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Review

Glutathione *s*-transferase polymorphisms (*GSTM1*, *GSTP1* and *GSTT1*) and the risk of acute leukaemia: A systematic review and meta-analysis

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Abstract

Glutathione *s*-transferase (GST) polymorphisms (*GSTM1*, *GSTP1* and *GSTT1*) have been considered as risk factors for developing acute leukaemia in a number of studies; however the overall results of such studies are inconsistent. To investigate a putative association of GST polymorphisms with the risk of acute leukaemia, we performed a systematic review and meta-analysis of 30 published case–control studies. To take into account the possibility of heterogeneity across the studies, a statistical test was performed. The pooled odds ratios (ORs) were assessed using both a fixed-effects and a random-effects model. The pooled OR of acute leukaemia risks associated with *GSTM1* null genotype, *GSTP1* Val105 allele and *GSTT1* null genotype were 1.22 (95% confidence interval (CI) 1.07–1.38), 1.07 (95% CI 1.00–1.13) and 1.19 (95% CI 1.00–1.41), respectively. Significantly increased risk of acute lymphoblastic leukaemia associated with *GSTM1* and *GSTT1* null genotypes was observed. Their pooled ORs were 1.24 (95% CI 1.17–1.31) and 1.30 (95% CI 1.06–1.60), respectively. We also found substantial evidence of heterogeneity between the studies. Our results suggest that *GSTM1* and *GSTT1*, but not *GSTP1* polymorphisms, appear to be associated with a modest increase in the risk of acute lymphoblastic leukaemia. It is conceivable that *GSTM1* and *GSTT1* null genotypes may thus play a role in leukemogenesis. A review of the 30 case–control studies indicates that greater attention should be paid to the design of future studies.

Keywords: Glutathione *s*-transferase; *GSTM1*; *GSTP1*; *GSTT1*; Acute leukaemia; Meta-analysis; Case–control study; Odds ratio

1. Introduction

Acute leukaemia, including acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML), is a frequent malignancy affecting both children and adults. Despite much investigation, the causes are not yet fully understood. Like many other cancers, acute leukaemia is considered to be a complex disease, which is determined by a combination of genetic and environmental factors [1,2]. There is increasing evidence that predisposition to acute leukaemia is associated with exposure to chemicals such as benzene and chemotherapeutic agents [3,4]. The enzymes involved in the metabolism of these carcinogens have thus received a reasonable level of attention.

Glutathione *s*-transferase (GST) M1, P1 and T1 are phase II enzymes that are involved in conjugation and detoxification of a wide range of xenobiotics, including environmental carcinogens and chemotherapeutic agents. GST polymorphisms have thus been considered

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as possible risk factors of acute leukaemia. Polymorphisms of *GSTM1*, *GSTP1*, and *GSTT1* exist in all populations. The GSTM1*0 (*GSTM1* null) and GSTT1*0 (*GSTT1* null) alleles represent deletions of *GSTM1* and *GSTT1* genes and result in a loss of enzymatic activity [5]. An increased frequency of *GSTM1* and *GSTT1* null genotypes has been associated with a number of human malignancies [6,7]. The 1578 A > G transition in *GSTP1* gives rise to the Ile105Val polymorphism, which confers reduced enzyme activity [8].

GST polymorphisms (*GSTM1*, *GSTP1* and *GSTT1*) were first reported as risk factors for acute leukaemia in 1997 [9,10]. Since then, a number of studies have confirmed or refuted an association between GST polymorphisms and the risk of acute leukaemia [2,4,11–28]. These disparate findings may be due to insufficient power in some studies, differences between cancer types, type of study populations and study design. To investigate the effect of GST polymorphisms and the risk of acute leukaemia, we performed a systematic review and meta-analysis of all the available published case–control studies from January 1997 to July 2004.

2. Methods

2.1. Identification of studies

Studies published between January 1997 and July 2004 with information on *GSTM1*, *GSTP1* or *GSTT1* status and the risk of acute leukaemia were identified using two electronic databases: MEDLINE (National Library of Medicine, Washington, DC, USA) and EMBASE (Elsevier Science, New York, USA), using the search terms '*GSTM1*' or '*GSTP1*' or '*GSTT1*' and 'acute leukaemia'. Additional articles were also checked using the references cited in these publications. Articles selected for analysis were studies with case–control design and their primary references, which had no obvious overlap of cancer cases with other studies. Selected articles should provide enough data to calculate an effect size. One study [29] was excluded because of lack of genotype data. Studies that included groups from different populations were considered separately in our analysis [9,15,21]. When studies had data on the different types of acute leukaemia (e.g., ALL and AML), they were treated as independent studies [13,17]. Chronic leukaemia studies were excluded from our analysis [30] since we focused on acute leukaemia. The application of these criteria yielded 30 case–control studies eligible for meta-analysis [2,4,9–28].

