RESEARCH ARTICLE

Quantitative Assessment of the Association between ABC Polymorphisms and Osteosarcoma Response: a Meta-analysis

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Abstract

Background: ABC proteins are one key type of transport su¬perfamilies which undertake majority of drug transport, which affect the osteosarcoma response to chemotherapeutics. Previous studies have suggested the association between ABC polymorphisms and osteosarcoma response. However, the results of previous studies remain controversial. Therefore, we perform a meta-analysis to get a more precise estimation of this association. The association between ABC polymorphisms and osteosarcoma response was assessed by odds ratios (ORs) together with their 95% confidence intervals (CIs). Three polymorphisms of ABC including ABCB1 rs1128503, ABCC3 rs4148416 and ABCC2 rs717620 polymorphism were investigated. Overall, significant association was observed between ABCC3 rs4148416 polymorphism and osteosarcoma response under allele contrast (T *vs.* C: OR=1.73, 95%CI=1.09-2.74, P=0.019), homozygote comparison (TT *vs.* CC: OR=2.00, 95%CI=1.25-3.23, *P*=0.004), recessive genetic model (TT vs. TC/CC: OR=1.80, 95%CI=1.14-2.84, *P*=0.011) and dominant genetic model (TT/TC *vs.* CC: OR=1.70, 95%CI=1.20-2.42, *P*=0.003). Moreover, significant association was also observed in Caucasian population rather than Asian population for ABCB1 rs1128503 polymorphism. We conclude that ABCC3 rs4148416 polymorphism was significantly associated with poor osteosarcoma response and ABCB1 rs1128503 polymorphism was significantly associated with good osteosarcoma response in Caucasian population rather than Asian population.

Keywords: ABC - polymorphism - osteosarcoma - tumor response - meta-analysis

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Introduction

Osteosarcoma, as one of the most common type of bone tumor in the world, is the leading cause of death in children less than fifteen years old (Ottaviani and Jaffe, 2009, He et al., 2014, Li et al., 2014). For the moment, the treatment of osteosarcoma consists of surgical operation and followed by chemotherapy. The main chemotherapy drugs include methotrexate, cisplatin, cyclophospamide, vincristine and adriamycin. However, the above treatment for osteosarcoma could not solve all the problems because about thirty percent of patients showed recurrence or metastasis within five years (Ottaviani and Jaffe, 2009).

Based on biomarkers, individualized chemotherapy may improve the response to chemotherapy and clinical outcome of patients. So that getting a comprehensive knowledge of pharmacogenetics plays an important role in establishing a personal chemotherapy, which will tremendously benefit the patients of osteosarcoma.

Drug absorption, distribution, metabolism and excretion are controlled by specific genes which affect the clinical response to chemotherapeutics. ABC proteins are one key type of transport superfamilies which undertake majority of drug transport (Zhou et al., 2008). The genetic polymorphism of the drugs metabolized and transport genes which encodes for ABC proteins will vastly influence clinical response to chemotherapeutics. Of these polymorphisms, ABCB1 rs1128503, ABCC3 rs4148416 and ABCC2 rs717620 polymorphism have been mostly investigated. However, previous results of the relationship between these polymorphisms and osteosarcoma response were controversial or inconsistent. The phenomenon can be caused by many reasons including different population background, small sample size or study design. Therefore, in the present study, we perform this meta-analysis on all published case-control studies to derive a more precise estimation of these polymorphisms with osteosarcoma response.

Materials and Methods

Search strategy

PubMed and CNKI (China National Knowledge Infrastructure) databases were searched using the terms as follows: ("*ABCB1*" or "*ABCC1*" or "*ABCC2*") in combination with ("*polymorphism*" or "*variant*" or "*mutation*") and in combination with "osteosarcoma" updated on May 2015 for all publications on the association

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between ABC polymorphisms and osteosarcoma response. In order to identify the relevant studies, additional literatures were identified through scanning the references of original studies. Review articles were also examined to find other eligible studies.

