Meta-Analysis of Observational Studies in Epidemiology

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Evaluating the role of *GSTP1* genetic polymorphism (rs1695, 313A>G) as a predictor in cyclophosphamide-induced toxicities

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Abstract

The association between Glutathione S-transferase Pi 1(*GSTP1*) genetic polymorphism (rs1695, 313A>G) and cyclophosphamide-induced toxicities has been widely investigated in previous studies, however, the results were inconsistent. This study was performed to further elucidate the association.

A comprehensive search was conducted in PubMed, Embase, Web of Science, China National Knowledge Infrastructure, and Wan Fang database up to January 5, 2020. Risk ratios (RRs) and 95% confidence intervals (95% CIs) were used to estimate the association between *GSTP1* rs1695 polymorphism and cyclophosphamide-induced hemotoxicity, gastrointestinal toxicity, infection, and neurotoxicity.

A total of 13 studies were eventually included. Compared with the *GSTP1* rs1695 AA genotype carriers, patients with AG and GG genotypes had an increased risk of cyclophosphamide-induced gastrointestinal toxicity (RR, 1.61; 95% CI, 1.18–2.19; $P = .003$) and infection (RR, 1.57; 95% CI, 1.00–2.48; $P = .05$) in the overall population. In the subgroup analyses, there were significant associations between *GSTP1* rs1695 polymorphism and the risk of cyclophosphamide-induced myelosuppression (RR, 2.10; 95% CI, 1.60–2.76; $P < .00001$), gastrointestinal toxicity (RR, 1.77; 95%CI, 1.25–2.53; $P = .001$), and infection (RR, 2.01; 95% CI, 1.14–3.54; $P = .02$) in systemic lupus erythematosus (SLE) or lupus nephritis syndrome patients, but not in cancer patients.

Our results confirmed an essential role for the *GSTP1* rs1695 polymorphism in the prediction of cyclophosphamide-induced myelosuppression, gastrointestinal toxicity, and infection in SLE or lupus nephritis syndrome patients. More studies are necessary to validate our findings in the future.

Abbreviations: 95% CI = 95% confidence interval, CNKI = China National Knowledge Infrastructure, *GSTP1* = glutathione S-transferase Pi 1, GSTs = glutathione S-transferases, LN = lupus nephritis, RR = risk ratio, SLE = systemic lupus erythematosus.

Keywords: cyclophosphamide, glutathione S-transferase Pi 1, polymorphism, rs1695, toxicity

1. Introduction

Cyclophosphamide is a widely used alkylating agent and plays an antitumor role by directly alkylating the bases on DNA and preventing cell division,[1] which is effective for malignant tumors such as breast cancer, lymphoma, and leukemia. Meanwhile, cyclophosphamide also acts as an immunosuppressant universally prescribed for patients with systemic lupus erythematosus (SLE) and lupus nephritis (LN).[2] However, it can also damage normal cells while performing pharmacological effects, leading to serious short-term side effects, such as hemotoxicity, gastrointestinal toxicity, infection, neurotoxicity, etc.[3] Severe side effects may be detrimental to the efficacy of cyclophosphamide and the quality of life of patients. The toxicities of cyclophosphamide vary among patients even though the regimen is identical. Previous studies suggested that the metabolic-associated gene polymorphism of cyclophosphamide is one of the reasons for the heterogeneity among patients.

As a prodrug, cyclophosphamide entered into the body is firstly metabolized by cytochrome P450s and activated into 4-hydroxycyclophosphamide.[4] Its tautomer aldophosphamide is then converted into potent alkylating agent phosphoramide mustard and acrolein through nonenzymatic β-elimination, which can play a cytotoxic role.[4,5] The metabolites are detoxified by the glutathione S-transferases (GSTs), forming

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water-soluble complexes and ultimately excreted from the body.[6] Glutathione S-transferase Pi 1 (GSTP1) is one of the members of the GSTs superfamily. *GSTP1* rs1695 (313 A>G, Ile105Val) is a widely studied polymorphism of *GSTP1* in which guanine (G) replaces adenine (A) at 313 bases of exon 5 in the *GSTP1* coding region, leading to the substitution of valine (Val) for isoleucine (Ile) at 105.[7] There were significant differences in GSTP1 enzyme activity among different genotypes.[8]

Specific recommendations for individualized treatment of cyclophosphamide are not available owing to the inconsistent evidence in the existing literatures. For example, there are evidences that the *GSTP1* rs1695 variant G allele reduced grade 3–4 neutropenia as well as leucopenia in breast cancer patients and increased the risk of myelosuppression, gastrointestinal toxicity in SLE patients,[9,10] while the results in several studies found no association between *GSTP1* rs1695 polymorphism and hemotoxicity, gastrointestinal toxicity, neurotoxicity, or infection.[11–13] Therefore, the purpose of this study was to comprehensively evaluate the relationship between *GSTP1* rs1695 polymorphism and the toxicities of cyclophosphamide utilizing meta-analysis.

2. Methods

2.1. Literature search

To investigate the relationship between the polymorphism of *GSTP1* rs1695 and the toxicities of cyclophosphamide, we searched PubMed, Embase, Web of Science, China National Knowledge Infrastructure (CNKI), and Wan Fang database. The search terms mainly included 2 aspects: Cyclophosphamide and *GSTP1* gene ("Glutathione S-Transferase Pi" or "*GSTP*" or "*GSTP1*"). A comprehensive search was conducted by using a combination of mesh words and free words. The publication year was set from the establishment year of the database (PubMed [1900], Embase [1967], Web of Science [1950], CNKI [1915], Wan Fang [1900]) to January 5, 2020. Besides, the references of the identified papers were also manually screened. This meta-analysis does not contain any studies with human participants or animals performed by any of the authors, thus ethical approval and informed consent are not required.

