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RESEARCH ARTICLE

Predictive potential of ABCB1, ABCC3, and GSTP1 gene polymorphisms on osteosarcoma survival after chemotherapy

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Abstract Genetic polymorphisms in drug metabolism and transport genes can influence the pharmacokinetics and pharmacodynamics of chemotherapy drugs. We investigated the role of genes involved in metabolic and transport pathways in response to chemotherapy and clinical outcome of osteosarcoma patients. The association between the eight polymorphisms with response to chemotherapy and clinical outcome of patients was carried out by unconditional logistic regression analysis and Cox proportional hazard models. Of 186 patients, 98 patients showed good response to chemotherapy, 64 died, and 97 showed progression at the end of the study. Patients carrying ABCB1 rs1128503 TT genotype and T allele were more likely to have a good response to chemotherapy. ABCC3 rs4148416 TT genotype and T allele and GSTP1 rs1695 GG genotype and G allele were associated with poor response to chemotherapy. In the Cox proportional hazards model, after adjusting for potential confounding factors, patients carrying ABCB1 rs1128503 TT genotype and T allele were associated with lower risk of progression-free survival (PFS) and overall survival (OS). ABCC3 rs4148416 TT genotype and T allele and GSTP1 rs1695 GG genotype and G allele were correlated with high risk of PFS and OS. The ABCB1 TT and GSTP1 GG genotypes were significantly associated with a shorter OS. In conclusion, variants of ABCB1 rs128503, ABCC3 rs4148416, and GSTP1 rs1695 are associated with response to chemotherapy and PFS and OS of osteosarcoma patients; these gene polymorphisms could help in the design of individualized therapy.

Keywords ATP-binding cassette · Glutathione S-transferases · Osteosarcoma · Survival

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Introduction

Osteosarcoma is a common malignant bone tumor and mainly occurs in adolescents and young adults worldwide [1]. The real mechanism of developing osteosarcoma is still not well understood. It is estimated that patients with localized osteosarcoma have about 60–80 % rate of long-term survival; however, those with metastatic disease show a poorer prognosis [2–4]. Most of the patients with osteosarcoma received neoadjuvant therapy before surgical resection of the primary tumor and received chemotherapy after operation [5]. However, more than 40 % patients show a poor response to chemotherapy, with an estimated survival of 45 to 55 % [6]. Thirty percent patients show recurrence or metastasis during a 5-year period [1]. Moreover, 60 % patients experience severe or disabling chronic health condition after receiving surgery [7].

Clinical response to chemotherapy drugs is influenced by both genetic and environmental factors. It is well known that anticancer therapies present a narrow therapeutic range, while a higher concentration in a patient's body can cause toxicity and a lower concentration decreases the efficacy of the drug. Interindividual differences in pharmacokinetics and pharmacodynamics can have an important role in the global response and toxicity profile of each drug. In the chemotherapy process, genes have a role in controlling drug absorption, distribution, metabolism, and excretion. Cytochrome P450 (CYP) enzymes have an effect on most of these metabolism reactions [8]. Glutathione S-transferases (GSTs) are involved in

metabolizing for chemotherapeutic agents, and they catalyze the conjugation of glutathione to a wide variety of xenobiotics [9]. Moreover, two kinds of transport superfamilies (ATP-binding cassette and ABC proteins) are responsible for most of the drug transport [10]. Most of the drug metabolizers and transporters show genetic polymorphisms and can cause the plasma concentration of chemotherapy drugs and thus influence the chemotherapy effectiveness of patients.

Previous studies have showed that genetic polymorphisms in drug metabolism and transport genes can influence the pharmacokinetics and pharmacodynamics of chemotherapy drugs [10]. Two previous studies reported the association between drug metabolism and transport gene polymorphisms and survival of osteosarcoma, but the results are inconsistent [11–14]. We conducted a study to investigate the role of eight common genes involved in metabolic and transport pathways in response to chemotherapy and clinical outcome of osteosarcoma patients. A better understanding of the prognostic markers for osteosarcoma can help design individualized therapy, and thus, patients can benefit more from treatment to prolong their life and improve their quality of life.

