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Genetic Polymorphism of Glutathione S-transferase T1, M1 and Asthma, A Meta-analysis of the Literature

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Abstract: Published studies have confirming or refusing an association between either glutathione S-transferase T1 (*GSTT1)* or M1 (*GSTM1*) polymorphism and asthma risk. Therefore the present meta-analysis was done. Literature-based meta-analysis was supplemented by tabular data from investigators of all relevant studies of two GST polymorphism (*GSTM1* and *GSTT1*) available before May 2006, with investigation of potential sources of heterogeneity. Included in the resent study were 14 studies, involving a total 2292 asthma patients and 5718 controls. We found substantial evidence of heterogeneity between the studies. Exclusion of two studies with lowest quality scores resulted in a dramatic decrease in heterogeneity. The overall OR of the asthma risk associated with *GSTM1* null genotype was 1.20 (95% CI: 1.08-1.35). Stratifying the meta-analysis by age and smoking status of subjects, the pooled ORs for *GSTM1* null genotype were 1.56 (95% CI: 1.25-1.94) in adults and 1.95 (95% CI: 1.21-3.13) in non-smokers. The *GSTT1* null genotype was associated with asthma risk in non-smoker adults (OR = 2.06, 95% CI: 1.21-3.71). To investigate whether profile of GST genotypes are associated with the risk of the asthma, further analysis combining the *GSTT1* and *GSTM1* genotypes were also carried out. Subjects with null genotypes for both *GSTM1* and *GSTT1* were at a significant higher risk for developing asthma (OR = 2.15, 95% CI: 1.39-3.33) compared with subjects who had both active genes. The trend in risk associated with zero, one and two putative high-risk genotypes was significant ($\chi^2 = 12.07$, df = 1, p = 0.0005). Overall, our present meta-analysis revealed that the null genotypes of *GSTM1* and *GSTT1* are associated with risk of asthma in adults especially in non-smoker ones. It might be suggested that chronic smoking carries such a high dose of toxins into the body that overloads the capacity of either GSTM1 or GSTT1 detoxification system. It seems that the GSTM1 and GSTT1 lack their protective values against development of asthma in adults with positive history of smoking. Present results also suggested that there is an additive effect for *GSTT1* and *GSTM1* genotypes.

Key words: Asthma, *GSTT1*, *GSTM1*, polymorphism, susceptibility, meta-analysis

INTRODUCTION

Combinations of genetic and environmental factors are important in the developing of asthma. Evidences for genetic contribution to asthma come from family aggregation and twin studies, also from genome wide searches and candidate gene approach (Blumenthal *et al*., 2006; Haagerup *et al*., 2001; Hakonarson and Halapi, 2002; Langefeld *et al*., 2001; Mauia, 1997; McCunney, 2005).

Inflammation is a central feature of asthma, as part of the inflammation, Reactive Oxygen Species (ROS) is formed. Genetic variations in enzymes that detoxify ROS and other products generated by several inflammatory cells may contribute to the exposure to an environmental trigger (Caramori and Papi, 2004; McCunney, 2005; Strange *et al*., 2001).

The members of the Glutathione S-Transferase (GST) (E.C. 2.5.1.18) super-family are potentially important in the protection of cells from ROS (McCunney, 2005; Strange *et al*., 2001). Members of the GST families have sequence similarity and shared catalytic properties for reaction of glutathione with reactive substrates. GSTs are well known as phase II xenobiotic detoxifying enzymes. One of the more recently recognized roles of GSTs is in oxidative defenses. Because antioxidants play a role in the pathobiology of a variety of disease and variants in the GST super-family are common, members of this super-family may be determinants of respiratory health (Strange *et al*., 2001).

Based on sequence homology and immunological cross-reactivity, human cytosolic GSTs have been grouped into eight distinct gene families designated GST Alpha, Mu, Pi, Sigma, Omega, Theta, Zeta and Kapa.

