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Review Article

Association of genetic polymorphisms of CYP2E1 and MPO genes with acute leukemia risk: a meta-analysis

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Abstract: There is a possible association between acute leukemia (AL) and polymorphisms in genes coding for cytochrome P4502E1 (CYP2E1) and myeloperoxidase (MPO), the enzymes both involved in the metabolism and bioactivation of xenobiotic compounds, including benzene especially. Previous studies reported conflicting results. Therefore, a meta-analysis of available molecular epidemiologic studies was performed to comprehensively investigate the association between *CYP2E1* RsaI/PstI polymorphism as well as *MPO* 463G>A genetic variants and AL risk. We systematically searched Pubmed, Web of Science, and Wanfang databases to identify eligible molecular epidemiologic studies up to March 1st, 2016. The effects of each polymorphism were pooled using either fixed or random effect models according to the heterogeneity of the studies. A total of 14 individual case-control studies from 13 articles, concerning polymorphism variants in these two genes (8 studies for *CYP2E1* and 6 studies for *MPO*) with risk to AL were included for analysis, with 2593 cases and 3442 controls involved. Overall, the c2 variant allele of *CYP2E1* RsaI/PstI polymorphism may slightly increase AL risk (dominant model: OR=1.46, 95% CI=1.05-2.02, *P*=0.02; heterozygous model: OR=1.44, 95% CI=1.03-2.00, *P*=0.03; allelic model: OR=1.42, 95% CI=1.05-1.92, *P*=0.02) despite a potential publication bias, while *MPO* 463G>A polymorphism was significantly associated with attenuated risk to AL (dominant model: OR=0.62, 95% CI=0.46-0.84, *P*=0.002; heterozygous model: OR=0.62, 95% CI=0.51-0.75, *P*<0.001; allelic model: OR=0.75, 95% CI=0.61-0.93, *P*=0.007). Subgroup analysis by AL type further showed that there was a significant association between *MPO* 463G>A polymorphism and decreased risk of acute myeloid leukemia (AML) (OR=0.25, 95% CI=0.15-0.43, *P*<0.001 for dominant model), rather than acute lymphoblastic leukemia (ALL), which was found associated with *CYP2E1* PstI polymorphism (OR=1.45, 95% CI=1.03-2.06, *P*=0.04 for dominant model). Conclusions: The meta-analysis suggests that *CYP2E1* RsaI/PstI polymorphism is associated with elevated AL risk, while *MPO* 463G>A allele may have a protective function against leukemogenesis.

Keywords: Myeloperoxidase, cytochrome P4502E1, acute leukemia, polymorphism, meta-analysis

Introduction

Acute leukemia (AL), a group of clonal hemopoietic disorders, has been reported to show increasing incidence and mortality rate worldwide in recent years [1]. Annually, AL accounts for about 4% of cancer cases in the United States [2]. Although treatment of the diseases has been improved with the advancement of modern chemotherapy, the causes of most leukemia, however, are not clarified and likely to involve an interaction between environmental and genetic factors [3, 4]. Among many human environmental carcinogens, benzene is a well-known carcinogenic agent that causes leukemia [5-9], a fact that was identified by the International Agency for Research on Cancer (IARC) early in 1982 [10]. Benzene is initially metabolized in the liver by the hepatic enzyme cytochrome P4502E1 (CYP2E1) to benzene oxides, which is spontaneously converted into phenol [11, 12]. Phenol is catalyzed by CYP2E1 to potentially toxic di- or trihydroxybenzenes such as hydroquinone, catechol, and 1,2,4-benzentriol [13, 14], which can be further converted in the bone marrow by myeloperoxidase (MPO) to benzoquinones [15], a potent hematotoxic and genotoxic compound that finally give a rise to leukemia development. As studied, CYP2E1 and MPO are both phase I xenobiotic metabolizing enzymes and involved in the bioactivation of benzene by forming intermedi-

ate reactive metabolites, such as hydroquinone and benzoquinones, which finally contribute to subsequent development of leukemia [16, 17]. However, further studies are needed to systematically access the role of these enzymes in leukemogenesis and the underlying mechanisms.

Increasing molecular epidemiologic researches, in recent years, reported a potential association between AL and gene polymorphisms. Polymorphisms in genes encoding for xenobiotic-metabolizing enzymes are largely responsible for the inter-individual differences in their ability to activate mutagenic or carcinogenic agents [18-20], and may thus influence individual susceptibility to leukemia. CYP2E1 is a cytochrome P450 isoform and shows genetic polymorphisms that can alert the gene expression and may be important in human carcinogenesis [21, 22]. Among *CYP2E1* polymorphisms, the most frequently studied are the *CYP2E1* RsaI/PstI polymorphism, which is also known as *CYP2E1** 5B polymorphism for locating in the 5'-flanking region of the gene [23]. Previous studies accessing the effect of *CYP2E1* RsaI/PstI polymorphisms on susceptibility to leukemia finally got contradictory results. Krajinovic et al. suggested an association of *CYP2E1** 5B variant alleles with ALL risk in Canadian children population [24]. On the contrary, Canalle et al. found no evidence supporting for such an association in Brazilian population [25]. MPO is also an important metabolizing enzyme involved in the catabolism of benzene [26]. A 463G/A polymorphism in the promoter region of the *MPO* gene can abolish the binding site for the SP1 transcription factor and thus results in reduced gene expression [18]. A case-control study carried by J. Zhang et al. found *MPO* 463G/A polymorphism was associated with a decrease risk of leukemia in mutant carriers due to diminished activation of carcinogens in the Chinese Han population [27], which is conflicting with previous results that no evidence of the involvement of *MPO* 463G/A polymorphism in AL susceptibility was found [24, 28].

