Research Article

Effects of MDR1 Gene Polymorphism on Efficacy and Hematotoxicity of Epirubicin-Based Regimen in Patients with Breast Cancer in Southwest China

Qiming Wei#, Xiaoyan Zhong#, Wanlong Zhu, Qingze Fan, Zhigui Wu, Changjing Xu, Mengya Li, Chengbin Zhang, Yuanzhi Liu, JunYi Zhao and Yilan Huang*

Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, Luzhou, China

#Contributed equally

ARTICLE INFO

Article history:
Received: 15 March, 2021
Accepted: 19 March, 2021
Published: 30 March, 2021

Keywords:
MDR1 gene polymorphism
breast cancer
Epirubicin
chemotherapy response
blood toxicity

ABSTRACT

Objective: To investigate the predictive value of multi-drug resistance gene (MDR1) polymorphism in the efficacy and hematological toxicity of chemotherapy regimen based on Epirubicin in patients with breast cancer in Southwest China.

Methods: Patients who received Epirubicin-based chemotherapy were included, and polymorphism of C1236T, G2677T/A and C3435T were detected by time-of-flight mass spectrometry (TOF-MS). The correlation between different genotypes and chemotherapy efficacy and blood toxicity were evaluated.

Results: A total of 102 patients were included, 44 of them were treated with neoadjuvant chemotherapy. There was no significant correlation between all genotypes and alleles of the three SNPs and the efficacy of neoadjuvant chemotherapy regimen containing Epirubicin in patients with breast cancer. There was a significant correlation between C3435T polymorphism and grade III-IV leukopenia in patients with breast cancer, the incidence of grade III-IV leukopenia in patients with C allele was significantly lower than that in patients with T allele.

Conclusion: T allele of C3435T polymorphism may be a risk factor for grade III-IV leukopenia in patients with breast cancer after chemotherapy.

Background

According to the latest statistical analysis of global cancer data, in 2018, the incidence of breast cancer is ranked first place as a deadly disease among female cancer patients [1]. Its incidence also ranks first in Chinese female population and exerts an increasing trend year by year [2, 3]. the number of new breast cancer cases and the number of deaths in China account for 12.2% and 9.6% per year [4]. Cytotoxic drug chemotherapy plays an important role in the treatment of breast cancer, in which the combination of Anthracycline and/or Taxanes is the first-line recommended [5].

Both anthracyclines and taxanes need to enter the tumor cells to act on the related targets and exert their anti-proliferation effects [6]. It has been confirmed that the transport of anthracyclines and taxanes is mediated by P-glycoprotein (P-gp), which is a transmembrane glycoprotein encoded by multi-drug resistance gene (MDR1) [7]. The gene polymorphism of MDR1 may affect the expression and functional activity of P-gp, thus affects the transport and even therapeutic effect of anthracyclines and taxanes, which is one of the important reasons for the occurrence of multi-drug resistance in tumor cells [8, 9]. At present, more than 50 single nucleotide polymorphisms (SNP) of MDR1 gene have been found, and the mutation frequencies of these SNPs are different in different regions or populations [10, 11]. So, there are some
differences in the expression, activity and function of P-gp in different organisms [12]. Among them, C1236T, G2677T/A and C3435T have been proved to be closely related [13]. Therefore, the purpose of this study is to explore the correlation between the polymorphisms of C1236T, G2677T/A and C3435T and the efficacy and hematotoxicity of Epirubicin-based regimens in patients with breast cancer in Southwest China, which may provide predictive indicators for clinical treatment options for breast cancer patients and promote individualized treatment.

Materials and Methods

I Research Objects

To collect female patients with breast cancer diagnosed by pathology and immunohistochemistry from the Affiliated Hospital of Southwest Medical University. Inclusion criteria: no antitumor drug therapy in the first treatment or in the previous 6 months; Eastern Cooperative Oncology Group (ECOG) score of physical condition is 0-2; No abnormal blood routine, heart, liver and kidney function. Excluding patients with clinical diagnosis, unclear primary tumor or complicated with other tumors, pregnant or lactating patients.

II Determination of Chemotherapy Regimen, Efficacy and Hematotoxicity

All the patients in the study were given Epirubicin-based chemotherapy regimens, including EC, TEC or ET regimens (E: Epirubicin, T: docetaxel, C: cyclophosphamide). Doxorubicin 90mg/m², intravenous drip (iv drip); cyclophosphamide 600mg, iv drip; docetaxel 75mg, iv drip. All patients took 21 days as a chemotherapy cycle. After 2-4 cycles of chemotherapy, the short-term efficacy of chemotherapy was judged according to the solid tumor efficacy evaluation standard RECIST1.1 [5]. The combination of complete remission (CR) and partial remission (PR) was judged to be effective, and stable disease (SD) and progression disease (PD) were judged to be ineffective. The hematotoxicity of chemotherapy, including leukopenia, neutropenia, thrombocytopenia and anemia, was evaluated according to the criteria for evaluating the toxicity of anticancer drugs issued by the World Health Organization (WHO). The lowest value monitored during chemotherapy was used as the test result. The efficacy and hematotoxicity of preoperative adjuvant chemotherapy were evaluated, while those of postoperative adjuvant chemotherapy were only monitored.