2.2. Statistical analysis

The odds ratio (OR) of acute leukaemia associated with GST polymorphisms was re-calculated for each study, and their corresponding 95% confidence intervals (CI) were estimated by Woolf's method [31,32]. The results might not be exactly the same as those of some studies as different criteria were used in the statistical analysis. We focused on the null alleles of the *GSTM1* and *GSTT1* genes and the Val105 allele of the *GSTP1* gene in this analysis. A low-risk genotype (presence of *GSTM1* or *GSTT1*, homozygous Ile for *GSTP1*) was used as the baseline for calculating ORs. Hardy–Weinberg equilibrium (HWE) was estimated among the controls for *GSTP1* using the χ^2 test. To take into account the possibility of heterogeneity across the studies, a statistical test for heterogeneity was performed based on the Q statistic, in which a P value greater than 0.05 suggested a lack of heterogeneity [33]. If heterogeneity between studies was present, a sensitivity analysis was performed based on the magnitude of Q statistic [34]. Studies contributing the most to the heterogeneity were removed sequentially until homogeneity was achieved.

We carried out meta-analyses using both a fixed-effects [35] and a random-effects model [33]. The fixed-effects model assumes no significant heterogeneity between the results of the individual studies being pooled, whereas the random-effects model allows for such heterogeneity, and they add an empirical estimate of the between-study variance τ^2 to the within-study variance [33,36]. We reported results from a random-effects model if heterogeneity between studies was observed. The analyses were also conducted on the subgroups of studies based on the age, geographic region and ethnic origin. Childhood acute leukaemia was defined as age less than 18 years of age at the time of diagnosis, while adult acute leukaemia cases were those aged 18 and over at the time of diagnosis. Geographic subgroups were defined as three regions (America, Europe and Asia), while ethnic subgroups were considered as three ethnic groups (white, black and Asian).

We assessed potential publication bias via funnel plots, Begg's test [37] and Egger's test [38]. The results of the small studies more widely scattered in the funnel plot than those of the larger studies. In the absence of publication bias, the plot will resemble a symmetrical inverted funnel [39]. To identify gene–gene interaction, the joint effect of GST polymorphisms was also evaluated [33,35]. Since most of the studies assessed the joint effect of *GSTM1* and *GSTT1* null genotypes on the risk of acute leukaemia, we focused on the pooled analysis for these two genotypes. Presence of both *GSTM1* and *GSTT1* genotypes was used as the control reference for each study. All analyses were conducted using KDE 1.9 software (InforSense, London).

3. Results

We identified 30 eligible studies, including nearly 12,000 subjects in relation to GST polymorphisms,

Table 1
Summary of studies on *GSTM1* status and the risk of acute leukaemia

First author (year) [reference]	Country	Cases (*n*)	%*GSTM1*[a] deficiency	Controls (*n*)	%*GSTM1*[b] deficiency	Control source	Tumour types	Age	Odds ratio (95% CI)
Chen (1997) white [9]	USA	163	55.21	213	53.50	Population	ALL	Childhood	1.07 (0.87–1.32)
Chen (1997) black [9]	USA	34	41.18	203	27.60	Population	ALL	Childhood	1.84 (1.25–2.69)
Krajinovic (1999) [11]	Canada	174	64.94	304	51.30	Population	ALL	Childhood	1.76 (1.44–2.14)
Sasai (1999) [12]	Japan	21	47.62	43	53.50	Population	AML	Adult	0.79 (0.46–1.35)
Sasai (1999) [12]	Japan	18	61.11	43	53.50	Population	t-AML	Adult	1.37 (0.77–2.42)
Lemos (1999) [13]	Portugal	22	63.64	128	57.80	Population	ALL	Adult	1.28 (0.79–2.06)
Lemos (1999) [13]	Portugal	18	55.56	128	57.80	Population	AML	Adult	0.91 (0.55–1.51)
Saadat (2000) [16]	Iran	38	55.26	75	32.00	Population	ALL	Childhood	2.63 (1.74–3.95)
Crump (2000) [14]	USA	297	53.54	152	49.30	Hospital	AML	Adult	1.18 (0.97–1.44)
Rollinson (2000) [17]	UK	475	54.32	826	49.30	Population	AML	Adult	1.22 (1.09–1.37)
Rollinson (2000) [17]	UK	70	50.00	113	48.70	Population	ALL	Adult	1.05 (0.78–1.43)
Naoe (2000) [18]	Japan	411	55.23	150	51.30	Population	AML	Adult	1.17 (0.97–1.42)
Woo (2000) white [15]	USA	40	37.50	160	56.90	Hospital	ALL	Childhood	0.45 (0.32–0.65)
Woo (2000) black [15]	USA	7	71.43	38	36.80	Hospital	ALL	Childhood	4.29 (1.74–10.56)
Woo (2000) hispanic [15]	USA	6	66.67	44	43.20	Hospital	ALL	Childhood	2.63 (1.05–6.59)
Davies (2000) white [19]	USA	232	63.79	153	47.10	Population	AML	Childhood	1.98 (1.60–2.45)
Arruda (2001) [2]	Brazil	38	73.68	276	37.00	Hospital	AML	Adult	4.78 (3.24–7.05)
Allan (2001) [4]	UK	417	54.92	1019	48.70	Population	AML	Adult	1.28 (1.14–1.44)
Allan (2001) [4]	UK	89	55.06	1019	48.70	Population	t-AML	Adult	1.29 (1.03–1.61)
Davies (2002) white [21]	USA	616	53.73	532	53.80	Hospital	ALL	Childhood	1.00 (0.89–1.12)
Davies (2002) black [21]	USA	35	40.00	201	31.80	Hospital	ALL	Childhood	1.43 (0.98–2.08)
Haase (2002) [20]	Germany	213	50.23	239	51.00	Population	AML	Adult	0.97 (0.80–1.17)
Alves (2002) [23]	Portugal	47	68.09	102	49.00	Population	ALL	Childhood	2.22 (1.53–3.21)
Balta (2003) [24]	Turkey	139	55.40	185	54.60	Population	ALL	Childhood	1.03 (0.82–1.29)
Balta (2003) [24]	Turkey	31	61.29	185	54.60	Population	ANLL	Childhood	1.32 (0.89–1.96)
Barnette (2004) [25]	USA	94	48.94	336	54.50	Population	ALL	Childhood	0.75 (0.59–0.95)
Canalle (2004) [26]	Brazil	113	42.48	221	45.70	Population	ALL	Childhood	0.88 (0.69–1.11)
Seedhouse (2004) [27]	UK	200	50.50	177	44.10	Population	AML	Adult	1.29 (1.05–1.59)
Seedhouse (2004) [27]	UK	42	45.24	177	44.10	Population	t-AML	Adult	1.05 (0.74–1.48)
Dalo (2004) [28]	Italy	193	42.49	273	46.90	Population	AML	Adult	0.84 (0.69–1.01)

