACTTCATCCACGTTCACC                        | 98 bp             |
|         | R: GAAGAGCCAAGGACAGGTAC                        |                   |

Note: F, forward; R, reverse; PCR, polymerase chain reaction; GST, glutathione-S-transferase.

PCR- restriction fragment length polymorphism (RFLP) was used to examine codon 105 genetic polymorphisms in GSTP1 exon 5. The primers for GSTP1 genotype were 10 pmol. The PCR reaction (25 μL) was prepared containing 2.0 μL of 4 × dNTP, 2.5 μL of 10 × buffer, 2.5 mmol/L MgCl₂, 1.0 U Taq DNA polymerase, 0.5 μL of DNA sample, 1 μL of forward primer and 1 μL of reverse primer. PCR reaction conditions were: pre-denaturation at 95°C for 2 min, 35 cycles (denaturation at 94°C for 30 s; annealing at 66°C for 30 s and another 2 min at 72°C), and extension at 72°C for 5 min. The PCR primers were designed and synthesized by Takara Biotechnology Co., Ltd. (Dalian, Liaoning, China) (Table 1). PCR products (5 μL) were mixed with 0.5 μL of Alw 26I (10 U/μL) and incubated at 37°C for 2 h. Genotypes were analyzed by restriction enzyme products in 2.5% agarose gel electrophoresis with ethidium bromide.

2.5. Treatment Regimens

All patients were positioned on a treatment bedstead and a vacuum cushion for CT scanning. The tumors were irradiated using Varian 2300CD linear accelerator (Varian Medical Systems, Inc., Palo Alto, CA, USA) at a dose of 2 Gy per time, five times a week, prescribed to 85% isodose curve. Each patient received 36~40 Gy for 18~20 times in total. Intraperitoneal perfusion was performed using ultrasound-guided abdominocentesis with silicone gel drainage placement. Intravenous chemotherapy was performed using B-mode ultrasound-guided Peripherally Inserted Central Catheter (PICC) in superior vena cava (SVC) via ulnar vein. Intravenous drip with 60 mg docetaxel and intraperitoneal perfusion with 20 mg cisplatin were performed on the 1st, 8th and 15th day of the first cycle and concurrent whole pelvic radiotherapy was carried out. On the 1st day of the succeeding 2nd, 3rd and 4th cycle, intravenous drip with 120 mg docetaxel and intraperitoneal perfusion with 60 mg cisplatin were performed again. One cycle consists of 21 days.

2.6. Evaluation of Efficacy

Anticancer drug toxicity was classified into Grade 0~IV according to the criteria set by World Health Organization (WHO) [23]. Patients were further examined three months after CCRT. Short-term efficacy was evaluated according to the Response Evaluation Criteria in Solid Tumor [24] 1, complete response (CR), no residual target lesions was detected; partial response (PR), sum of the longest diameters in target lesions reduced by at least 30%; progressive disease (PD), sum of the longest diameters in target lesions increased by 20% or more, or new target lesion was detected; stable disease (SD), sum of the longest diameters in target lesions was between PR and PD. Patients with CR and PR were considered respondent to CCRT and those with SD and PD non-respondent.

Tumor recurrence and metastasis evaluation: further review was carried out every two or three months in the first year following the treatment. A year later, review was conducted every six months to monitor the recurrence and metastasis of cancer cells.

Long-term efficacy evaluation: the 3-year survival rate and mean survival time of patients were checked. No patient lost to follow up and the follow-up rate was 100%.

2.7. Statistical Analysis

All the data was processed using SPSS 21.0 (IBM Corp. Armonk, NY, USA). DNA genotyping was tested with Hardy-Weinberg equilibrium assumption. p > 0.05 indicated that samples had reached genetic equilibrium and were representative. Enumeration data was presented in percentage or rate and comparison was conducted using the χ² test. Mean survival time and survival rate were checked with the Kaplan-Meier analysis and Log-rank test. Non-conditional logistic regression model was used to calculate odds ratio (OR) with 95% confidence interval (CI). p < 0.05 was accounted as statistically significant.
3. Results

