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Genetic polymorphisms in Glutathione S-transferase Omega (GSTO) and cancer risk: a meta-analysis of 20 studies

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Glutathione S-transferase Omega (GSTO) plays an important role in the development of cancer. Recently, a number of studies have investigated the association between single nucleotide polymorphisms on GSTO and susceptibility to cancer; however, the results remain inconclusive. We performed a meta-analysis of 20 studies, involving 4770 cases and 5701 controls to identify the strength of association by pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Overall, the pooled results revealed a significantly increased risk of susceptibility for GSTO2 polymorphism (GG vs. AA: OR = 1.20, 95%CI: 1.02–1.41, $P_{heterogeneity}$ = 0.116), but no significant association was found for GSTO1 polymorphism. Subgroup analysis showed that GSTO2 polymorphism significantly increased cancer risk in Caucasian population (GG vs. AA: OR = 1.32, 95%CI 1.06–1.64, $P_{heterogeneity}$ = 0.616) and GSTO2 polymorphism was significantly associated with elevated risk of breast cancer (GG vs. AA OR = 1.37, 95%CI: 1.06–1.77; $P_{heterogeneity}$ = 0.281). This meta-analysis demonstrates that GSTO2 polymorphism may significantly increase cancer risk in Caucasian population and is associated with elevated risk of breast cancer; while GSTO1 polymorphism is not associated with cancer risk.

Glutathione S-transferases (GSTs) are a family of Phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to various endogenous and exogenous electrophilic compounds[1]. Up to now, human cytosolic GST super family contains at least 16 genes subdivided into eight distinct classes designated as: Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta[2,3]. GSTs possess both enzymatic and non-enzymatic functions and are involved in many important cellular processes, such as, phase II metabolism, stress response, cell proliferation, apoptosis, oncogenesis, tumor progression and drug resistance[4]. Many studies have explored the association between single nucleotide polymorphisms (SNPs) of GSTs and susceptibility to various cancers. Clinical association studies have shown that genetic alterations within the human GST isozymes may play a key role in cancer susceptibility and treatment[5]. For example, GSTM1 and GSTP1 genetic polymorphisms are associated with increased risk of breast cancer[6] and hepatocellular carcinoma[7].

As a member of GSTs, glutathione S-transferase Omega (GSTO) has two members, named GSTO1 and GSTO2. Three polymorphisms in hGSTO genes: hGSTO1*A140D, hGSTO1*E155del and hGSTO2*N142D have been identified[8]. Numerous case-control studies have been performed to investigate the association between hGSTO1*A140D and hGSTO2*N142D and cancer risk in the last decades. But the results were inconsistent. Several investigators have reported an increased risk of breast cancer, hepatocellular carcinoma, bile duct carcinoma, urothelial cancer, acute lymphoblastic leukemia and non-small cell lung cancer for the GSTO1 A140D[9–12]. However, Granja et al.[13] and Marahatta et al.[14] did not find any significant association in thyroid and colorectal cancers. In addition, there was no evidence for association of GSTO1 or GSTO2 polymorphism and breast cancer risk in the study performed by Irena E. Andonova et al.[15]. After literature research, we did not find any previous genome-wide association studies (GWAS) relevant to the polymorphism of GSTO.

Therefore, we performed this meta-analysis to explore the association strength of GSTO1 and GSTO2 polymorphism with cancer risk.