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Polymorphism has been described in many genes in these families though to date, most alteration has focused on allelism in the Pi, Mu and Theta families. Glutathione S-transferase M1 (*GSTM1*; a member of class μ) has two functional alleles (named *GSTM1-A* and *GSTM1-B*) and a non-functional null allele (named *GSTM1-0*). Homozygosity for the null-allele (null genotype) expresses no *GSTM1*. Glutathione S-transferase T1 (*GSTT1*; a member of class θ) has a functional (*GSTT1-1*) and a non-functional null-allele (*GSTT1-0*). Homozygous for non-functional allele of *GSTT1* (null genotype), cause an absence of *GSTT1* enzyme activity (Strange *et al*., 2001).

Published articles in the literature have confirming or refusing the association between genetic polymorphism of *GSTM1* and/or *GSTT1* and asthma risk (Ebrahimi *et al*., 2004; Freidin *et al*., 2002; Fryer *et al*., 2000; Gilliland *et al*., 2002a; Holla *et al*., 2006; Ivaschenko *et al*., 2002; Kabesch *et al*., 2004; Lee *et al*., 2005; Li *et al*., 2006; Makarova *et al*., 2004; Piirila *et al*., 2001; Saadat *et al*., 2004a; Tamer *et al*., 2004; Vavilin *et al*., 2005; Zhang *et al*., 2004). To clarify an association between genotypes and asthma risks, sample size is considered to be a crucial factor in the design of studies. Several studies evaluating *GSTT1* and/or *GSTM1* polymorphism as risk factors for asthma had small sample size (Gilliland *et al*., 2002a; Ivaschenko *et al*., 2002; Saadat *et al*., 2004a). In order to clarify the effect of *GSTM1* and *GSTT1* genotypes on the risk of developing asthma, we carried out a meta-analysis using published data from 2000 up to the May 2006, to obtain more precise estimates of risk.

MATERIALS AND METHODS

Identification of studies: Studies published between 2000 and May 2006 with information of *GSTT1* and/or *GSTM1* genetic polymorphism and the risk of asthma were identified using electronic databases, MEDLINE (National Library of Medicine, Washington, DC, USA), EBSCO host Research Databases, ProQuest and CAB Abstract. Search terms were *GSTT1* or glutathione S-transferase T1, *GSTM1* or glutathione S-transferase M1 and asthma. Additional articles were also checked using the references cited in these publications.

Articles selected for analysis were studies with case-control or cross sectional designs and their primary references, which had no obvious overlap of cases with other studies. Study of Gilliland *et al.* (2002a) that included different ethnical groups were considered separately in our analysis. Five studies (Freidin *et al*., 2002; Li *et al*., 2006; Makarova *et al*., 2004; Vavilin *et al*., 2005; Zhang *et al*., 2002) were excluded, because in one of them (Li *et al*., 2006) there was overlapping data with the other paper of the investigators (Gilliland *et al*., 2002a) and in the other two studies (Makarova *et al*., 2004; Vavilin *et al*., 2005), there were no data for genotypes in control subjects and finally Freidin *et al.* (2002) and Zhang *et al.* (2004) published their article in Russian and Chinese, respectively. The application of these criteria yielded 14 studies eligible for meta-analysis (Ebrahimi *et al*., 2004; Fryer *et al*., 2000; Gilliland *et al*., 2002a; Holla *et al*., 2005; Ivaschenko *et al*., 2002; Kabesch *et al*., 2004; Lee *et al*., 2005; Piirila *et al*., 2001; Saadat *et al*., 2004a; Tamer *et al*., 2004). In all of the studies, *GSTM1* and *GSTT1* polymorphism were determined by PCR assays; many studies reported quality control measurements (Fryer *et al*., 2000; Gilliland *et al*., 2002a; Holla *et al*., 2006; Kabesch *et al*., 2004; Lee *et al*., 2005; Piirila *et al*., 2001; Saadat *et al*., 2004a; Tamer *et al*., 2004).

Quality score assessment: The quality of studies was also independently assessed by the same two reviewers who used quality assessment scores that were modified from previous meta-analysis of molecular association studies. These scores were based on both traditional epidemiologic considerations and genetic issues (Thakkinstian *et al*., 2005). Total scores ranged from 0 (worst) to 12 (best).

