

GSTT1 and *GSTM1* polymorphisms and myelodysplastic syndrome risk: a systematic review and meta-analysis

Issa J. Dahabreh¹, Stavroula Giannouli², Vaia Gota³ and Michael Voulgarelis⁴

¹Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Tufts University School of Medicine, Boston, MA, USA

²Department of Hematology, Medical School, National University of Athens, Athens, Greece

³Haematology Research Laboratory, Biomedical Research Foundation, Academy of Athens, Athens, Greece

⁴Department of Pathophysiology, Medical School, National University of Athens, Athens, Greece

Glutathione-S-transferase polymorphisms may make hematopoietic lineage cells susceptible to genotoxicity following exposure to heavy metals or benzene. We conducted a systematic review and meta-analysis to define the effect of *GSTM1* and *GSTT1* null polymorphisms on MDS risk. We searched the PubMed and SCOPUS databases to identify peer-reviewed published case-control studies investigating the association between *GSTT1* and/or *GSTM1* null genotypes and development of MDS. Between-study heterogeneity was assessed using Cochran's Q statistic and the I^2 statistic. Odds ratios from individual studies were pooled using fixed and random effects models. Thirteen studies were considered eligible for the *GSTT1* meta-analysis (1471 cases, 1907 controls) and 10 were considered eligible for the *GSTM1* meta-analysis (1161 cases, 1668 controls). For the *GSTT1* polymorphism, there was moderate between study heterogeneity ($p_Q = 0.01$; $I^2 = 52.3\%$) and the null genotype was significantly associated with increased risk of MDS development, random effects OR = 1.43 (95% CI, 1.09–1.89); $p = 0.01$. For the *GSTM1* polymorphisms there was moderate between-study heterogeneity ($p = 0.07$; $I^2 = 43.1\%$) and the random effects OR = 1.02 (95% CI, 0.82–1.28) was non-significant ($p = 0.85$). The *GSTT1* null genotype is a significant risk factor for MDS development. Gene-environment interactions need to be further explored.

The myelodysplastic syndromes (MDS) are clonal myeloid disorders characterized by ineffective hematopoiesis, bone marrow dysplastic changes and an increased risk of transformation to acute leukemia. The generally accepted mechanism of primary MDS pathogenesis involves an initial deleterious genetic event within a hematopoietic stem cell, subsequent development of an excessive cytokine/inflammatory response leading to a pro-apoptotic/proliferative state, and resultant peripheral cytopenias despite the presence of a hypercellular bone marrow. The presence of detectable cytogenetic abnormalities in approximately 40–70% of patients with primary MDS and over 80% of those with secondary MDS, as well as

the validated prognostic value of cytogenetic data have been considered to support the theory of an inciting genetic event.¹

Recent studies, have demonstrated that the recurrent cytogenetic abnormalities associated with MDS, previously considered the “primary” cause of disease, are actually “secondary” events that arise due to cytogenetically cryptic initiating lesions in an established clonal hematopoietic stem cell population.² Such initiating events are likely to be heterogeneous and could either be inherited or result from acquired DNA damage, genomic instability, defective DNA repair, or perturbations in cellular signaling pathways that give rise to stem cell clones with a proliferative or survival advantage. While genetic and familial mapping studies have clearly demonstrated that inherited mutations in specific genes, such as AML1, NF1, or genes mediating DNA repair, can predispose to the acquisition of secondary cytogenetic abnormalities and MDS, it is likely that highly penetrant inherited mutations will account for only a minority of MDS cases.^{3–5} Furthermore, the majority of MDS cases are “sporadic” and several epidemiologic investigations have demonstrated associations between MDS and exposure to a variety of environmental toxins.⁶ In light of these findings, a complex MDS pathogenetic model has been proposed, whereby genetic predisposition to the disease, mediated by low penetrance polymorphisms in DNA repair genes, cooperates with environmental carcinogens.^{7,8}

Among the most extensively studied inherited genetic risk factors for MDS are variants of glutathione S-transferases (GSTs, Enzyme Commission (EC) number 2.5.1.18). Among the GST substrates, there are several environmental

Key words: glutathione-S-transferase, *GSTT1*, *GSTM1*, myelodysplastic syndrome, meta-analysis

Abbreviations: FAB: French-American-British; MDS: myelodysplastic syndrome; OR: odds ratio; RA/RARS: refractory anemia/refractory anemia with an excess of blasts; RAEB/RAEB-t: refractory anemia with an excess of blasts/refractory anemia with an excess of blasts in transformation; WHO: World Health Organization