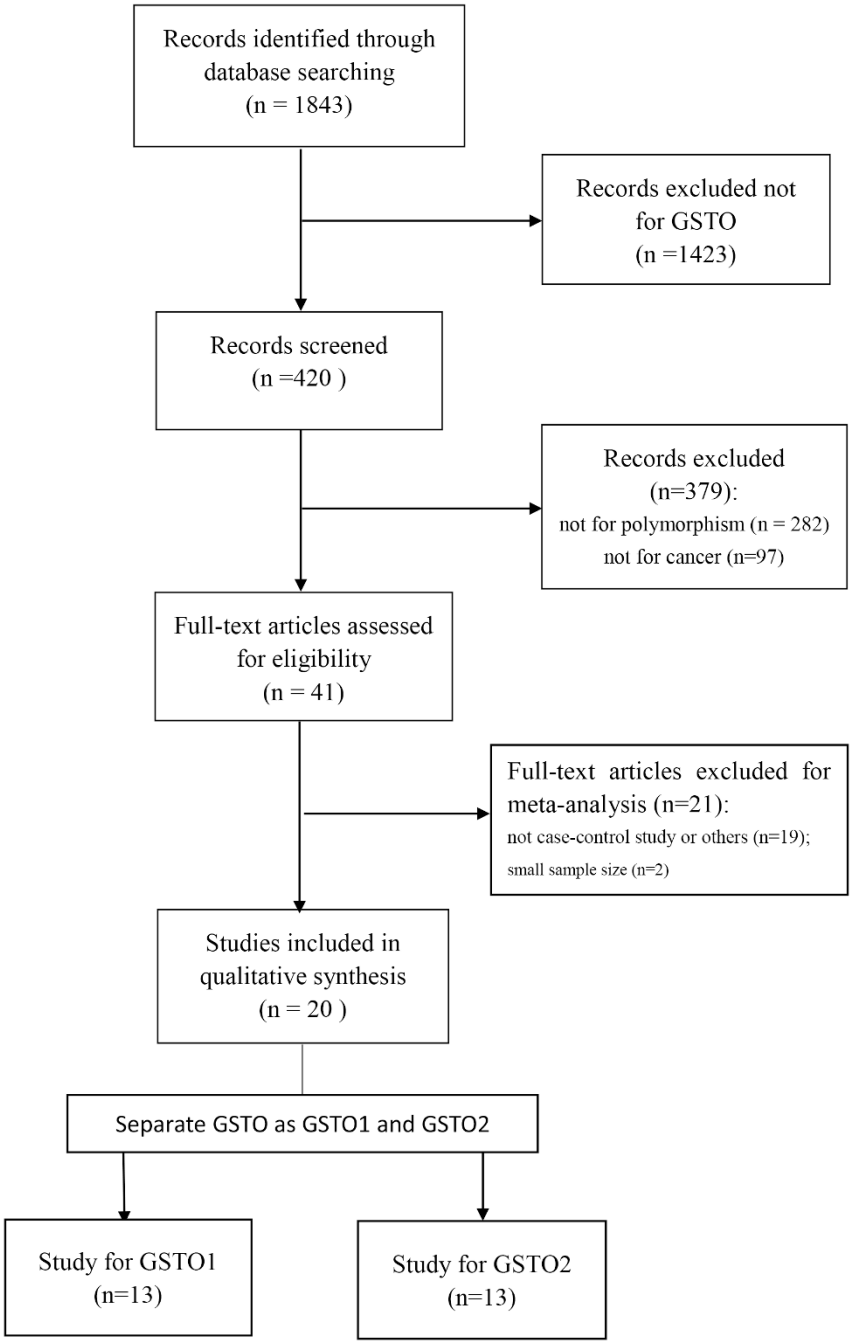

Figure 1 | Flow chart of studies in the analysis.

Results

Characteristics of eligible studies. In total, 20 articles[9–29] were identified according to the inclusion and exclusion criteria. The flow chart of literature search and study selection was illuminated in Figure 1. After deleting the duplicate articles, 1843 articles were obtained in total. We read the title and abstract, and then screened out 420 studies for GSTO. Among these, 379 articles not for polymorphism (282) or not for cancer (97) were excluded. Then, the remaining 41 articles underwent further identification and 19 articles were not included owing to not case-control-designed study or not enough information on the association between GSTO polymorphism and cancer risk. Two articles[14,30] were excluded for the reason of not for cancer susceptibility and small sample size (less than 50). Among the rest of 20 articles, some researched both GSTO1 and GSTO2 polymorphism, so we regarded one study as two separate ones. Specifically, 13 studies for GSTO1 and 13 studies for GSTO2 were analyzed in our meta-analysis. Table 1 shows the detailed characteristics of the eligible studies included in this meta-analysis.

Among the 20 articles, 5 of them were studies of Caucasian[9,12,15,19,27], 10 studies were of Asian[10,11,17,18,20,24–26,28,29] and the rest were study of different races (white and non-white groups)[13,16,21–23]. Cancer cases were all diagnosed histologically or pathologically in these studies. Polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) assay, TaqMan genotyping assay, matrix assisted laser desorption/ionizationtime-of-flight mass spectrometry (MALDI-TOF MS) assay, a custom Illumina Golden Gate 96SNP panel array, and polymerase chain reaction–single strand conformation polymorphism (PCR-SSCP)-sequencing approach were used as genotyping methods in 15, 1, 1, 1 and 2 articles respect-