With regard to the role of *CYP2E1* PstI genetic variant and MPO 463G>A polymorphism in the susceptibility to AL, inconclusive results were reported in term of the low sample size of single study and different characteristics among studies like ethnicity and leukemia types [24, 27-31]. The aim of our study was to conduct a meta-analysis of related molecular epidemiologic studies in a comprehensive over viewing of all available knowledge to provide an more thorough assessment summarizing the possible relationship between polymorphisms in genes encoding CYP2E1 and MPO and AL risk.

Materials and methods

Literature searching strategy

A systematical review of literatures concerning the associations between the *MPO* or *CYP2E1* polymorphism and risk of leukemia was conducted by searching PubMed, Web of Science and Wanfang databases up to March 1st 2016. Language was limited in English and Chinese, with no restriction on country. The search terms were: ("polymorphism" or "variant" or "genotype" or "mutation" or "SNPs") AND ("Myeloperoxidase" or "MPO" or "rs2333227" or "CYP2E1" or "rs2031920" or "rs3813867") AND ("leukemia" or "ALL" or "AML" OR "Hematological Malignancies"). Furthermore, a manual search was carried out according to reference lists of retrieved original articles and recent reviews to avoid from omission of any usable data. However, we did not check the grey literature.

Inclusion and exclusion criteria

Studies conforming to the following criteria were selected for inclusion in our meta-analysis: (1) case-control or cohort studies that evaluated the association between polymorphism in genes coding for MPO or CYP2E1 and risk of AL; (2) study in human only; (3) presenting original and sufficient data for calculating and estimating odds ratios (ORs) and the corresponding 95% confidence intervals (CIs). When multiple publications reported the same or overlapping data, only the most reliable report with the largest sample size was chosen for the final analysis. Case-only studies, editorials, systematic reviews (including meta-analysis) and studies with subjects who had Down's Syndrome or any hematological malignancies other than AL were excluded. For reliability, all the identified citations were reviewed independently by two authors (Wu and Liu), and studies met the above criteria were finally included in the present meta-analysis. Divergence of views was settled by reaching a consensus between the two authors.

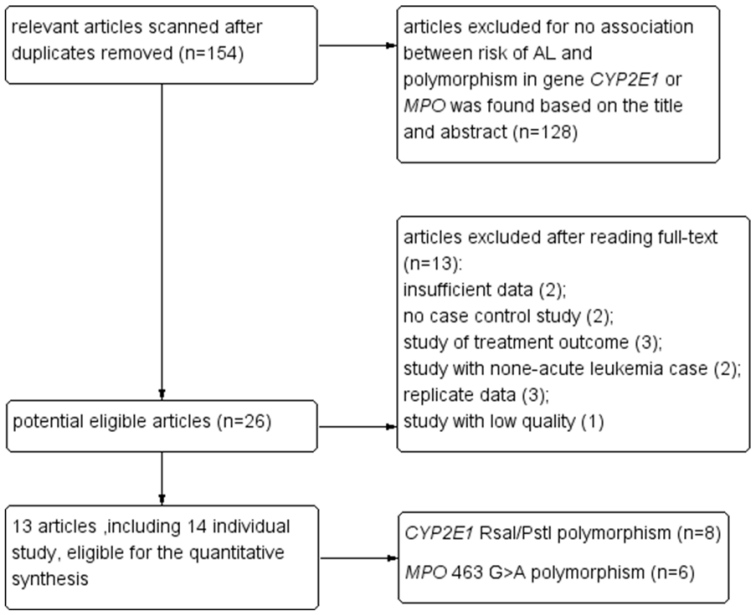

Figure 1. The flow diagram of selecting eligible studies.

Data extraction and quality assessment

Information was also extracted independently by two researchers (Wu and Liu) from studies included, and any disagreements were resolved by discussion. The following terms of data were collected: first author, year of publication, study design, AL subtypes (AML or ALL), genotyping methods, country and ethnicity investigated, number of cases and controls, genotypes data, and the confirmation of Hardy-Weinberg equilibrium (HWE) in control. We assessed paper quality based on the requirements and items of the Newcastle-Ottawa-Scale (NOS) for case-control studies [32], considering studies with scores of 0-3, 4-6, 7-9 as low, moderate and high quality, respectively. Two authors contributed in carrying out the quality assessment independently, resolving any arguments by discussion.