2.2. Selection criteria

Publications were considered for inclusion in this meta-analysis if they conformed to the following criteria: clinical trial study; patients received cyclophosphamide-based treatment; at least one of the toxicities of cyclophosphamide was reported, such as hemotoxicity (leukopenia, neutropenia, thrombocytopenia, anemia, myelosuppression), gastrointestinal toxicity, infection, and neurotoxicity; the frequency or percentage of patients with each *GSTP1* rs1695 genotype was available; the occurrence frequency of various toxicities stratified by genotypes could be obtained or calculated. If any of the above is not applicable, the study was excluded.

2.3. Data extraction

The following items as author's name, publication year, disease type, region, race, sample size, age, the ratio of male to female, drug administration, the total number of carriers of each genotype, and the corresponding number of each grade ≥3 toxic event, study design, study period, genotyping method, etc were extracted from the included literatures.

2.4. Statistical analysis

A meta-analysis was conducted to evaluate the correlation between the *GSTP1* rs1695 polymorphism and the various toxicities of cyclophosphamide. Due to the low frequency of the G allele, the patients were divided into mutant type (AG and GG genotypes) and wild type (AA genotype). All data were analyzed using the Cochrane Review Manager software (version 5.0, the Cochrane Collaboration, Oxford, the United Kingdom). Risk ratios (RRs) and 95% confidence intervals (95% CIs) were calculated, and a bilateral probability value ≤ 0.05 was considered statistically significant. Heterogeneity between different studies was evaluated by calculating the I^2 statistic and the Cochrane Q (chi-squared) statistic.[14] The fixed-effect model (Mantel–Haenszel) was used when $I^2 < 50\%$, otherwise, the random effect model was applied.[15]

3. Results

3.1. Selection and characterization of studies

As observed in Fig. 1, a total of 387 publications were retrieved from the databases, and 260 articles left after removing the duplicates. An additional 118 articles were excluded by initial review of title and abstract (39 reviews, 36 studies mainly focused on animals or cells, 20 pharmacokinetic studies, 17 conference abstracts, 5 case reports, 1 letter). Another 129 articles were excluded by careful review of the full text (93 focused on efficacy or prognosis other than toxicity, 18 without cyclophosphamide-based treatment, 9 without *GSTP1* rs1695 polymorphism, 9 without sufficient data).

A total of 13 studies were eventually included in our study,[8–13,16–22] and the main information and characteristics of the included studies were summarized in Table 1. Overall, more than half of the subjects were Asians. Of the included studies, 3 studies enrolled lymphoma patients, 7 enrolled patients with breast cancer, and 3 with SLE or lupus nephritis syndrome. All studies reported at least one type of toxicity (13 studies of participants with hemotoxicity, 3 studies with gastrointestinal toxicity, 3 studies with infections, and 2 studies with neurotoxicity).

3.2. GSTP1 rs1695 polymorphism and hemotoxicity

Among the included publications, there were 8, 9, 5, and 4 studies with the relationship between *GSTP1* rs1695 polymorphism and the risk of leukopenia, neutropenia, thrombocytopenia as well as anemia, respectively. And only Thu et al's[18] study involved non-cancer patients. The incidence rate of leukopenia, neutropenia, thrombocytopenia, and anemia for carries of AA genotype was 38.0%, 47.7%, 18.4%, 28.2%, while that for AG and GG genotypes was 37.5%, 46.2%, 12.9%, 32.2%, respectively. No significant heterogeneity across the studies was found ($I^2 = 44\%$ and $P_{\text{heterogeneity}} = .08$ for leukopenia, $I^2 = 2\%$ and $P_{\text{heterogeneity}} = .42$ for neutropenia, $I^2 = 0\%$ and $P_{\text{heterogeneity}} = .93$ for thrombocytopenia and $I^2 = 0\%$ and $P_{\text{heterogeneity}} = .71$ for anemia), so the pooled results were calculated by a fixed-effect model. The *GSTP1* rs1695 polymorphism was not associated with grade 3–4 hematological toxicities, including leukopenia (RR, 0.94; 95% CI, 0.84–1.05; $P = .27$, Fig. 2A), neutropenia (RR, 0.95; 95% CI, 0.87–1.04; $P = .30$, Fig. 2B), thrombocytopenia (RR, 1.02; 95% CI, 0.83–1.25; $P = .86$, Fig. 2C) and anemia (RR, 1.11; 95% CI, 0.87–1.41; $P = .40$, Fig. 2D).