III Extraction of DNA from Peripheral Venous Blood

Before chemotherapy, 4mL of peripheral fasting venous blood was collected with disodium etylenediamine tetraacetate (EDTA-Na₂) purple anticoagulant tube. DNA was extracted by commercial Kit (Beijing BioTeKe Company). OD value was detected by NanoDrop2000 instrument, and 1.25% agarose gel electrophoresis was detected. DNA was stored in the refrigerator at-20°C after passing quality test.

IV The Primer Design of MDR1 Gene SNP

The primer design and synthesis of MDR1 gene SNP was performed by Huada Genome Technology Co., Ltd. C1236T (rs1128503): forward- ACGTTGGATGATCCTGCTGTTCATTCCAA CC; G2677T/A (rs2032582): forward- ACGTTGGATGGCAGCTACATGGCCACCTTTA TG; reverse- ACGTTGGATGACTGCTGTGGGTTCTAAAG; C3435T (rs1045642); forward- ACGTTGGATGTTGCGTATGGAGACAACGC; reverse- ACGTTGGATGTAAGCGTATGGTGCCCT.

V SNPs Determination of MDR1 Gene

SNPs genotyping was performed by Agena Mass ARRAY system. Briefly, the target DNA fragment was amplified by polymerase chain reaction (PCR). The total mixture of PCR reaction was 5μl, containing reaction solution 4μl and DNA sample standardized to 20ng/μl. The specific reaction procedure was as follows: pre-denaturation at 94°C for 5 min, annealing at 56°C for 30s, extension at 72°C for 1 min, cycle 45 times, complete extension at 72°C for 3 min, and end the reaction. The amplification results were verified by 1.25% agarose gel electrophoresis. Then, under the action of shrimp alkaline phosphatase (SAP), the excess deoxyribonucleotide (dNTPs), was removed and the SAP reaction solution was increased by 2μl. The reaction procedure was set as follows: 37°C 20 min/85°C 5 min, and the reaction was finished. Then the single base extension reaction was carried out and the extension reaction solution was increased by 2μl. The specific reaction procedure was as follows: pre-denaturation at 94°C, denaturation at 94°C for 5s, annealing at 52°C for 5s, extension at 80°C for 5s, cycle 45 times, complete extension at 72°C for 3 minutes, and end the reaction. Finally, the product was purified by resin and detected by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

VI Statistical Analysis

Statistical analysis was carried out by SPSS 22.0 software. Fisher test and Fisher exact probability method were used to test the genetic balance between gene polymorphism and Hardy-Weinberg, and to analyse the relationship between MDR1 gene polymorphism and basic clinical characteristics, pathological stage, molecular classification, chemotherapeutic efficacy and chemotoxicity. The relative risk of different genotypes and hematotoxicity of breast cancer chemotherapy was compared by binary logistic regression analysis, and the odds ratio (OR) and 95% confidence interval (CI) were calculated. P < 0.05 was considered to be statistically significant.

Results

I MDR1 Gene Polymorphism Typing

A total of 102 patients with breast cancer were included in this study. The results of MDR1 gene polymorphism detection showed that there were three genotypes of C1236T: CC, CT and TT, and the variation frequency of T alleles was 66.7%. Three genotypes of C1236T: GG, GT and TT, and the variation frequency of T allele was 53.9%. Three genotypes of C3435T: CC, CT and TT, and the variation frequency of T alleles was 45.6%. By χ² test, the genotypic distribution of the three SNPs loci conformed to the Hardy-Weinberg genetic balance (P > 0.05), as shown in (Table 1).
Table 1: C1236T, G2677T/A and C3435T polymorphisms and the results of Hardy-Weinberg genetic balance test.