Table 1 | Characteristics of eligible studies

Author	Year	Country	Ethnicity	Cancer	Sources of control	Genotyping method	Cases	Controls	Case			Control			P_{hwe}
GSTO1 polymorphism									CC	CA	AA	CC	CA	AA	
Granja[13]	2005	Brazil	mixed	TC	PB	PCR-SSCP	93	173	74	17	2	146	19	8	<0.001
Bufalo[21]	2006	Brazil	mixed	TC	PB	PCR-RFLP	73	184	59	14		159	25		
Olsen[9]	2008	Denmark	Caucasian	BC	PB	PCR-RFLP	396	396	121	199	76	123	210	63	0.089
Lima-Jr[16]	2008	Brazil	mixed	PC	HB	PCR-SSCP	125	100	116	6	3	92	4	4	<0.001
Pongstaporn[10]	2009	Thailand	Asian	ALL	HB	PCR-RFLP	99	100	59	40	0	76	23	1	0.607
Wang[11]	2009	Taiwan	Asian	UC	HB	PCR-RFLP	520	520	368	135	17	356	153	11	0.243
Andonova[15]	2010	Germany	Caucasian	BC	PB	MALDI-TOF MS	1000	992	396	509	95	429	456	107	0.384
Chariyalertsak[17]	2009	Thailand	Asian	BC	HB	PCR-RFLP	101	151	80	20	1	117	33	1	0.414
Chung[18]	2011	Taiwan	Asian	UC	HB	PCR-RFLP	149	251	107	41	1	166	78	7	0.546
Beebe-Dimmer[15]	2012	USA	Caucasian	UC	PB	Taqman	212	266	97	98	17	123	113	30	0.601
Sanguansin[20]	2012	Thailand	Asian	HNSCC	HB	PCR-RFLP	300	299	231	67	2	234	62	3	0.619
Guzide ADA[12]	2013	Turkey	Caucasian	LC	PB	PCR-RFLP	172	214	82	77	13	104	87	23	0.456
Min-Che Tung[29]	2014	Taiwan	Asian	UC	HB	PCR-RFLP	300	233	230	70		150	79		
GSTO2 polymorphism									AA	AG	GG	AA	AG	GG	
Morari[22]	2006	Brazil	mixed	OC	PB	PCR-RFLP	69	222	24	37	8	87	104	31	0.993
Leite[23]	2007	Brazil	mixed	SC	PB	PCR-RFLP	88	124	37	38	13	40	62	22	0.811
Masoudi[24]	2009	Iran	Asian	GC	PB	PCR-RFLP	67	134	33	29	5	54	61	19	0.791
Pongstaporn[10]	2009	Thailand	Asian	ALL	HB	PCR-RFLP	99	100	57	34	8	63	30	7	0.208
Wang[11]	2009	Taiwan	Asian	UC	HB	PCR-RFLP	520	520	298	175	47	294	195	31	0.859
Masoudi[25]	2010	Iran	Asian	BC	PB	PCR-RFLP	181	181	74	75	32	80	78	23	0.561
Andonov[15]	2010	Germany	Caucasian	BC	PB	MALDI-TOF MS	1004	997	425	456	123	442	453	102	0.371
Chariyalertsak[17]	2009	Thailand	Asian	BC	HB	PCR-RFLP	101	151	59	38	4	86	60	5	0.155
Chung[15]	2011	Taiwan	Asian	UC	HB	PCR-RFLP	149	251	88	59	2	134	104	13	0.207
Masoud[26]**i**	2011	Iran	Asian	CRC	PB	PCR-RFLP	63	126	30	25	8	52	55	19	0.482
Lesseur[27]	2012	USA	Caucasian	UC	PB	CIGG	658	928	255	321	82	398	439	91	0.057
Sanguansin[20]	2012	Thailand	Asian	HNSCC	HB	PCR-RFLP	300	299	170	112	18	171	109	19	0.771
Sohail[28]	2013	Pakistan	Asian	BC	PB	PCR-RFLP	100	102	0	10	90	7	28	67	0.105

PB: population-based; HB: hospital-based; P_{hwe}: Hardy-Winberg equilibrium; TC: thyroid carcinoma; BC: breast cancer; PC: prostate carcinoma; ALL: acute lymphoblastic leukemia; UC: urothelial carcinoma; HNSCC: head and neck squamous cell carcinoma; LC: lung cancer; OC: ovarian carcinoma; SC: skin cancer; GC: gastric cancer CRC: colorectal cancer; CIGG: custom Illumina Golden Gate 96 SNP panel array.