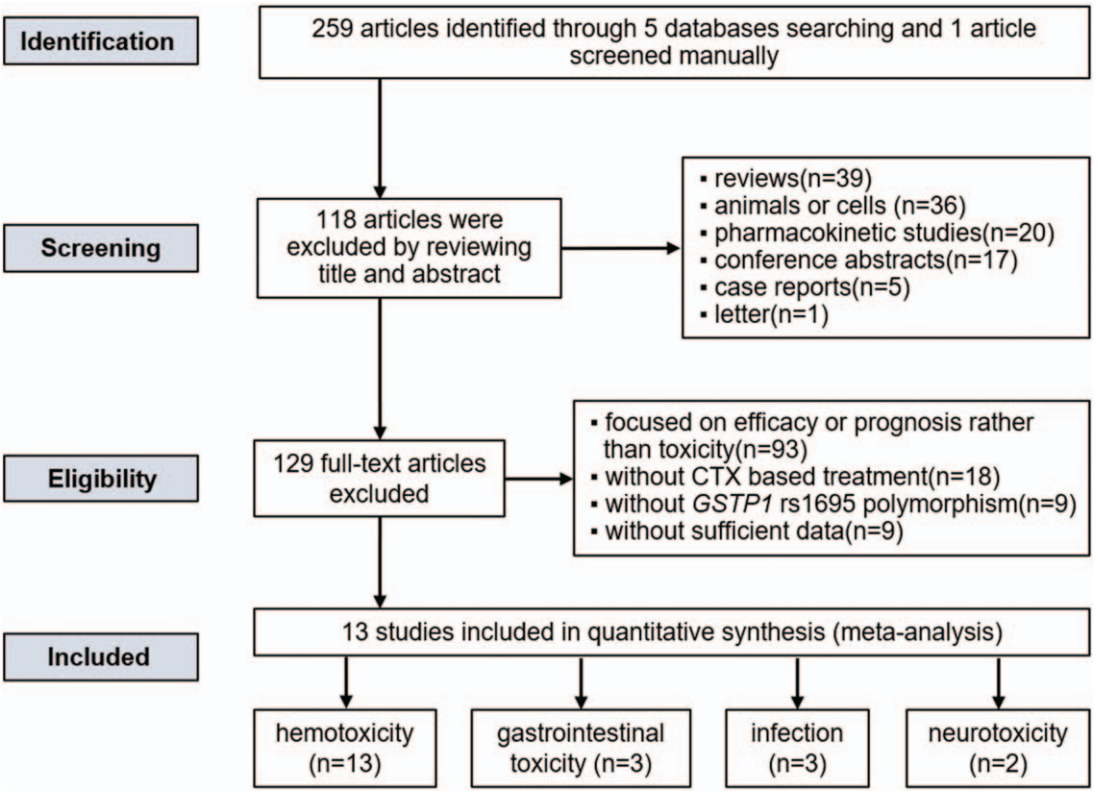

Figure 1. Flow diagram of literature selection. CTX = cyclophosphamide, GSTP1 = glutathione S-transferase Pi 1.

Table 1

The characteristics of the 13 eligible studies.

Study or subgroup	Country	Disease	Sample	Treatment	Genotyping	Toxic type	Genotype frequency	Reference
Sugishita 2016	Japan	BC	102	FEC/EC	TaqMan	Neutropenia	AA (70/68.6%); AG (26/25.5%); GG (6/5.9%)	[8]
Zhong 2006	China	SLE	102	CTX	PCR-RFLP	Myelosuppression; GI; infection	AA (64/62.7%); AG and GG (38/37.3%)	[9]
Yao 2010	North America	BC	405	FAC or CMF ± TAM	MALDI-TOF-MS	Leukopenia; neutropenia; myelosuppression	AA (190/47%); AG (174/43%); GG (41/10%)	[10]
Islam 2015	Bangladesh	BC	256	FEC	PCR-RFLP	Leukopenia; neutropenia; thrombocytopenia; anemia; GI	AA (131/51.2%); AG (98/38.3%); GG (27/10.5%)	[11]
Cho 2010	Korea	DLBCL	90	R-CHOP	PCR	Leukopenia; neutropenia; thrombocytopenia; anemia; GI; infection; neurotoxicity	AA (53/59%); AG and GG (37/41%)	[12]
Ludovini 2017	Italy	BC	242	CMF/FAC	PCR-RFLP	Leukopenia; neutropenia;	AA (145/59.9%); AG and GG (97/40.1%)	[13]
Abo-Bakr 2017	Egypt	ALL	97	CHOPACM	PCR	Myelosuppression; neurotoxicity	AA (49/50.5%); AG and GG (48/49.5%)	[16]
Hasni 2016	Indonesia	BC	91	FAC/FEC	PCR-RFLP	Leukopenia; neutropenia	AA (55/60.4%); AG (27/29.7%); GG (9/9.9%)	[17]
Thu 2019	Myanmar	LN	67	CTX	PCR-RFLP	Leukopenia; thrombocytopenia; myelosuppression	AA (34/50.7%); AG and GG (49.3%)	[18]
Liu 2014	China	BC	124	FAC/AC	PCR-HRM	Leukopenia; neutropenia; thrombocytopenia; anemia	AA (69/55.6%); AG and GG (55/44.4%)	[19]
Wei 2009	China	RNS	163	PC	PCR-RFLP	Myelosuppression; GI; infection	AA (98/60.1%); AG (58/35.6%); GG (7/4.3%)	[20]
Zhang 2018	China	lymphoma	83	CVB	FISH	Leukopenia; neutropenia; thrombocytopenia; anemia;	AA (55/66.3%); AG (25/30.1%); GG (3/3.6%)	[21]
Tsuji, 2016	Japan	BC	100	AC	PCR-RFLP	Neutropenia	AA (68/68%); AG and GG (32/32%)	[22]

AC = doxorubicin, cyclophosphamide, ALL = acute lymphoblastic leukemia, BC = breast cancer, CHOPACM = cyclophosphamide, doxorubicin, vincristine, prednisone, L-asparaginase, cyclophosphamide, cytarabine, and 6-mercaptopurine, CMF = cyclophosphamide, methotrexate, 5-fluorouracil, CTX = cyclophosphamide, CVB = cyclophosphamide, carmustine, etoposide, DLBCL = diffuse large B-cell lymphoma, EC = epirubicin, cyclophosphamide, FAC = 5-fluorouracil, doxorubicin, cyclophosphamide, FEC = 5-fluorouracil, epirubicin, cyclophosphamide, FISH = fluorescence in situ hybridization, LN = lupus nephritis, MALDI-TOF-MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, PC = cyclophosphamide, prednisone, PCR-RFLP = PCR-restriction fragment length polymorphism (RFLP), R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone, RNS = refractory nephrotic syndrome, SLE = systemic lupus erythematosus, TAM = tamoxifen.