Material and methods

Patients, treatments, and clinical variables

A total of 186 consecutive patients diagnosed with osteosarcoma were selected from the First Affiliated Hospital of Xi'an Jiaotong University between January 2008 and December 2009. Clinical data were recorded when patients were enrolled into study, including sex, age, tumor location, metastatic events, and relapses. Blood samples were obtained from all patients. Written informed consent was provided by all patients. Our study was approved by the ethics committee of the First Affiliated Hospital of Xi'an Jiaotong University.

All osteosarcoma patients received chemotherapy before surgery. The chemotherapy regimen was intravenous 25–30 mg/m^2 doxorubicin (three courses on days 1 to 3), 14 mg/m^2 methotrexate (four courses on day 1), and intra-arterial 35 mg/m^2 cisplatin (three courses on days 1 to 3). After surgery, the chemotherapy regimen was 10 mg/m^2 methotrexate on day 1 and alternate cycles of i.v. 0.45 mg/m^2 cisplatin or actinomycin D and 500/ and 1.5 mg/m^2 vincristine on day 1. The chemotherapy duration was conducted for up to 48 weeks. One course of chemotherapy was defined as treatment with a series of chemotherapy drugs. The treatment was repeated every 3 weeks for a maximum of six courses. The toxicity assessment was conducted before each cycle. The treatment would not be continued when patient presented progressive disease or experienced unacceptable toxicity. Response to chemotherapy treatment was determined by the extent of tumor necrosis after nonadjuvant chemotherapy. The poor responders were defined as patients with less than 90 % necrosis, and good responders were defined as those with 90 % necrosis or more [15]. Overall survival (OS) was calculated at the time of diagnosis until death or last known date alive. Progression-free survival (PFS) was calculated at the time of diagnosis until disease recurrence, development of lung or bone metastases, or death. All the patients were followed up every month by telephone until death or the end of follow-up (30 December 2012).

Genotyping

TIANGEN DNA kit (Tiangen Biotech, Beijing, China) was taken to extract genetic DNA from peripheral blood. Genotyping of ABCB1 rs1128503, ABCB1 rs414737, ABCB1 rs10276036, ABCC1 rs246240, ABCC3 rs4148416, GSTP1 rs1695, GSTT1 deletion, and GSTM1 deletion were carried out on a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Sequenom Assay Design 3.1 software (Sequenom®) was conducted to design primers for polymerase chain reaction amplification and single base extension assays. PCR reaction was performed in a total volume of 25 μL, containing 50 ng of genomic DNA, 0.1 μl dNTP, 1.25 U Taq DNA polymerase (Promega Corporation, Madison, WI, USA), and 21 μl forward and reverse primers. The cycling program involved preliminary denaturation at 95 °C for 2 min, followed by 45 step cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 5 min. The PCR products were analyzed by 1.0 % agarose gel electrophoresis. For quality control, 10 % of subjects were randomly selected, and the results of repeated samples showed 100 % concordance.

Statistical analysis

Continuous variables were shown by mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages. The odds ratios (OR) and corresponding 95 % confidence intervals (CIs) were calculated by unconditional logistic regression analysis and utilized to assess the potential association between ABCB1 rs1128503, ABCB1 rs414737, ABCB1 rs10276036, ABCC1 rs246240, ABCC3 rs4148416, GSTP1 rs1695, GSTT1 deletion and GSTM1 deletion, and response to chemotherapy. The homozygote for the most frequent allele was regarded as reference group. The Cox proportional hazard models were used to evaluate the effect of ABCB1 rs1128503, ABCB1 rs414737,

ABCB1 rs10276036, ABCC1 rs246240, ABCC3 rs4148416, GSTP1 rs1695, GSTT1 deletion and GSTM1 deletion, and OS and PFS of osteosarcoma. The OR (95 % CI) and HR (95 % CI) were adjusted for sex, age, tumor location, and subtypes. Kaplan-Meier method was used to plot the PFS and OS curves. SPSS® statistical package, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows® was used for statistical analyses. All P values were two-tailed, and a difference was considered statistically significant when $P<0.05$.