|                  | Cases | C1236T Genotype | Allele gene | \(\chi^2\) | \(P\) |
|------------------|-------|-----------------|-------------|------------|-----|
|                  |       | CC              | CT          | TT         | C   | T   |
| Actual frequency | 102   | 15              | 38          | 49         | 68  | 136 | 1.37 | 0.50 |
| Theoretical      | 102   | 11.3            | 45.4        | 45.3       |     |     |     |     |
|                  |        | G2677T/A Genotype | Allele gene | \(\chi^2\) | \(P\) |
|                  |       | GG              | GT          | TT         | G   | T/A |
| Actual frequency | 102   | 28              | 38          | 36         | 94  | 110 | 3.16 | 0.21 |
| Theoretical      | 102   | 21.7            | 50.7        | 29.6       |     |     |     |     |
|                  |        | C3435T Genotype | Allele gene | \(\chi^2\) | \(P\) |
|                  |       | CC              | CT          | TT         | C   | T   |
| Actual frequency | 102   | 32              | 47          | 23         | 111 | 93  | 0.32 | 0.85 |
| Theoretical      | 102   | 30.2            | 50.6        | 21.2       |     |     |     |     |

II Relationship between MDRI Gene Polymorphism and Clinicopathology

All the patients included in the study were Han women, ranging in age from 23 to 71-year-old, with an average age of 49-year-old. The genotypes of C1236T, G2677T/A and C3435T were compared with the baseline of clinicopathological characteristics by \(\chi^2\) test, including age, menstrual status, clinical stage, degree of tissue differentiation and molecular typing, as shown in (Table 2).

Table 2: Baseline clinical characteristics of patients with different MDRI genotypes.

|                  | Cases | C1236T | G2677T/A | C3435T |
|------------------|-------|--------|----------|--------|
|                  |       | CC     | CT       | TT     | GG   | GT | TT | CC | CT | TT |
| Age              |       |        |         |        |      |    |    |    |    |    |
| \(\leq 35\)      | 6     | 2      | 3       | 1      | 0.25 | 1   | 3  | 2  | 0.96 | 2 | 1 | 3 | 0.40 |
| 36-50            | 51    | 8      | 15      | 28     | 14   | 19  | 18 | 16 | 26  | 9  |
| >50              | 45    | 5      | 20      | 20     | 13   | 16  | 16 | 14 | 20  | 11 |
| Menopausal state |       |        |         |        |      |    |    |    |    |    |
| Premenopausal    | 48    | 7      | 17      | 24     | 0.93 | 12  | 18 | 18 | 0.85 | 17 | 22 | 9  | 0.59 |
| Menopause        | 54    | 8      | 21      | 25     | 16   | 20  | 18 | 15 | 25  | 14 |
| Clinical staging |       |        |         |        |      |    |    |    |    |    |
| I                | 12    | 2      | 1       | 9      | 0.13 | 2   | 7  | 3  | 0.45 | 4 | 6 | 2 | 0.37 |
| II               | 39    | 8      | 15      | 16     | 12   | 15  | 12 | 11 | 15  | 13 |
| III              | 51    | 5      | 22      | 24     | 14   | 16  | 21 | 17 | 26  | 8  |
| Histological grading |     |        |         |        |      |    |    |    |    |    |
| I                | 29    | 2      | 10      | 17     | 0.57 | 8   | 15 | 6  | 0.14 | 10 | 12 | 7  | 0.84 |
| II               | 61    | 11     | 24      | 26     | 15   | 19  | 27 | 19 | 30  | 12 |
| III              | 12    | 2      | 4       | 6      | 5    | 4   | 3  | 3  | 5   | 4  |
| Molecular type   |       |        |         |        |      |    |    |    |    |    |
| triple-negative type | 83 | 11    | 29      | 43     | 0.57 | 25  | 33 | 27 | 0.37 | 27 | 41 | 15 | 0.22 |
| non-triple-negative type | 16 | 3     | 7       | 6      | 4    | 4   | 8  | 4  | 6   | 6  |

III The Relationship between MDRI Gene Polymorphism and the Efficacy of Chemotherapy

In this study, a total of 44 breast cancer patients received neoadjuvant chemotherapy. The evaluation results of chemotherapy efficacy were: CR: 2 cases, PR: 22 cases, SD: 15 cases and PD: 5 cases. The overall response rate (CR+PR) of chemotherapy was 54.5%. The results of correlation analysis between C1236T, G2677T/A and C3435T polymorphisms and chemotherapeutic efficacy showed that the genotypes or alleles of the three SNPs were not related to the chemotherapeutic efficacy of Epirubicin-based regimen in breast cancer patients (all \(P > 0.05\)), as shown in (Table 3).