Statistical analysis

Firstly, we tested the conformation of genotype distribution in controls to Hardy-Weinberg equilibrium (HWE) by the chi-square test [33]. The strength of the associations between gene polymorphisms and risk of AL was estimated by ORs and 95% CIs. *Z*-test was used to determine the statistical significance of the summary OR. Heterogeneity between studies was estimated by Cochran's Q test and I^2 statistic, and a *P* value less than 0.10 or I^2>50% was considered indicating the existence of significant heterogeneity [34]. If no significant heterogeneity across studies was observed, the fixed-effects model (Mantel-Haenszel methods) [35] was used prior to evaluate the pooled ORs. Otherwise, the random-effects model (DerSimonian and Laird's method) [36] was chosen. Additionally, the Begg's and Egger's funnel plot asymmetry tests were conducted to check the publication bias [37, 38].

In the present meta-analysis, the pooled ORs were calculated under 5 genetic models of each polymorphism, *CYP2E1* RsaI/PstI polymorphism (dominant model: c2c2+c1c2 vs. c1c1; recessive model: c2c2 vs. c1c1+c1c2; heterozygous model: c1c2 vs. c1c1; homozygous model: c2c2 vs. c1c1; allelic model: c2 vs. c1) and *MPO* 463G>A polymorphism (dominant model: aa+ga vs. gg; recessive model: aa vs. ga+gg; heterozygous model: ga vs. gg; homozygous model: aa vs. gg; allelic model: a vs. g). Besides, we conducted stratify analyses to assess the covariate effects, in which ethnicities were categorized into two groups (Asian and Caucasian for *MPO* 463G>A, Asian and no-Asian for *CYP2E1* c2 alleles), AL types were categorized into AML and ALL and ages were grouped into adult and childhood. Studies that didn't specify leukemia types and those without clear age bracket were not included for the stratification analyses. The meta-analysis was performed mainly using Review Manager version 5.2 (Cochrane Collaboration, Oxford, England) except that Begg's and Egger's tests were conducted by Stata version 11.2 (Stata Corporation, College Station, TX, USA). All *P* values were two-sided and *P*<0.05 was considered statistically significant, unless stated otherwise.

Results

Identification and characteristics of included studies

In total, 154 potentially individual abstracts were screened from the Pubmed, Web of

Table 1. Main characteristics of studies included in the meta-analysis

First author	Year	Leukemia subtype	Country (Ethnicity)	Genotyping method	Number (case/control)	Case			Control			OR (95% CI)	NOS	HWE
CYP2E1 RsaI/PstI polymorphism						c1c1	c1c2	c2c2	c1c1	c1c2	c2c2			
Wang Shumei	2014	ALL	China (Asian)	PCR-RFLP	91/283	51	35	5	192	84	7	1.66 (1.02-2.68)	4	0.20
Audrey Bonaventure	2012	AML, ALL	France (Caucasian)	Genome-wide genotyping[a]	493/549	462	26	0	500	36	0	0.80 (0.50-1.40)[b]	8	0.42
Xi Yaming	2011	AML, ALL	China (Asian)	PCR-LDR	150/150	105	40	5	111	36	3	1.22 (0.74-2.02)	6	0.97
Gulen Ulusoy	2007	ALL	Turkey (Caucasian)	PCR-RFLP	168/207	156	12	0	199	8	0	1.91 (0.76-4.80)	6	0.78
Pascual Bolufer	2007	AML, ALL	Spain (Caucasian)	Real-Time PCR	343/390	321	21	1	367	23	0	1.09 (0.60-2.00)	7	0.55
Muge Aydin-Sayitoglu	2006	AML, ALL	Turkey (Caucasian)	PCR-RFLP	249/140	213	36	0	136	6	0	3.34 (1.55-9.20)	7	0.80
Renata Canalle	2004	ALL	Brazil (Caucasian)	PCR-RFLP	113/221	99	14	0	197	23	1	1.16 (0.58-2.34)	8	0.71
Maji Krajinovic	2002	ALL	Canada (Caucasian)	PCR-ASO	174/302	160	14	0	293	9	0	2.85 (1.21-6.73)	8	0.79
MPO 463G>A polymorphism						gg	ga	aa	gg	ga	aa			
Jia Mingfeng	2012	AML, ALL	China (Asian)	PCR-LDR	150/150	100	45	5	72	76	2	0.46 (0.29-0.74)	5	<0.01
Shi Xiu-E	2010	AML, ALL	China (Asian)	PCR-LDR	100/100	69	24	7	46	52	2	0.38 (0.22-0.68)	5	<0.01
Vanessa da Silva Silveira	2010	ALL	Brazil (mixed)	PCR-RFLP	124/300	71	46	7	159	124	17	0.84 (0.55-1.28)[c]	6	0.26
Zhang Juan	2007	AL	China (Asian)	PCR-RFLP	135/187	94	39	2	108	74	5	0.58 (0.36-0.92)	7	0.06
Zhu Fangyan	2006	AML, ALL	China (Asian)	PCR-RFLP	139/139	87	47	5	65	69	5	0.53 (0.33-0.85)	5	<0.01
Maji Krajinovic	2002	ALL	Canada (Caucasian)	PCR-ASO	169/337	105	55	9	212	114	11	1.03 (0.71-1.51)	8	0.36