ALL, acute lymphoblastic leukaemia; ANLL, acute non-lymphoblastic leukaemia; AML, acute myeloid leukaemia; t-AML, therapy-related AML.
[a] Frequencies of *GSTM1* deficiency in cases.
[b] Frequencies of *GSTM1* deficiency in controls.

Table 2
Summary of studies on *GSTP1* Val status and the risk of acute leukaemia

First author (year) [reference]	Country	Cases (*n*)	%*GSTP1*[a] Val allele	Controls (*n*)	%*GSTP1*[b] Val allele	Control source	Tumour types	Age	Odds ratio (95% CI)
Rollinson (2000) [17]	UK	472	32.63	823	32.62	Population	AML	Adult	1.01 (0.90–1.13)*
Rollinson (2000) [17]	UK	66	34.85	112	34.82	Population	ALL	Adult	0.97 (0.71–1.32)
Allan (2001) [4]	UK	89	40.45	1015	32.41	Population	t-AML	Adult	1.63 (1.30–2.05)*
Allan (2001) [4]	UK	414	32.97	1015	32.41	Population	AML	Adult	1.01 (0.90–1.13)*
Krajinovic (2002) [23]	Canada	278	33.63	302	31.62	Population	ALL	Childhood	1.26 (1.06–1.49)
Balta (2003) [24]	Turkey	136	23.90	185	24.59	Population	ALL	Childhood	0.99 (0.79–1.24)
Balta (2003) [24]	Turkey	33	25.76	185	24.59	Population	ANLL	Childhood	0.93 (0.63–1.36)
Barnette (2004) [25]	USA	83	33.73	288	34.72	Population	ALL	Childhood	0.91 (0.71–1.17)
Canalle (2004) [26]	Brazil	113	32.30	221	31.45	Population	ALL	Childhood	1.04 (0.83–1.31)

ALL, acute lymphoblastic leukaemia; ANLL, acute non-lymphoblastic leukaemia; AML, acute myloid leukaemia; t-AML, therapy-related AML.
[a] Frequencies of *GSTP1* Val allele in cases.
[b] Frequencies of *GSTP1* Val allele in controls.
* Departure from Hardy–Weinberg equilibrium ($P < 0.05$).

which are summarised in Tables 1–3. Thirteen studies were carried out in European countries, 14 in American countries and 3 in Asian countries. Hospital-based controls were used in 7 studies (Table 1). The numbers in the case–control studies varied considerably (range 45–1436 individuals). The frequencies of genotypes varied in the control participants: the frequency of *GSTM1* null genotype was 32.0–53.5% in Asians, 44.1–57.8% in Europeans and 27.6–56.9% in Americans. The frequency of *GSTP1* Val genotype was 65.2–75.4% in Europeans,