DOI: 10.1002/ijc.24940

History: Received 28 Jul 2009; Accepted 10 Sep 2009; Online 8 Oct 2009

Correspondence to: Michael Voulgarelis, Department of Pathophysiology, Medical School, National University of Athens, 75 Mikras Asias St., 11527 Athens, Greece, Tel: +30 210 7462648, Fax: +30 210 7462664, E-mail: mvoulgar@med.uoa.gr

carcinogens found in food, air, or medications, such as polycyclic aromatic hydrocarbons, found in combustion products, diet, and tobacco smoke. Polycyclic aromatic hydrocarbons are activated by members of the phase 1 cytochrome P-450 supergene family to epoxide-containing metabolites (e.g., benzo[a]pyrene-7,8-diol-9,10-oxide), which are substrates for the mu, alpha, theta and pi GST classes.⁹ *GSTT1* and *GSTM1* are good candidate cancer susceptibility genes because of their involvement in the metabolism of chemicals such as methylating agents, pesticides, and industrial solvents. In vitro studies suggest that both *GSTT1* and *GSTM1* enzymes protect cells from the toxic products of phase 1 detoxification reactions.¹⁰ However, *GSTT1*-catalyzed reactions can also increase the toxicity of some compounds, such as dichloromethane. GSTs also conjugate isothiocyanates, which are potent inducers of enzymes that detoxify environmental mutagens. The conjugation process diverts the isothiocyanates from the enzyme induction pathway into excretion, leading to elimination of these anticarcinogenic substances and thus decreasing their potential chemopreventive effect. The pleiotropy of GST enzyme functions indicates that their role in human carcinogenesis is complex.

The most common polymorphism in *GSTT1* consists of a deletion of the whole gene (null genotype), resulting in the lack of active enzyme.^{11,12} Complete deletion at the *GSTT1* locus was hypothesized by observing the phenotypic variation in glutathione-related detoxification of halomethanes by human erythrocytes, resulting in “conjugator” and “nonconjugator” phenotypes. Individuals with homozygous deletions of either the *GSTM1* locus or the *GSTT1* locus have no enzymatic functional activity of the respective enzyme. This has been confirmed by phenotype assays that have demonstrated 94% or greater concordance between phenotype and genotype. Many genotoxins, including benzene and cytotoxic drugs, act directly *in vivo* on bone marrow cells.¹³ Bone marrow cell protection from these insults depends upon intact detoxification pathways and DNA repair systems to prevent cellular mutagenesis/apoptosis.¹⁴ Exposure to environmental toxins, such as benzene or heavy metals, has been implicated in the etiology of MDS.¹⁵ Therefore, it has been proposed that the decreased production of *GSTM1* and *GSTT1*, characteristic of the null genotypes, may be associated with an increased MDS risk in the presence of environmental mutagens and/or carcinogens exposure.

Individual genetic association studies, such as those that have evaluated selected GST polymorphisms and risk of MDS, are frequently extremely underpowered and often report small or variable effects. We therefore conducted a systematic review and meta-analysis to more accurately define the effect of *GSTM1* and *GSTT1* polymorphisms on risk for MDS.

Material and Methods

Study identification, eligibility criteria, and data extraction

We used the PubMed (MEDLINE) and SCOPUS databases to identify genetic association studies published before April

30th, 2009, investigating the association between the *GSTT1* and/or *GSTM1* null genotypes and MDS. We also used the National Institutes of Health (NIH) Genetic Association Database (GAD, last search: April 30th, 2009). Computer searches were conducted using keywords relevant the genes of interest (e.g., “*GSTT1*,” “*GSTM1*” and “glutathione S-transferase”) in combination with words related to “myelodysplastic syndrome” and “myelodysplasia.” To increase the yield of our search, we also scanned the reference lists of all relevant studies and review articles, manually searched relevant journals and consulted with experts in the field of MDS. Case-control studies that employed validated genotyping methods and examined the prevalence of *GSTT1* and/or *GSTM1* null genotypes were eligible for inclusion. Family based studies were excluded owing to different design considerations. The search was limited to studies published in English.

The following information was abstracted from each study: first author, journal, year of publication, study design, matching, geographical location, ethnicity of participants, definition and numbers of cases and controls, source of controls, DNA extraction and genotyping methods, frequency of GST null and normal genotypes, MDS classification criteria (French-American-British, FAB or World Health Organization, WHO), morphological classification (refractory anemia with or without ringed sideroblasts, RA/RARS; refractory anemia with an excess of blasts, RAEB; refractory anemia with an excess of blasts in transformation, RAEB-t).

Evidence synthesis

For *GSTM1* and *GSTT1*, since only two genotypes are possible, we compared genotype frequencies (null versus normal) between cases and controls. All associations were presented as odds ratios (OR) with the corresponding 95% confidence interval (CI). Between-study heterogeneity was assessed using Cochran's Q statistic and inconsistency quantified using the I^2 statistic, which describes the proportion of variation in the log odds ratios that is attributable to genuine differences across studies rather than to random error.¹⁶ Pooled OR was estimated based on the individual OR using fixed- (Mantel-Haenszel) and random-effects (DerSimonian and Laird) models.^{17–20} Cumulative meta-analysis was carried out for each polymorphism to evaluate the trend of random effects OR over time.^{21,22}

Assessment of bias and subgroup analysis

Source of controls (e.g., population versus hospital based) and ethnicity (individuals of East Asian origin versus all other) were pre-specified as characteristics for assessment of heterogeneity by subgroup analysis. The differential magnitude of effects in large versus small studies was assessed using the Egger and Begg tests.^{23,24} A test for interaction was used to compare the OR of the first study with the pooled OR of subsequent studies, as well as the pooled ORs between subgroups.²⁵ The following subgroup comparisons were pre-specified: source of controls (healthy versus hospital), MDS