OR: Odd ratio; CI: Confidence interval; NOS: Newcastle-Ottawa-Scale; HWE: Hardy-Weinberg equilibrium; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; AL: Acute leukemia; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; LDR: Ligase detection reaction; ASO: Allele specific oligonucleotide hybridization assays. a: Genome-wide genotyping was based on a high-throughput platform and imputation. b: Adjusted by the gender, age quota variable, parental professional category, maternal educational level, birth order, and breastfeeding. c: Adjusted by age, sex, and ethnic group.

Table 2. Meta-analysis of *CYP2E1* RsaI/PstI and *MPO* 463G>A polymorphisms on acute leukemia risk

Overall and subgroup	N	Case/ control	Dominant model OR (95% CI)	*P*	I^2 (%)	Recessive model OR (95% CI)	*P*	I^2 (%)	Heterozygous model OR (95% CI)	*P*	I^2 (%)	Homozygous model OR (95% CI)	*P*	I^2 (%)	Allelic model OR (95% CI)	*P*	I^2 (%)
CYP2E1 RsaI/PstI																	
Total	8	1776/2229	1.46 (1.05-2.02)	0.02	51%	1.91 (0.83-4.43)	0.13	0%	1.44 (1.03-2.00)	0.03	51%	2.08 (0.89-4.87)	0.09	0%	1.42 (1.05-1.92)	0.02	49%
Ethnicity																	
Asian	2	241/433	1.43 (1.01-2.03)	0.04	0%	2.01 (0.80-5.04)	0.14	0%	1.37 (0.95-1.96)	0.09	0%	2.22 (0.87-5.66)	0.09	0%	1.41 (1.04-1.89)	0.03	0%
No-Asian	6	1535/1796	1.53 (0.94-2.48)	0.09	63%	1.52 (0.20-11.63)	0.69	0%	1.53 (0.94-2.49)	0.09	63%	1.54 (0.20-11.82)	0.68	0%	1.50 (0.94-2.39)	0.09	61%
Leukemia type																	
AML	4	472/1216	1.41 (0.71-2.79)	0.32	59%	2.43 (0.63-9.42)	0.20	0%	1.35 (0.66-2.76)	0.41	60%	2.61 (0.67-10.17)	0.17	0%	1.43 (0.77-2.65)	0.25	55%
ALL	8	1295/2229	1.45 (1.03-2.06)	0.04	46%	1.68 (0.63-4.46)	0.30	0%	1.44 (1.02-2.04)	0.04	45%	2.18 (0.88-5.42)	0.09	0%	1.41 (1.02-1.95)	0.04	45%
Age group																	
Adult	2	236/290	1.91 (0.67-5.47)	0.23	72%	1.69 (0.40-7.20)	0.48	-	1.89 (0.63-5.61)	0.25	73%	1.69 (0.40-7.20)	0.48	-	1.85 (0.69-4.96)	0.22	70%
Children	6	1197/1689	1.64 (1.04-2.60)	0.03	62%	1.98 (0.66-5.94)	0.23	0%	1.71 (1.03-2.84)	0.04	67%	1.98 (0.66-5.94)	0.23	0%	1.58 (1.02-2.43)	0.04	61%
MPO 463G>A																	
Total	6	817/1,213	0.62 (0.46-0.84)	0.002	62%	1.37 (0.86-2.19)	0.19	0%	0.62 (0.51-0.75)	<0.001	66%	1.15 (0.70-1.89)	0.57	0%	0.75 (0.61-0.93)	0.007	44%
Ethnicity																	
Asian	4	524/576	0.50 (0.39-0.63)	<0.001	0%	1.48 (0.73-3.00)	0.28	13%	0.47 (0.36-0.60)	<0.001	6%	1.05 (0.49-2.25)	0.91	0%	0.63 (0.52-0.78)	<0.001	0%
Caucasian	2	293/637	0.94 (0.71-1.25)	0.68	0%	1.28 (0.68-2.41)	0.44	0%	0.91 (0.68-1.22)	0.51	0%	1.24 (0.65-2.37)	0.52	0%	0.99 (0.79-1.25)	0.95	0%
Leukemia type																	
AML	2	180/250	0.25 (0.15-0.43)	<0.001	29%	3.18 (0.96-10.52)	0.06	0%	0.21 (0.13-0.34)	<0.001	0%	1.88 (0.56-6.28)	0.31	0%	0.40 (0.28-0.58)	<0.001	7%
ALL	4	363/887	0.99 (0.72-1.36)	0.24	28%	1.42 (0.79-2.55)	0.24	0%	0.95 (0.70-1.31)	0.77	25%	1.38 (0.76-2.49)	0.29	0%	1.04 (0.84-1.28)	0.72	6%

N: number of studies; OR: odd ratio; CI: confidence interval; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia. I^2 is the statistic that measure the degree of heterogeneity between studies. The pooled odd ratio and 95% CI in the article were either from fixed effects model or random effects model according to heterogeneity measured by I^2 statistics, with I^2>50% indicating a significant heterogeneity existed between in studies.