Table 2 | Meta-analysis results of GSTO1 polymorphism

	N	Case/Control	AA vs CC	P_h	CA vs CC	P_h	AA vs CA/CC	P_h	AA/CA vs CC	P_h	A vs C	P_h
Total	12	3240/3646	0.95(0.78,1.16)	0.692	1.09(0.97,1.21)	0.176	0.92(0.76,1.11)	0.474	1.01(0.87,1.18)	0.049	1.02(0.94,1.11)	0.601
Cancer												
BC	3	1497/1539	1.05(0.82,1.34)	0.639	1.12(0.96,1.31)	0.357	1.00(0.80,1.26)	0.293	1.11(0.96,1.29)	0.618	1.06(0.95,1.18)	0.888
UC	3	881/1037	0.87(0.54,1.39)	0.147	0.91(0.74,1.11)	0.293	0.86(0.55,1.36)	0.117	0.82(0.70,0.98)	0.162*	0.91(0.78,1.07)	0.563
Others	6	862/1070	0.65(0.37,1.14)	0.992	1.30(1.03,1.65)*	0.985	0.61(0.35,1.07)	0.985	1.22(0.98,1.52)	0.391	1.07(0.88,1.30)	0.346
Sources of control												
PB	6	1946/2225	1.01(0.81,1.25)	0.328	0.98(0.82,1.18)	0.568	0.92(0.75,1.12)	0.264	1.12(0.99,1.27)	0.852	1.04(0.94,1.14	0.887
HB	6	1294/1421	0.99(0.54,1.82)	0.473	1.08(0.59,2.00)	0.107	0.95(0.54,1.65)	0.500	0.92(0.71,1.21)	0.026	0.98(0.84,1.14)	0.240
Ethnicity												
Asian	5	1169/1231	0.99(0.54,1.82)	0.473	1.03(0.77,1.39)	0.062	1.02(0.56,1.86)	0.432	0.93(0.69,1.24)	0.014	0.99(0.84,1.16	0.166
Caucasian	4	1780/1868	0.97(0.78,1.21)	0.445	1.13(0.99,1.30)	0.682	0.93(0.76,1.14)	0.218	1.10(0.96,1.26)	0.842	1.03(0.94,1.14)	0.788
Mixed	3	291/457	0.54(0.18,1.61)	0.867	1.60(0.86,3.00)	0.600	0.51(0.17,1.52)	0.812	1.31(0.85,2.03)	0.685	1.01(0.63,1.61)	0.483
Sample size												
Large	4	2216/1439	1.08(0.85,1.36)*	0.606	1.00(0.79,1.25)	0.118	1.03(0.83,1.28)	0.284	0.95(0.76,1.18)	0.022	1.04(0.95,1.15)	0.885
Small	8	1024/2207	0.64(0.42,0.98)*	0.946	0.57(0.37,0.88)*	0.879	0.62(0.41,0.93)*	0.948	1.08(0.90,1.29)	0.239	0.96(0.83,1.13)	0.327

N: number of studies included; OR: odds ratio; P_h: p value for heterogeneity; BC: breast cancer; UC: urothelial carcinoma; PB: population-based; HB: hospital-based;
*OR with statistical significance.

ively. Blood sample was used for genotyping in all studies. In our meta-analysis, 2 studies about GSTO1*A140D polymorphism[9,13] were deviated from HWE.

The GSTO1 polymorphism. 13 eligible studies, involving 3540 cancer cases and 3879 controls, were pooled for the analysis of GSTO1 polymorphism. No significant association of GSTO1 polymorphism with cancer risk was observed in any of the five comparison models (Table 2). Similarly, In the subgroup analysis by ethnicity, sources of control or cancer types, we did not find any significant association between the GSTO1 polymorphism and cancer risk, except that an increased cancer risk was found in the heterozygote comparison model (CA vs. CC: OR = 1.30, 95%CI 1.03–1.65, $P_{heterogeneity}$ = 0.985) for other cancers and a decreased cancer risk was found in the dominant model (AA/AC vs. CC: OR = 0.82, 95%CI 0.70–0.98, $P_{heterogeneity}$ = 0.985) for urothelial carcinoma.

The GSTO2 polymorphism. By pooling 13 eligible studies with 3399 cancer cases and 4135 controls, we observed a significantly increased risk of cancer susceptibility in homozygote comparison model (GG vs. AA: OR = 1.20, 95%CI: 1.02, 1.41, $P_{heterogeneity}$ = 0.116; Figure 2) for GSTO2 polymorphism, but no significant association was found in other comparison models (Table 3).

Then we performed subgroup analyses to investigate the effect of ethnicity, cancer types and sources of control. As for cancer types, there was a statistically increased cancer risk for breast cancer (GG vs. AA: OR = 1.37, 95%CI: 1.06–1.77; $P_{heterogeneity}$ = 0.281; Figure S1). As for ethnicity, increased cancer risk was found in Caucasian in the homozygote comparison model (GG vs. AA: OR = 1.32, 95%CI 1.06–1.64, $P_{heterogeneity}$ = 0.616; Figure S2), recessive comparison model (GG vs. AG/AA: OR = 1.26, 95%CI 1.02–1.55, $P_{heterogeneity}$ = 0.757; Figure S3) and allelic comparison model (G vs. A: OR = 1.12, 95%CI 1.02–1.24, $P_{heterogeneity}$ = 0.556; Figure S4).