Statistical analysis: The odds ratio (OR) of asthma associated with *GSTM1* and *GSTT1* genetic polymorphism was re-calculated for each study and their corresponding 95% Confidence Intervals (CI) were estimated. The results might not be exactly the same as those of some studies as different criteria were used in the statistical analysis. The low-risk genotypes (presence of *GSTM1* and presence of *GSTT1*) were used as the baselines for calculation ORs.

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 (DerSimonian and Laird, 1986). We carried out meta-analysis using a fixed-effects model and a random-effects model. 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. The fixed-effects and random-effects models were used by Mantel-Haenszel method (Mantel and Haenszel, 1959) and DerSimonian and Laird method (DerSimonian and Laird, 1986), respectively.

The analyses were also conducted on the subgroups of studies based on the score of study quality (after excluding two studies having lowest quality scores), smoking behavior of subjects and age of subjects

(childhood and adulthood). Because *GSTT1* and *GSTM1* genotypes may show additive effects in the development of the asthma, further analysis combining the *GSTM1* and *GSTT1* genotypes was carried out.

RESULTS

Association between polymorphism of GSTM1 and asthma risk: We identified 14 eligible studies, including 8010 subjects (2292 cases and 5718 controls) in relation to *GSTM1* polymorphism and risk of asthma, which are summarized in Table 1. From these, 4, 5 and 5 studies were carried out in Asian, European and American countries, respectively. Case-control and cross sectional designs were 7 and 7 studies, respectively (Table 1). The number of subjects in these studies varied considerably (range 114 to 3054 individuals).

The frequencies of *GSTM1* null genotype varied in the control participants, from 23.5 to 54.5% (Table 2). The distribution of *GSTM1* polymorphism among control individuals is in agreements with other reports (La Torre *et al*., 2005; Ye and Sang, 2005).

Test for heterogeneity between studies showed a significant heterogeneity (Q statistic = 45.09, df = 13, $p<0.0001$). The overall OR of the asthma risk associated with *GSTM1* null genotype was 1.21 (95% CI: 1.08-1.35). Exclusion of two studies with lowest quality scores (Ebrahimi *et al*., 2004; Ivaschenko *et al*., 2002 (Table 1) resulted in a dramatic decrease in Q statistic (45.09 to 27.68). Therefore, in order to reduce the heterogeneity, in further analysis these two studies were excluded.

Table 3 also summarizes the results of the stratified meta-analysis. Subgroup analysis, regarding age of subjects and smoking status of subjects were carried out.

Stratifying the meta-analysis by age of subjects, the pooled ORs for *GSTM1* null genotype were 1.56 (95% CI: 1.25-1.94) in adults and 1.10 (95% CI: 0.97-1.25) in childhood. Although there was no identifiable evidence of heterogeneity in the analysis of *GSTM1* and asthma in childhood, there was a borderline heterogeneity between studies in adulthood (Table 3).

Considering that cigarette smoking is an obvious risk factor for asthma and the GST genes are involved in the metabolism of various compounds present in cigarette

Table 1: Studies used in the meta-analysis

Quality score	Cases (n)	Controls (n)	Smoking status	Age	Study design	Ethnicity	Country	References
12	125	44	Non-smokers	Adulthood	Case-control	Caucasian	UK	Fryer *et al.* (2000)
12	109	73	Both	Adulthood	Cross-sectional	Caucasian	Finland	Piirila *et al.* (2001)
12	663	1050	-	Childhood	Cross-sectional	Non-Hispanic	USA	Gilliland *et al.* (2002a)
	220	454	-	Childhood		Hispanic		”
	41	73	-	Childhood		African		”
	20	98	-	Childhood		Asian		”
	68	94	-	Childhood		Others		”
5	109	90	Both	Adulthood and Childhood	Case-control	Caucasian	Russia	Ivaschenko *et al.* (2002)
8	85	85	Non-smokers	Adulthood	Case-control	Caucasian	Iran	Saadat *et al.* (2004a)
10	101	103	Both	Adulthood	Case-control	Caucasian	Turkey	Tamer *et al.* (2004)
5	95	253	ND	Adulthood	Case-control	Caucasian	Ukraine	Ebrahimi *et al.* (2004)
12	268	2786	-	Childhood	Cross-sectional	Caucasian	Germany	Kabesch *et al.* (2004)
11	82	184	-	Childhood	Case-control	Asian	Taiwan	Lee *et al.* (2005)
9	306	331	Both	Adulthood	Case-control	Caucasian	Czech	Holla *et al.* (2006)