Table 1. Characteristics of studies included in the meta-analysis

Study, Year	Ethnicity	MDS cases	Secondary MDS (n, %)	MDS classification	Controls	Source of controls	Matching	Genotyping method	GSTT1 null cases (n, %)	GSTT1 null controls (n, %)	GSTM1 null cases (n, %)	GSTM1 null controls (n, %)
Chen, 1996	Caucasian	90	NR	FAB	190 for GSTT1; 201 for GSTM1	Cancer free patients attending urology clinics	No	Multiplex PCR	41 (45.6)	30 (15.8)	38 (42.2)	105 (47.8)
Atoyebi, 1997	NR	166	NR	NR	112	Laboratory staff and general medical outpatients	No	PCR	35 (21.1)	18 (16.1)	91 (54.8)	54 (48.2)
Basu, 1997	NR	57	NR	NR	100	Laboratory staff, general medical patients, women attending the antenatal clinic	No	PCR	16 (28.1)	23 (23)	NA	NA
Okada, 1997	East Asian	59	NR	NR	50	NR	No	PCR	27 (45.8)	23 (46)	NA	NA
Preudhomme, 1997	Caucasian	174	16 (9.2%)	FAB	100	Blood donors	No	Multiplex PCR	39 (22.4)	19 (19)	NA	NA
Sasai, 1999	East Asian	116	0	FAB	43	Healthy volunteers	No	Multiplex PCR	62 (53.4)	13 (30.2)	64 (55.2)	23 (53.5)
Davies, 2000	Caucasian	13	0	FAB	153	Blood donors + participants in an epidemiological study	No	Multiplex PCR	2 (15.4)	23 (15.0)	9 (69.2)	72 (47.1)
Tsabouri, 2000	Caucasian	54	0	FAB	60	Cancer-free patients from the internal medicine department, malignant and hematological disease patients were excluded	Age, smoking, sex	Multiplex PCR	6 (11.1)	4 (6.7)	31 (57.4)	20 (33.3)
Arruda, 2001	NR	49	0	FAB	276	Newborns from the same university hospital as controls	No	Multiplex PCR	7 (14.3)	44 (15.9)	18 (36.7)	102 (37.0)
Haase, 2002	NR	128	14 (10.9%)	FAB	239	Healthy individuals	No	Multiplex PCR	28 (21.9)	38 (15.9)	68 (53.1)	122 (51)
Varkonyi, 2008	Caucasian	86	0	WHO	99	Hospitalized patients from the same university hospital	Age, sex	Multiplex PCR	17 (19.8)	25 (25.3)	41 (47.7)	48 (48.5)
Stavropoulou, 2008	Caucasian	323 for GSTT1; 302 for GSTM1	0	FAB	330	Healthy donors	Age, sex	Multiplex PCR	58 (18.0)	52 (15.8)	107 (35.4)	140 (42.4)
Fabiani, 2009	Caucasian	156 for GSTT1; 157 for GSTM1	0	WHO	155	Individuals with no history of previous malignancies and normal peripheral blood counts	Age, sex	Multiplex PCR	38 (24.4)	28 (18.1)	81 (51.6)	92 (59.4)

Studies are listed by year of publication.

Abbreviations: FAB, French-American-British classification; MDS, myelodysplastic syndrome; NA, not applicable; NR, not reported; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; WHO, World Health Organization classification.

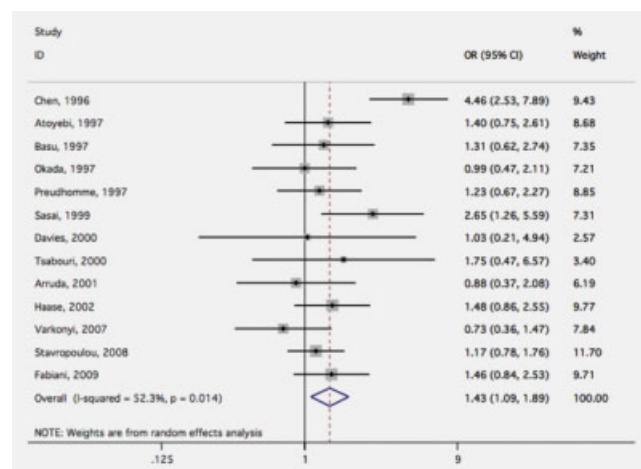


Figure 1. Forest plot for *GSTT1* null polymorphism and MDS risk; meta-analysis using a random effects model. Each study is shown by the point estimate of the odds ratio (square proportional to the weight of each study) and 95% confidence interval for the odds ratio (extending lines); the summary odds ratio and 95% confidence intervals by random effects calculations is depicted as a diamond. For all figures, values higher than 1 indicate that the null genotype is associated with increased risk of MDS development. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

classification (FAB versus WHO), inclusion of secondary MDS cases (yes versus no), ethnicity of participants (Caucasian versus East Asian) and morphological diagnosis (RA/RARS versus RAEB/RAEB-t).