Table 3
Summary of studies on *GSTT1* status and the risk of acute leukaemia

First author (year) [reference]	Country	Cases (*n*)	%*GSTT1*[a] deficiency	Controls (*n*)	%*GSTT1*[b] deficiency	Control source	Tumour types	Age	Odds ratio (95% CI)
Chen (1997) white [9]	USA	163	14.11	213	15.0	Population	ALL	Adult	0.93 (0.69–1.25)
Chen (1997) black [9]	USA	34	35.29	203	24.1	Population	ALL	Adult	1.71 (1.16–2.54)
Basu (1997) [10]	UK	200	21.50	100	23.0	Hospital	AML	Adult	0.92 (0.68–1.23)
Krajinovic (1999) [11]	Canada	176	15.91	274	17.2	Population	ALL	Childhood	0.91 (0.70–1.19)
Sasai (1999) [12]	Japan	22	31.82	43	30.2	Population	AML	Adult	1.08 (0.61–1.90)
Sasai (1999) [12]	Japan	18	66.67	43	30.2	Population	t-AML	Adult	4.62 (2.53–8.41)
Crump (2000) [14]	USA	297	16.16	152	17.1	Hospital	AML	Adult	0.93 (0.72–1.22)
Rollinson (2000) [17]	UK	482	18.46	826	15.1	Population	AML	Adult	1.27 (1.09–1.48)
Rollinson (2000) [17]	UK	70	21.43	113	8.0	Population	ALL	Adult	3.15 (2.00–4.96)
Naoe (2000) [18]	Japan	411	47.98	150	54.0	Population	AML	Adult	0.78 (0.65–0.95)
Woo (2000) white [15]	USA	40	21.98	160	13.8	Hospital	ALL	Childhood	1.33 (0.83–2.14)
Woo (2000) black [15]	USA	7	17.50	38	31.6	Hospital	ALL	Childhood	2.89 (1.25–6.69)
Woo (2000) hispanic [15]	USA	6	57.14	44	18.2	Hospital	ALL	Childhood	2.25 (0.87–5.82)
Davies (2000) white [19]	USA	232	33.33	153	15.0	Population	AML	Childhood	1.59 (1.21–2.10)
Arruda (2001) [2]	Brazil	38	34.21	276	15.9	Hospital	AML	Adult	2.74 (1.88–4.01)
Allan (2001) [4]	UK	417	18.94	1019	13.7	Population	AML	Adult	1.47 (1.26–1.71)
Allan (2001) [4]	UK	89	21.35	1019	13.7	Population	t-AML	Adult	1.70 (1.30–2.24)
Davies (2002) white [21]	USA	616	15.58	532	16.4	Population	ALL	Childhood	0.94 (0.80–1.11)
Davies (2002) black [21]	USA	35	17.14	201	27.9	Population	ALL	Childhood	0.54 (0.33–0.86)
Haase (2002) [20]	Germany	213	23.00	239	15.9	Population	AML	Adult	1.58 (1.24–2.01)
Alves (2002) [23]	Portugal	47	19.15	102	25.5	Population	ALL	Childhood	0.69 (0.45–1.07)
Balta (2003) [24]	Turkey	139	20.86	185	22.7	Population	ALL	Childhood	0.90 (0.68–1.18)
Balta (2003) [24]	Turkey	31	6.45	185	22.7	Population	ANLL	Childhood	0.23 (0.11–0.50)
Barnette (2004) [25]	USA	79	11.11	300	22.0	Population	ALL	Childhood	0.44 (0.30–0.65)
Canalle (2004) [26]	Brazil	113	22.12	221	19.5	Population	ALL	Childhood	1.18 (0.89–1.56)
Dalo (2004) [28]	Italy	193	29.02	273	19.0	Population	AML	Adult	1.73 (1.39–2.17)

ALL, acute lymphoblastic leukaemia; ANLL, acute non-lymphoblastic leukaemia; AML, acute myeloid leukaemia; t-AML, therapy-related AML.
[a] Frequencies of *GSTT1* deficiency in cases.
[b] Frequencies of *GSTT1* deficiency in controls.

65.2–68.4% in Americans. The frequency of *GSTT1* null genotype was 30.2–54.0% in Asians, 8.0–25.5% in Europeans, and 13.8–31.6% in Americans. Of the 30 case–control studies selected for meta-analysis, most of studies had sufficiently detailed methods sections. Methods of recruitment, total numbers and inclusion criteria were generally stated clearly. In all of the studies, GST status was determined by polymerase chain reaction (PCR) assays; many studies reported quality control measurements. The distribution of GST genotypes among control individuals is in agreement with HWE in most of the studies. However, the control groups in three studies [4,17] show departure from HWE, which suggests possible bias in selection of controls [17].

Figs. 1–3 show plots of the ORs (95% CI) of acute leukaemia associated with GST polymorphisms. The funnel plots were symmetrical. Both Egger's test (weighted regression, $P = 0.22$ for *GSTM1* status; $P = 0.59$ for *GSTP1* Val status; $P = 0.80$ for *GSTT1* status) and Begg's test (rank correlation method, $P = 0.77$ for *GSTM1* status; $P = 0.60$ for *GSTP1* Val status; $P = 0.74$ for *GSTT1* status) showed no evidence of publication bias. The overall ORs of the acute leukaemia risks associated with *GSTM1* null genotype, *GSTP1* Val genotype, and *GSTT1* null genotype were 1.22 (95% CI 1.07–1.38), 1.08 (95% CI 1.00–1.13) and 1.19 (95% CI 1.00–1.41), respectively (Table 4). Tests for heterogeneity between studies showed evidence of heterogeneity related to *GSTM1* ($P < 0.01$) and *GSTT1* ($P < 0.01$) status. For *GSTM1* status, exclusion of two outlying studies [2,15] resulted in a Q statistic that was no longer statistically significant (a test of sensitivity). The amended OR was 1.21 (95% CI 1.15–1.26). Similarly, for *GSTT1* status, exclusion of three outlying studies [2,12,25] resulted in a Q statistic that was no longer statistically significant, the amended OR was 1.14 (95% CI 1.09–1.22).