Inclusion and exclusion criteria

The following were inclusion criteria for the literature selection: (a) a case-control study; (b) evaluation of association between ABC polymorphisms and osteosarcoma response; (c) providing distribution of alleles, genotypes or other information to calculate ORs and 95% CIs. Accordingly, studies were excluded if one of the following exclusion criteria existed: (a) studies that contained overlapping data; (b) Not offering the distribution of alleles, genotypes or other necessary information; (c) studies in which family members had been investigated because of linkage disequilibrium.

Table 1. General Characteristics of Studies Included in the Meta-Analysis

First Author	Year	Country	Ethnicity	Genotyping Methods	Sample size	Investigated SNP
Windsor	2011	United Kingdom	Caucasian	Microarray	58	rs1128503
Caronia	2011	Spain	Caucasian	Microarray	90	rs1128503, rs717620
Yang	2013	China	Asian	MassARRAY	208	rs1128503, rs4148416, rs717620
Li	2014	China	Asian	MassARRAY	162	rs1128503
Liu	2014	China	Asian	MassARRAY	186	rs1128503, rs4148416
Sun	2014	China	Asian	MassARRAY	182	rs1128503, rs4148416
Wei	2006	China	Asian	MassARRAY	100	rs1128503

Table 2. Meta-analysis of the ABC polymorphisms with osteosarcoma response

Comparison	Ethnicity	N	Test of association			Mode	Test of heterogeneity		
			OR	95%CI	P		χ^2	P	I^2
rs1128503									
T vs. C	Overall	7	1.18	0.72-1.96	0.509	R	45.68	0	86.9
	Caucasian	2	0.58	0.40-0.85	0.005	F	0.33	0.567	0
	Asian	5	1.57	0.90-2.72	0.111	R	28.69	0	86.1
TT vs. CC	Overall	7	1.15	0.48-2.75	0.751	R	34.15	0.022	47.3
	Caucasian	2	0.24	0.10-0.61	0.003	F	0.14	0.705	0
	Asian	5	1.92	0.81-4.53	0.137	R	19.34	0	82.4
TC vs. CC	Overall	7	1.08	0.65-1.81	0.761	R	19.07	0.004	68.5
	Caucasian	2	0.44	0.23-0.82	0.009	F	0.36	0.546	0
	Asian	5	1.41	0.87-2.27	0.16	R	8.84	0.065	54.8
TT vs. TC/CC	Overall	7	1.1	0.54-2.21	0.796	R	25.81	0	76.8
	Caucasian	2	0.29	0.12-0.70	0.006	F	0.15	0.701	0
	Asian	5	1.64	0.84-3.21	0.149	R	13.86	0.008	71.1
TT / TC vs. CC	Overall	7	1.22	0.71-2.12	0.475	R	27.58	0	78.2
	Caucasian	2	0.58	0.34-0.98	0.044	F	0.12	0.73	0
	Asian	5	1.61	0.87-2.97	0.127	R	18.13	0.001	77.9
rs4148416									
T vs. C	Overall	3	1.73	1.09-2.74	0.019	R	5.63	0.06	64.5
TT vs. CC	Overall	3	2	1.25-3.23	0.004	F	2.96	0.227	32.5
TC vs. CC	Overall	3	1.45	0.95-2.21	0.084	F	0.83	0.661	0
TT vs. TC/CC	Overall	3	1.8	1.14-2.84	0.011	F	2.99	0.224	33.2
TT / TC vs. CC	Overall	3	1.7	1.20-2.42	0.003	F	2.61	0.271	23.5
rs717620									
T vs. C	Overall	2	0.37	0.05-2.50	0.307	R	24.92	0	96
TT vs. CC	Overall	2	0.87	0.44-1.33	0.255	R	16.88	0.004	85.7
TC vs. CC	Overall	2	1.44	0.87-3.27	0.096	R	8.6	0.078	45.9
TT vs. TC/CC	Overall	2	0.45	0.09-2.57	0.477	R	19.88	0.009	78.4
TT / TC vs. CC	Overall	2	0.98	0.37-3.12	0.123	R	16.88	0.006	81.3

Data extraction

All the data were independently reviewed and extracted by two investigators (Xu Chen and Guohao Gu). And the result was reviewed by a third investigator (Min Jiang). From each study, the following information was recorded: first author, year of publication, country of origin, ethnicity of the population; the number of cases and controls, allele frequency, and genotype distribution in cases and controls. Different ethnic descents were categorized as Caucasians, Asian, African or Mixed. For studies comprising subjects of different ethnic populations, data were separately extracted for each independent ethnic group.