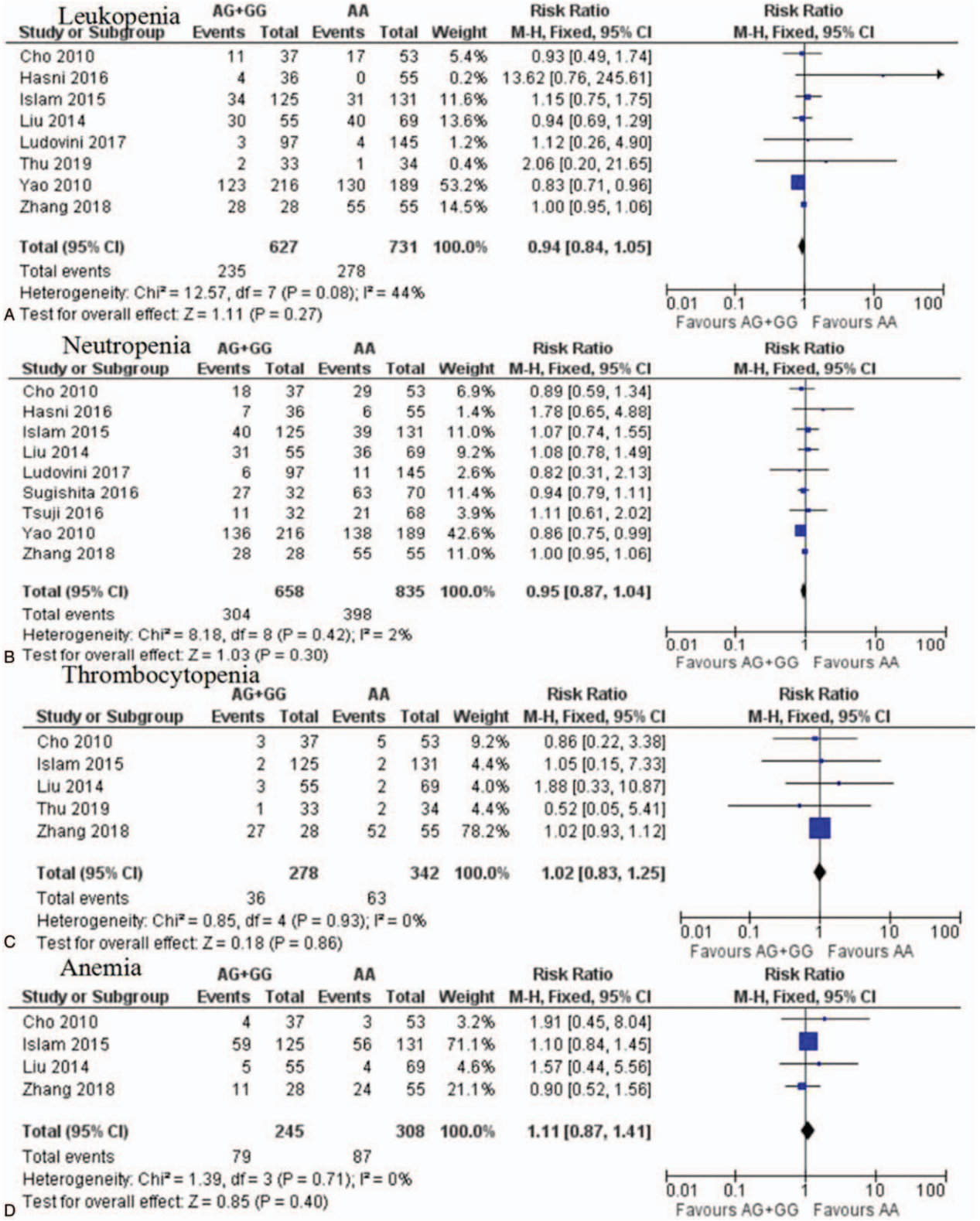

Figure 2. Risk of (A) leukopenia, (B) neutropenia, (C) thrombocytopenia, and (D) anemia for carriers of AG and GG genotypes in comparison with carriers of AA genotype.

In total, data from 5 studies were available for meta-analysis to evaluate the association between the GST*P1* rs1695 polymorphism and myelosuppression. Myelosuppression events occurred in 38.5% of AA genotype carriers and 45.0% of AG and GG genotypes, respectively. However, no significant association was found between myelosuppression and the polymorphism of *GSTP1* rs1695 in the overall population (RR, 1.34; 95% CI, 0.79–2.29; $P=.28$, Fig. 3A). Due to the high between-study heterogeneity ($I^2=87\%$, $P_{\text{heterogeneity}}<.00001$), subgroup analysis stratified by diseases was used to detect the possible sources of heterogeneity. Interestingly, the significant heterogeneity was eliminated in the 2 subgroups ($I^2=0\%$, $P_{\text{heterogeneity}}=.58$ for the subgroup of SLE or lupus nephritis syndrome patients; $I^2=0\%$, $P_{\text{heterogeneity}}=.96$ for the subgroup of cancer patients). The *GSTP1* rs1695 variant G allele was associated with a higher risk of myelosuppression in SLE and lupus nephritis syndrome patients (RR, 2.10; 95% CI, 1.60–2.76; $P<.00001$, Fig. 3B). Conversely, the *GSTP1* rs1695 variant G allele was associated with a lower risk of myelosuppression in cancer patients, while there was no statistically significant (RR, 0.85; 95% CI, 0.70–1.03; $P=.10$, Fig. 3C).