Science, and Wanfang databases. After checking the abstracts and reviewing the full texts of possible studies, 14 individual case-control studies from 13 publications [24, 25, 27, 28, 31, 39-46] were finally included into the meta-analysis. However, no cohort study met our inclusion criteria. The details of study selection process are presented in the flow diagram (**Figure 1**). The 14 studies identified with 6,035 subjects (2,593 cases and 3,442 controls) were then read thoroughly to record the basic information, including AL subtypes, country of origin, ethnicity, the number of cases and controls of each study, with the detailed characteristics summarized in **Table 1**.

Overall, 7 studies [25, 31, 40-43, 45] focused on the association between AL risk and *CYP2E1* RsaI/PstI polymorphism, and 5 studies [27, 28, 39, 44, 46] targeted on that of *MPO* 463G>A polymorphism, while only one study [24] tried to explore the impact of both two gene variants on AL risk. In the total 14 studies, 6 studies focused on ALL patients [24, 25, 28, 40, 43], and 7 studies targeted at ALL and AML patients together [31, 39, 41, 42, 44-46], but only one article didn't specify the type of AL [27]. The controls were free of leukemia or any leukemia related diseases in all studies. As to ethnicity, 8 studies were conducted in Caucasian population [24, 25, 28, 31, 41-43] and 6 studies in Asian population [27, 39, 40, 44-46]. In term of the quality assessment of NOS, 7 studies were in high quality [24, 25, 31, 39, 41, 42] and 7 were in moderate quality [28, 39, 40, 43-46]. Genotype distributions among the controls were in agreement with HWE in all studies, except for three studies [39, 44, 46] (**Table 1**).

Meta-analysis

Pooling data in 8 studies which have examined the c2 carriers of the *CYP2E1* gene suggested a significant association between *CYP2E1* PstI polymorphism and elevated risk to AL under dominant model (OR=1.46, 95% CI=1.05-2.02, *P*=0.02), heterozygous model (OR=1.44, 95% CI=1.03-2.00, *P*=0.03), and allelic model (OR=1.42, 95% CI=1.05-1.92, *P*=0.02) (**Table 2**; **Figure 2**). There was, however, no evidence for a relationship under homozygous and recessive model (**Table 2**). Additionally, subgroup analyses were performed to access the effects of different ethnics, leukemia subtypes and age groups. The result further indicated that there was a significant association between *CYP2E1* c2 gene variants and increased risk of ALL (for dominant model, OR=1.45, 95% CI=1.03-2.06, *P*=0.04; for allelic model, OR=1.41, 95% CI=1.02-1.95, *P*=0.04), but no such relationship observed in AML population (**Table 2**). In ethnicity subgroup analysis, the RsaI/PstI polymorphism of *CYP2E1* significantly increased AL risk in Asian population when using dominant (OR=1.43, 95% CI=1.01-2.03, *P*=0.04) and allelic model analysis (OR=1.41, 95% CI=1.04-1.89, *P*=0.03), rather than in non-Asian population. Pooled data from 6 studies evaluating children leukemia [24, 25, 31, 40, 41, 43] provided evidence for an association between the variant and children AL risk under three main models (dominant model: OR=1.64, 95% CI=1.04-2.60, *P*=0.03; heterozygous model: OR=1.71, 95% CI=1.03-2.84, *P*=0.04; allelic model: OR=1.58, 95% CI=1.02-2.43, *P*=0.04) (**Table 2**).

6 studies have evaluated *MPO* 463G>A polymorphism as a risk factor for AL. Pooling data from the six studies showed that there was a significant association between *MPO* 463G>A polymorphism and attenuated AL risk under three genetic models (dominant model: OR=0.62, 95% CI=0.46-0.84, *P*=0.002; heterozygous model: OR=0. 62, 95% CI=0.51-0.75, *P*<0.001; allelic model: OR=0.75, 95% CI=0.61-0.93, *P*=0.007) (**Table 2**; **Figure 2**). There was evidence of population stratification in the study authored by Shi Xiu-E et al. with controls found deviating from HWE (*P*=0.003). Nevertheless, almost similar result was yielded excluding the study from the meta-analysis (Additional File 1). Subsequent stratified analysis by AL types suggested a relationship between this variant and AML, but not ALL (**Table 2**), though two studies excluding for analysis due to not specifying the types of AL [27, 39]. In another subgroup analysis, pooling data from four studies evaluating the effect of different ethnics provided a support for a significant association between *MPO* 463G>A polymorphism and elevated AL risk in Asian population, while no evidence of similar relationship in Caucasian population was observed in a pooled analysis of other two studies (**Table 2**).