Heterogeneity. Heterogeneity between studies in each comparison model was shown in Table 2 and 3. No significant heterogeneity was found for GSTO1 polymorphism, but for GSTO2, obvious heterogeneity was detected in two comparison models (GG vs. GA/AA, P = 0.019; G vs. A, P = 0.002). Meta-regression revealed that ethnicity, cancer types, sample size and sources of control did not contributed to the source of heterogeneity ($\tau^2 > 0.05$).

Sensitivity analysis. To examine the stability and reliability of our meta-analysis results, we performed sensitivity analyses by repeatedly deleting the single studies each time from pooled analysis. Our analysis showed that the omission of individual studies did not materially alter the results because the recalculated ORs and 95%CIs were not quantitatively changed, suggesting that the results were robust and convincing. (Figures not shown).

Publication bias. Begg's funnel plot and Egger's test were performed to assess the publication bias of literatures, and we did not find any publication bias for GSTO1 or GSTO2 polymorphism in all compassion models.

Discussion

GSTs are a family of phase II detoxifying enzymes that catalyze the conjugation of glutathione to a wide variety of electrophilic compounds. Besides detoxifying electrophilic xenobiotics such as chemical carcinogens, environmental pollutants, and antitumor agents, these transferases inactivate endogenous alpha, beta-unsaturated aldehydes, quinones, epoxides, and hydroperoxides formed as secondary metabolites during oxidative stress[31]. GSTs play important roles in the protection of cells against foreign compounds and cellular stress, and may consequently play a role in the development of cancer[32]. And due to high expression of GSTs in tumors when com-