3.3. GSTP1 rs1695 polymorphism and gastrointestinal toxicity

Figure 4 shows the relationship between the polymorphism of *GSTP1* rs1695 and gastrointestinal toxicity. Taken together, gastrointestinal toxicity occurred in 18.4% of AA genotype carriers and 27.2% of AG and GG genotypes, respectively. Evidence from the heterogeneity test indicated no heterogeneity among the 3 studies ($I^2=0\%$; $P_{\text{heterogeneity}}=.61$). In the overall group, we observed an association between *GSTP1* rs1695 polymorphism and gastrointestinal toxicity (RR, 1.61; 95% CI, 1.18–2.19; $P=.003$). After subgroup analysis, it was interesting to note that the *GSTP1* rs1695 variant G allele was associated with a higher risk of gastrointestinal toxicity in SLE and lupus nephritis syndrome (RR, 1.77; 95% CI, 1.25–2.53; $P=.001$), but not in cancer patients (RR, 1.26; 95% CI, 0.66–2.38; $P=.48$).

3.4. GSTP1 rs1695 polymorphism and infection

Three publications were enrolled for the meta-analysis between the *GSTP1* rs1695 polymorphism and infection. There was no heterogeneity in these 3 studies ($I^2=13\%$; $P_{\text{heterogeneity}}=.32$),

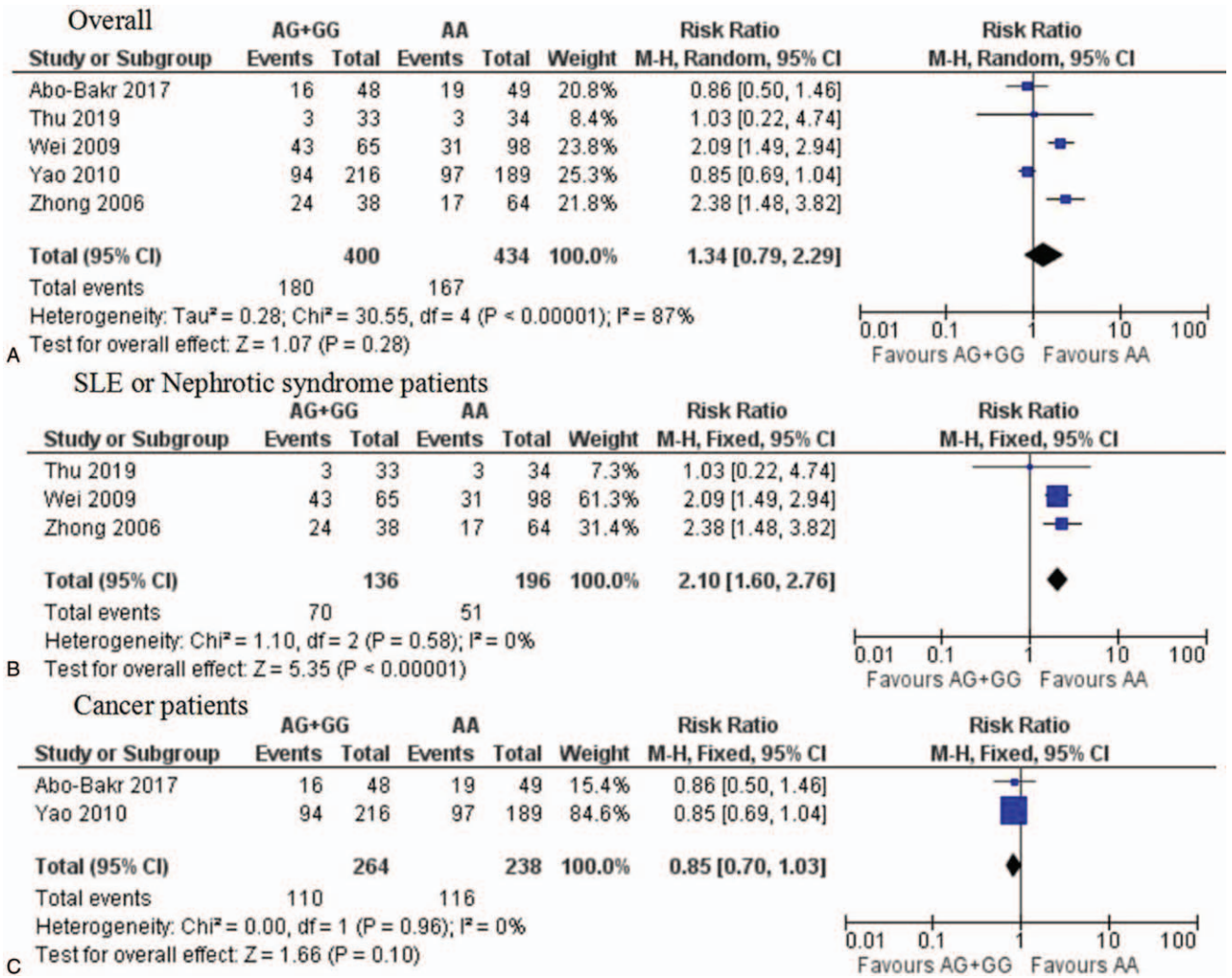

Figure 3. Risk of myelosuppression for carriers of AG and GG genotypes in comparison with carriers of AA genotype.