Results

The distributions of selected characteristics of study subjects were shown in Table 1. The mean age of the osteosarcoma subjects was 15.7±12.4 years old (ranging 8.5 to 47.3 years old). Of 186 osteosarcoma patients, 108 (59.34 %) patients were males, 78 (42.86 %) were females, 89 (48.90 %) presented tumor location at lower limb, 69 (37.91 %) showed tumor location at tibia/fibula, 100 (54.95 %) were osteoblastic subtype of osteosarcoma, 98 (53.85 %) showed good response to chemotherapy, and 38 (20.88 %) showed metastasis at diagnosis. At the end of follow-up, 64 patients (34.41 %) died.

Table 1 Clinical and pathological characteristics of included subjects

Characteristics	Patients, *N*	Percent
Median (range)	15.7 (8.5–47.3)	
<15	82	45.05
≥15	104	57.14
Sex		
Male	108	59.34
Female	78	42.86
Tumor location		
Femur	89	48.91
Tibia/fibula	69	37.91
Arm	13	7.14
Central	15	8.24
Histological response		
Good	98	53.85
Poor	88	48.35
Metastasis at diagnosis		
No	148	81.32
Yes	38	20.88
Subtype		
Osteoblastic	100	54.95
Chondroblastic	39	21.43
Other	47	25.82

Ninety-eight patients showed good response to chemotherapy, with a response rate of 53.85 % (Table 2). Patients were classified into good and poor responders, and significantly, different genetic distributions of ABCB1 rs1128503, ABCC3 rs4148416, and GSTP1 rs1695 were observed between these groups (Table 2). Patients carrying ABCB1 rs1128503 TT genotype and T allele were more likely to have a good response to chemotherapy when compared with CC genotype, with ORs (95 % CI) of 2.54 (1.10–5.96) and 1.77 (1.14–2.75), respectively. Those carrying ABCC3 rs4148416 TT genotype and T allele were associated with poorer response to chemotherapy when compared with CC genotype (OR, 0.43; 95 % CI, 0.19–0.97 for TT genotype; OR, 0.53; 95 % CI, 0.33–0.84 for T allele). Moreover, we found that those carrying GSTP1 rs1695 GG genotype and G allele were more likely to have a poorer response to chemotherapy when compared with AA genotype, with ORs (95 % CI) of 0.82 (0.35–1.91) and 0.57 (0.36–0.88), respectively. However, we did not find any association between ABCB1 rs414737, ABCB1 rs10276036, ABCC1 rs246240, GSTT1 or GSTM1, and response to chemotherapy.

During the follow-up period, 64 patients (34.41 %) died and 97 patients (52.15 %) showed progression at the end of the study. The median survival time and progression-free survival time were 38.5 months (ranging from 2 to 60 months) and 31.4 months (ranging from 1 to 60 months), respectively.

In the Cox proportional hazards model, after adjusting for potential confounding factors, patients carrying ABCB1 rs1128503 TT genotype and T allele were associated with lower risk of PFS and OS when compared with CC genotype (Table 3; Fig. 1). Moreover, we found that those carrying ABCC3 rs4148416 TT genotype and T allele and GSTP1 rs1695 GG genotype and G allele were correlated with higher risk of PFS and OS when compared with homozygote of the most frequent genotype (Table 3; Figs. 2 and 3). However, we did not find significant association between polymorphisms in ABCB1 rs414737, ABCB1 rs10276036, ABCC1 rs246240, GSTT1 and GSTM1, and risk of PFS and OS of osteosarcoma patients.