Heterogeneity test

As showed in **Table 2**, We observed no significant heterogeneity across the studies either on

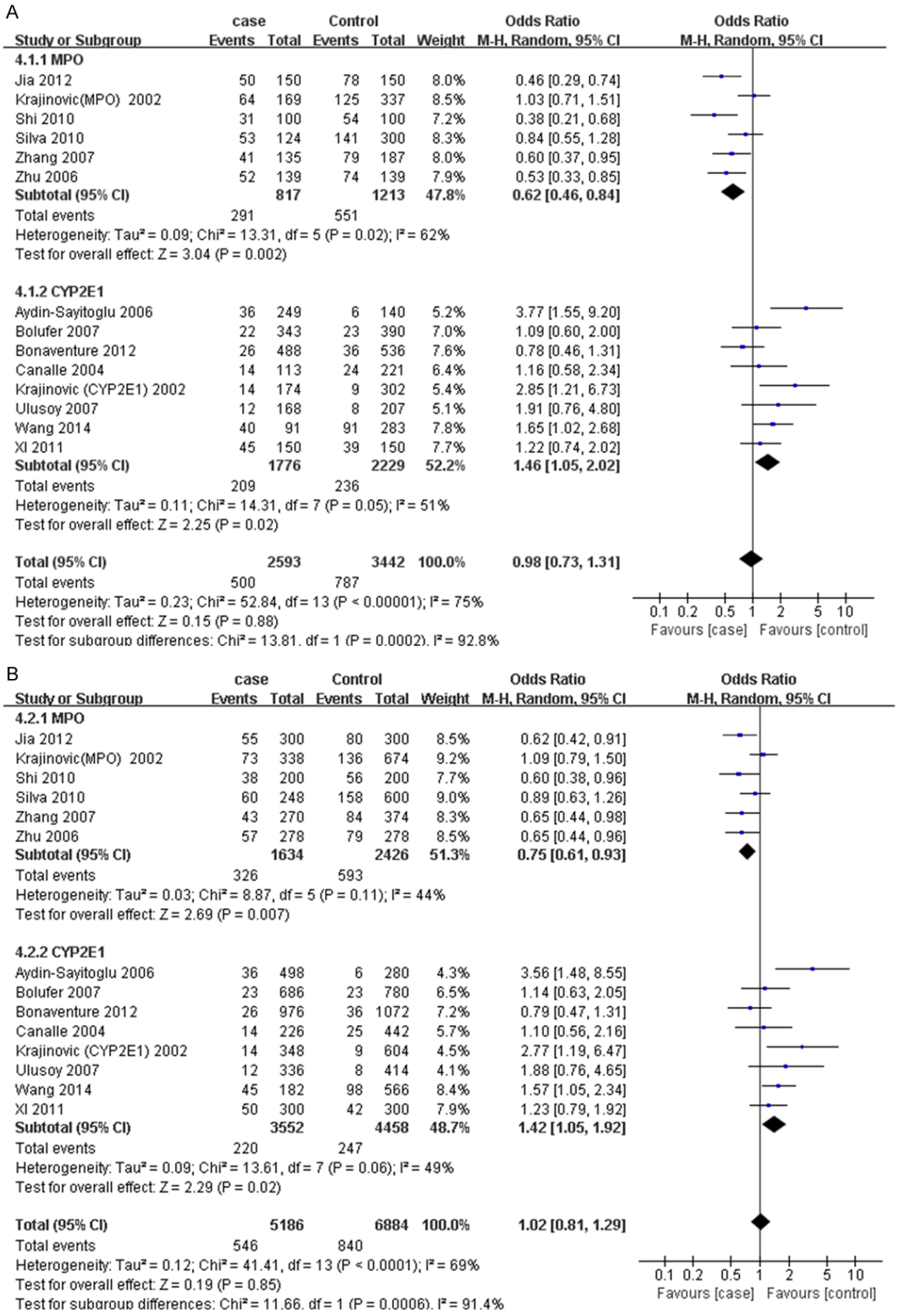

Figure 2. Forest plots of meta-analysis of *CYP2E1* RsaI/PstI and *MPO* 463G>A Polymorphisms on risk of acute leukemia. A: Dominant model; B: Allelic model.

CYP2E1 RsaI/PstI polymorphism or *MPO* 463G>A polymorphism with AL risk under recessive and homozygous model (P>0.10 and I^2<50%) and the fixed-effects model was applied to calculate the pooled ORs. However, in other three genetic models, heterogeneity was demonstrated (P<0.10 or I^2>50%), so the random-effects model was subsequently adopted for the meta-analysis. Attempts were made to explore the sources of between-study heterogeneity by subgroup analysis, and the results showed ethnicity, age groups and types of AL were the key factors that affected the homogeneity (**Table 2**).