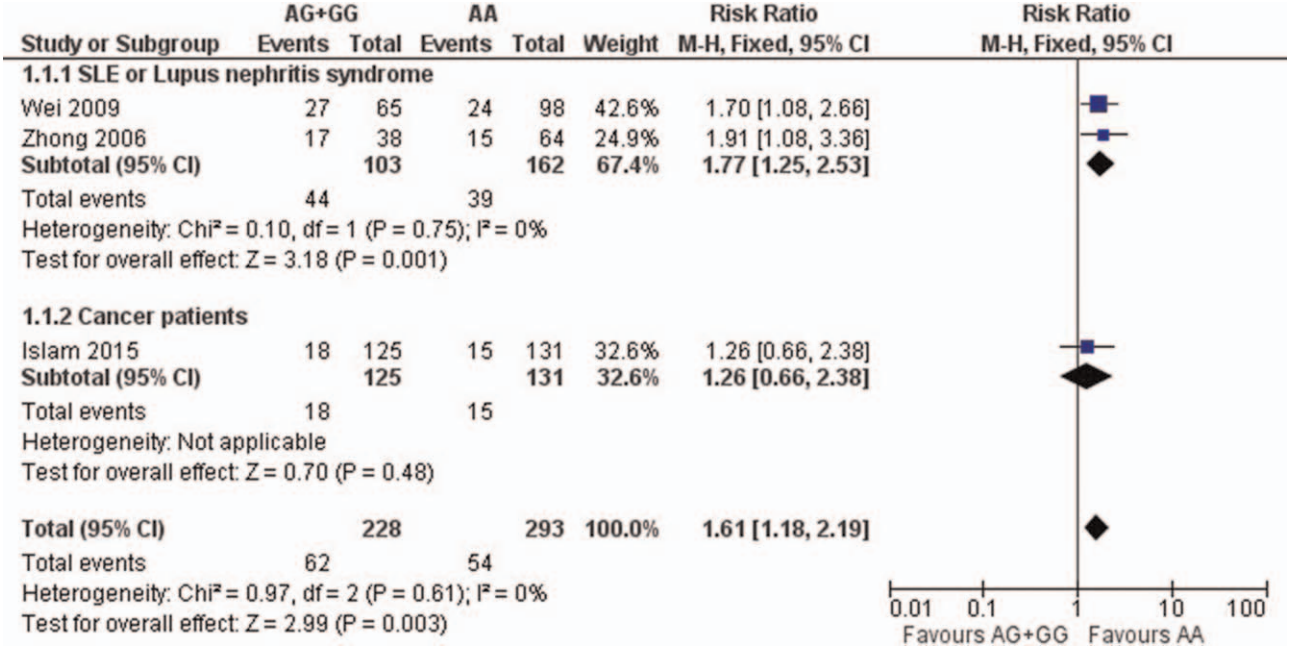

Figure 4. Risk of gastrointestinal toxicity for carriers of AG and GG genotypes in comparison with carriers of AA genotype.

thus the fixed-effect model was selected. In the overall population, *GSTP1* rs1695 polymorphism showed an association with the incidence of infection, where carriers of variant G allele had a higher risk of infection than non-carriers (RR, 1.57; 95% CI, 1.00–2.48; $P = .05$). It is noteworthy that this association was still observed in the subgroup of SLE or lupus nephritis syndrome patients (RR, 2.01; 95% CI, 1.14–3.54; $P = .02$), but not observed in the subgroup of cancer patients (RR, 0.95; 95% CI, 0.43–2.10; $P = .91$) (Fig. 5).

3.5. GSTP1 rs1695 polymorphism and neurotoxicity

Only 2 literatures that both involving lymphoma patients reported the association between the polymorphism of *GSTP1*

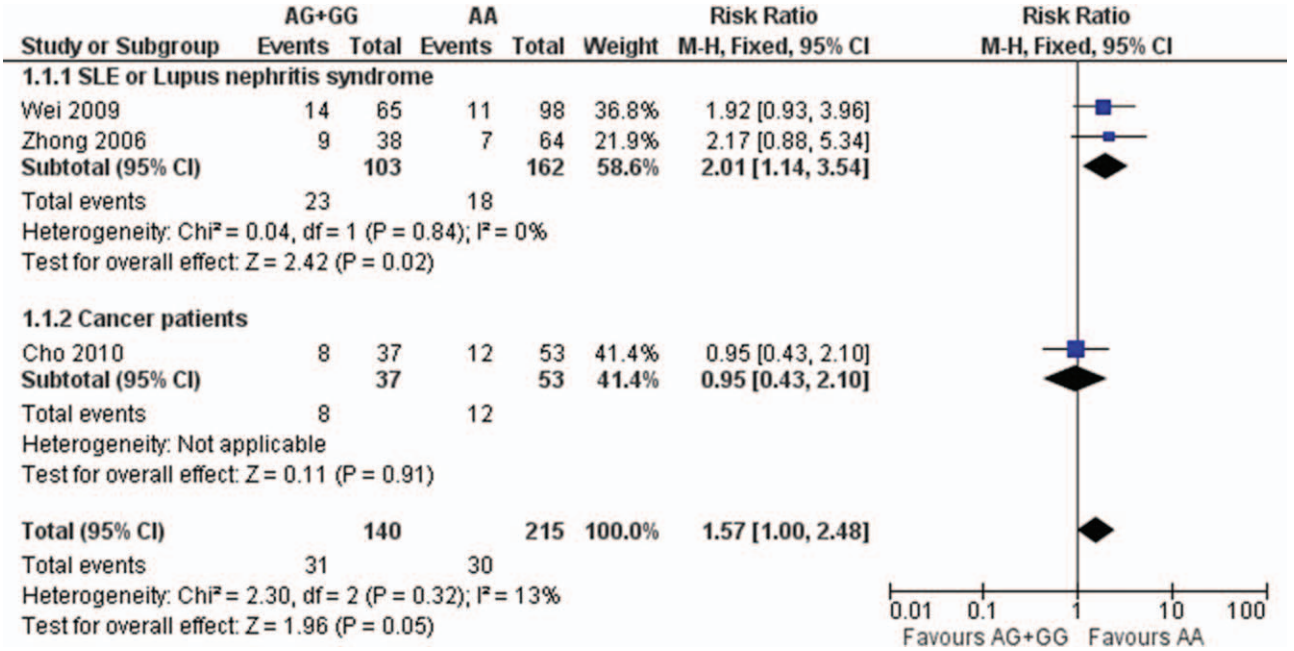

Figure 5. Meta-analysis of the association between *GSTP1* rs1695 polymorphism and infection.