Table 4 summarises the results of the stratified meta-analysis. Several subgroup analyses were carried out, i.e., geographical region; ethnicity; cancer types; sample sizes and control sources (Table 4). In all the subgroup analyses, there was no identifiable evidence of heterogeneity in the analyses of *GSTP1* Val genotype and acute leukaemia risk. However, we observed distinct differences in the analysis of ethnic groups, cancer types and sample sizes associated with *GSTM1* and *GSTT1* status. Thirteen pooled analyses of *GSTM1* and *GSTT1* status showed significant heterogeneity between studies (Table 4). Restricting analyses to ethnic groups, the pooled ORs for *GSTM1* status were 1.15 (95% CI 1.02–1.30)

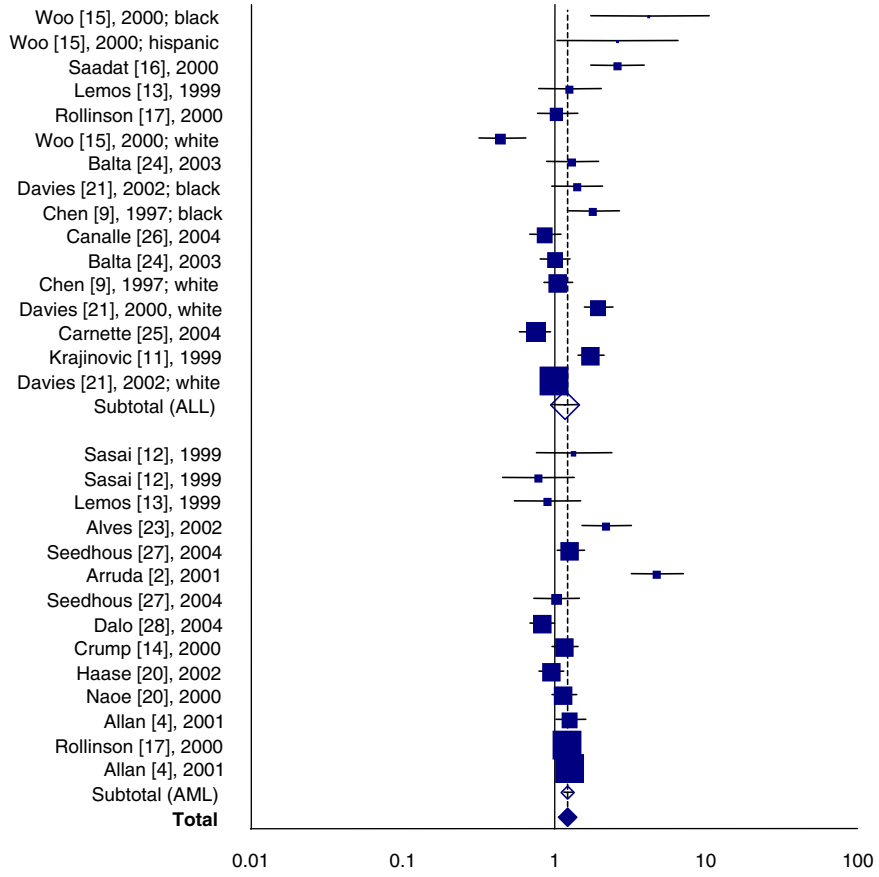

Fig. 1. Funnel plot of odds ratio (OR) of *GSTM1* deficiency and the risk of developing acute leukaemia. Studies stratified by sample size and leukaemia type and are plotted according to the variance of the log (OR). Each box represents the OR estimate and its area is proportional to the weight of the study. The smallest study has a sample size of 45; the largest study has a sample size of 1436.

in whites, 1.76 (95% CI 1.35–2.26) in blacks and 1.29 (95% CI 1.10–1.51) in Asians. There was evidence of heterogeneity between studies in whites ($P < 0.05$). However, when one outlying study was excluded [15], the Q statistic was no longer statistically significant. After excluding this study, the OR was 1.20 (95% CI 1.14–1.25). The overall ORs for *GSTT1* status were 1.19 (95% CI 1.14–1.29) in whites, 1.13 (95% CI 0.90–1.59) in blacks and 1.45 (95% CI 0.46–4.54) in Asians. There was an evidence of heterogeneity across the Asians studies ($P < 0.05$). Exclusion of one outlying Asian study [12] reduced the OR to 0.81 (95% CI 0.68–0.97).

Restricting analyses to cancer types, the pooled ORs for *GSTM1* status were 1.19 (95% CI 0.96–1.47) in ALL and 1.24 (95% CI 1.16–1.32) in AML. Heterogeneity between studies was shown for both ALL ($P < 0.01$) and AML ($P < 0.025$). For ALL, exclusion of one outlying study [15] resulted in a Q statistic that was no longer statistically significant and an amended OR of 1.16 (95% CI 1.08–1.24). Similarly, for AML, exclusion of one outlying study [2] made the Q statistic non-significant, but did not change the OR. For *GSTT1* status, the ORs were 0.97 (95% CI 0.90–1.08) for ALL and 1.30 (95% CI 1.06–1.60) for AML. Stratifying the meta-analysis by sample size, the pooled ORs for *GSTM1* status were 1.17 (95% CI 1.12–1.22) in studies with at least 100 cases and 100 controls and 1.80 (95% CI 1.39–2.34) in studies with fewer than 100 cases and 100 controls. For *GSTT1* status, the pooled ORs were 1.16 (95% CI 1.09–1.24) with at least 100 cases and 100 controls and 2.25 (95% CI 1.60–3.19) with fewer than 100 cases and 100 controls. There was no evidence of heterogeneity in strata of this subgroup analysis.