3.1. Distribution of GST Family (GSTM1, GSTT1, and GSTP1) Genotype in the Case and Control Groups

The distribution of GST family (GSTM1, GSTT1, and GSTP1) genotypes was consistent with Hardy-Weinberg equilibrium in all the 161 patients with advanced OC, indicating that the selected groups were representative. Both non-null and null genotypes were found in GSTM1 and GSTT1. In the case group, GSTM1 non-null was found in 52% and GSTM1 null in 48%, while in the control group, GSTM1 non-null was found in 54% and GSTM1 null in 46% \((p > 0.05)\). In the case group, GSTT1 non-null was found in 51% and GSTT1 null in 49%, while in the control group, GSTT1 non-null was found in 41% and GSTT1 null in 59% \((p < 0.05)\). There were three genotypes in GSTP1, AA, AG and GG, among which AA accounted for 45%, AG for 41% and GG for 14%. The frequency of GG genotype between the case group and control group had significant differences \((p < 0.05)\) (Table 2).

### Table 2. Distribution of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms in the case and control groups.

| Genotype/allele | Case group (n = 161) | Control group (n = 163) | \(\chi^2\) | p value | OR (95% CI) |
|-----------------|----------------------|------------------------|-----------|---------|-------------|
| GSTM1           |                      |                        |           |         |             |
| GSTM1 (+)       | 84 (0.52)            | 89 (0.54)              |           |         | Ref.        |
| GSTM1 (-)       | 77 (0.48)            | 74 (0.46)              | 0.192     | 0.661   | 0.91 (0.59-1.40) |
| GSTT1           |                      |                        |           |         |             |
| GSTT1 (+)       | 83 (0.51)            | 66 (0.41)              |           |         | Ref.        |
| GSTT1 (-)       | 78 (0.49)            | 97 (0.59)              | 3.990     | 0.045   | 0.64(0.41-0.99) |
| GSTP1           |                      |                        |           |         |             |
| AA              | 72 (0.45)            | 93 (0.57)              |           |         | Ref.        |
| AG              | 65(0.41)             | 59 (0.36)              | 2.190     | 0.139   | 1.42 (0.89-2.27) |
| GG              | 24 (0.14)            | 11 (0.07)              | 7.193     | 0.007   | 3.34 (1.29-6.13) |
| A               | 209 (0.65)           | 245 (0.75)             |           |         | Ref.        |
| G               | 113 (0.35)           | 81 (0.25)              | 8.109     | 0.004   | 1.64 (1.16-2.29) |

Notes: +, non-null; -, null; OR, odds ratio; CI, confidence interval; Ref., reference; GST, glutathione-S-transferase.

3.2. GSTP1 Genetic Polymorphism is Related to Hematologic Toxicity of Advanced OC

As shown in Table 3, in 161 patients with advanced OC, 39% \((64/161)\) suffered from grade 0-II neutropenia and 61% \((97/160)\) suffered from grade III-IV neutropenia after receiving CCRT. GSTP1 genetic polymorphisms were found to be significantly relevant to the neutropenia caused by CCRT in advanced OC patients \((p < 0.05)\), and exhibited no relation with leucopenia, decrease in hemoglobin or thrombocytopenia \((all p > 0.05)\). GSTM1/GSTT1 genetic polymorphisms were not relevant to the hematologic toxicity resulted from CCRT in advanced OC patients \((both p > 0.05)\).

### Table 3. Associations of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms with hematologic toxicity in patients with advanced OC.

| Hematologic toxicity | n | GSTM1 | | GSTT1 | | GSTP1 | |
|----------------------|---|-------|---|-------|---|-------|---|
|                      |   | Non-null |   | Non-null |   | AA | AG | GG | p |
| Leucopenia           |   | 0.209 | | 0.253 | | 0.752 |
| 0-II                 | 71 | 41 | 30 | 33 | 38 | 30 | 31 | 10 |
| III-IV               | 90 | 43 | 47 | 50 | 40 | 42 | 34 | 14 |
| Neutropenia          |   | 0.245 | | 0.521 | | 0.006 |
| 0-II                 | 64 | 37 | 27 | 31 | 33 | 38 | 17 | 9 |
| III-IV               | 97 | 47 | 50 | 52 | 45 | 34 | 48 | 15 |
| Decrease in hemoglobin |   | 0.876 | | 0.189 | | 0.875 |
| 0-II                 | 149 | 78 | 71 | 79 | 70 | 66 | 61 | 22 |
| III-IV               | 12 | 6 | 6 | 4 | 8 | 6 | 4 | 2 |
| Thrombocytopenia     |   | 0.914 | | 0.450 | | 0.460 |
| 0-II                 | 155 | 81 | 74 | 79 | 76 | 68 | 64 | 23 |
| III-IV               | 6 | 3 | 3 | 4 | 2 | 4 | 1 | 1 |