Statistical analyses were performed using STATA (version 10/SE, Stata Corp., College Station, Texas). Studies in the figures are listed by the year of publication.

Results

Eligible studies

Out of 98 studies retrieved by our initial search strategy, 13 were considered eligible for this systematic review and were included in the meta-analysis; all studies investigated the *GSTT1* and 10 also investigated the *GSTM1* polymorphism (Table 1).^{7,26–37}

Studies had been conducted over a period of thirteen years (1996 to 2009). The vast majority of the studies (11 of 13) used the French-American-British (FAB) criteria to classify patients and only two used the more recent World Health Organization Criteria (WHO). Ten of the studies were conducted in Caucasian populations, two in East Asian populations, and one in a Latin American population. Hospital controls were used in seven studies and healthy controls (healthy volunteers, blood donors or newborns) were used in six studies. In general, studies were relatively small: mean number of cases was 113.15 (SD = 80.4, range: 13–323) and the mean number of controls was 146.7 (SD = 89.57, range: 43–330). Nine studies did not match cases to controls; controls were matched to cases for age and gender and in three

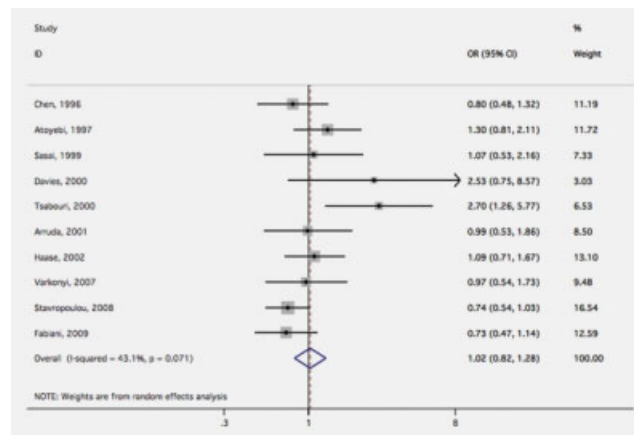


Figure 2. Forest plot for *GSTM1* polymorphism and MDS risk; meta-analysis using a random effects model. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

studies, for age, gender and smoking in one study. All studies used validated polymorphism detection methods, such as PCR or PCR-RFLP, with the majority employing multiplex PCR protocols to identify the *GSTT1* and *GSTM1* null genotypes along with control genes for reaction quality control.

The frequency of the *GSTT1* null genotype among controls ranged from 30.2% to 46% in studies of East Asian populations and from 6.7% to 25% in studies of non-East Asian populations. The control group frequency of the *GSTM1* null genotype was 53.5% in the one East Asian study that provided information and ranged from 33.3% to 59.4% in studies of non-East Asian populations.

Evidence synthesis

The studies investigating the *GSTT1* polymorphism reported on a total of 1471 cases and 1907 controls. 376 cases (25.6%) had null genotypes compared to 340 (17.8%) controls. There was moderate between study heterogeneity ($p_Q = 0.01$) and inconsistency ($I^2 = 52.3\%$). Overall, the *GSTT1* null genotype was associated with increased risk of MDS, fixed-effects OR = 1.44 (95% CI, 1.21–1.72; $p < 0.0001$). Random effects models were also consistent with an association between the *GSTT1* null genotype and MDS risk, OR = 1.43 (95% CI, 1.09–1.89; $p = 0.01$). Figure 1 demonstrates the results of the meta-analysis of *GSTT1* studies.

The studies investigating the *GSTM1* polymorphism reported on a total of 1161 cases and 1668 controls. There was moderate between study heterogeneity ($p_Q = 0.07$) and inconsistency ($I^2 = 43.1\%$). Overall, the *GSTM1* null genotype was not associated with increased risk of MDS, fixed effects OR = 0.97 (95% CI, 0.83–1.14); $p = 0.69$. Random effects models also did not demonstrate an association between *GSTM1* genotypes and MDS risk, OR = 1.02 (95% CI, 0.82–1.28); $p = 0.69$. Figure 2 demonstrates the results of the meta-analysis of *GSTM1* studies.

Table 2. Results of meta-analysis and subgroup analysis. Significant results are highlighted in bold. All odds ratios (OR) are calculated using random effects models