We also estimated the joint effect of GST genotypes on the risk of acute leukaemia. Only one study evaluated the joint effect of the three GST loci on the risk of acute leukaemia (OR = 2.8; 95% CI 1.1–6.9) [21]. Most of studies assessed the joint effect of *GSTM1* and *GSTT1* null genotypes on the risk of acute leukaemia. Fifteen two-loci case–control studies were identified. One additional study was excluded because of an extreme result (OR = 7.50; 95% CI; 4.36–12.89). The pooled OR for the 15 studies was 1.14 (95% CI 1.04–1.26) (Fig. 4).

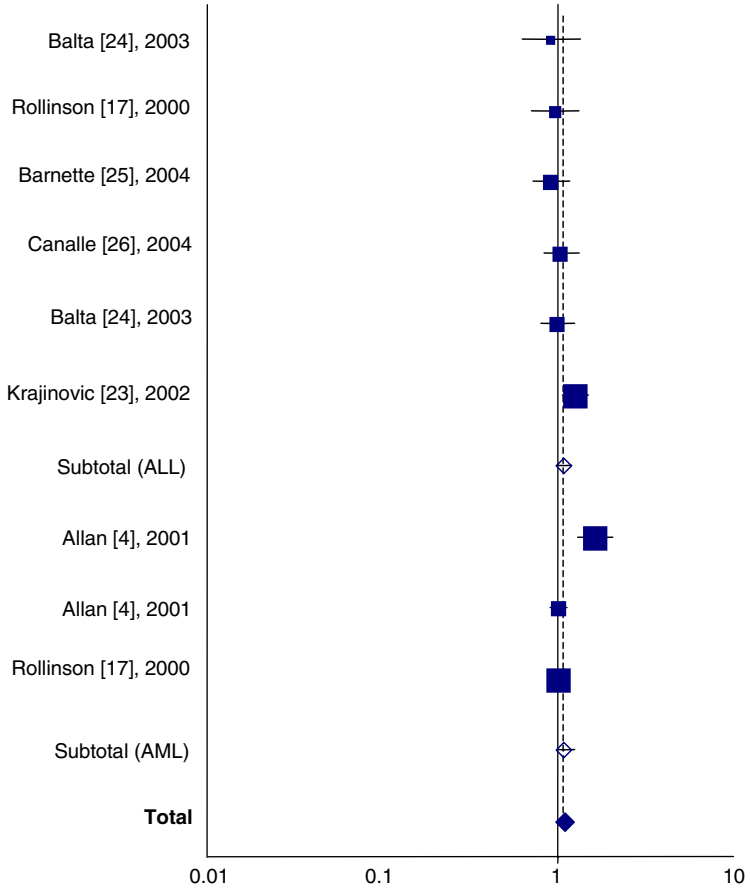

Fig. 2. Funnel plot of odds ratio (OR) of *GSTP1* Val genotype and the risk of developing acute leukaemia. Studies stratified by sample size and leukaemia type and are plotted according to the variance of the log (OR). Each box represents the OR estimate and its area is proportional to the weight of the study. The smallest study has a sample size of 178; the largest study has a sample size of 1429.

4. Discussion

GST polymorphisms (*GSTM1*, *GSTP1* and *GSTT1*) have been evaluated as risk factors for cancers in a number of studies. Molecular epidemiological studies indicate that individuals lacking the genes *GSTM1* and *GSTT1* are more likely to develop cancer than those having these genes [5]. Based upon this meta-analysis of 30 case–control studies, *GSTM1* and *GSTT1*, but not *GSTP1* polymorphisms, appear to be associated with a modest increase in the risk of acute lymphoblastic leukaemia (AML). It is conceivable that *GSTM1* and *GSTT1* null genotypes may thus play a role in leukemogenesis. The pooled analysis of both *GSTM1* and *GSTT1* null genotypes produced a similar risk estimate.

There is evidence of heterogeneity between studies in this systematic review and meta-analyses. Four studies were the major sources of heterogeneity [2,12,15,25], but the reasons for this are unclear. It might be due to uncontrolled confounding and inherent bias of study design. In some studies, the study design has important oversights, for example, some studies used small sample sizes [2,12,15]. Two studies used samples with highly heterogeneous ethnic origin [2,12]. Some studies recruited subjects from multi-centre and blood donors [9,11,15,18–22] or hospital-based controls were used [15]. Selection bias may be a major source of heterogeneity, therefore such bias was reduced by removing studies in the sensitivity analyses. Although there is some evidence of heterogeneity across studies, which will produce an overestimate of the true association, studies that contribute to heterogeneity do not significantly alter the estimate of the overall OR and result in a Type I error.