Notes: GST, glutathione-S-transferase
3.3. Relationship between GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms and short-term efficacy of CCRT in patients with advanced OC

Subsequently, the relationship between GST family (GSTM1, GSTT1, and GSTP1) gene polymorphisms and short-term efficacy of CCRT in 161 patients with advanced OC. CR was found in 23 cases, PR in 64, SD in 34 and PD in 41 cases. The effective rate of CCRT for patients with GSTM1 non-null genotype was 48.8% and for those with GSTM1 null genotype was 58.4% \((p > 0.05)\). The effective rate of CCRT for patients with GSTT1 null genotype was 65.4%, much higher than that of GSTT1 non-null genotype \((p < 0.05)\). The effective rate of the CCRT for GSTP1 AA + AG genotype was significantly higher than that of GSTP1 GG genotype \((p < 0.05)\) (Table 4).

Table 4 Associations of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms with short-term efficacy of CCRT in patients with advanced OC.

| Genotype | n  | CR + PR | PD + SD | \(p\) | OR (95%CI) |
|----------|----|---------|---------|------|------------|
| GSTM1    |    |         |         |      |            |
| GSTM1 (+)| 84 | 41 (0.488) | 43 (0.512) |    | Ref.      |
| GSTM1 (-)| 77 | 45 (0.584) | 32 (0.416) | 0.221 | 0.68 (0.36-1.27) |
| GSTT1    |    |         |         |      |            |
| GSTT1 (+)| 83 | 35 (0.422) | 48 (0.578) |    | Ref.      |
| GSTT1 (-)| 78 | 52 (0.654) | 27 (0.346) | 0.003 | 0.39 (0.2-0.73) |
| GSTP1    |    |         |         |      |            |
| AA + AG  | 137| 78 (0.569) | 59 (0.431) |    | Ref.      |
| GG       | 24 | 8 (0.333) | 16 (0.667) | 0.003 | 2.64 (1.06-6.59) |

Notes: +, non-null; -, null; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease; OR, odds ratio; CI, confidence interval; Ref., reference; GST, glutathione-S-transferase.

3.4. Association between GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms and recurrence and metastasis in patients with advanced OC

Afterwards, the associations of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms in patients with OC recurrence and metastasis during the follow-up were investigated (Table 5). For those with GSTM1 non-null genotype, 49 patients (58.3%) exhibited recurrence and metastasis and for those with GSTM1 null genotype, 39 patients (50.6%) showed symptoms of recurrence and metastasis \((p > 0.05)\). For those with GSTT1 null genotype, 36 patients (46.1%) showed recurrence and metastasis, which was significantly lower than that of GSTT1 non-null genotype \((p < 0.05)\). For those with GSTP1 AA + AG genotype, 70 patients (51.1%) showed recurrence and metastasis and for those with GSTP1 GG genotype, 18 patients (75%) showed symptoms of recurrence and metastasis \((p < 0.05)\).

Table 5 Associations of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms with recurrence and metastasis in patients with advanced OC.

| Genotype | n  | Recurrence or metastasis | \(p\) | OR (95%CI) |
|----------|----|--------------------------|------|------------|
| GSTM1    |    | Positive                 | Negative |         |            |
| GSTM1 (+)| 84 | 49 (0.583)               | 35 (0.417) |    | Ref.      |
| GSTM1 (-)| 77 | 39 (0.506)               | 38 (0.494) | 0   | 0.73 (0.39-1.37) |
| GSTT1    |    | Positive                 | Negative |      |            |
| GSTT1 (+)| 83 | 52 (0.627)               | 31 (0.373) |    | Ref.      |
| GSTT1 (-)| 78 | 36 (0.461)               | 42 (0.539) | 0   | 0.51 (0.27-0.96) |
| GSTP1    |    | Positive                 | Negative |      |            |
| AA + AG  | 137| 70 (0.511)               | 67 (0.489) |    | Ref.      |
| GG       | 24 | 18 (0.750)               | 6 (0.250) | 0   | 2.87 (1.07-7.67) |

Notes: +, non-null; -, null; OR, odds ratio; CI, confidence interval; Ref., reference; GST, glutathione-S-transferase.
3.5. Association Between GST family (GSTM1, GSTT1, and GSTP1) Genetic Polymorphisms and Survival Time of Patients with Advanced OC