Publication bias

In this analysis, we tested funnel plot asymmetry using both Begg's and Egger's tests in a comparison model of the carrier status (carriers vs wild-type) for *CYP2E1* c2 allele (P values of 0.048 and 0.048, respectively) and *MPO* 463G>A variant (P values of 0.851 and 0.951, respectively) individually. The results, however, revealed evidence of publication bias for *CYP2E1* PstI allelic variants, indicating our results concerning relationship between *CYP2E1* PstI polymorphism and elevated AL risk may not be robust due to a relatively high false positive result reported in the 8 studies included for the analysis. No evidence of publication bias was observed in the analysis of *MPO* 463G>A polymorphism on risk of AL (Additional File 2).

Discussion

Acute leukemia is still a huge threat to human health worldwide, despite advances in survival and quality of life have been achieved due to improved modern chemotherapy for the diseases [1, 47]. Though substantial efforts were involved in, the exact causes of AL remain unclear. However, most studies agree on a combined risk from the environmental and genetic factors altogether [3, 4]. Many environmental carcinogens contribute to the leukemogenesis, among which benzene is one ubiquitous occupational hematotoxin and leukemogen that clearly identified by IARC [10, 48]. Last decades, emerging epidemiologic investigations have revealed several genetic variants as risk factors for AL, highlighting a new sight for leukemia pathogenesis and etiology research. Considering potential role of allelic variants of genes for xenobiotic-metabolizing enzymes in the susceptibility to AL, we carried out a meta-analysis targeting two gene polymorphisms (*CYP2E1* RsaI/PstI and *MPO* 463G>A), in an attempt to better define the association between polymorphism in genes encoding for environmental carcinogens bioactivation enzymes and risk of AL. As previous studies [24, 25, 27, 28, 31, 39-46] focusing on the relationship between these two single nucleotide polymorphisms (SNPs) and AL risk reported conflicting results, our study aim to provide more reliable results by combining analysis of all these studies and in a larger sample size (1776 cases and 2,229 controls for *CYP2E1* RsaI/PstI polymorphism, 817 cases and 1,213 controls for *MPO* 463G>A polymorphism).

In the present meta-analysis, we found evidence supporting for a significant association between RsaI/PstI polymorphism of *CYP2E1* and elevated AL risk under three main comparison models (allelic, heterozygous and dominant), which is consistent with evidence suggesting that *CYP2E1* RsaI/PstI polymorphism was associated with increased transcriptional activity, thus enhancing ability to activate carcinogens [23]. Additionally, similar conclusion has been concluded for children acute lymphoblastic leukemia in previous meta-analysis under heterozygous and dominant models [30]. The SNPs on the *CYP2E1* promoter region were too complicated, here we only studied *CYP2E1* RsaI/PstI polymorphism, covering two SNPs (RsaI/C-1055T/rs2031920; PstI/G-1295C/rs3813867), which are both located in the 5'-flanking region of the gene and most frequently investigated as a factor of human carcinogenesis [21, 22]. A case-control study revealed the c2 allele carriers of the *CYP2E1* gene significantly increased the risk of benzene poisoning when combined with other SNPs in genes involved in benzene metabolism [49], providing us a hint for exploring a potential role of the SNPs in leukemogenesis. Moreover, subgroup analyses by ethnicity and AL types, mainly AML and ALL, suggested a relationship between *CYP2E1* PstI polymorphism and increased ALL risk, especially in Asian population. In addition, stratified studies by age groups further showed that children carrying *CYP2E1* c2 genotypes were at higher risk of AL. However, these data should be interpreted with cautions as the association may not be robust with a potential publication bias.

As another important phase I metabolizing enzymes, MPO is involved in oxidative stress response to environmental carcinogens, such as benzene [26]. Differing from *CYP2E1* RsaI/PstI polymorphism, a single G to A transition at position 463 in the promoter region of the MPO gene can abolish the binding site for the SP1 transcription factor, resulting in reduced gene expression [18]. Interestingly, statistically significant association was observed between *MPO* 463G>A and attenuated AL risk for dominant, heterozygous and allelic models in our analyses. Similar relationship of the *MPO* genetic variant with other cancers and disorders were demonstrated [50-53]. Additionally, we further demonstrated that people in Asian ethnic bearing *MPO* 463G>A variant are more susceptible to AL, especially AML, in the subsequent subgroup analysis. However, our analyses provide no evidence for relationship between *MPO* 463G>A variant and risk of AL in Caucasians.