Statistical analysis

The odds ratio (OR) and its 95% confidence interval (95%CI) were calculated to assess the association strength between ABC polymorphisms with osteosarcoma response. We evaluated the ABC polymorphisms polymorphism with osteosarcoma response by allele contrast, heterozygote comparison, homozygote comparison, dominant genetic model and recessive genetic model. The heterogeneity between the studies was assessed by the χ^2-test based Q-statistic and I^2 statistics (Higgins and Thompson, 2002). If there was no obvious heterogeneity, the fixed-effects model (the Mantel-Haenszel method) would be used to estimate summary OR(Mantel and Haenszel, 1959). Otherwise, the random-effects model (DerSimonian and Laird method) would be applied (DerSimonian and Laird, 1986). To detect the possible source of heterogeneity, we did logistic meta-regression analyses. We examined the sources of heterogeneity including publication year, genotyping method and sample size.

Sensitivity analysis was performed to assess the stability of the results and identify potentially influential studies. Any single study from the meta-analysis was deleted each time to calculate the influence of the individual data set to the pooled OR. Funnel plots and Egger's linear regression test were applied to detect the potential publication bias (Begg and Mazumdar, 1994). An asymmetric plot infers a possible publication bias. The significance of the intercept was determined by the Student t test suggested by Egger (P<0.05 was considered representative of statistically significant publication bias). All analyses were conducted using Stata software (version 12.0; StataCorp LP, College Station, TX, USA).

Results

Eligible studies

A flow diagram of the search process is shown in Figure 1. Based on the predefined search strategy and inclusion criteria, a total of 7 studies were finally included for our meta-analysis (Wei et al., 2006, Caronia et al., 2011, Windsor et al., 2012, Yang et al., 2013, Li et al., 2014, Liu et al., 2014, Xiaohui et al., 2014). 5studies focus on ABCB1 rs1128503 polymorphism, while 3studies focus on ABCC3 rs4148416 polymorphism and 2studies focus on ABCC2 rs717620 polymorphism. Of all studies, 5 studies investigate the East Asian population and 2 studies investigate the Caucasian population. Several genotyping methods were used in the eligible studies such as Microarray, MassARRAY. The main characteristics of all the case-control studies included in our meta-analysis were listed in Table 1.

Quantitative synthesis of data

Summary results of this meta-analysis for the association between ABC polymorphisms and osteosarcoma response are shown in Table 2. Overall, significant association was observed between ABCC3 rs4148416 polymorphism and osteosarcoma response under allele contrast (T *vs*. C: OR=1.73, 95%CI=1.09-2.74, P=0.019), homozygote comparison (TT *vs*. CC: OR=2.00, 95%CI=1.25-3.23, P=0.004), recessive genetic model (TT *vs*. TC/CC: OR=1.80, 95%CI=1.14-2.84, P=0.011) and dominant genetic model (TT/TC *vs*. CC: OR=1.70, 95%CI=1.20-2.42, P=0.003). In the stratified analysis by ethnicity, significantly decreased risk was found among Caucasians under allele contrast (T *vs*. C: OR=0.58, 95%CI=0.40-0.85, P=0.005, Figure 2), homozygote comparison (TT *vs*. CC: OR=0.24, 95%CI=0.10-0.61, P=0.003), heterozygote comparison (TC *vs*. CC: OR=0.44, 95%CI=0.23-0.82, P=0.009), recessive genetic model (TT *vs*. TC/CC: OR=0.29, 95%CI=0.12-0.70, P=0.006) and dominant genetic model (TT/TC *vs*. CC: OR=0.58, 95%CI=0.34-0.98, P=0.044). Forest plots of ABCB1 rs1128503 polymorphism and ABCC3 rs4148416polymorphism with osteosarcoma response were shown in Figure 2 and Figure 3.

Test of heterogeneity

There was significant heterogeneity in ABCB1 rs1128503 polymorphism with osteosarcoma response. To explore the sources of heterogeneity, we evaluated allele contrast by considering possible sources including publication year, genotyping methods and sample size. We found that ethnicity (χ^2=4.39; df=1; P=0; I^2=93.8%) could substantially influence the initial heterogeneity. Additionally, meta-regression analyses revealed the sources of heterogeneity. The sample size could explain almost 80% of I^2 in meta-analysis.