To clarify an association between genotypes and cancer risks, sample size is considered to be a crucial factor in the design of case–control studies. Some case–control studies evaluating GST polymorphisms as risk factors for acute leukaemia had small sample sizes. Since the pooled ORs in studies with larger sample size ($\geqslant$100 cases and controls) are approximately the same in all the studies (Table 4), the studies with small sample size (<100 cases and controls) appear to overestimate the true association due to lack of sufficient power to detect such an association. Although it is known that a well-defined small study can provide a more accurate assess-

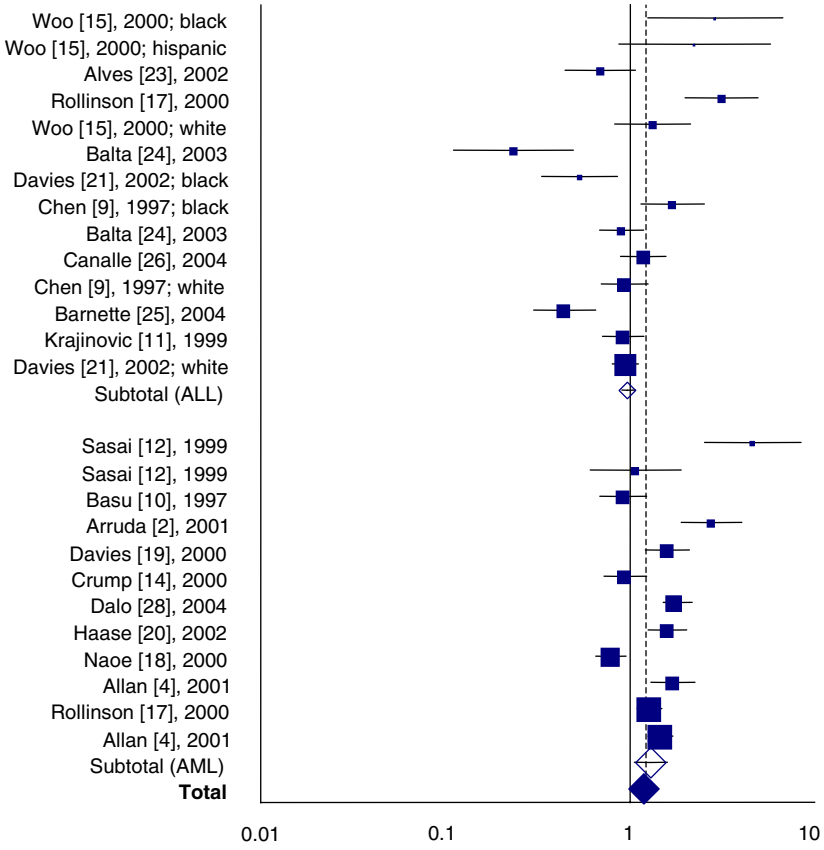

Fig. 3. Funnel plot of odds ratio (OR) of *GSTT1* deficiency and the risk of developing acute leukaemia. Studies stratified by sample size and leukaemia type and are plotted according to the variance of the log (OR). Each box represents the OR estimate and its area is proportional to the weight of the study. The smallest study has a sample size of 45; the largest study has a sample size of 1436.

ment of risk than a poorly designed large study, large sample size with adequate power is one of the important factors in the design of case–control studies and will better reflect the association of genotypes and cancer risks.

It is clear from this overview that the design of some of case–control studies is suboptimal. Some of the studies analysed were based upon a comparison of the cancer cases and hospital-based controls. The pooled ORs for studies using hospital-based controls were higher than those studies using population-based controls (Table 4). Since it is conceivable that *GSTM1* and *GSTT1* genes might confer susceptibility to non-cancer disease, their genotype frequencies might be different between the population-based and hospital-based controls (non-cancer diseases) and this might introduce heterogeneity between studies. The use of population-based controls is, therefore, more appropriate in the association studies.

It is now widely accepted that differences in the distribution of various ethnicities between cases and controls may be a source of confounding when pooling studies [40]. The association between cancer and a particular polymorphic site in one population might be of limited value as a biomarker for cancer in another population. In the pooled analysis, the frequencies of GST genotypes showed distinct differences in whites, blacks and Asians (Tables 1–3). Stratification by ethnicity was considered in the meta-analysis of studies of association between GST genotypes and the risk of acute leukaemia. For example, the result of the pooled OR associated with *GSTM1* polymorphism is the highest in blacks, followed by Asians and whites (Table 4).

To the best of our knowledge, all the available case–control studies of GST polymorphisms associated with acute leukaemia risk published prior to July 2004 have been included in this meta-analysis. Based upon these studies, we carried out comprehensive meta-analyses of GST polymorphisms and the risk of acute leukaemia. It was noted that only one study evaluated the association between GST polymorphisms by subtype of acute leukaemia (i.e., B-lineage ALL and T-lineage ALL) [21]. We were unable effectively to assess such an association based upon such limited data available. Hence, more studies including information on the subtype of acute leukaemia will be required to clarify the relationship between the GST polymorphisms and subtype of acute leukaemia. In addition, publication bias, which can occur when studies with null or unexpected results

Table 4
Meta-analysis of case–control studies of *GSTM1*, *GSTP1* and *GSTT1* status and the risk of acute leukaemia