Note: Both means subjects were smokers and non-smokers

Table 2: Genetic polymorphism of *GSTM1* and risk of asthma

		Controls		Cases			
95% CI	Ethnicity	M1+	M1-	M1+	M1-	OR	References
0.54-2.39	Caucasian	20	24	53	72	1.13	Fryer *et al.* (2000)
0.94-3.42	Caucasian	44	29	50	59	1.79	Piirila *et al.* (2001)
0.94-1.14	Non-Hispanic	569	481	336	327	1.15	Gilliland *et al.* (2002a)
0.60-1.19	Hispanic	260	194	135	85	0.84	”
0.43-2.77	African	53	20	29	12	1.10	”
0.94-8.64	Asian	59	39	7	13	2.81	”
0.34-1.31	Others	43	51	38	30	0.67	”
1.83-6.69	Caucasian	47	43	26	83	3.49	Ivaschenko *et al.* (2002)
1.57-6.47	Caucasian	65	20	43	42	3.17	Saadat *et al.* (2004a)
1.38-4.60	Caucasian	61	42	37	64	2.51	Tamer *et al.* (2004)
0.41-1.13	Caucasian	125	128	56	39	0.68	Ebrahimi *et al.* (2004)
0.90-1.52	Caucasian	1358	1428	120	148	1.17	Kabesch *et al.* (2004)
0.76-2.34	Asian	87	97	33	49	1.33	Lee *et al.* (2005)
0.85-1.63	Caucasian	165	166	140	166	1.18	Holla *et al.* (2006)

Table 3: Summary of meta-analysis of *GSTM1* polymorphism and the risk of asthma

Groups	Q-statistic	df	OR	95% CI
All of studies	45.09	13	1.21	1.08-1.35
Excluding two studies	27.68	11	1.20	1.08-1.35
Adulthood	11.36	4	1.56	1.25-1.94
Childhood	9.27	6	1.10	0.97-1.25
Non-smokers	4.49	1	1.95	1.21-3.13
Smokers and non-smokers	5.81	2	1.47	1.14-1.88

smoke, further analysis regarding smoking status of subjects were carried out in adulthood subjects. Unfortunately, only few studies reported the smoking status of their subjects and mentioned that their subjects were non-smokers (Fryer *et al*., 2000; Saadat *et al*., 2004a) other studies reported that some of their subjects were smokers and others were non-smokers (Holla *et al*., 2006; Ivaschenko *et al*., 2002; Piirila *et al*., 2001; Tamer *et al*., 2004). Unfortunately, there were no raw data about smoking behavior of the subjects in the studies using mixed of smoker and non-smoker subjects (Holla *et al*., 2006; Ivaschenko *et al*., 2002; Piirila *et al*., 2001; Tamer *et al*., 2004). After grouping according to smoking status, the *GSTM1* null genotype was associated with an increased risk of asthma in either non-smokers (OR=1.95, 95% CI: 1.21-3.13) or mixed subjects of smokers and non-smokers (OR=1.47, 95% CI: 1.14-1.88).

Association between polymorphism of *GSTT1* and asthma risk: We identified 8 eligible studies, including 4965 subjects (3765 controls and 1200 patients) in relation to *GSTT1* polymorphism and risk of asthma, which are summarized in Table 4. From these, 2 and 6 studies were carried out in Asian and European countries, respectively. The numbers of subjects in these studies varied considerably (range 170 to 3054 individuals).

The frequencies of *GSTT1* null genotype varied in the control participants, from 8.2 to 41.1% (Table 4). The distribution of *GSTT1* polymorphism among control individuals is in agreements with other reports (La Torre *et al*., 2005; Saadat 2006; Ye and Sang, 2005).