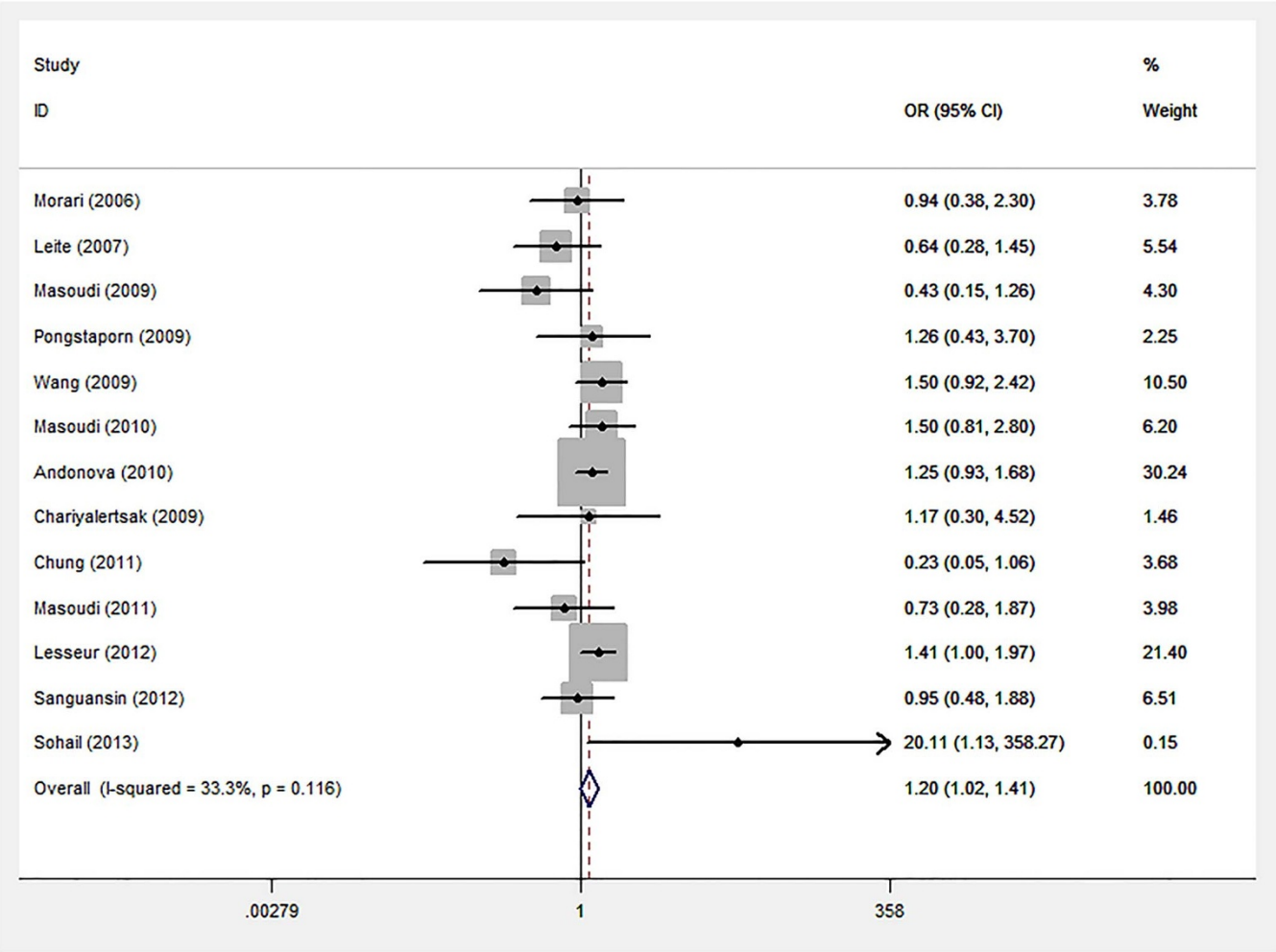

Figure 2 | Forest plot of homozygote comparison for overall comparison (GG vs. AA).

pared to normal tissues and their high level in plasma from cancer patients, these enzymes were considered to be cancer markers[33]. Recently, many studies have demonstrated the association between SNPs of GSTs and cancer risk. Study conducted by Safarinejad, M.R et al.[34] suggested that the GSTP1 polymorphism and its combination with GSTM1, and GSTT1 may be associated with bladder cancer susceptibility. Yang et al.[35] also found that GSTT1 null genotype contributes to lung cancer risk in Asian populations.

Unlike other GSTs, GSTO has an active site cysteine that is able to form a disulfide bond with GSH and exhibits glutathione dependent dehydroascorbate reductase and thiol transferase activities, reminiscent of glutaredoxin and thioredoxin enzymes[36]. Expression of GSTO is abundant in a wide range of normal tissues, including the liver, colon, heart, ovary, pancreas, prostate and spleen. The widespread distribution of GSTO suggests that it has important biological functions[8]. GSTO participates in cellular signalling and overexpression of GSTO has been reported to be linked with the induction of apoptosis involving the development of cancer[37]. Additionally, GSTO was shown to promote activation of the pro-inflammatory cytokine, interleukin-1β (IL-1β) by post-translational processing[38]. Thus, association between GSTO and cancer risk has been explored in some studies. Marahatta et al.[14] demonstrated that GSTO1*A140D polymorphism could play an important role for the development of cholangiocarcinoma, breast cancer and hepatocellular carcinoma, and Mohammad Masoudi[26] and his colleagues indicated that GSTO2 NN genotype increase the risk of colorectal cancer. On the contrary, GSTO1 and GSTO2 variants were not associated with breast cancer risk in some study[17].

Given the inconsistent results from individual studies, we decided to explore the association between GSTO polymorphisms and cancer risk. In the present meta-analysis, 20 eligible studies including 4770 cases and 5701 controls, were identified and analyzed. Our results showed that there was no significant association between the GSTO1 polymorphism and susceptibility to cancer. Similarly, subgroup analyses by cancer type, source of control or ethnicity did not suggest a significantly different result. As for GOTS2 polymorphism, we can observe an increased risk of overall cancer and breast cancer. In addition, it is worth noting that the association between GSTO2 polymorphism and cancer risk was significant in Caucasian populations. We demonstrated an increased cancer risk in Caucasian for GSTO2 polymorphism, specifically in the homozygote comparison model, recessive comparison model and allelic comparison model. However, in our meta-analysis for GSTO2, only two study were conducted in Caucasian race totally. Corina Lesseur et al.[27] found bladder cancer risk overall was associated with GSTO2 Asn142Asp. Whereas Irena E. Andonova et al.[15] did not find any evidence for GSTO2 in breast cancer risk. So, a conclusion the GOST2 polymorphism increasing the cancer risk in Caucasian may not be convincing that much.