Table 3: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy response in patients with breast cancer.

| Genes     | Genotype | Cases | Effective (CR+PR) | Invalid (SD+PD) | Efficiency | \(\chi^2\) | \(P\) value |
|-----------|----------|-------|------------------|-----------------|------------|------------|-------------|
| C1236T    | CC       | 6     | 2                | 4               | 33.3%      | 1.57       | 0.46        |
|           | CT       | 17    | 9                | 8               | 52.9%      |            |             |
|           | TT       | 21    | 13               | 8               | 61.9%      |            |             |
|           | C        | 29    | 13               | 16              | 44.8%      | 1.65       | 0.26        |
|           | T        | 59    | 35               | 24              | 59.3%      |            |             |
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Clinical Oncology & Research doi:10.31487/j.COR.2021.04.04 Volume 4(4): 4-8

G2677T/A

| Genotype | Cases | Leukopenia |
|----------|-------|------------|
| GG       | 10    | 6          | 4          | 60.0% | 0.58 | 0.75 |
| GT       | 19    | 11         | 8          | 57.9% |
| TT       | 15    | 7          | 8          | 46.7% |
| G        | 39    | 23         | 16         | 59.0% | 0.55 | 0.52 |
| T        | 49    | 25         | 24         | 51.0% |

C3435T

| Genotype | Cases | Leukopenia |
|----------|-------|------------|
| CC       | 16    | 10         | 6          | 62.5% | 2.37 | 0.31 |
| CT       | 21    | 12         | 9          | 57.1% |
| TT       | 7     | 2          | 5          | 28.6% |
| C        | 53    | 32         | 21         | 60.4% | 1.83 | 0.20 |
| T        | 35    | 16         | 19         | 45.7% |

IV Relationship between MDR1 Gene Polymorphism and Hematotoxicity

The results of blood toxicity evaluation of all 102 patients were as follows: the incidence of severe leukopenia: 18.6% (n=19); severe neutropenia: 35.3% (n=36); severe thrombocytopenia: 3.9% (n=4); severe anemia: 4.9% (n=5). The correlations between C1236T, G2677T/A and C3435T genotypes and alleles and blood toxicity were analysed. The results showed that C3435T polymorphism was associated with severe leukopenia. Further allele analysis showed that the incidence of severe leukopenia in patients with C allele was 12.6%, which was lower than that in patients with T allele, as shown in (Table 4). The C3435T polymorphism was associated with severe neutropenia, and the incidence of severe neutropenia in patients with C allele was significantly lower than that in patients with T allele, and the incidence of severe neutropenia in patients with C allele was significantly lower than that in patients with T allele. The genotypes or alleles of other SNPs were not associated with severe hematological toxicity, as shown in (Tables 5-7).

Table 4: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced leukopenia.

| Genes   | Genotype | Cases | Leukopenia |
|---------|----------|-------|------------|
|         |          |       | 0-II | III-IV | \( \chi^2 \) | P value |
| C1236T  | CC       | 15    | 11   | 4     | 1.40 | 0.50 |
|         | CT       | 38    | 30   | 8     |       |
|         | TT       | 49    | 42   | 7     |       |
|         | C        | 68    | 52   | 16    | 1.62 | 0.25 |
|         | T        | 136   | 114  | 22    |       |
| G2677T/A| GG       | 28    | 23   | 5     | 1.19 | 0.55 |
|         | GT       | 38    | 29   | 9     |       |
|         | TT       | 36    | 31   | 5     |       |
|         | G        | 94    | 75   | 19    | 0.29 | 0.59 |
|         | T        | 110   | 91   | 19    |       |
| C3435T  | CC       | 32    | 29   | 3     | 5.85 | 0.05 |
|         | CT       | 47    | 39   | 8     |       |
|         | TT       | 23    | 15   | 8     |       |
|         | C        | 111   | 97   | 14    | 5.81 | 0.02*|
|         | T        | 93    | 69   | 24    |       |

Note: *P < 0.05

Table 5: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced neutropenia.

| Genes   | Genotype | Cases | Neutropenia |
|---------|----------|-------|-------------|
|         |          |       | 0-II | III-IV | \( \chi^2 \) | P value |
| C1236T  | CC       | 15    | 10   | 5     | 4.10 | 0.13 |
|         | CT       | 38    | 20   | 18    |       |
|         | TT       | 49    | 36   | 13    | 3.65 | 0.06 |
|         | C        | 68    | 40   | 28    |       |
|         | T        | 136   | 112  | 44    |       |
| G2677T/A| GG       | 28    | 20   | 8     | 2.10 | 0.35 |
|         | GT       | 38    | 26   | 12    |       |
|         | TT       | 36    | 20   | 16    | 2.32 | 0.14 |
|         | G        | 94    | 66   | 28    |       |
|         | T        | 110   | 66   | 44    |       |
| C3435T  | CC       | 32    | 27   | 5     | 8.83 | 0.01*|
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Table 6: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced thrombocytopenia.