Test for heterogeneity showed a significant heterogeneity between studies (Q statistic = 43.56, df = 7, p>0.001). Using the random effects model the overall OR of the asthma risk associated with *GSTT1* null genotype was 1.36 (95% CI: 1.11-1.63). Exclusion of two studies with lowest quality scores (Ebrahimi *et al*., 2004; Ivaschenko *et al*., 2002; Table 1) resulted in a dramatic decrease in Q statistic (43.36 to 11.27). Therefore, in order to reduce the heterogeneity, in further analysis these two studies were excluded.

Table 5 also summarizes the results of the stratified meta-analysis. Subgroup analysis based on age and smoking status of subjects was carried out. In all the

Table 4: Genetic polymorphism of *GSTT1* and risk of asthma

	Controls		Cases			
95% CI	T1+	T1-	T1+	T1-	OR	References
0.46-3.45	37	7	103	24	1.23	Fryer *et al*. (2000)
0.50-4.73	67	6	96	13	1.51	Piirila *et al*. (2001)
3.39-13.2	69	21	36	73	6.66	Ivaschenko *et al*. (2002)
1.35-5.12	50	35	30	55	2.62	Saadat *et al*. (2004a)
0.58-2.24	78	25	74	27	1.14	Tamer *et al*. (2004)
1.16-3.98	217	36	70	25	2.15	Ebrahimi *et al*. (2004)
0.61-1.26	2300	486	226	42	0.88	Kabesch *et al*. (2004)
0.56-1.26	258	73	247	59	0.84	Holla *et al*. (2006)

Table 5: Summary of meta-analysis of *GSTT1* polymorphism and the risk of asthma

Groups	Q-statistic	df	OR	95% CI
All of studies	43.560	7	1.36	1.11-1.63
Excluding two studies	11.270	5	1.06	0.86-1.31
Adulthood	9.510	4	1.18	0.90-1.54
Non-smokers	1.771	1	2.06	1.21-3.71
Smokers and non-smokers	1.477	2	0.96	0.70-1.33

subgroup analyses, there was no identifiable evidence of heterogeneity in the analyses of *GSTT1* and asthma risk. It should be mentioned that using reminder 6 studies, the overall OR of the asthma risk associated with *GSTT1* null genotype was 1.06 (95% CI: 0.86-1.31). The *GSTT1* null genotype was associated with asthma risk in non-smoker adults (OR = 2.06, 95% CI: 1.21-3.71). Other associations were not significant.

Combination of *GSTM1* and *GSTT1* genotypes and risk of asthma: GSTs have overlapping substrate specificities. Therefore, deficiency of an individual GST isoenzyme may be compensated by other isoforms (Hayes and Pulford, 1995; Strange *et al*., 2001). Therefore, simultaneous determination of all GST genotypes appears to be a prerequisite for reliable interpretation of the role of the GST family in asthma development. To investigate whether profile of GST genotypes are associated with the risk of the asthma, further analysis combining the *GSTT1* and *GSTM1* genotypes were also carried out. The reference group consisted of individuals with two putative low-risk genotypes, i.e. the presence of *GSTM1* and *GSTT1* functional alleles. Four studies have examined the relation between asthma risk and combination of *GSTM1* and *GSTT1* polymorphism (Holla *et al*., 2006; Ivaschenko *et al*., 2002; Saadat *et al*., 2004a; Tamer *et al*., 2004). These studies included 1210 subjects (601 cases and 609 controls) (Table 6). It should be mentioned that the observed frequencies for genotype combinations in control groups did not show significant difference with the expected frequencies according to the Hardy-Weinberg equilibrium (the values of χ^2 in Russia, Iran, Turkey and Czech were equal to 0.22, 1.21, 2.11 and 1.86, respectively (for all comparisons df = 2, p>0.30). Table 6 displays the risk of asthma associated with each