Figure 1 shows that a three-year follow-up for all the patients was conducted after treatment, which was completed by September 2015. Of all 161 OC patients, 96 died from OC and 65 survived. Three-year survival rate of all the patients in the case group was 40%. No significant difference was found between the mean survival time and rate of patients with GSTM1 null and non-null genotypes (p > 0.05). Mean survival time and rate of patients with GSTT1 null genotype was significantly greater than that of patients with GSTT1 non-null genotype (p < 0.05). Compared with patients with GG genotype, those with GSTP1 AA + AG genotype also had greater mean survival time and rate (p < 0.05) (Table 6).

![Figure 1](image)

**Figure 1.** Survival curve of the advanced OC patients indicates that GSTT1 null genotype and GSTP1 AA + AG genotype had a higher survival rate of all the patients. OC, ovarian cancer.

| Genotype | n   | Mean survival time (month) | Survival rate | p     |
|----------|-----|-----------------------------|---------------|-------|
| GSTM1 (+) | 84/31 | 28.27                      | 36.90%        |       |
| GSTM1 (-) | 77/34 | 29.88                      | 44.10%        | 0.193 |
| GSTT1 (+) | 83/23 | 26.95                      | 27.70%        |       |
| GSTT1 (-) | 78/42 | 31.27                      | 53.80%        | < 0.001 |
| GSTP1 AA + AG | 137/60 | 29.07 | 43.80% |       |
| GSTP1 GG   | 24/5  | 19.63                      | 20.80%        | < 0.001 |

Notes: +, non-null; -, null; OC, ovarian cancer; GST, glutathione-S-transferase.

Table 6 Associations of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms with survival time of OC patients in patients with advanced OC

4. Discussion

OC is the most aggressive gynecological malignancy causing the highest number of gynecological cancer-related deaths, which is especially associated with delayed diagnosis at an advanced stage [25]. Constant breakthroughs have been made in treatments since its identification in 1959 by Dr. Martin [26]. This study was performed to explore the correlations of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms with short- and long-term efficacy of CCRT in patients with advanced OC. Collectively, the results revealed that polymorphisms within GSTT1 and GSTP1 but not in GSTM1 is associated with short- and long-term efficacy of CCRT in patients with advanced OC.

Initially, the current study demonstrated that frequencies of GSTT1 non-null genotype and homozygous mutation type GG of GSTP1 in the case group were remarkably high. GSTs are the most important enzymes of the phase II metabolizing xenobiotic pathway, detoxifying several cytotoxic compounds and thus protect organisms from DNA damage or protect chromosomes from oxidative damage [27,28]. Both GSTT1 and GSTM1 genes of the GST super gene family exhibit null or deletion polymorphism; individuals homozygous for the null allele lack GST enzyme activity and hence have an increased risk of cancer [29]. Previous researches have also revealed the potential role of GST polymorphisms as markers of susceptibility to type 2 diabetes, risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia, lung cancer and breast cancer [30–33].

In addition, the present study displayed that patients with GSTT1 null genotype and GSTP1 AA + AG genotype
appeared to have a better chemoradiotherapy efficacy and higher survival rate when compared with those with GSTT1 non-null genotype and GSTP1 GG genotype respectively. Our result is in line with the research conducted by Tahara et al., who found that gastric cancer patients with GSTT1 null genotype showed significantly better survival than patients with GSTT1 non-null genotype [29]. It was suggested that GSTT1 null genotype has lower enzyme activity and thus greater drug availability due to absent polymorphisms, leading to better clinical outcomes [34]. The cancer research community has also been focusing on GSTP1, evaluating its potential as a cancer biomarker [35]. For example, Lu et al. found in his research that GSTP1 exon 6 variant genotype is associated with improved survival among patients with Stage III and IV non-small cell lung carcinoma after platinum-based chemoradiotherapy and chemotherapy [36]. Islam et al. found that AG and AG + GG genotypes of GSTP1 were more likely to have a good response in breast cancer patients after chemotherapy in a Bangladeshi population [37]. A possible explanation is the assumption that GSTP1 105Val variant (G) enzyme causes reduced ability to detoxify chemotherapeutic agents, leading to lower clearance and reduced efficacy [38].