As we mentioned before, two studies[9,13] were deviated from HWE and two study could not be calculated for HWE due to its incomplete data[21,29]. Traditionally speaking, any study that deviated from HWE should have been removed. However, Minelli et al.[39] pointed out that unless there are other grounds for doubting the quality of the study, studies that appear to deviate from HWE should be investigated further rather than just excluded. Until now, it is still inconclusive

Table 3 | Meta-analysis results of GSTO2 polymorphism

	N	Case/Control	GG vs AA	P_h	AG vs AA	P_h	GG vs AG/AA	P_h	GG/AG vs AA	P_h	G vs A	P_h
Total	13	3399/4135	1.20(1.02,1.41)*	0.116	1.01(0.92,1.12)	0.735	1.18(0.91,1.52)	0.019	1.04(0.95,1.15)	0.331	1.04(0.91,1.19)	0.002
Cancer type												
BC	4	1386/1431	1.37(1.06,1.77)*	0.281	1.04(0.89,1.23)	0.697	1.76(0.98,3.18)	0.015	1.10(0.95,1.28)	0.294	1.38(0.93,2.04)	0.001
GC	2	130/260	0.57(0.28,1.16)	0.469	0.78(0.50,1.23)	0.978	0.65(0.33,1.27)	0.456	0.73(0.48,1.12)	0.807	0.76(0.55,1.04)	0.616
UC	3	1327/1699	1.18(0.68,2.07)	0.066	1.01(0.86,1.17)	0.249	1.19(0.70,2.02)	0.071	1.05(0.91,1.21)	0.167	1.03(0.85,1.25)	0.092
Others	4	556/745	0.89(0.59,1.35)	0.780	1.03(0.81,1.31)	0.388	0.90(0.60,1.33)	0.939	1.01(0.80,1.27)	0.365	0.98(0.82,1.17)	0.505
Sources of control												
PB	8	2230/2814	1.11(0.82,1.51)	0.092	1.05(0.93,1.18)	0.52	1.22(0.87,1.69)	0.011	1.08(0.97,1.22)	0.154	1.08(0.89,1.32)	<0.001
HB	5	1169/1321	1.12(0.80,1.57)	0.217	0.94(0.80,1.12)	0.832	1.15(0.82,1.59)	0.199	0.97(0.83,1.14)	0.77	1.00(0.88,1.14)	0.515
Ethnicity												
Asian	9	1580/1864	1.03(0.68,1.56)	0.074	0.94(0.81,1.09)	0.879	1.18(0.75,1.85)	0.006	0.90(0.91,1.29)	0.452	0.86(0.69,1.08)	0.019
Caucasian	2	1662/1925	1.32(1.06,1.64)*	0.616	1.09(0.94,1.25)	0.550	1.26(1.02,1.55)*	0.757	1.13(0.99,1.29)	0.515	1.12(1.02,1.24)*	0.556
Mixed	2	157/346	0.76(0.41,1.39)	0.539	0.93(0.62,1.42)	0.121	0.81(0.46,1.40)	0.992	0.89(0.60,1.33)	0.135	0.89(0.68,1.18)	0.276
Sample size												
Large	4	2482/2744	1.31(1.08,1.59)*	0.708	1.04(0.92,1.16)	0.531	1.28(1.06,1.53)*	0.649	1.08(0.97,1.21)	0.640	1.10(1.01,1.19)*	0.780
Small	9	917/1391	0.95(0.69,1.29)	0.101	0.95(0.78,1.14)	0.686	1.04(0.63,1.72)	0.004	0.95(0.79,1.13)	0.270	1.04(0.80,1.35)	<0.001

N: number of studies included; OR: odds ratio; P_h: p value for heterogeneity; BC: breast cancer; UC: urothelial carcinoma; GC: gastrointestinal cancer; PB: population-based; HB: hospital-based;
*OR with statistical significance.

whether studies deviated from HWE should be included or excluded in conducting meta-analysis[40]. When deleting the four studies in the sensitivity analysis, the pooled results did not change significantly. Meta-regression results revealed that ethnicity, cancer types, sources of control and sample size did not contributed to the source of heterogeneity.

Several limitations should be acknowledged in this meta-analysis. Firstly, the studies were full text in English and some inevitable publication bias might exist in the publications. Secondly, our results were based on single-factor estimates without adjustment for other risk factors such as age, family history and environment factors, should be conducted if possible. These factors may explain the heterogeneity. Beyond that, the number of studies for subgroup analysis was small.

In conclusion, we demonstrate that GSTO2 polymorphism may significantly increase cancer risk in Caucasian population and is associated with elevated risk of breast cancer; while GSTO1 polymorphism is not associated with cancer risk. To further confirm the results, large scale case-control studies with different ethnic groups and multiple cancer types are needed.

Methods

Identification of eligible studies. We extracted Eligible case-control studies by searching databases and manual search of references of relative reviews and articles. To identify all the studies that examined the association of GSTO polymorphism and cancer risk, we conducted a computerized literature search of Embase, Web of Science, PubMed and China National Knowledge Infrastructure (CNKI). The combination of the following key words were used as search terms: GST (e.g.: "Glutathione S-transferase"); cancer (e.g.: "carcinoma", "tumor" or "neoplasms") and polymorphism (e.g.: "single nucleotide polymorphism", "SNP" or "variation"). There was no limitation of research and the last research was carried out on Aug 13, 2014. To explore potentially additional studies, we also examined the references of articles and reviews.