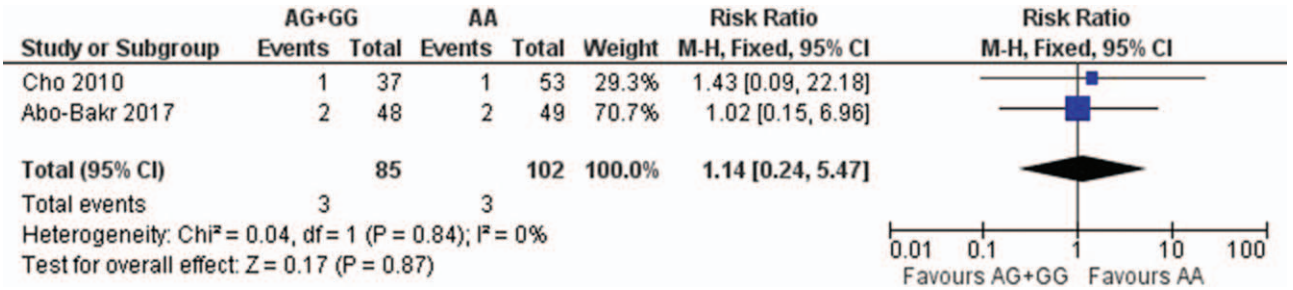

Figure 6. Meta-analysis of the association between *GSTP1* rs1695 polymorphism and neurotoxicity.

rs1695 and neurotoxicity, and the incidence of neurotoxicity in G allele (AG and GG genotypes) and non-G allele (AA genotype) carriers was 3.5% and 2.9%, respectively. We chose the fixed-effect model due to the absence of heterogeneity in the 2 studies ($I^2=0\%$; $P_{\text{heterogeneity}}=.84$). The association between *GSTP1* rs1695 polymorphism and neurotoxicity was not significant. (RR, 1.14; 95% CI, 0.24–5.47; $P=.87$) (Fig. 6).

4. Discussion

Cyclophosphamide, despite its widespread use in clinical practice, many patients have to reduce doses or switch to other treatment regimens since cyclophosphamide as a cytotoxic drug lacks target specificity leading to various toxicities.[3,23] Searching for genetic markers that can identify patients with severe toxicities in advance will help optimize the treatment of cyclophosphamide. GSTP1, belonging to phase II metabolic enzymes, plays a crucial role in the detoxification of cyclophosphamide by catalyzing the conjugation of electrophilic substances with glutathione. Genetic changes may alter the function of the GSTP1 metabolic enzyme.[24] In recent years, some studies have investigated the role of *GSTP1* rs1695 polymorphism in cyclophosphamide-induced toxicities. However, the true significance of *GSTP1* rs1695 polymorphism in predicting cyclophosphamide-induced toxicities is controversial, we thus carried out an integrated analysis to investigate whether *GSTP1* rs1695 polymorphism was associated with cyclophosphamide-induced toxicities. In the present study, we found that *GSTP1* rs1695 polymorphism was only associated with cyclophosphamide-induced gastrointestinal toxicity and infection in the overall population. The same results were confirmed in the subgroup analyses of SLE and lupus nephritis syndrome patients but not cancer patients. In addition, we identified a significant association between *GSTP1* rs1695 polymorphism and cyclophosphamide-induced myelosuppression in the subgroup of SLE and lupus nephritis syndrome patients (Table 2).

Compared to the *GSTP1* rs1695 AA genotype, the AG and GG genotypes conferred to a significantly increased risk of cyclophosphamide-induced gastrointestinal toxicity, and infection. The *GSTP1* rs1695 variant G has poor thermal stability and catalytic activity, and the enzyme activity of GG genotypes is lower than that of AG genotype, that is, base variation makes the substitution of valine (Val) for isoleucine (Ile) and results in decreased or even lost enzyme activity of GSTP1.[9,25] This low enzyme activity leads to decreased detoxification ability of GSTP1 and increased accumulation of toxic substances, thus increasing the likelihood of toxic events.[26] Additionally, GSTP1 can interact with c-Jun N-terminal kinase (JNK), a stress-activated protein kinase that plays an important role in regulating cell growth and apoptosis, to form a GSTP-JNK protein complex to inhibit the release and phosphorylation of JNK, and prevent apoptosis-mediated cell death.[27,28] The *GSTP1* rs1695 variant G allele may increase the activity of JNK and impair the cellular protective function. Therefore, these functions of GSTP1 might in part account for why variant G allele of GSTP1 rs1695 increases the risk of cyclophosphamide-induced toxicities in SLE or lupus nephritis syndrome patients.

We performed subgroup analyses by disease stratification of cyclophosphamide-induced myelosuppression, gastrointestinal toxicity, and infection, respectively. The results of gastrointesti-

Table 2

Summary of RRs and 95% CIs of *GSTP1* genetic polymorphism (rs1695, 313A>G) and cyclophosphamide-induced toxicities.