Sensitivity analysis

Sensitivity analysis was performed by sequential omission of individual studies. In the present meta-

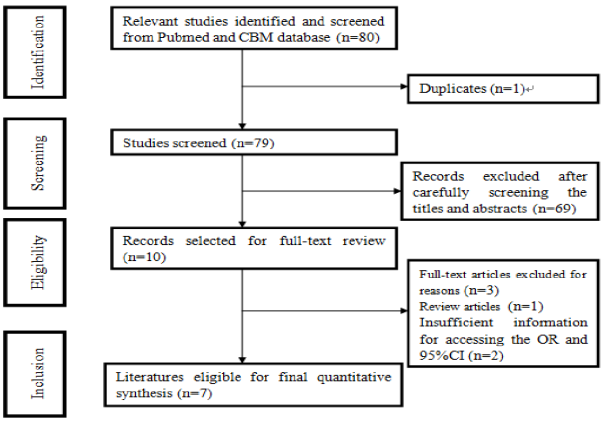

Figure 1. Flow diagram for Identification of Eligible Studies for this Meta-Analysis

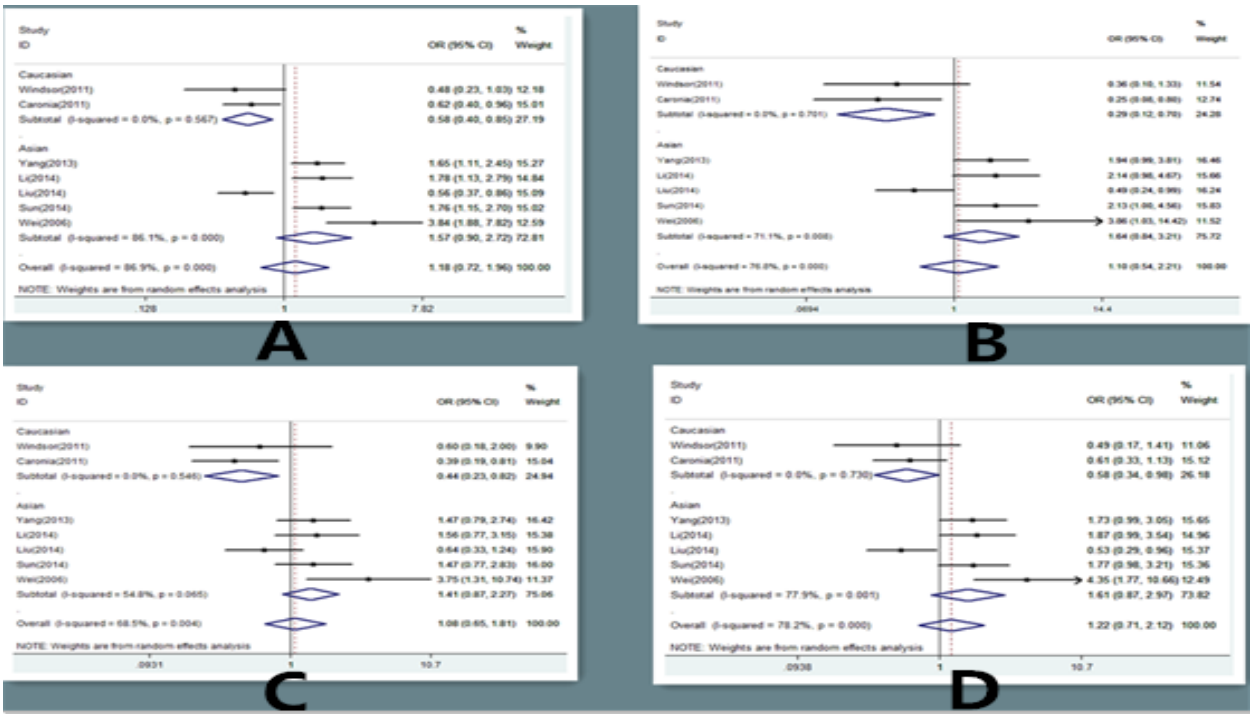

Figure 2. Forest Plot of ABCB1 rs1128503 Polymorphism with Osteosarcoma Response. (A: allele contrast T *vs*. C; B: homozygote comparison TT *vs*. CC; C: recessive genetic model TT *vs*. TC/CC; D: dominant genetic model TT/TC *vs*. CC)