| Genes   | Genotype | Cases | Thrombocytopenia | χ² | P value |
|---------|----------|-------|-----------------|----|---------|
|         |          |       | 0-II | III-IV |       |
| C1236T  | CC       | 15    | 14   | 1     | 0.94 | 0.63   |
|         | CT       | 38    | 36   | 2     |       |        |
|         | TT       | 49    | 48   | 1     |       |        |
|         | C        | 68    | 64   | 4     | 1.04 | 0.45   |
|         | T        | 136   | 132  | 4     |       |        |
| G2677T/A| GG       | 28    | 28   | 0     | 2.86 | 0.24   |
|         | GT       | 38    | 35   | 3     |       |        |
|         | TT       | 36    | 35   | 1     |       |        |
|         | G        | 94    | 91   | 3     | 0.25 | 0.73   |
|         | T        | 110   | 105  | 5     |       |        |
| C3435T  | CC       | 32    | 32   | 0     | 2.07 | 0.36   |
|         | CT       | 47    | 44   | 3     |       |        |
|         | TT       | 23    | 22   | 1     |       |        |
|         | C        | 111   | 108  | 3     | 0.96 | 0.47   |
|         | T        | 93    | 88   | 5     |       |        |

Note: *P < 0.05; **P < 0.01

Table 7: Relationship between C1236T, G2677T/A and C3435T polymorphism and chemotherapy-induced anemia.

| Genes   | Genotype | Cases | Anemia | χ² | P value |
|---------|----------|-------|--------|----|---------|
|         |          |       | 0-II | III-IV |       |
| C1236T  | CC       | 15    | 13   | 2     | 3.16 | 0.21   |
|         | CT       | 38    | 36   | 2     |       |        |
|         | TT       | 49    | 48   | 1     |       |        |
|         | C        | 68    | 62   | 6     | 3.37 | 0.09   |
|         | T        | 136   | 132  | 4     |       |        |
| G2677T/A| GG       | 28    | 27   | 1     | 1.19 | 0.55   |
|         | GT       | 38    | 35   | 3     |       |        |
|         | TT       | 36    | 35   | 1     |       |        |
|         | G        | 94    | 89   | 5     | 0.07 | 1.0    |
|         | T        | 110   | 105  | 5     |       |        |
| C3435T  | CC       | 32    | 31   | 1     | 0.45 | 0.80   |
|         | CT       | 47    | 44   | 3     |       |        |
|         | TT       | 23    | 22   | 1     |       |        |
|         | C        | 111   | 106  | 5     | 0.08 | 1.0    |
|         | T        | 93    | 88   | 5     |       |        |

With three SNPs as covariates, hematotoxicity as a dependent variable, binary logistic regression analysis of leukocytes and neutrophils was carried out. The results showed that there was a correlation between C3435T polymorphism and severe leukopenia (P < 0.05). Patients with CC genotype had a significantly lower risk of severe leukopenia than patients with other genotypes [OR=6.40, 95%CI (1.032-39.662)], as shown in (Table 8). However, the results did not show that C3435T polymorphism was a risk factor for severe neutropenia after chemotherapy (Table 9). The goodness-of-fit test results of binary logistic regression analysis showed that the calibration of the risk research model was high, and the accuracy of the prediction model was high.
Table 8: Binary Logistic analysis of C1236T, G2677T/A and C3435T polymorphisms and chemotherapy-induced leukopenia.

| Genes   | β    | SE   | Wald | P   | OR  | 95% CI          |
|---------|------|------|------|-----|-----|-----------------|
| C1236T  | -0.546 | 0.692 | 0.623 | 0.43 | 0.579 | 0.149-2.247    |
| G2677T/A| -1.085 | 0.85  | 1.629 | 0.202 | 0.338 | 0.064-1.788    |
| C3435T  | 1.856  | 0.931 | 3.976 | 0.046 | 6.399 | 1.032-39.662   |

Note: Nagelkerke (pseudo) R²=0.086; Goodness-of-fit test: χ²=6.558 (P=0.087).

Table 9: Binary Logistic analysis of C1236T, G2677T/A and C3435T polymorphisms and chemotherapy-induced neutropenia.

| Genes   | β    | SE   | Wald | P   | OR  | 95% CI          |
|---------|------|------|------|-----|-----|-----------------|
| C1236T  | -1.244 | 0.642 | 3.760 | 0.052 | 0.288 | 0.082-1.013    |
| G2677T/A| -1.136 | 0.673 | 2.854 | 0.091 | 0.321 | 0.086-1.200    |
| C3435T  | 0.139  | 0.685 | 0.041 | 0.840 | 1.149 | 0.300-4.398    |

Note: Nagelkerke (pseudo) R²=0.134; Goodness-of-fit test: χ²=3.377 (P=0.337).