It was also suggested in the study that GSTP1 genetic polymorphisms were significantly relevant to neutropenia resulted from CCRT in advanced OC patients. Our data revealed that patients with GSTP1 AG + GG genotype were more likely to suffer from grade III-IV neutropenia than those with GSTP1 AA type. This result is actually quite in line with the above two. The high frequency and poor detoxifying capacity of GSTP1 105 Val variant (G) in OC patients led to unsatisfying efficacy, as well as adverse drug reactions (ADRs), which, in this case, III-IV neutropenia. ADRs are defined as “any response to a drug which is noxious, unintended and occurs at doses used in man for prophylaxis, diagnosis or therapy” and the most common are nausea, vomiting, neutropenia, anemia and leukopenia [39]. Studies exist about possible relations between genetic polymorphisms and ADRs. For example, it was found that SNP (rs9561778) in ABCC4 might be applicable in predicting the risk of ADRs in patients receiving cyclophosphamide-based combination chemotherapy [40]. Prophylactic treatment with granulocyte-colony stimulating factors is available to reduce the neutropenia risk induced by chemotherapy-, for example, filgrastim (including approved biosimilars), pegfilgrastim or lenograstim [41].

In conclusion, it is proved that GSTT1 null and GSTP1 (AA + GG) genotype could possibly improve the short- and long-term efficacy of CCRT in advanced OC. Nonetheless, results of this research need to be validated by further studies incorporating more treatment and response data before application to determine the predictive and prognostic significance of GST family (GSTM1, GSTT1, and GSTP1) genetic polymorphisms in patients with advanced OC.

**Author Contributions:** All authors have read and agreed to the published version of the manuscript. Xu X and Zhong Z. Conceptualization, and Xu X, Zhong Z and Dou DM; methodology, Xu X and Li F; software, Zhong Z and Dou DM; validation, Xu X, Zhong Z and Dou DM; formal analysis, writing—original draft preparation.

**Funding:** None.

**Conflicts of Interest:** All authors declare no conflict of interest.

**Copyright Statement**

©2020 the authors. This article is an open access article licensed under the terms and conditions of the CREATIVE COMMONS ATTRIBUTION (CC BY) LICENSE (http://creativecommons.org/licenses/by/4.0/).

**References**

1. Qu CP, Sun GX, Yang SQ, Tian J, Si JG, et al. Toxicities of different first-line chemotherapy regimens in the treatment of advanced ovarian cancer: A network meta-analysis. *Medicine (Baltimore)*, 2017, 96: e5797.
2. Kim A, Ueda Y, Naka T, Enomoto T. Therapeutic strategies in epithelial ovarian cancer. *Journal of Experimental and Clinical Cancer Research*, 2012, 31: 14.
3. McLemore MR, Miaskowski C, Aouizerat BE, Chen LM, Dodd MJ. Epidemiological and genetic factors associated with ovarian cancer. *Cancer Nursing*, 2009, 32: 281–288.
4. Lukesova S, Vroblova V, Tosner J, Kopecky J, Sedlakova I, et al. Comparative study of various subpopulations of cytotoxic cells in blood and ascites from patients with ovarian carcinoma. *Contemp Oncol (Poln)*, 2015, 19: 290–299.
5. Han X, Zhen S, Ye Z, Lu J, Wang L, et al. A Feedback Loop Between miR-30a/c-5p and DNMT1 Mediates Cisplatin Resistance in Ovarian Cancer Cells. *Cellular Physiology and Biochemistry*, 2017, 41: 973–986.
6. Mousavi A, Karimi-Zarchi M, Behrash N, Modares-Gilani M, Mokhtari-Gorgani M, et al. The Role of Intraperitoneal Carboplatin as Consolidation Chemotherapy in Women with Ovarian Carcinoma: Report of Our Experience and Systematic Review. *International Journal of Biomedical Science*, 2016, 12: 120–124.
7. Xu C, Zhang LH, Chen YP, Liu X, Zhou GQ, et al. Chemoradiotherapy Versus Radiotherapy Alone in Stage II Nasopharyngeal Carcinoma: A Systemic Review and Meta-analysis of 2138 Patients. *Journal of Cancer*, 2017, 8: 287–297.
8. Choi Y, Oh DY, Kim K, Chie EK, Kim TY, et al. Concurrent Chemoradiotherapy Versus Chemotherapy Alone for Unresectable Locally Advanced Pancreatic Cancer: A Retrospective Cohort Study. *Cancer Research and Treatment*, 2016, 48: 1045–1055.
Hsu PK, Chen HS, Huang CS, Liu CC, Hsieh CC, et al. Patterns of recurrence after oesophagectomy and postoperative chemoradiotherapy versus surgery alone for oesophageal squamous cell carcinoma. *British Journal of Surgery*, 2017, 104: 90–97.