Discussion

It is generally known that traditional selection of chemotherapy regimen could improve response and survival of cancer patients. Personalized chemotherapy according to molecular biomarkers could further augment response rates and improve survival of osteosarcoma. Our study assessed the association between eight single nucleotide polymorphisms (SNPs) of GSTs and ATP-binding cassette and response to chemotherapy and PFS and OS of osteosarcoma patients. Our study suggests that polymorphisms

Table 2 Correlation between eight polymorphisms and tumor response

Genotype		Patients	Percent	Tumor response				OR (95 % CI)[a]	*P* value
				Good	%	Poor	%		
ABCB1 rs1128503	CC	81	43.5	32	32.65	42	47.73	1.0 (Ref.)	–
	CT	65	35.1	37	37.76	31	35.23	1.57 (0.77–3.21)	0.18
	TT	40	21.4	29	29.59	15	17.05	2.54 (1.10–5.96)	0.02
Allele	C	227	61.05	101	51.53	115	65.34	1.0 (Ref.)	–
	T	145	38.95	95	48.47	61	34.66	1.77 (1.14–2.75)	0.007
ABCB1 rs414737	AA	68	36.5	39	39.80	31	35.23	1.0 (Ref.)	
	AG	97	52.3	49	50.00	47	53.41	0.83 (0.43–1.61)	0.55
	GG	21	11.2	10	10.20	10	11.36	0.79 (0.26–2.44)	0.65
Allele	A	233	62.65	127	64.80	109	61.93	1.0 (Ref.)	–
	G	139	37.35	69	35.20	67	38.07	0.88 (0.57–1.38)	0.57
ABCB1 rs10276036	TT	58	31.2	35	35.71	25	28.41	1.0 (Ref.)	
	TC	116	62.3	58	59.18	55	62.50	0.75 (0.38–1.48)	0.38
	CC	12	6.5	5	5.10	8	9.09	0.45 (0.10–1.78)	0.19
Allele	T	232	62.35	128	65.31	105	59.66	1.0 (Ref.)	–
	C	140	37.65	68	34.69	71	40.34	0.79 (0.50–1.22)	0.26
ABCC1 rs246240	AA	116	62.1	65	66.33	54	61.36	1.0 (Ref.)	–
	AG	42	22.7	21	21.43	21	23.86	0.83 (0.39–1.79)	0.61
	GG	28	15.2	12	12.24	13	14.77	0.77 (0.29–2.00)	0.55
Allele	A	274	73.45	151	77.04	129	73.30	1.0 (Ref.)	–
	G	98	26.55	45	22.96	47	26.70	0.82 (0.50–1.35)	0.40
ABCC3 rs4148416	CC	118	63.4	65	66.33	45	51.14	1.0 (Ref.)	–
	CT	39	21.2	18	18.37	19	21.59	0.66 (0.29–1.48)	0.27
	TT	29	15.4	15	15.31	24	27.27	0.43 (0.19–0.97)	0.03
Allele	C	275	74	148	75.51	109	61.93	1.0 (Ref.)	–
	T	97	26	48	24.49	67	38.07	0.53 (0.33–0.84)	0.005
GSTP1 rs1695	AA	76	40.6	48	48.86	30	33.67	1.0 (Ref.)	–
	AG	72	38.8	37	37.50	36	40.82	0.64 (0.32–1.29)	0.18
	GG	38	20.6	13	13.64	22	25.51	0.82 (0.35–1.91)	0.63
Allele	A	224	60	133	67.61	96	54.08	1.0 (Ref.)	–
	G	148	40	63	32.39	80	45.92	0.57 (0.36–0.88)	0.008
GSTT1	Present	88	47.5	48	48.86	40	44.90	1.0 (Ref.)	–
	Null	98	52.5	50	51.14	48	55.10	0.87 (0.47–1.61)	0.63
GSTM1	Present	106	57.2	59	60.23	48	55.10	1.0 (Ref.)	–
	Null	80	42.8	39	39.77	40	44.90	0.79 (0.42–1.48)	0.44

[a] Adjusted for sex, age, tumor location, and subtypes

of ABCB1 rs1128503, ABCC3 rs4148416, and GSTP1 rs1695 can influence the response to chemotherapy and are associated with PFS and OS of osteosarcoma patients. The three variants could be useful as prognostic markers in osteosarcoma patients and help in the design of individualized therapy.