Discussion

MDR1 gene, also known as ABCB1 gene, is located on the long arm of human chromosome 7 q21.1 and encodes the P-gp [14-16]. P-gp can bind to ATP through a specific region and pump “harmful substances” out of the cell, which plays an important role in the absorption, distribution and excretion of its substrates including drugs [17]. Some studies have shown that MDR1 gene-related SNPs polymorphism may affect the Area Under Curve (AUC) or clearance rate of docetaxel [18, 19]. At present, it has been found that there exists more than 50 SNPs in MDR1 gene, of which exon 12 (C1236T), 21 (G2677T/A) and 26 (C3435T) have high mutation frequency [20-23]. Liu et al. found that breast cancer patients with T allele at C3435T locus had better chemotherapeutic efficacy than patients with C allele, but no significant difference was found in C1236T and G2677T/A polymorphism [24]. While in breast cancer patients in Saudi Arabia, Alsaif et al. thought that the CT/TT type of C1236T gene had a low response rate to anthracycline and/or yew chemotherapy regimens [25]. Chang et al. obtained the opposite results in the study of paclitaxel monotherapy for breast cancer [26]. They found that in patient with CC type of C3435T gene, the disease control rate was significantly higher than that with mutant type, and the GG type of G2677T/A locus was related to paclitaxel resistance. In a Meta-analysis, Adela et al. considered that under the existing evidence, the above three SNPs did not have a significant correlation with the chemotherapeutic efficacy in patients with breast cancer, which did not support it as an effective predictor [27]. Considering that the gene polymorphism may affect different drugs, the inconsistency of these findings may be attributed to the different chemotherapy regimens. This study included breast cancer patients in southwest China as the study population and chooses Epirubicin-based chemotherapy regimen, which is different from other taxus-based regimens, and has a reference value for the clinical treatment in this area.

The effect of MDR1 gene polymorphism on anthracycline and taxane drug transport may also lead to differences in drug toxicity. Tran et al. found that docetaxel-treated patients with T allele (C3435T) had a significantly higher frequency of leukopenia than those with C allele [28]. Meanwhile, Ji et al. found that T allele carriers may have a higher risk of neutropenia [29]. However, the study of Chang et al. failed to detect a significant correlation between MDR1 gene polymorphism and the toxicity of paclitaxel chemotherapy [30]. All the above results of this study showed that C3435T polymorphism was a risk factor for severe leukopenia in breast cancer patients treated with Epirubicin. Meanwhile, the judgment of blood toxicity in this study is based on the lowest monitoring value during the study period, and the impact of doctors’ immediate treatment of adverse reactions on the results cannot be ruled out.

Since multiple SNPs of MDR1 gene may jointly affect the function and activity of P-gp, multivariate analysis may be more convincing than independent studies of single SNPs. Wang et al. used multiple SNPs combination analysis to analyse the combination of pairwise haploid and three haploids [31]. The results showed that the effective rate of chemotherapy in 3435T-2677T-1236T haplotype carriers was lower than that in other haplotype carriers. In addition, Ji et al. analysed the combined effect of GSTP1 gene and MDR1 gene on the prognosis of chemotherapy in Chinese breast cancer patients, suggesting the predictive value of these genes in tumor therapy [32]. In this study, after excluding the influence of three SNPs factors in binary Logistic regression analysis, C3435T polymorphism cannot act as a risk factor for severe neutropenia.

Conclusion

To sum up, this study suggests that C3435T polymorphism may be a risk factor for grade III-IV leukopenia in breast cancer patients treated with Epirubicin regimen in Southwest China. The incidence of grade III-IV leukopenia in patients with T allele was significantly higher than that with C allele after chemotherapy. So, breast cancer patients with T allele should be strengthened to actively prevent the occurrence of leukopenia in clinical monitoring. The above results need to be further confirmed by large sample, multicenter clinical studies.

Acknowledgement

We sincerely thank the Professor Bin Wu and Dr. Mingquan Huang (Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, Luzhou, China) to diagnosed the breast cancer cases, it’ s our pleasure to acknowledge their contributions.
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Author Contributions
Q.W., Y.H., and X.Z. designed this research and wrote the manuscript; Q.F. and W. Z. collected blood samples; Z.W., C.X., M.L., C.Z., Y.L. and J.Z. performed data collection and pre-processing of data.

Funding
This study was supported by research funding from the Science and Technology Planning Project of Sichuan Province (No.2019YFS0180), Science and Technology Program of Luzhou (No.2018-JYJ-41, 2015LZCYD-SO2-9/11), the Foundation for Young Scholars of Southwest Medical University (2019-ZQN-125, 2019-ZQN-151, 2018-ZQN-098), the Foundation of southwest medical university (2017-ZRZD-003).

Availability of Data and Materials
All data generated or analysed in this study are included in this published article.

Ethics Approval and Consent to Participate
All of the patient samples used in this study was approved by the ethics committee of The Affiliated Hospital of Southwest Medical University (Luzhou city, Sichuan Province, China) before we start the experiment.

Consent for Publication
Not applicable.

Conflicts of Interest
None.