Table 6: Combination genotypes of *GSTM1* and *GSTT1* and asthma risk

		Combinations of genotypes					
Country		M1+/T1+ [a]	M1+/T1- [b]	M1-/T1+ [b]	M1-/T1- [c]	Total	References
Russia	Controls	37	10	32	11	90	Ivaschenko *et al.* (2002)
	Cases	12	14	24	59	109	
Iran	Controls	36	29	14	6	85	Saadat *et al.* (2004a)
	Cases	17	26	13	29	85	
Turkey	Controls	43	18	35	7	103	Tamer *et al.* (2004)
	Cases	26	11	48	16	101	
Czech	Controls	123	177d	D	31	331	Holla *et al.* (2006)
	Cases	116	155d	d	35	306	

Note: The trend for none, one and two putative high-risk genotypes was significant using 4 studies $\chi^2 = 37.53$, df = 1, $p<0.00001$ and using 3 studies after excluding study from Russia $\chi^2 = 12.07$, df = 1, p = 0.00051, [a] Reference genotype. [b] After combination these two genotypes using 4 studies Q statistic = 9.92, df = 3; OR=1.29, 95% CI: 0.99-1.67 and using 3 studies after excluding study from Russia Q statistic=5.77, df=2; OR=1.18, 95% CI: 0.89-1.56. [c] Using 4 studies Q statistic = 29.616, df = 3; OR = 3.14, 95% CI: 2.12-4.66 and using 3 studies after excluding study from Russia Q statistic = 14.07, df = 2; OR = 2.15, 95% CI: 1.39-3.33.d There is no data about M1+/T1- and M1-/T1+ genotypes

combination of genotypes as well as the trend in risk associated with zero, one and two putative high-risk genotypes. Subjects with null genotypes for both *GSTM1* and *GSTT1* were at a significant higher risk for developing asthma (OR = 3.14, 95% CI: 2.12-4.66; Q statistic = 29.616, df = 3) compared with subjects who had both active genes. The trend in risk associated with zero, one and two putative high-risk genotypes was significant ($\chi^2 = 37.53$, df = 1, $p<0.00001$). If the study of Ivaschenko *et al.* (2002) was excluded from the analysis the heterogeneity decrease and subjects with null genotypes for both *GSTM1* and *GSTT1* were at a significant higher risk for developing asthma (OR = 2.15, 95% CI: 1.39-3.33; Q statistic=14.07, df = 2) compared with subjects who had both active genes. The trend in risk associated with zero, one and two putative high-risk genotypes was significant ($\chi^2 = 12.07$, df = 1, p = 0.0005). Therefore there is an additive effect for *GSTT1* and *GSTM1* genotypes.

DISCUSSION

The overall goal of meta-analysis is to combine the results of previous studies to arrive at summary conclusions about a body of research. It is most useful in summarizing prior research when individual studies are small and they are individually too small to yield a valid conclusion. In the present study, 14 studies were found eligible for meta-analysis (Ebrahimi *et al.*, 2004; Fryer *et al.*, 2000; Gilliland *et al.*, 2002a; Holla *et al.*, 2005; Ivaschenko *et al.*, 2002; Kabesch *et al.*, 2004; Lee *et al.*, 2005; Piirila *et al.*, 2001; Saadat *et al.*, 2004a; Tamer *et al.*, 2004). There was considerable heterogeneity between studies in this meta-analysis in our initial results (Table 3, 5 and 6). Heterogeneity can be an important drawback of meta-analysis when a pooled estimate is the main objective. Other wise, heterogeneity can be also a major goal in meta-analysis: finding the variables associated with variation across the primary studies can help future research on a topic. In the present meta-analysis, two studies (Ebrahimi *et al.*, 2004; Ivaschenko *et al.*, 2002) were the major sources of heterogeneity. It might be due to uncontrolled confounding and inherent bias of study design. One study (Ivaschenko *et al.*, 2002) used subjects with highly heterogeneous with respect to the age of subjects (adulthood and childhood). Selection bias may be a major source of heterogeneity; therefore such bias was reduced by removing the studies. 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 of the asthma risk associated with *GSTM1* null genotype (All studies: Q statistic = 45.09, df = 13; OR = 1.21, 95%CI: 1.08-1.35; After removing the two studies: Q statistic = 27.68, df = 11; OR=1.20, 95%CI: 1.08-1.35).