Studies	<i>GSTT1</i> Number of studies (cases/controls) OR (95% CI); p-value	Interaction p-value	<i>GSTM1</i> Number of studies (cases/controls) OR (95% CI); p-value	Interaction p-value
All studies	13 studies, 1471/1907 1.43 (1.09–1.89) <i>p</i> = 0.01	NA	10 studies, 1161/1668 1.02 (0.82–1.28) <i>p</i> = 0.85	NA
No matching	9 studies, 852/1263 1.57 (1.09–2.27) <i>p</i> = 0.02	0.21	6 studies, 562/1024 1.08 (0.86–1.36) <i>p</i> = 0.52	0.77
Matched design	4 studies, 619/644 1.17 (0.88–1.56) <i>p</i> = 0.29		4 studies, 599/644 1.0 (0.63–1.57) <i>p</i> = 0.98	
Healthy controls	6 studies, 831/1057 1.32 (1.03–1.71) <i>p</i> = 0.03	0.66	5 studies, 524/711 0.85 (0.66–1.11) <i>p</i> = 0.23	0.13
Hospital controls	7 studies, 640/850 1.49 (0.93–2.41) <i>p</i> = 0.10		5 studies, 637/957 1.17 (0.84–1.62) <i>p</i> = 0.35	
FAB classification	11 studies, 1229/1653 1.52 (1.12–2.07) <i>p</i> = 0.007	0.36	8 studies, 918/1414 1.10 (0.84–1.46) <i>p</i> = 0.49	0.18
WHO classification	2 studies, 242/254 1.07 (0.54–2.10) <i>p</i> = 0.85		2 studies, 243/254 0.81 (0.57–1.16) <i>p</i> = 0.25	
Exclusively de novo MDS	11 studies, 1169/1568 1.44 (1.03–2.03) <i>p</i> = 0.03	0.86	9 studies, 1033/1429 1.02 (0.79–1.33) <i>p</i> = 0.86	0.80
Includes secondary MDS	2 studies, 302/339 1.37 (0.91–2.05) <i>p</i> = 0.13		1 study, 128/239 1.09 (0.71–1.67) <i>p</i> = 0.79	
Caucasian	10 studies, 1247/1538 1.45 (1.06–1.99) <i>p</i> = 0.02	0.83	8 studies, 996/1349 1.04 (0.79–1.37) <i>p</i> = 0.78	0.94
East Asian	2 studies, 175/93 1.62 (0.62–4.26) <i>p</i> = 0.33		1 study, 116/43 1.07 (0.53–2.16) <i>p</i> = 0.85	
RA/RARS	6 studies, 360/825 1.68 (1.05–2.71) <i>p</i> = 0.03	NA	3 studies, 558/643 0.89 (0.64–1.25) <i>p</i> = 0.51	NA
RAEB/RAEB-t	7 studies, 342/978 1.31 (0.69–2.48) <i>p</i> = 0.42		4 studies, 571/796 0.98 (0.67–1.45) <i>p</i> = 0.93	

Abbreviations: FAB, French-American-British; MDS, myelodysplastic syndrome; OR, odds ratio; RA/RARS, refractory anemia/refractory anemia with an excess of blasts; RAEB/RAEB-t, refractory anemia with an excess of blasts/refractory anemia with an excess of blasts in transformation; WHO, World Health Organization.

Subgroup analysis

The results of subgroup analysis by ethnicity, control selection and morphological characteristics are presented in Table 2. The relatively small number of studies per subgroup and small number of participants did not allow for accurate estimation of ORs. The point estimates appeared consistent between subgroups and nominal significance was achieved for several subgroups regarding the *GSTT1* polymorphisms. In general, ORs for the *GSTT1* polymorphisms were consistent across subgroups and favored an increased risk of MDS for patients carrying the null genotype.

Potential for bias

For the *GSTT1* polymorphism there was no evidence of small study effects (Begg-Mazumdar's test $p = 0.67$ and Egger's test $p = 0.79$). For the *GSTT1*, the odds ratio of the first published study³⁴ (OR = 4.46, 95% CI 2.53–7.89) was statistically significantly different from the pooled odds ratio of all subsequent studies (random effects OR = 1.27, 95% CI 1.06–1.54; interaction test $p < 0.0001$). Interestingly, removal of the first study also eliminated between-study heterogeneity ($p_Q = 0.679$; $I^2 = 0\%$). Over time the random effects OR for *GSTT1* followed a decreasing trend (Figure 3). For the *GSTM1* polymorphism there was some evidence of small study effects (Begg-Mazumdar's test $p = 0.03$ and Egger's test $p = 0.02$) but there was no evidence that the first study differed significantly from subsequent evidence.

Discussion

This report is the first meta-analysis examining the effect of *GSTT1* and *GSTM1* polymorphisms on the risk of MDS. We found the null variant of *GSTT1* to be associated with an increased MDS risk. Although less evidence is available, there was no indication for an association between *GSTM1* and MDS. These polymorphisms have been investigated as potential risk factors for the development of many solid tumors, and convincing associations have been demonstrated for lung, prostate, colorectal, bladder, head and neck cancer as well as hepatocellular carcinoma.^{38–43} A systematic review that investigated the association of GST null polymorphisms with acute leukemia, both myeloid and lymphoblastic found that *GSTM1* and *GSTT1*, but not *GSTP1* polymorphisms, appear to be associated with an increase in the risk of acute leukemia.⁴⁴ Previous studies have established that the prevalence of the *GSTT1* null genotype is lower among Caucasians (10–20 percent) compared with Asians (50–60 percent).⁴⁵ MDS in Asian populations occurs at a younger age compared to Western populations, indicating a stronger genetic component.^{46–48} We speculate that this difference may be partly ascribed to the higher prevalence of the *GSTT1* null genotype among Asian populations.