Nevertheless, some limitations should be noted to better understand the current findings. Firstly, limited number of eligible studies made us unable to provide sufficient sample size in the analysis, especially for subgroup analyses, and may thus weaken the statistical power to draw conclusions upon the effect of these two polymorphisms on AL risk. And some studies included for our analysis failed to specify leukemia types [27, 39] and age groups [39, 42, 44, 46] simply aggravated the problems. In this respect, more well-designed molecular epidemiologic studies with large sample size and more precise estimation of impact of *CYP2E1* RsaI/PstI and *MPO* 463G>A polymorphisms on AL risk are required to confirm these findings. Secondly, we observed obvious publication bias in the analysis of *CYP2E1* c2 variant under the dominant model, so conclusions drawn upon the relationship concerning *CYP2E1* RsaI/PstI with risk of AL may not be robust and should be interpreted with some cautions. Thirdly, heterogeneity that may arise from differences in leukemia types, age groups and ethnicity in the studies was detected in the present meta- analysis. Finally, we didn't, however, incorporate the effect of the two genetic alleles on AL risk for lacking sufficient data to permit the assessment of interaction effect. Nevertheless, it might be worthwhile to investigate the interaction relationship of the two polymorphisms with AL risk in further studies since both CYP2E1 and MPO are phase I metabolizing enzymes, involved in benzene metabolism and bioactivation. In brief, the systematic review of literatures focusing on association between AL and genetic polymorphisms suggested that *CYP2E1* RsaI/PstI polymorphism is associated with increased riskof AL, while MPO 463G>A may have a protective function against individual susceptibility to AL underdominant, heterozygous and allelic models, respectively. Additional studies covering a large number of samples with different age groups and ethnic origins are warranted to validate the results and to facilitate a further understanding of the pathogenesis underlying acute leukemia.

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Disclosure of conflict of interest

None.

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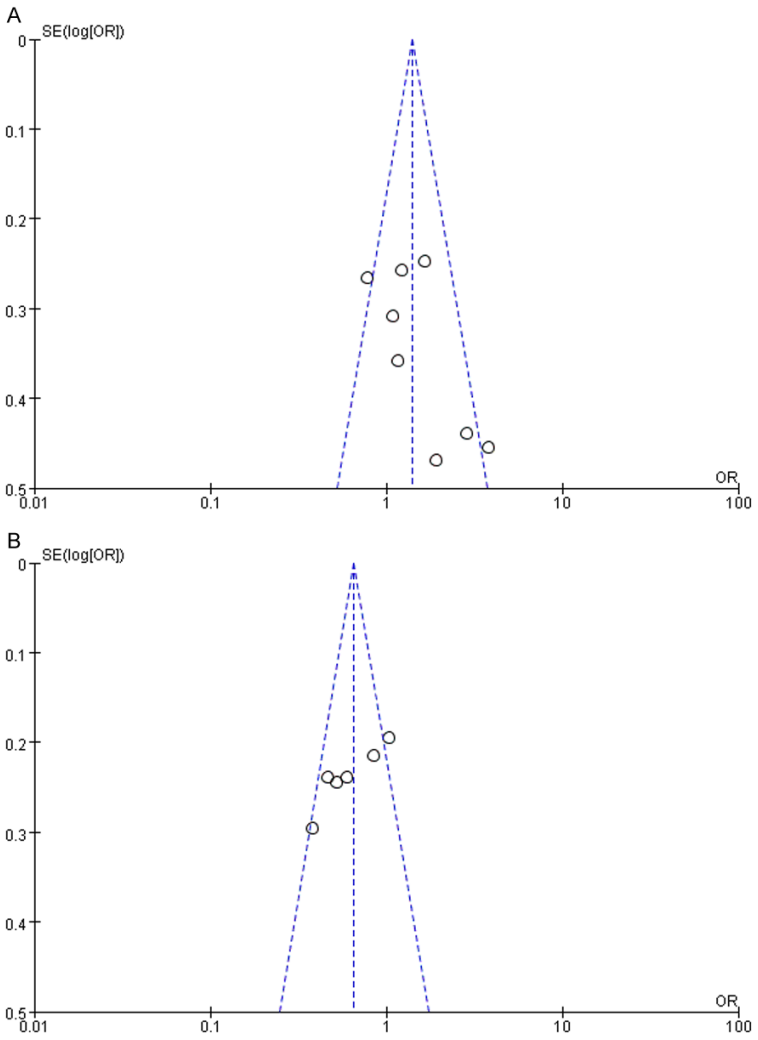
Additional File 1. The meta-analysis of studies concerning the association between MPO polymorphism and AL risk after excluded the study that deviation from HWE

Overall and subgroup	Overall	Study of Shi Xiu-E et al. excluded
N	6	5
Subjects (case/control)	817/1,213	717/1,113
Alleles model OR (95% CI)	0.77 (0.66-0.90)	0.79 (0.67-0.93)
P	0.0008	0.005
I^2 (%)	44%	48%
Heterozygous model OR (95% CI)	0.62 (0.51-0.75)	0.67 (0.55-0.82)
P	0.0008	<0.001
I^2 (%)	66%	56%
Homozygous model OR (95% CI)	1.15 (0.70-1.89)	1.07 (0.64-1.80)
P	0.57	0.79
I^2 (%)	0%	0%
Dominant model OR (95% CI)	0.62 (0.46-0.84)	0.67 (0.50-0.92)
P	0.002	0.01
I^2 (%)	62%	59%
Recessive model OR (95% CI)	1.37 (0.86-2.19)	1.21 (0.73-2.00)
P	0.19	0.46
I^2 (%)	0%	0%

OR, Odd ratio; CI, Confidence interval; HWE, Hardy-Weinberg equilibrium.



Additional File 2. Funnel plots. A: Funnel plot for CYP2E1 polymorphism. B: Funnel plot for MPO polymorphism.