ABCB1 (P-glycoprotein, multidrug resistance 1) is expressed both in normal human tissues and multidrug-resistant cancer cells, and this protein contributes to the absorption and distribution of xenobiotics, toxins, and drugs. It is reported that ABCB1 acts as an energy-dependent drug efflux pump for chemotherapeutic drugs, including platinum-based chemotherapy drugs [16]. ABCB1 eliminates the parent drug through hepatobiliary and intestinal secretion and plays an important role in the proliferation and survival of epithelial cells and malignant cells during tumorigenesis [16, 17]. It is well known that there are more than 50 SNPs in the ABCB1 gene, and some SNPs are involving in altered expression and transporter activity of P-glycoprotein (P-gp) [16, 17]. The SNPs can increase the efflux of chemotherapeutic

Table 3 Cox regression analysis of genes and polymorphisms with PFS and OS of osteosarcoma patients

Genotype		Patients	Events		PFS[a]		Events		OS[a]	
			n	%	HR (95 % CI)[a]	*P* value	*n*	%	HR (95 % CI)[a]	*P* value
ABCB1 rs1128503	CC	81	51	52.58	1.0 (Ref.)	–	34	52.31	1.0 (Ref.)	–
	CT	65	32	32.99	0.57 (0.28–1.17)	0.1	21	32.31	0.66 (0.31–1.38)	0.23
	TT	40	14	14.43	0.32 (0.13–0.75)	0.004	9	15.38	0.40 (0.15–1.01)	0.04
Allele	C	227	134	69.07	1.0 (Ref.)	–	90	68.46	1.0 (Ref.)	–
	T	145	60	30.93	0.49 (0.31–0.76)	<0.001	38	63.08	0.54 (0.33–0.87)	0.008
ABCB1 rs414737	AA	68	33	34.02	1.0 (Ref.)	–	21	32.81	1.0 (Ref.)	–
	AG	97	51	52.58	1.18 (0.60–2.29)	0.61	34	53.13	1.21 (0.59–2.48)	0.58
	GG	21	13	13.4	1.72 (0.57–5.43)	0.28	9	14.06	1.68 (0.53–5.12)	0.31
Allele	A	233	117	120.62	1.0 (Ref.)	–	76	118.75	1.0 (Ref.)	–
	G	139	77	79.38	1.47 (0.93–2.33)	0.08	52	81.25	1.23 (0.78–1.96)	0.35
ABCB1 rs10276036	TT	58	28	28.87	1.0 (Ref.)	–	18	28.13	1.0 (Ref.)	–
	TC	116	61	62.89	1.19 (0.60–2.35)	0.59	41	64.06	1.21 (0.59–2.55)	0.57
	CC	12	8	8.25	2.14 (0.50–10.72)	0.25	5	7.81	1.59 (0.35–6.70)	0.48
Allele	T	232	117	120.62	1.0 (Ref.)	–	76	120.31	1.0 (Ref.)	–
	C	140	78	79.38	1.24 (0.79–1.93)	0.32	52	79.69	1.21 (0.76–1.92)	0.39
ABCC1 rs246240	AA	116	56	57.73	1.0 (Ref.)	–	37	57.81	1.0 (Ref.)	–
	AG	42	23	23.71	1.30 (0.60–2.81)	0.47	15	23.44	1.19 (0.52–2.63)	0.65
	GG	28	18	18.56	1.93 (0.76–5.08)	0.13	12	18.75	1.60 (0.62–4.02)	0.27
Allele	A	274	136	139.18	1.0 (Ref.)	–	89	139.06	1.0 (Ref.)	–
	G	98	58	60.82	1.47 (0.90–2.42)	0.1	39	60.94	1.37 (0.83–2.27)	0.19
ABCC3 rs4148416	CC	118	52	53.61	1.0 (Ref.)	–	34	53.13	1.0 (Ref.)	–
	CT	39	23	23.71	1.82 (0.82–4.09)	0.11	15	23.44	1.54 (0.67–3.50)	0.26
	TT	29	22	22.68	3.99 (1.49–11.83)	0.002	14	23.44	2.31 (0.92–5.73)	0.05
Allele	C	275	127	130.93	1.0 (Ref.)	–	84	129.69	1.0 (Ref.)	–
	T	97	68	69.07	2.73 (1.62–4.66)	<0.001	44	70.31	1.89 (1.14–3.12)	0.008
GSTP1 rs1695	AA	76	30	30.93	1.0 (Ref.)	–	20	31.25	1.0 (Ref.)	–
	AG	72	39	40.21	1.81 (0.90–3.67)	0.07	26	40.63	1.58 (0.74–3.40)	0.2
	GG	38	28	28.87	4.29 (1.70–11.28)	<0.001	19	28.13	2.8 (1.14–6.85)	0.01
Allele	A	224	100	102.06	1.0 (Ref.)	–	65	103.13	1.0 (Ref.)	–
	G	148	94	97.94	2.16 (1.38–3.38)	<0.001	63	96.88	1.81 (1.15–2.87)	0.007
GSTT1	Present	88	44	45.36	1.0 (Ref.)	–	28	43.75	1.0 (Ref.)	–
	Null	98	53	54.64	1.18 (0.64–2.18)	0.58	36	56.25	1.24 (0.65–2.40)	0.48
GSTM1	Present	106	53	54.64	1.0 (Ref.)	–	35	54.69	1.0 (Ref.)	–
	Null	80	44	45.36	1.22 (0.66–2.29)	0.5	29	45.31	1.15 (0.60–2.22)	0.65