The association of environmental toxin exposure and MDS development strongly supports the existence of gene environment interactions. MDS and acute leukemia form a pathogenetic and clinical continuum, with pro-apoptotic

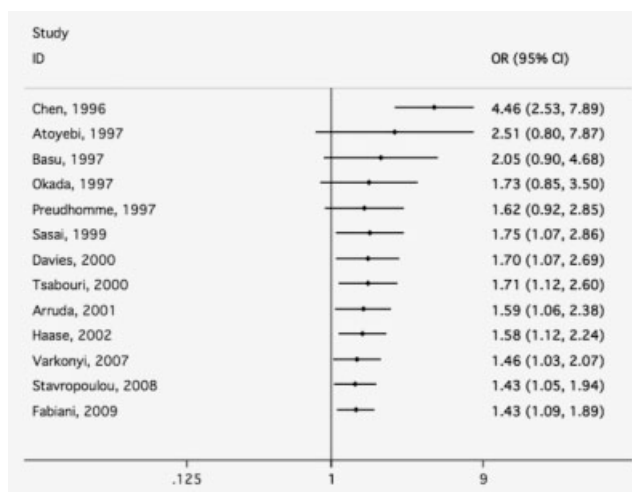


Figure 3. Cumulative meta-analysis for *GSTT1* polymorphism and MDS risk using a random effects model. Each point estimate with corresponding confidence intervals presents the meta-analysis results as each study was published. Pooled odds ratios are depicted as circles with extending lines representing 95% CI.

effects and cytopenias on one end and increased proliferation, blocked differentiation and high blast counts on the other. Given its significant association with both acute leukemia and MDS, the *GSTT1* null genotype appears as a potential initiating risk factor for both conditions. Based on our analysis, the association of the *GSTT1* null genotype with MDS is strengthened by the consistency of results across ethnic and morphological subtypes of disease.

Functional studies provide further support for the association between the *GSTT1* null genotype and MDS. Benzene exposure has been associated with a variety of disorders, including MDS.⁴⁹ The effects of benzene may be mediated by intermediate genotoxic and cytotoxic metabolites that induce DNA damage, including chromosomal aberrations and sister chromatid exchanges.^{50,51} Some benzene metabolites accumulate in the bone marrow where they undergo autooxidation, producing highly reactive oxygen species which are responsible for cell damage.⁵² Both *GSTM1* and *GSTT1* are involved in the detoxification of benzene oxide to *s*-phenylmercapturic acid.⁵³ Several studies have examined the role of *GSTM1* and *GSTT1* polymorphisms on biomarkers of benzene toxicity. Kim *et al.* reported an increased occurrence of chromosomal aberrations in subjects carrying the null genotype of both *GSTM1* and *GSTT1* relative to the heterozygotes or non-null individuals.⁵¹ In addition, the frequency of diepoxybutane-induced sister chromatid exchanges was significantly increased in *GSTT1* null subjects.⁵⁴

This study is limited by the unavailability of individual patient data that would allow the identification of gene-environment interactions as well as the potential association of GST polymorphisms with specific disease characteristics such as cytogenetic abnormalities or somatic mutations. Be that as it may, by pooling the results of all

published independent studies this review has increased power to detect the association between GST polymorphisms and MDS risk. Also, by including studies published over a long period of time, it has been possible to demonstrate that, apart from the somewhat exaggerated effect estimate of the first published study, the available evidence supports an association between the *GSTT1* null genotype and MDS risk.

In conclusion, this review demonstrates that null polymorphisms in *GSTT1*, but not in *GSTM1*, are associated with increased odds of MDS development. Given the strong association between *GSTT1* null genotype and MDS risk, as well as the established role of GSTs in environmental carcinogen detoxification and acute leukemia pathogenesis, studies to investigate potential gene-environment interactions are necessary across the myelodysplasia-leukemia spectrum.