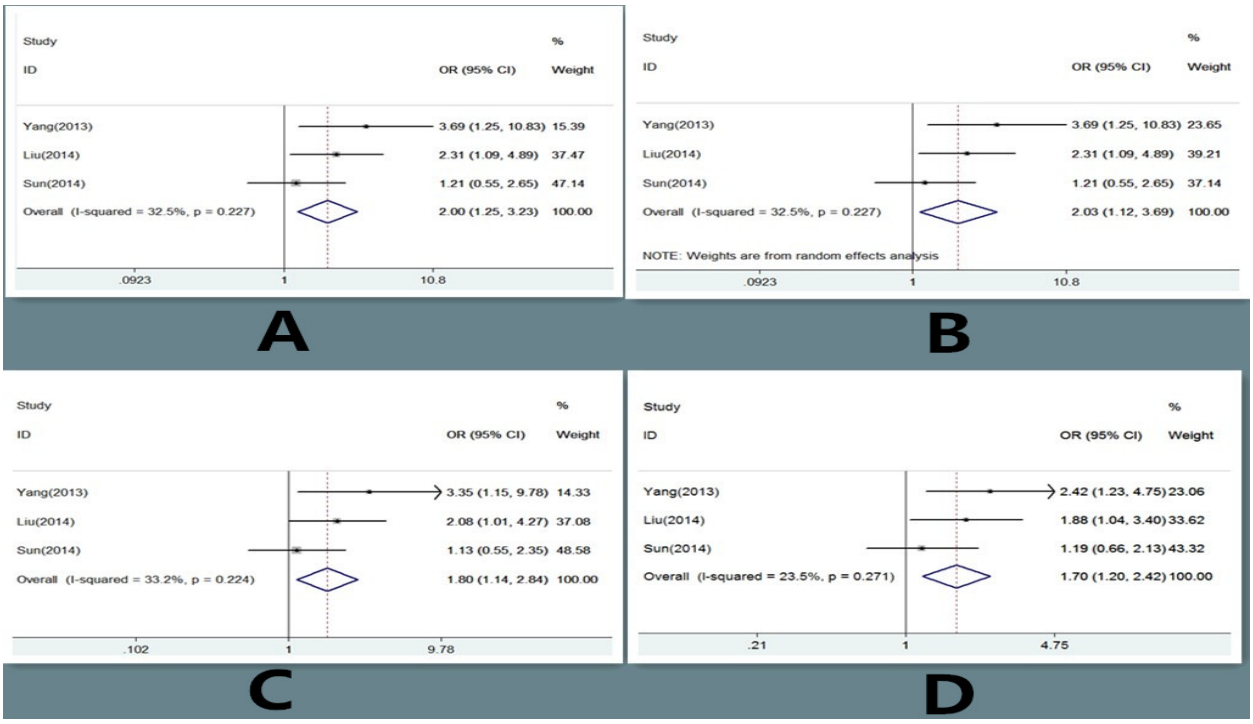

Figure 3. Funnel Plot of ABCC3 rs4148416 Polymorphism with Osteosarcoma Response. (A: allele contrast T *vs*. C; B: homozygote comparison TT *vs*. CC; C: recessive genetic model TT *vs*. TC/CC; D: dominant genetic model TT/TC *vs*. CC)

analysis, any single study could not influence the overall results qualitatively, indicating robustness and reliability of our results.

Publication bias

Begg funnel plot was created to assess the publication bias of selected literatures. The shapes of the funnel plots did not show any evidence of obvious asymmetry. Then, the Egger test was used to provide statistical evidence of funnel plot symmetry. The results also suggested the absence of publication bias (data not shown).