Abbreviations

MDRI gene: Multi-Drug Resistance Gene 1
P-gp: P-glycoprotein
SNP: Single Nucleotide Polymorphisms
ECOG: Eastern Cooperative Oncology Group
EC: Epirubicin + Cyclophosphamide
TEC: Docetaxel+ Epirubicin+ Cyclophosphamide
ET: Epirubicin+ Docetaxel
RECIST: Response Evaluation Criteria in Solid Tumors
CR: Complete Remission
PR: Partial Remission
SD: Stable Disease
PD: Progression Disease
WHO: World Health Organization
EDTA-Na2: Ethylenediaminetetraacetic Acid Disodium Salt
PCR: Polymerase Chain Reaction
SAP: Shrimp Alkaline Phosphatase
dNTPs: Deoxyribonucleotide
MALDI-TOFMS: Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry
OR: The Odds Ratio

CI: Confidence Interval
AUC: Area Under Curve
GSTP1: Glutathione S-Transferase P1

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA et al. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424. [Crossref]
2. Fan L, Strasser Weippl K, Li JJ, Louis JS, Finkelstein DM et al. (2014) Breast cancer in China. Lancet Oncol 15: e279-289. [Crossref]
3. Feng F, Wei Y, Zheng K, Li Y, Zhang L et al. (2018) Comparison of epidemiological features, clinicopathological features, and treatments between premenopausal and postmenopausal female breast cancer patients in western China: a retrospective multicenter study of 15,389 female patients. Cancer Med 7: 2753-2763. [Crossref]
4. Huang Z, Wen W, Zheng Y, Gao YT, Wu C et al. (2016) Breast cancer incidence and mortality: trends over 40 years among women in Shanghai, China. Ann Oncol 27: 1129-1134. [Crossref]
5. Breast Cancer Professional Committee of China Anti-Cancer Association (2017) Guidelines and norms for diagnosis and treatment of Breast Cancer of China Anti-Cancer Association (2017 Edition). China Oncology 27: 695-759.
6. Turner N, Biganzoli L, Di Leo A (2015) Continued value of adjuvant anthracyclines as treatment for early breast cancer. Lancet Oncol 16: e362-e369. [Crossref]
7. Duran GE, Derdau V, Weitz D, Philippe N, Blankenstein J et al. (2018) Cabazitaxel is more active than first-generation taxanes in ABCB1(+) cell lines due to its reduced affinity for P-glycoprotein. Cancer Chemother Pharmacol 81: 1095-1103. [Crossref]
8. Taheri M, Mahjoubi F, Omranipour R (2010) Effect of MDR1 polymorphism on multi drug resistance expression in breast cancer patients. Genet Mol Res 9: 34-40. [Crossref]
9. Fung KL, Pan J, Ohnuma S, Lund PE, Pickney JN et al. (2014) MDR1 synonymous polymorphisms alter transporter specificity and protein stability in a stable epithelial monolayer. Cancer Res 74: 598-608. [Crossref]
10. Kimchi Sarfaty C, Oh JM, Kim W, Sauna ZE, Calcagno AM et al. (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315: 525-528. [Crossref]
11. Elmansy D, Koyuturk M (2019) Cross-population analysis for functional characterization of type II diabetes variants. BMC Bioinformatics 20: 320. [Crossref]
12. Oghara T, Mizoi K, Kamioka H, Yano K (2020) Physiological Roles of ERM Proteins and Transcriptional Regulators in Supporting Membrane Expression of Efflux Transporters as Factors of Drug Resistance in Cancer. Cancers (Basel) 12: 3352. [Crossref]
13. Shan XX, Qu Y, Xie WW, Wu RR, Yu Y et al. (2019) ABCB1 Gene Is Associated With Clinical Response to SNRIs in a Local Chinese Han Population. Front Pharmacol 10: 761. [Crossref]
14. Nieuwint AW, Baas F, Wiegant J, Joenje H (1992) Cyto genetic alterations associated with P-glycoprotein- and non-P-glycoprotein-mediated multidrug resistance in SW-1573 human lung tumor cell lines. Cancer Res 52: 4361-4371. [Crossref]
15. Wang YC, Juric D, Francisco B, Yu RX, Duran GE et al. (2006) Regional activation of chromosomal arm 7q with and without gene amplification in taxane-selected human ovarian cancer cell lines. *Genes Chromosomes Cancer* 45: 365-374. [Crossref]

16. De Vera AA, Gupta P, Lei Z, Liao D, Narayanan S et al. (2019) Immuno-oncology agent IPI-549 is a modulator of P-glycoprotein (P-gp, MDR1, ABCB1)-mediated multidrug resistance (MDR) in cancer: In vitro and in vivo. *Cancer Lett* 442: 91-103. [Crossref]