Numerous studies have reported an association between Environmental Tobacco Smoke (ETS) exposure or cigarette smoking and asthma risk (Cook and Stranchan, 1997; Cook *et al.*, 1998). Unfortunately, only two studies (Fryer *et al.*, 2000; Saadat *et al.*, 2004a) mentioned that their subjects were adults and non-smokers. In other articles, authors did not report the GST polymorphism in smoker and non-smoker subjects (Holla *et al.*, 2006; Ivaschenko *et al.*, 2002; Piirila *et al.*, 2001; Tamer *et al.*, 2004). Overall our present meta-analysis revealed that the null genotype of *GSTM1* was associated with risk of asthma in adults (OR = 1.56, 95% CI: 1.25-1.94) especially in non-smoker ones (OR = 1.95, 95% CI: 1.21-3.13). Also the null genotype of *GSTT1* was associated with increased risk of asthma in non-smoker adults (OR = 2.06, 95% CI: 1.21-3.71). It might be suggested that chronic smoking carries such a high dose of toxins into the body that overloads the capacity of either *GSTM1* or *GSTT1* detoxification system. Therefore, the GSTM1 and *GSTT1* lack their protective roles against

development of asthma in adults with positive history of smoking. Interestingly, there are same story between risk of developing cataract and polymorphism of *GSTM1* in non-smoker females (Saadat *et al*., 2004b). However, this result is not consistent with a report investigated association between *in utero* exposure to maternal smoking and prevalence of many asthmatic phenotypes with respect to the *GSTM1* genotype of children. The authors reported that among children with null genotype of *GSTM1*, *in utero* exposure was associated with increased prevalence of early onset asthma, asthma with recurrent symptoms, persistent asthma, lifetime history of wheezing, wheezing with exercise, wheezing requiring medication and emergency room visits in the past year (Gilliland *et al*., 2002b).

It should be noted that the *GSTM1* and *GSTT1* are involved in detoxification of a variety of compounds, some that overlap between enzymes and some that are highly specific (Hayes and Pulford 1995; Strange *et al*., 2001). We found four studies that reported the combination genotypes of *GSTM1* and *GSTT1* in control subjects and asthma patients (Holla *et al*., 2005; Ivaschenko *et al*., 2002; Saadat *et al*., 2004a; Tamer *et al*., 2004). Statistical analysis showed that there is significant trend in the risk associated with zero, one and two putative high-risk genotypes (Table 6). Those who had null genotypes of *GSTM1* and *GSTT1* had an increased risk compared with those who had both active genes (OR = 3.14, 95% CI: 2.12-4.66). Because both *GSTM1* and *GSTT1* showed substrate overlapping (Hayes and Pulford 1995; Strange *et al*., 2001) and detoxify several compounds involved in development of asthma (Caramori and Papi, 2004; Mauia, 1997; McCunney, 2005), the observed additive effect might be interpreted. It should be mentioned that such additive effect of *GSTM1* and *GSTT1* was reported for developing colorectal (Saadat and Saadat, 2001) and gastric cancers (Saadat, 2006) and senile cataract (Saadat *et al*., 2004b).

The mRNA level of *GSTT1* in lung tissue of women was affected positively by the serum level of cotinine (a metabolite of nicotine). Also the *GSTT1* mRNA level was decreased as a function of age (Spivack *et al*., 2003). There are some indications of ethnic differences in the effects of *GSTM1* genotype on FVC and FEV1 growth (Carroll *et al*., 2005; Gilliland *et al*., 2007). Taken together, future research in this field should take great care in the interaction between risk factors and combination genotypes of GSTs (such as *GSTT1*, *GSTM1*, *GSTP1*, etc) with respect to age and ethnicity of subjects. Also additive effect of involved genes should be investigated. Therefore, stratification of subjects according to their age, sex, ethnicity, smoking behavior and GST genotypes (including combination of genotypes) in future studies is necessary.

Finally in this meta-analysis, only published studies were used and in some analysis some studies were excluded because the raw data was not available. Therefore, publication bias is an issue.

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