References

- Rund D, Ben-Yehuda D. Therapy-related leukemia and myelodysplasia: evolving concepts of pathogenesis and treatment. *Hematology* 2004;9:179–87.
- Raskind WH, Steinmann L, Najfeld V. Clonal development of myeloproliferative disorders: clues to hematopoietic differentiation and multistep pathogenesis of cancer. *Leukemia* 1998;12:108–16.
- Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, Ratajczak J, Resende IC, Haworth C, Hock R, Loh M, Felix C, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet* 1999;23:166–75.
- Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, Lange BJ, Freedman MH, McCormick F, Jacks T, Shannon K. Loss of NF1 results in activation of the ras signaling pathway and leads to aberrant growth in haematopoietic cells. *Nat Genet* 1996;12:144–8.
- Luna-Fineman S, Shannon KM, Atwater SK, Davis J, Masterson M, Ortega J, Sanders J, Steinhilber P, Weinberg V, Lange BJ. Myelodysplastic and myeloproliferative disorders of childhood: a study of 167 patients. *Blood* 1999;93:459–66.
- Nisse C, Lorthois C, Dorp V, Eloy E, Haguenoer JM, Fenaux P. Exposure to occupational and environmental factors in myelodysplastic syndromes. preliminary results of a case-control study. *Leukemia* 1995;9:693–9.
- Preudhomme C, Nisse C, Hebbard M, Vanrumbeke M, Brizard A, Lai JL, Fenaux P. Glutathione S transferase theta 1 gene defects in myelodysplastic syndromes and their correlation with karyotype and exposure to potential carcinogens. *Leukemia* 1997;11:1580–2.
- Perera FP. Environment and cancer: who are susceptible? *Science* 1997;278:1068–73.
- Strange RC, Spiteri MA, Ramachandran S, Fryer AA. Glutathione-S-transferase family of enzymes. *Mutat Res* 2001 Oct 1;482:21–6.
- Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005;45:51–88.
- Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000;61:154–66.
- Norppa H. Cytogenetic biomarkers and genetic polymorphisms. *Toxicol Lett* 2004;149:309–34.
- Ross D. Metabolic basis of benzene toxicity. *Eur J Haematol Suppl* 1996;60:111–8.
- Wang L, Groves MJ, Hepburn MD, Bowen DT. Glutathione S-transferase enzyme expression in hematopoietic cell lines implies a differential protective role for T1 and A1 isoenzymes in erythroid and for M1 in lymphoid lineages. *Haematologica* 2000;85:573–9.
- West RR, Stafford DA, White AD, Bowen DT, Padua RA. Cytogenetic abnormalities in the myelodysplastic syndromes and occupational or environmental exposure. *Blood* 2000;95:2093–7.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Bmj* 2003;327:557–60.
- Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719–48.
- Mantel N. Chi-square tests with one degree of freedom: extensions of the mantel-haenszel procedure. *J Am Statist Assoc* 1963;58:690–700.
- DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–88.
- Dahabreh IJ, Economopoulos K. Meta-analysis of rare events: an update and sensitivity analysis of cardiovascular events in randomized trials of rosiglitazone. *Clin Trials* 2008;5:116–20.
- Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. *Ann Intern Med* 1997;127:820–6.
- Lau J, Schmid CH, Chalmers TC. Cumulative meta-analysis of clinical trials builds evidence for exemplary medical care. *J Clin Epidemiol* 1995;48:45,57; discussion 59–60.
- Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50:1088–101.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj* 1997;315:629–34.
- Altman DG, Bland JM. Interaction revisited: the difference between two estimates. *Bmj* 2003;326:219.
- Atoyebi W, Kusec R, Fidler C, Peto TE, Boultonwood J, Wainscoat JS. Glutathione S-transferase gene deletions in myelodysplasia. *Lancet* 1997;349:1450–1.
- Basu T, Gale RE, Langabeer S, Linch DC. Glutathione S-transferase theta 1 (*GSTT1*) gene defect in myelodysplasia and acute myeloid leukaemia. *Lancet* 1997;349:1450.
- Arruda VR, Lima CS, Grignoli CR, de Melo MB, Lorand-Metze I, Alberto FL, Saad ST, Costa FF. Increased risk for acute myeloid leukaemia in individuals with glutathione S-transferase mu 1 (*GSTM1*) and theta 1 (*GSTT1*) gene defects. *Eur J Haematol* 2001;66:383–8.
- Varkonyi J, Szakaly D, Janoskuti L, Hosszufalusi N, Panczel P, Karadi I, Schoket B. Glutathione S-transferase enzyme polymorphisms in a hungarian myelodysplasia study population. *Pathol Oncol Res* 2008;14:429–33.
- Stavropoulou C, Sambani C, Rigana H, Georgakakos VN, Voutsinas G, Manola KN, Pantelias GE, Makropoulos V, Hellenic MDS Study Group. Low frequency of the glutathione-S-transferase T1-null genotype in patients with primary myelodysplastic syndrome and 5q deletion. *Leukemia* 2008;22:1643–6.
- Sasai Y, Horiike S, Misawa S, Kaneko H, Kobayashi M, Fujii H, Kashima K, Taniwaki M. Genotype of glutathione S-transferase and other genetic configurations in myelodysplasia. *Leuk Res* 1999;23:975–81.
- Okada M, Okamoto T, Wada H, Takemoto Y, Kakishita E. Glutathione S-transferase theta 1 gene (*GSTT1*) defect in Japanese patients with myelodysplastic syndromes. *Int J Hematol* 1997;66:393–4.
- Haase D, Binder C, Bunger J, Fonatsch C, Streubel B, Schnittger S, Griesinger F, Westphal G, Schoch C, Knopp A, Berkovicz D, Krieger O, et al. Increased risk for therapy-associated hematologic malignancies in patients with carcinoma of