17. Kodan A, Futamata R, Kimura Y, Kioka N, Nakatsu T et al. (2020) ABCB1/MDR1/P-gp employs an ATP-dependent twist-and-squeeze mechanism to export hydrophobic drugs. *FEBS Lett.* [Crossref]

18. Bosch TM, Huitema AD, Doodeman VD, Jansen R, Witteveen E et al. (2006) Pharmacogenetic screening of CYP3A and ABCB1 in relation to population pharmacokinetics of docetaxel. *Clin Cancer Res* 12: 5786-5793. [Crossref]

19. Fajac A, Gligorov J, Rezai K, Lévy P, Lévy E et al. (2010) Effect of ABCB1 C3435T polymorphism on docetaxel pharmacokinetics according to menopausal status in breast cancer patients. *Br J Cancer* 103: 560-566. [Crossref]

20. Rinaldi I, Nova R, Widjastuti R, Priambodo R, Instiaty I et al. (2019) Association between C1236T Genetic Variant of ABCB1 Gene and Molecular Response to Imatinib in Indonesian Chronic Myeloid Patients. *Asian Pac J Cancer Prev* 20: 3331-3334. [Crossref]

21. Gervasini G, Carrillo JA, Garcia M, San Jose C, Cabanillas A et al. (2006) Adenosine triphosphate-binding cassette B1 (ABCB1) (multidrug resistance 1) G2677T/A gene polymorphism is associated with high risk of lung cancer. *Cancer* 107: 2850-2857. [Crossref]

22. Potocnik U, Ravnik Glavac M, Glavac D (2002) Functional MDR1 polymorphisms (G2677T and C3435T) and TCF4 mutations in colorectal tumors with high microsatellite instability. *Cell Mol Biol Lett* 7: 92-95. [Crossref]

23. Yin OQ, Tomlinson B, Chow MS (2009) Effect of multidrug resistance gene-1 (ABCB1) polymorphisms on the single-dose pharmacokinetics of cloxacillin in healthy adult Chinese men. *Clin Ther* 31: 999-1006. [Crossref]

24. LIU XL, Zhang HX, ZhaoYY, Jing M (2016) Influence of ABCB1 gene polymorphisms on breast cancer with paclitaxel-based chemotherapy. *J Xiu' an Jiaotong Univ (Med Sci)* 37: 383-387.

25. Alsaif AA, Hasan TN, Shafi G, Syed NA, Alsaif MA et al. (2013) Association of multiple drug resistance-1 gene polymorphism with multiple drug resistance in breast cancer patients from an ethnic Saudi Arabian population. *Cancer Epidemiol* 37: 762-766. [Crossref]

26. Chang H, Rha SY, Jeung HC, Im CK, Ahn JB et al. (2009) Association of the ABCB1 gene polymorphisms 2677G>T/A and 3435C>T with clinical outcomes of paclitaxel monotherapy in metastatic breast cancer patients. *Ann Oncol* 20: 272-277. [Crossref]

27. Madrid Paredes A, Canadas Garre M, Sanchez Pozo A, Exposito Ruiz M, Calleja Hernandez MA (2017) ABCB1 gene polymorphisms and response to chemotherapy in breast cancer patients: A meta-analysis. *Surg Oncol* 26: 473-482. [Crossref]

28. Tran A, Jullien V, Alexandre J, Rey E, Rabillon F et al. (2006) Pharmacokinetics and toxicity of docetaxel: role of CYP3A, MDR1, and GST polymorphisms. *Clin Pharmacol Ther* 79: 570-580. [Crossref]

29. Ji MH, Zha WW, Yun W, Li J, Zhao JH et al. (2012) MDR1 polymorphism predict response and hematologic toxicities in breast cancer patients treated with preoperative chemotherapy. *Chin J Cancer Treat* 19: 735-737.

30. Priyadarshini R, Raj GM, Kayal S, Ramesh A, Shewade DG (2019) Influence of ABCB1 C3435T and C1236T gene polymorphisms on tumour response to docetaxel-based neo-adjuvant chemotherapy in locally advanced breast cancer patients of South India. *J Clin Pharm Ther* 44: 188-196. [Crossref]

31. Wang J, Tan JH, Zhong SL et al. (2011) Association Between MDR1 Gene Polymorphisms and Curative Effect of Taxane-Anthracycline Chemotherapy in Breast Cancer. *Chin J Clin Oncol* 38: 15-19.

32. Ji M, Tang J, Zhao J, Xu B, Qin J et al. (2012) Polymorphisms in genes involved in drug detoxification and clinical outcomes of anthracycline-based neoadjuvant chemotherapy in Chinese Han breast cancer patients. *Cancer Biol Ther* 13: 264-271. [Crossref]