Fu ZZ, Li K, Peng Y, Zheng Y, Cao LY, et al. Efficacy and toxicity of different concurrent chemoradiotherapy regimens in the treatment of advanced cervical cancer: A network meta-analysis. *Medicine (Baltimore)*, 2017, 96: e5853.

Zitvogel L, Kepp O, Kroemer G. Immune parameters affecting the efficacy of chemotherapeutic regimens. *Nature Reviews: Clinical Oncology*, 2011, 8: 151–160.

Lehouriitis P, Cummins J, Stanton M, Murphy CT, McCarthy FO, et al. Local bacteria affect the efficacy of chemotherapeutic drugs. *Scientific Reports*, 2015, 5: 14554.

Liu JH, Xi P, Chai YL, Wang J, Wang T, et al. Association of DNA repair gene polymorphisms with response to cisplatin-based concurrent chemoradiotherapy in patients with cervical carcinoma. *DNA Repair (Anst)*, 2016, 41: 69–72.

Yang PW, Huang YC, Hsieh CY, Hua KT, Huang YT, et al. Association of miRNA-related genetic polymorphisms and prognosis in patients with esophageal squamous cell carcinoma. *Annals of Surgical Oncology*, 2014, 21 Suppl 4: S601–609.

Chen PC, Chen YC, Lai LC, Tsai MH, Chen SK, et al. Use of germline polymorphisms in predicting concurrent chemoradiotherapy response in esophageal cancer. *International Journal of Radiation Oncology, Biology, Physics*, 2012, 82: 1996–2003.

Abbas M, Kushwaha VS, Srivastava K, Banerjee M, Glutathione S-Transferase Gene Polymorphisms and Treatment Outcome in Cervical Cancer Patients under Concomitant Chemoradiation. *Plos One*, 2015, 10: e0142501.

Gorukmez O, Yakut T, Gorukmez O, Sag SO, Topak A, et al. Glutathione S-transferase T1, M1 and P1 Genetic Polymorphisms and Susceptibility to Colorectal Cancer in Turkey. *Asian Pacific Journal of Cancer Prevention*, 2016, 17: 3855–3859.

Kang HW, Song PH, Ha YS, Kim WT, Kim YJ, et al. Glutathione S-transferase M1 and T1 polymorphisms: susceptibility and outcomes in muscle invasive bladder cancer patients. *European Journal of Cancer*, 2013, 49: 3010–3019.

Song Z, Shao C, Feng C, Lu Y, Gao Y, et al. Association of glutathione S-transferase T1, M1, and P1 polymorphisms in the breast cancer risk: a meta-analysis. *Therapeutics and Clinical Risk Management*, 2016, 12: 763–769.

Xiao HL, Yang ZT, Han F, Wei HX. Association of glutathione S-transferase (GST) genetic polymorphisms with treatment outcome of cisplatin-based chemotherapy for advanced non-small cell lung cancer in a Chinese population. *Genetics and Molecular Research*, 2016, 15.

Pereira D, Assis J, Gomes M, Nogueira A, Medeiros R. Improvement of a predictive model in ovarian cancer patients submitted to platinum-based chemotherapy: implications of a GST activity profile. *European Journal of Clinical Pharmacology*, 2016, 72: 545–553.

Kanter M, Turan G, Usta C, Esen HH, et al. Survivin and cyclin D1 expressions are associated with malignant potential in mucinous ovarian neoplasms. *Journal of Molecular Histology*, 2016, 47: 145–152.

Giovinazzo H, Kumar P, Sheikh A, Brooks KM, Ivanovic M, et al. Technetium Tc 99m sulfur colloid photonic probe for the pharmacokinetics and pharmacodynamics of PEGylated liposomal doxorubicin in women with ovarian cancer. *Cancer Chemotherapy and Pharmacology*, 2016, 77: 565–573.

Kroeger DR, Milne K, Nelson BH. Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer. *Clinical Cancer Research*, 2016, 22: 3005–3015.