[a] Adjusted for sex, age, tumor location, and subtypes

agents from tumor cells and their elimination from the body, reduce the plasma concentrations, and thus can have effect on therapeutic efficacy of cancer patients.

In our study, we found that ABCB1 polymorphism was associated with poor response to chemotherapy and better PFS and OS of osteosarcoma patients. Our study is in line with previous studies on prognosis of osteosarcoma patients [12, 18]. A recent study conducted in Spain reported that three SNPs of ABCB1 (rs4148737, rs128503, and rs10276036) may affect osteosarcoma treatment efficacy, and these variants could be used as genetic predictors of clinical outcome in treatment of osteosarcoma [12]. However, two studies reported inconsistent results with ours [11, 19]. Yang et al. reported that ABCB1 rs128503 was significantly associated with shorter DFS and OS [11]. Windsor et al. did not find that ABCB1 rs128503 variant can influence toxicity and clinical outcome of osteosarcoma patients [19]. The discrepancy of the results may be explained by the differences in ethnicities, source of cases, sample size, and also by chance.

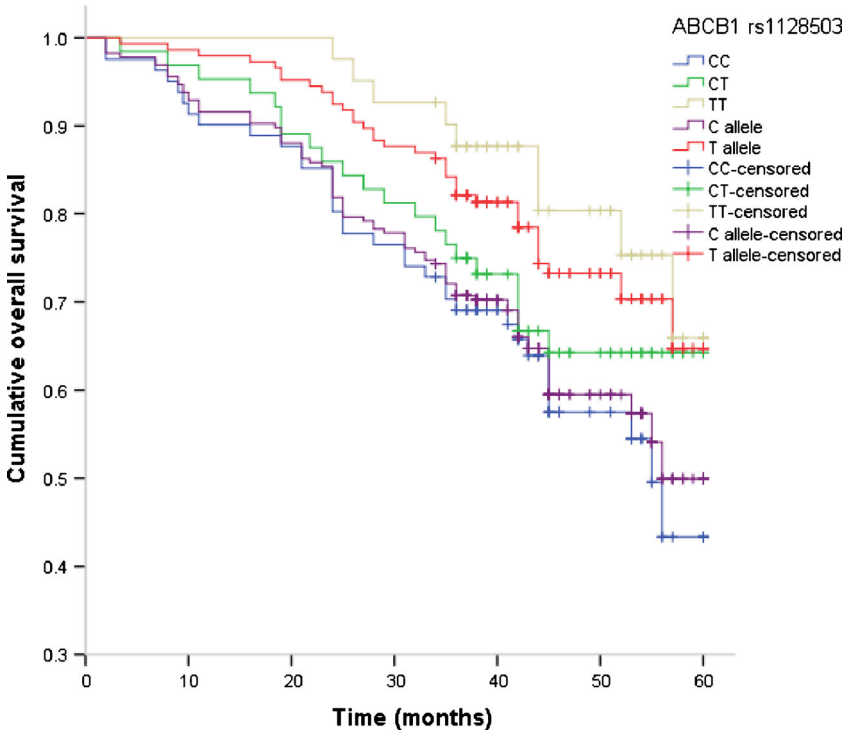

Fig. 1 Kaplan-Meier survival curves for osteosarcoma patients for ABCB1 rs1128503

ABCC3 is a member of the multidrug resistance protein family and is expressed in liver, gallbladder, kidney, and gut [20, 21]. The main substrates of ABCC3 are bile salts, but it has a role in transporting anticancer drugs [22]. It is reported that the expression of ABCC3 mRNA is associated with drug resistance, and SNPs of ABCC3 can influence the expression and therapeutic efficacy. Only two studies reported the association between ABCC3 rs4148416 and osteosarcoma survival after chemotherapy [11, 12], which is inconsistent with our results.

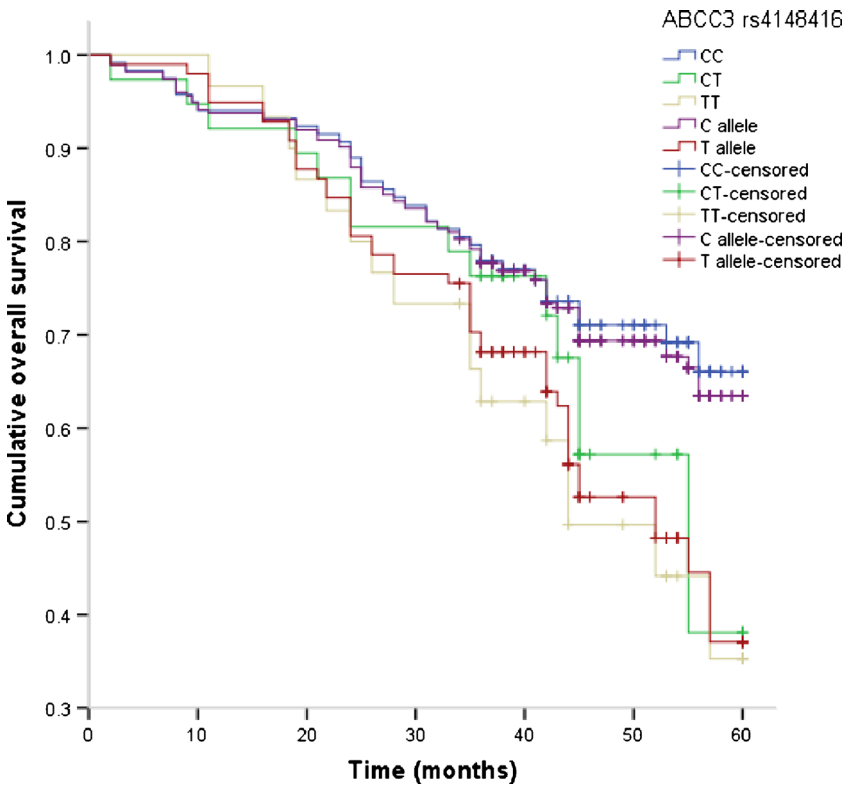

Fig. 2 Kaplan-Meier survival curves for osteosarcoma patients for ABCC3 rs4148416