Discussion

Nowadays the treatment and prognosis of osteosarcoma has been improved to a certain degree. However, about 50% patients show a poor tumor response to chemotherapy and only 40%-50% estimated overall survival rate has

been reached (Bielack et al., 2002). The tumor response to chemotherapy drugs can be affected by various factors, including genetic factors and environment factors. The therapeutic range should be in a proper concentration when conducting anticancer therapies. But it is not easy for clinical physicians to select a proper concentration. Because a higher concentration of chemotherapy drugs brings toxicity and a lower concentration reduce the efficacy of drugs. Furthermore, the difference between individuals plays an important role in tumor response to chemotherapy drugs. As I said before, specific genes can influence and control drug absorption, distribution, metabolism and excretion. For example, Cytochrome P450 (CYP) enzymes have an impact on many metabolism reactions (Redlich et al., 2008). And Glutathione S-transferases (GSTs) participate in metabolizing for chemotherapeutic agents(Hayes and Pulford, 1995). ABC proteins are one key type of transport superfamilies which undertake majority of drug transport. The genetic polymorphism of the drugs metabolized and transport genes which encodes for ABC proteins will vastly influence clinical response to chemotherapeutics. Of these polymorphisms, ABCB1 rs1128503, ABCC3 rs4148416 and ABCC2 rs717620 polymorphism have been mostly investigated. However, previous results of the relationship between these polymorphisms and osteosarcoma response were controversial or inconsistent. Therefore, it is necessary to explore the relationship between ABC polymorphisms and osteosarcoma response.

To the best of our knowledge, this is the first meta-analysis which investigates the relationship between ABC polymorphisms and osteosarcoma response. Overall, we found that ABCC3 rs4148416 polymorphism was significantly associated with poor osteosarcoma response. In the stratified analysis by ethnicity, ABCB1 rs1128503 polymorphism was significantly associated with good osteosarcoma response in Caucasian population rather than Asian population. It is very common for this phenomenon to happen. Different ethniticities have different genetic polymorphism of specific genes. The population of United Kingdom and Spain belongs to Caucasians and the people of China are part of Asians.

The heterogeneity is an important factor when performing meta-analysis. Finding the sources of heterogeneity and solving the heterogeneity is of significance to our results. In our study, we found that significant heterogeneity existed in in ABCB1 rs1128503 polymorphism with osteosarcoma response. So that we calculated the overall population by method of random effect model. Then, we conducted meta-regression and subgroup analysis. Sensitive analysis demonstrated that any single study could not affect the final result of the present meta-analysis, indicating that our results were stable and reliable. The publication bias is another important factor that may influence the reliability of meta-analysis. We failed to find any evidence to demonstrate the existence of publication bias, suggesting that publication bias have little effect on the results in our study and the results of our meta-analysis are relatively stable.

Although comprehensive meta-analysis was conducted to demonstrate the association between ABC polymorphisms and osteosarcoma response, there are still some limitations that should be pointed out. Firstly, the primary studies included in our meta-analysis mainly investigated Asian population. Since ABC polymorphisms substantially varies across different ethnicities, more primary studies which focused on other ethnicities such as African population, mixed population should be carried out. Secondly, we should be cautious to the results because only seven eligible studies were included in our meta-analysis and the studies of Caucasians consist of only 2 studies. The sample size was relatively small. Thirdly, because of lack of sufficient primary data, hence, subgroup analysis according to age, gender, radiation exposure, and other confounding factors could not been performed in the present meta-analysis.

In spite of the limitations above, our meta-analysis had also several advantages. Firstly, a meta-analysis of the association of ABC polymorphisms with osteosarcoma response is statistically more powerful than any other single study. Secondly, the quality of our eligible studies met our inclusion criteria and was satisfactory, and the sensitivity analysis and publication bias analysis suggested the stability and credibility of the meta-analysis, which leads to a more convincing result. More important, the process of literature selection, data extraction and data analysis in the meta-analysis was well designed and conducted.

In conclusion, this is the first meta-analysis which investigates the association between ABC polymorphisms and osteosarcoma response. We conclude that ABCC3 rs4148416 polymorphism was significantly associated with poor osteosarcoma response and ABCB1 rs1128503 polymorphism was significantly associated with good osteosarcoma response in Caucasian population rather than Asian population. However, more primary large scale and well-design studies are still required to further evaluate the interaction of ABC polymorphisms with osteosarcoma response.

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