Bacalbasa N, Balescu I, Dima S, Popescu I. Long-term Survivors After Liver Resection for Ovarian Cancer Liver Metastases. *Anticancer Research*, 2015, 35: 6919–6923.

Mills K, Fuh K. Recent Advances in Understanding, Diagnosing, and Treating Ovarian Cancer. *FI000Res*, 2017, 6: 84.

Negovan A, Iancu M, Moldovan V, Mocan S, Banescu C. The Interaction between GSTT1, GSTM1, and GSTP1 Ile105Val Gene Polymorphisms and Environmental Risk Factors in Premalignant Gastric Lesions Risk. *Genom Data*, 2017, 7: 6923.

Yadav P, Chatterjee A, Bhattacharjee A. Identification of deleterious nsSNPs in alpha, mu, pi and theta class of GST family and their influence on protein structure. *Genom Data*, 2014, 2: 66–72.

Tahara T, Shibata T, Nakamura M, Yamashita H, Yoshioka D, et al. Effect of genetic polymorphisms related to DNA repair and the xenobiotic pathway on the prognosis and survival of gastric cancer patients. *Anticancer Research*, 2011, 31: 705–710.

Mastana SS, Kaur A, Hafiz R, Lindey MR. Influence of glutathione S-transferase polymorphisms (GSTT1, GSTM1, GSTP1) on type-2 diabetes mellitus (T2DM) risk in an endogamous population from north India. *Molecular Biology Reports*, 2013, 40: 7103–7110.

Stanulla M, Schrappe M, Brechlin AM, Zimmermann M, Welte K. Polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: a case-control study. *Blood*, 2000, 95: 1222–1228.

Sharma N, Singh A, Singh N, Behera D, Sharma S. Genetic polymorphisms in GSTM1, GSTT1 and GSTP1 genes and risk of lung cancer in a North Indian population. *Cancer Epidemiology*, 2015, 39: 947–955.

Kim L, Ghatak S, Yadav RP, Chhuani L, Lallawmzuali D, et al. Relevance of GSTM1, GSTT1 and GSTP1 Gene Polymorphism to Breast Cancer Susceptibility in Mizoram Population, Northeast India. *Biochemical Genetics*, 2016, 54: 41–49.

Tulsyan S, Chaturvedi P, Agrawal G, Lal P, Agrawal S, et al. Pharmacogenetic influence of GST polymorphisms on anthracycline-based chemotherapy responses and toxicity in breast cancer patients: a multi-analytical approach. *Molecular Diagnosis & Therapy*, 2013, 17: 371–379.

Schnekenburger M, Karius T, Diedrich M. Regulation of epigenetic traits of the glutathione S-transferase P1 gene: from
detoxification toward cancer prevention and diagnosis. *Frontiers in Pharmacology*, 2014, 5: 170.
36. Lu C, Spitz MR, Zhao H, Dong Q, Truong M, et al. Association between glutathione S-transferase pi polymorphisms and survival in patients with advanced nonsmall cell lung carcinoma. *Cancer*, 2006, 106: 441–447.
37. Islam MS, Islam MS, Parvin S, Ahmed MU, Bin Sayeed MS, et al. Effect of GSTP1 and ABCC4 gene polymorphisms on response and toxicity of cyclophosphamide-epirubicin-5-fluorouracil-based chemotherapy in Bangladeshi breast cancer patients. *Tumour Biology*, 2015, 36: 5451–5457.
38. Zhang BL, Sun T, Zhang BN, Zheng S, Lu N, et al. Polymorphisms of GSTP1 is associated with differences of chemotherapy response and toxicity in breast cancer. *Chinese Medical Journal*, 2011, 124: 199–204.
39. Wahlang JB, Laishram PD, Brahma DK, Sarkar C, Lahon J, et al. Adverse drug reactions due to cancer chemotherapy in a tertiary care teaching hospital. *Therapeutic Advances in Drug Safety*, 2017, 8: 61–66.
40. Low SK, Kiyotani K, Mushiroda T, Daigo Y, Nakamura Y, et al. Association study of genetic polymorphism in ABCC4 with cyclophosphamide-induced adverse drug reactions in breast cancer patients. *Journal of Human Genetics*, 2009, 54: 564–571.
41. Aapro MS, Bohlius J, Cameron DA, Dal Lago L, Donnelly JP, et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. *European Journal of Cancer*, 2011, 47: 8–32.