	Total (AG+GG vs AA)			SLE and lupus nephritis syndrome (AG+GG vs AA)			Cancers (AG+GG vs AA)		
Type of toxicities	**RR**	**95%CI**	***P***	**RR**	**95%CI**	***P***	**RR**	**95%CI**	***P***
Gastrointestinal toxicity	1.61	1.18–2.19	**.003**	1.77	1.25–2.53	**.001**	1.26	0.66–2.38	.48
Infection	1.57	1.00–2.48	**.05**	2.01	1.14–3.54	**.02**	0.95	0.43–2.10	.91
Myelosuppression	1.34	0.79–2.29	.28	2.10	1.60–2.76	**<.00001**	0.85	0.70–1.03	.10
Leukopenia	0.94	0.84–1.05	.27	NA	NA	NA	NA	NA	NA
Neutropenia	0.95	0.87–1.04	.30	NA	NA	NA	NA	NA	NA
Thrombocytopenia	1.02	0.83–1.25	.86	NA	NA	NA	NA	NA	NA
Anemia	1.11	0.87–1.41	.40	NA	NA	NA	NA	NA	NA
Neurotoxicity	1.14	0.24–5.47	.87	NA	NA	NA	NA	NA	NA

Bold values are significant.

95%CI = 95% confidence interval, NA = not available, RR = risk ratio, SLE = systemic lupus erythematosus.

nal toxicity and infection in the subgroup of SLE or lupus nephritis syndrome patients but not cancer patients were consistent with the overall group. This may be due to the small sample size in cancer patients, as only one study was included in these 2 subgroup analyses. In SLE and lupus nephritis syndrome patients, the association between *GSTP1* rs1695 polymorphism and cyclophosphamide-induced myelosuppression was also observed, which was not evident in the overall group or the subgroup of cancer patients. Myelosuppression was reported to be a feature of SLE disease activity and it was difficult to tell whether myelosuppression was disease-related or caused by cyclophosphamide.[29]

The current meta-analyses did not indicate any associations between *GSTP1* rs1695 polymorphism and cyclophosphamide-induced grade 3–4 hemotoxicity, including leukopenia, neutropenia, thrombocytopenia, and anemia. However, Tulsyan et al[30] reported a positive association between AG as well as AG+GG genotypes of *GSTP1* rs1695 and grade 2–4 anemia in 207 North Indian breast cancer patients, which is inconsistent with our result of anemia. With regard to leukopenia, neutropenia, and thrombocytopenia, it should be pointed out that Tsuji et al[22] and Zhang et al[21] focused on grade 4 neutropenia and grade 4 leukopenia, neutropenia, and thrombocytopenia, respectively, whereas other studies have investigated grade 3–4 toxicities, but the omission of these 2 studies could not substantially alter our conclusions. The incidence of cyclophosphamide-induced grade 3–4 hemotoxicity in cancer patients did not vary in the *GSTP1* rs1695 AA genotype and G allele carriers (AG and GG genotypes), which may be due to the fact that cancer patients were treated with cyclophosphamide in combination but not a single agent. In addition to cyclophosphamide, patients in 9 studies were treated with anthracyclines (doxorubicin or epirubicin), which have been reported to cause common side effects similar to cyclophosphamide, including leukopenia and myelosuppression.[31,32] Moreover, the degree of toxicity may vary when exposed to cyclophosphamide at different dosage and duration. Patients who received relatively low doses of cyclophosphamide and shorter cycles of chemotherapy tended to develop lower levels of toxicity,[3,33] whereas we only included grade $\geq$3 toxicities in the analysis.

Our study revealed that *GSTP1* rs1695 polymorphism was not associated with cyclophosphamide-induced neurotoxicity in lymphoma patients. A previous study has shown that 2- and 3-dechloroethyl-chloroacetaldehyde is neurotoxic by-products of cyclophosphamide, which is catalyzed by CYP2B6,[34] suggesting that *CYP2B6* polymorphism could be responsible for cyclophosphamide-induced neurotoxicity. In addition, neurotoxicity was reported in only 2 of the included studies, which seemed to have occurred infrequently in cyclophosphamide. The combination chemotherapy regimens in both 2 studies contained vincristine. Unlike cyclophosphamide, the most common side effect of vincristine is neurotoxicity, with neuropathic symptoms beginning within a few weeks of administration.[35] Thus we speculate that the detected neurotoxicity may be mainly contributed by vincristine rather than cyclophosphamide.

The major strength of our meta-analysis was that there was no between-study heterogeneity except for high heterogeneity in the overall analysis of myelosuppressive. Furthermore, the heterogeneity was eliminated after stratified analysis by diseases, indicating that the type of disease contributes to the high heterogeneity. The limitations should also be acknowledged in our study. First, only 3 studies using a single cyclophosphamide regimen were included in this meta-analysis and we did not consider the influence of adverse reactions from other drugs on our results. Second, the sample size was small in some subgroup analyses. Hence, further studies are required to verify our findings. Last, the pooled data were not adjusted by potential confounders such as participant characteristics, cyclophosphamide dose, and the length of follow-up, etc, which could influence our exploration of associations between *GSTP1* rs1695 polymorphism and cyclophosphamide-induced toxicities. However, the lack of reported data hampers our further analysis to some extent.

5. Conclusions

In conclusion, the current meta-analysis indicated that *GSTP1* rs1695 polymorphism was associated with an increased risk of cyclophosphamide-induced myelosuppression, gastrointestinal toxicity, and infection in SLE or lupus nephritis syndrome patients, suggesting that genotyping of *GSTP1* rs1695 may help to predict the risk of cyclophosphamide-induced toxicities. However, *GSTP1* rs1695 polymorphism was not associated with the cyclophosphamide-induced toxicities in cancer patients. Given the aforementioned limitations, our results should be interpreted with caution. So more pharmacogenomics studies are needed to further validate our findings to make better recommendations for the individualized application of cyclophosphamide.

Author contributions

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