	GSTM1			*GSTP1*			*GSTT1*		
	Number of studies	OR for null genotype (95% CI)	Heterogeneity across studies	Number of studies	OR for Val allele (95% CI)	Heterogeneity across studies	Number of studies	OR for null genotype (95% CI)	Heterogeneity across studies
All studies	30	1.22 (1.07–1.38)	Yes	9	1.08 (1.00–1.13)	No	26	1.19 (1.00–1.41)	Yes
Europe only	13	1.17 (1.10–1.24)	No	6	1.08 (1.01–1.15)	No	10	1.32 (1.25–1.45)	No
America only	13	1.30 (1.00–1.72)	Yes	3	1.11 (0.90–1.36)	No	13	1.04 (0.98–1.16)	No
Asia only	4	1.29 (1.10–1.51)	No				3	1.41 (0.52–3.87)	Yes
Ethnic groups									
Whites	20	1.15 (1.02–1.30)	Yes	8	1.09 (1.01–1.16)	No	18	1.19 (1.14–1.29)	No
Blacks	3	1.76 (1.35–2.26)	No				3	1.13 (0.90–1.59)	No
Asians	4	1.29 (1.10–1.51)	No				3	1.45 (0.46–4.54)	Yes
Cancer types									
ALL	16	1.19 (0.96–1.47)	Yes	6	1.07 (0.97–1.19)	No	14	0.97 (0.90–1.08)	No
AML	14	1.24 (1.16–1.32)	Yes	3	1.07 (0.99–1.15)	No	12	1.30 (1.06–1.60)	Yes
Childhood									
ALL	13	1.21 (0.94–1.55)	Yes	5	1.07 (0.97–1.19)	No	13	0.93 (0.86–1.03)	No
AML	1	1.98 (1.60–2.45)	No				1	1.59 (1.21–1.20)	No
Adults									
ALL	2	1.12 (0.86–1.44)	No	1	0.97 (0.71–1.32)	No	1	3.15 (2.00–4.96)	No
AML	14	1.20 (1.13–1.26)	No	3	1.07 (0.99–1.15)	No	11	1.36 (1.08–1.71)	Yes
Number of cases and controls									
<100 cases and <100 controls	5	1.80 (1.39–2.34)	No				4	2.25 (1.60–3.19)	No
$\geqslant$100 cases and $\leqslant$100 controls	12	1.17 (1.12–1.22)	No	6	1.05 (0.98–1.12)	No	12	1.16 (1.09–1.24)	No
Control sources									
Population-based	23	1.21 (1.15–1.26)	No	9	1.08 (1.00–1.13)	No	20	1.16 (0.95–1.41)	Yes
Hospital-based	7	1.40 (0.86–2.27)	Yes				6	1.22 (1.06–1.46)	No

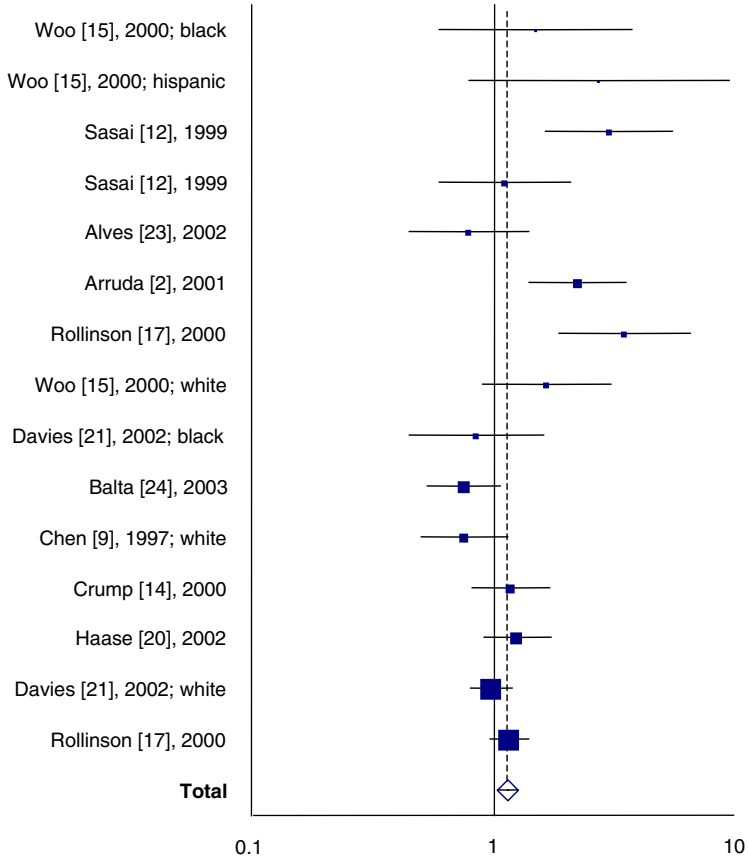

Fig. 4. Funnel plot of odds ratio (OR) of both *GSTM1* and *GSTT1* deficiency and the risk of developing acute leukaemia. Studies stratified by sample size and are plotted according to the variance of the log (OR). Each box represents the OR estimate and its area is proportional to the weight of the study. The smallest study has a sample size of 45; the largest study has a sample size of 1301.

are not published, is of concern. Therefore, we cannot exclude the effect of potential publication bias on our meta-analysis.

Conflict of interest statement

None declared.

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