Inclusion and exclusion criteria. The following criteria were used for the literature selection: (a) information on the association of cancer risk with GSTO1 or GSTO2 polymorphism; (b) participants more than fifty; (c) sufficient genotype data to calculate the odds ratios (ORs) with 95% confidence intervals (CIs). The major exclusion criteria were: (a) overlapping study populations; (b) non case-control design; (c) without detailed data on genotype distribution. Titles and abstracts of searching records were screened and full text papers were further evaluated to confirm the eligibility. According to the inclusion criteria, two reviewers (Xu and Wang) extracted eligible studies independently, and disagreement between the two reviewers was settled by discussing with the third reviewer (Qiu).

Data extraction. According to the selection criteria mentioned above, the following date was extracted from each study independently by two authors (Xu and Wang): name of first author, year of publication, country where the study was conducted, ethnicity of participants, methods for genotyping, sources of control, cancer types, genotype frequency in cases and controls. Different ethnicities were defined as Asian, Caucasian and mixed races. All eligible studies were categorized as population-based (PB) and hospital-based (HB) according to the sources of control. Cancer types were classified as breast cancer, urothelial carcinoma (including bladder cancer), gastrointestinal cancer (gastric cancer and colorectal cancer) only for GSTO2 and other cancers (thyroid carcinoma, gastric cancer, colorectal cancer, ovarian cancer, basal cell skin carcinoma, prostate carcinoma, head and neck cancer, acute lymphoblastic leukemia and non-small cell lung cancer). Chi-square test was used to examine the Hardy–Weinberg equilibrium (HWE) based on the two polymorphisms genotyping distribution in controls ($p < 0.05$ indicated significant deviation from HWE). Two reviewers reached consensus on each item.

Statistical analysis. We utilized ORs with 95% CIs to assess the strength of the association between GSTO1 or GSTO2 polymorphism and cancer risk. The estimated pooled ORs were achieved by calculating a weighted average of OR from each study. Pooled ORs were calculated for homozygote comparison (AA vs. CC for GSTO1; GG vs. AA for GSTO2), heterozygote comparison (AC vs. CC for GSTO1; GG vs. GA for GSTO2), allelic comparison (A vs. C for GSTO1; G vs. A for GSTO2), recessive model (AA vs. AC/CC for GSTO1; GG vs. GA/AA for GSTO2) and dominant model (AA/AC vs. CC for GSTO1; GG/GA vs. AA for GSTO2), respectively. A 95% CI without 1 for OR indicating a significant increased or reduced cancer risk.

The statistical significance of pooled ORs was determined by Z-test ($P < 0.05$ indicated statistically significant). We used a chi-square based Q-test to check the heterogeneity among the studies. Q-test results of $P < 0.10$ suggested significant heterogeneity among studies, so the pooled OR of all studies was calculated using the random-effects model based on DerSimonian-Laird method[41]; Otherwise, the fixed-effects model based on Mantel-Haenszel method was conducted[42]. Afterwards, subgroup analyses were conducted to test the effects of ethnicity, cancer type and sources

of control. Meta-regression was performed to detect the source of heterogeneity by cancer type, ethnicity, sources of control and sample size (studies with less 500 participants were categorized as "small", and studies with more than 500 participants were categorized as "large"). Between study, variance Tau-squared ($\tau 2$) value was used to evaluate the degree of heterogeneity and the τ^2 was used to describe the extent of heterogeneity explained[43]. Sensitivity analysis conducted by sequential deleting a single study each time was carried out to identify the effect of data from each study on pooled ORs.

Both the Begg's funnel plot and the Egger's linear regression test were used to evaluate publication bias across the literatures and a $p < 0.05$ was considered significant[44]. All p values were two sided. All of the statistical analyses were performed using STATA software version 12.0 (STATA Corporation, College Station, TX, USA).

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Author contributions

Conceived and designed the experiments: Y.T.X., J.W., R.Y. and L.X. Performed the experiments: Y.T.X., J.W., M.T.Q., L.X., J.W., R.Y. and L.X. Analyzed the data: Y.T.X., J.W. and M.T.Q. Contributed reagents/materials/analysis tools: Y.T.X., J.W., M.T.Q., R.Y. and L.X. Wrote the paper: Y.T.X., J.W., M.T.Q., J.W. and R.Y. Access to full-text articles: L.X. and J.W.

Additional information

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