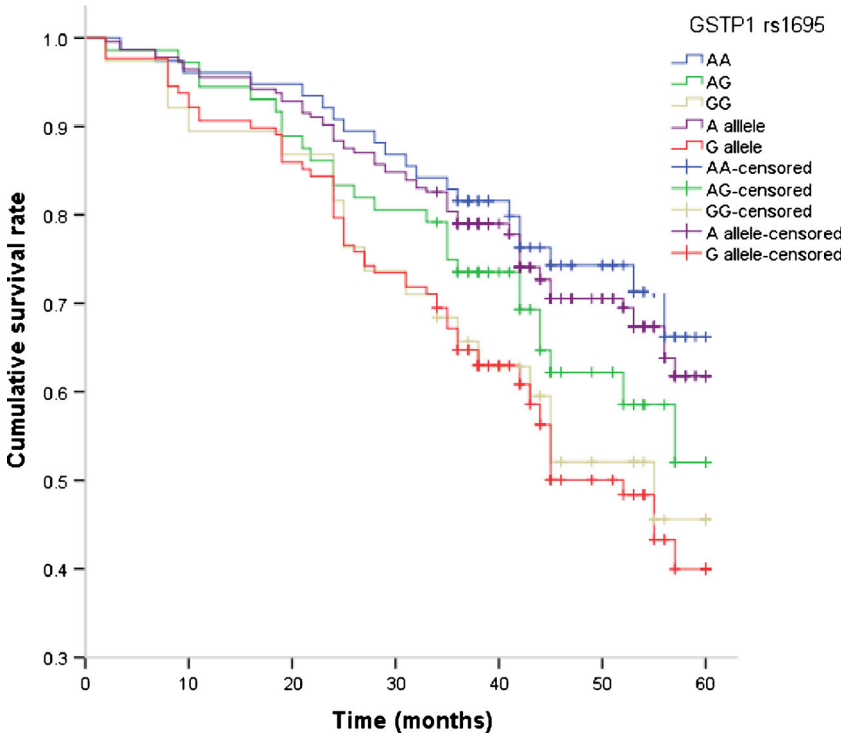

Fig. 3 Kaplan-Meier survival curves for osteosarcoma patients for GSTP1 rs1695

Glutathione S-transferases (GSTs) are a family of cytosolic enzymes, and they play an important role in catalyzing detoxifying endogenous reactions with GSH and protect cellular macromolecules from damage caused by a wide variety of endogenous and exogenous molecules, including cytotoxic, mutagens, carcinogens, and chemotherapeutic agents [23, 24]. Previous studies reported a significant association between high GSTP1 expression of tumor cells and reduced sensitivity to chemotherapy [25, 26]. Previous studies reported an effective role of GSTP1 rs1695 in clinical outcome of osteosarcoma patients [13, 18, 27], which was in line with our results. Our study suggests that genetic variation of GSTP1 rs1695 could be used as a prognostic factor to identify osteosarcoma patients who might benefit from chemotherapy.

There were several limitations in our study. First, cases were selected from one hospital, which may not be representative of the general population. Second, other genetic polymorphisms may influence the prognosis of osteosarcoma besides the ATP and GSTs proteins. Third, the sample size of our study is relatively small, which may reduce the statistical power to find the difference between groups. Therefore, further large sample, multicenter studies including different ethnicities are warranted to investigate the role of GSTs and ATP-binding cassette genes on the prognosis of osteosarcoma.

In conclusion, our study found that variants of ABCB1 rs128503, ABCC3 rs4148416, and GSTP1 rs1695 are associated with response to chemotherapy and PFS and OS of osteosarcoma patients. Our study suggests that ABCB1 rs128503, ABCC3 rs4148416, and GSTP1 rs1695 may be used as potential prognostic biomarkers for osteosarcoma, which could help in the design of individualized therapy.

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