- the breast and combined homozygous gene deletions of glutathione transferases M1 and T1. *Leuk Res* 2002;26:249–54.
34. Chen H, Sandler DP, Taylor JA, Shore DL, Liu E, Bloomfield CD, Bell DA. Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (GSTT1) gene defect. *Lancet* 1996 Feb 3;347:295–7.
 35. Davies SM, Robison LL, Buckley JD, Radloff GA, Ross JA, Perentesis JP. Glutathione S-transferase polymorphisms in children with myeloid leukemia: a children's cancer group study. *Cancer Epidemiol Biomarkers Prev* 2000; 9:563–6.
 36. Tsabouri SE, Georgiou I, Alamanos I, Bourantas KL. Increased prevalence of GSTM(1) null genotype in patients with myelodysplastic syndrome: a case-control study. *Acta Haematol* 2000;104:169–73.
 37. Fabiani E, D'Alo F, Scardocci A, Greco M, Di Ruscio A, Criscuolo M, Fianchi L, Pagano L, Hohaus S, Leone G, Voso MT. Polymorphisms of detoxification and DNA repair enzymes in myelodysplastic syndromes. *Leuk Res* 2009;33:1068–71.
 38. Bull LM, White DL, Bray M, Nurgalieva Z, El-Serag HB. Phase I and II enzyme polymorphisms as risk factors for Barrett's esophagus and esophageal adenocarcinoma: a systematic review and meta-analysis. *Dis Esophagus* 2009 Feb 13.
 39. Ye Z, Song H, Higgins JP, Pharoah P, Danesh J. Five glutathione s-transferase gene variants in 23,452 cases of lung cancer and 30,397 controls: meta-analysis of 130 studies. *PLoS Med* 2006 Apr;3(4): e91.
 40. White DL, Li D, Nurgalieva Z, El-Serag HB. Genetic variants of glutathione S-transferase as possible risk factors for hepatocellular carcinoma: a HuGE systematic review and meta-analysis. *Am J Epidemiol* 2008;167:377–89.
 41. Hashibe M, Brennan P, Strange RC, Bhisey R, Cascorbi I, Lazarus P, Oude Ophuis MB, Benhamou S, Foulkes WD, Katoh T, Coutelle C, Romkes M, et al. Meta- and pooled analyses of GSTM1, GSTT1, GSTP1, and CYP1A1 genotypes and risk of head and neck cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:1509–17.
 42. Raimondi S, Botteri E, Iodice S, Lowenfels AB, Maisonneuve P. Gene-smoking interaction on colorectal adenoma and cancer risk: review and meta-analysis. *Mutat Res* 2009 Jul 7.
 43. Raimondi S, Paracchini V, Autrup H, Barros-Dios JM, Benhamou S, Boffetta P, Cote ML, Dialyna IA, Dolzan V, Filiberti R, Garte S, Hirvonen A, et al. Meta- and pooled analysis of GSTT1 and lung cancer: a HuGE-GSEC review. *Am J Epidemiol* 2006;164:1027–42.
 44. Ye Z, Song H. Glutathione s-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukaemia: a systematic review and meta-analysis. *Eur J Cancer* 2005;41:980–9.
 45. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, et al. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001;10:1239–48.
 46. Chen B, Zhao WL, Jin J, Xue YQ, Cheng X, Chen XT, Cui J, Chen ZM, Cao Q, Yang G, Yao Y, Xia HL, et al. Clinical and cytogenetic features of 508 Chinese patients with myelodysplastic syndrome and comparison with those in western countries. *Leukemia* 2005;19:767–75.
 47. Matsuda A, Germing U, Jinnai I, Misumi M, Kuendgen A, Knipp S, Aivado M, Iwanaga M, Miyazaki Y, Tsushima H, Sakai M, Bessho M, et al. Difference in clinical features between Japanese and German patients with refractory anemia in myelodysplastic syndromes. *Blood* 2005 Oct 15;106:2633–40.
 48. Kuendgen A, Matsuda A, Germing U. Differences in epidemiology of MDS between western and eastern countries: ethnic differences or environmental influence? *Leuk Res* 2007;31:103–4.
 49. Sorahan T, Kinlen LJ, Doll R. Cancer risks in a historical UK cohort of benzene exposed workers. *Occup Environ Med* 2005; 62:231–6.
 50. Erexson GL, Wilmer JL, Kligerman AD. Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites in vitro. *Cancer Res* 1985;45:2471–7.
 51. Kim SY, Choi JK, Cho YH, Chung EJ, Paek D, Chung HW. Chromosomal aberrations in workers exposed to low levels of benzene: association with genetic polymorphisms. *Pharmacogenetics* 2004;14: 453–63.
 52. Levay G, Ross D, Bodell WJ. Peroxidase activation of hydroquinone results in the formation of DNA adducts in HL-60 cells, mouse bone marrow macrophages and human bone marrow. *Carcinogenesis* 1993;14: 2329–34.
 53. Snyder R, Witz G, Goldstein BD. The toxicology of benzene. *Environ Health Perspect* 1993;100: 293–306.
 54. Xu X, Wiencke JK, Niu T, Wang M, Watanabe H, Kelsey KT, Christiani DC. Benzene exposure, glutathione S-transferase theta homozygous deletion, and sister chromatid exchanges. *Am J Ind Med* 1998; 33:157–63.