Pathol. Oncol. Res. (2008) 14:429–433
DOI 10.1007/s12253-008-9008-1

ORIGINAL PAPER

# Glutathione *S*-Transferase Enzyme Polymorphisms in a Hungarian Myelodysplasia Study Population

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Received: 18 May 2007 / Accepted: 14 December 2007 / Published online: 21 May 2008


**Abstract** *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* that are members of the *GST* gene family encode for Phase II drug/xenobiotic metabolizing enzymes, primarily with detoxifying function, and are polymorphic in humans. *GSTM1* and *GSTT1* homozygous deletion genotypes do not express the enzymes. It has been hypothesised that individuals with homozygous deletion of the *GSTM1* and/or *GSTT1* gene may have lower detoxification capacity towards genotoxic agents therefore those individuals may be at increased risk of myelodysplastic syndrome which is a preleukemic condition. Genetic polymorphism of *GSTM1*, *GSTT1* and *GSTP1 Ile105Val was* investigated in a case–control study in a Hungarian patient population comprising 86 patients with myelodysplastic syndrome and 99 hospital-based controls. There were no statistically significant differences between cases and controls for the *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* genotype frequencies for any of the three genes separately and in various combinations. This suggests that these genetic polymorphisms may not be strong risk factors, if any, for myelodysplastic syndrome.

**Keywords** Myelodysplastic syndrome · *GSTM1* · *GSTT1* · *GSTP1* · Genetic polymorphism

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## Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterised by pancytopenia and a high risk for transformation into acute leukemia. A distinction can be made among MDS with reference to the prior patient history. When patients have records of previous chemotherapy or radiotherapy or of occupational exposure to toxic substances, the disease is defined as secondary MDS (sMDS), whereas in absence of such history the disease is considered primary MDS. In sMDS the most frequently involved drugs include alkylating agents, epipodophyllotoxins and anthracyclines. Cytogenetically, single chromosome aberrations are typical for primary MDS, whereas multiple changes are more frequently seen in secondary disorders. The most common karyotype abnormalities are the partial or complete loss of genetic material of either part or all of chromosome 7 and/or 5 (7q/-7, /5q-, -5). sMDS has a rapid course and a short survival [1].

Glutathione *S*-transferases (GSTs) are a large family of Phase II drug-metabolizing enzymes that are important in the cellular defence mechanisms against chemicals many of which have carcinogenic potential [2, 3]. Several representatives of the *GST* gene family include polymorphic loci and it is hypothesised that allelic variants associated with less efficient or adversely altered detoxification capacity may increase susceptibility to cancer. The homozygous deletion (null) genotypes of *GSTM1* and *GSTT1* are variants that do not express the enzyme [4]. In view of the likely importance of environmental carcinogens in the etiology of several malignancies, it can be expected that individuals with the null genotype of *GSTM1* and *GSTT1* may be at increased risk of cancer [5, 6]. Previous studies revealed that *GSTP1 Ile105Val* substitution modifies the substrate affinity of the GSTP1 enzyme, and the homozy-

gous Val105 variants may have altered substrate-dependent catalytic enzyme activity as compared to the Ile105 wild-type homozygous genotypes [7, 8]. In particular, it was found that the *GSTP1* 105Val allele was more active in the metabolism in oxiplatin-based chemotherapy than the wild-type allele [9].

In the study of Chen et al. *GSTT1* null genotype individuals were found in higher frequency among 96 MDS patients (46%) than among 201 age- and sex-matched cancer-free hospital based controls (16%), however there was no statistically significant difference between *GSTM1* positive and null genotypes [10]. In our present study we investigated the association of genetic polymorphisms of *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* with primary MDS in a Hungarian patient population.

## Materials and Methods

The study population comprised 86 primary MDS patients and 99 hospitalized controls, matched for age and sex, investigated in our university hospital between February 2000 and December 2004. Subtype distribution of MDS was the following according to the WHO classification: Refractory anemia ($n$=30), refractory anemia with ring sideroblasts ($n$=21), refractory anemia with excess of blasts ($n$=20), 5q- ($n$=6), and unclassifiable MDS ($n$=9) including hypoplastic form ($n$=3) and MDS/MPS ($n$=6) (MPS: myelo-proliferative disease). Controls were diagnosed for various unrelated diseases including ischaemic heart disease ($n$=17), second type diabetes with/or without disease complications ($n$=17), chronic obstructive lung disease ($n$=4), obliterative arteriosclerosis ($n$=10), Parkinson's disease ($n$=6), reflux disease, GI haemorrhage and disorders of the gall bladder ($n$=7), atrial fibrillation ($n$=11), hypertension ($n$=14), deep vein thrombosis ($n$=2), gout and disorder related to alcohol consumption ($n$=8), and deficiency anemias ($n$=3). Exclusion criteria for controls were malignancies and immunopathological disorders. Mean age was 68 years for both the patients and the controls with a range of 37–91, and 52–87 years, respectively. The sex ratio was the same in the two groups, male/female=45%/55%.

Blood samples were taken from patients and controls with their informed consent and approval from the scientific ethical committee ETT TUKEB 12236-45/2004-1018EKU.

DNA was isolated from peripheral blood according to the standard salt extraction method. *GSTM1* and *GSTT1* genotyping were carried out by a multiplex PCR essentially according to Lin et al. [5]. *GSTP1 Ile105Val* genotypes were identified according to the method by Ozawa et al. except that Thermoprime Plus DNA polymerase (Abgene, Epsom, Surrey, UK) was used for catalysing the PCR reaction [11].

Statistical analyses were performed by chi-square/Fisher's exact test and contingency tables using GraphPad software (GraphPad Sowftware Inc, San Diego, CA, USA). Two-tailed $p$-values were calculated for the determination of statistical significance.

## Results

Frequency of *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* genotypes was similar in the MDS patient population and in the control group as shown in Table 1. The study population was in Hardy–Weinberg equilibrium for *GSTP1 Ile105Val* gene polymorphism. The *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* genotype frequency distributions were similar in both cases and controls to those that have been reported for various Caucasian study populations [6]. The association of the combined genotypes of *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* was investigated for pairs of the genes and for the three genes together. Figure 1 illustrates the frequency of the combined *GSTM1–GSTT1* genotypes in the patient and in the control group. The *GSTT1* genotype frequencies were similar in both MDS patients and controls in both the *GSTM1* positive and the null subgroup, and the calculations concluded to the lack of statistically significant differences. The p values of difference were higher than 0.05. A small difference in the percent values was observed between the *GSTM1* positive and null genotype subgroups within the MDS group (odds ratio=2.62, 95% confidence interval (CI)=0.83–8.23), however, the association was not significant ($p$=0.11).

The genotype frequency distributions of the combinations of *GSTP1 Ile105Val* with *GSTM1, GSTT1* and with both *GSTM1* and *GSTT1*, respectively, are shown in Fig. 2a,b and c for the MDS patients and for the controls. The proportions of the different combined *GST* genotypes were similar in the cases and in the controls. The $p$ values of difference were higher than 0.05 in all comparisons. The only notable difference in the percent values of the *GSTP1* allelic frequencies was seen in the MDS group in the comparison between the *GSTT1* gene carriers and homozygous deletion variants, however, the numbers of subjects are very small, and the association was not significant either ($p$=0.14).

## Discussion

There is a rapidly growing literature on the possible association between pharmacogenetic polymorphisms and cancer risk [2, 12]. The major goal of those studies was to identify genetic susceptibility factors, that may increase risk for cancer development in association with particular

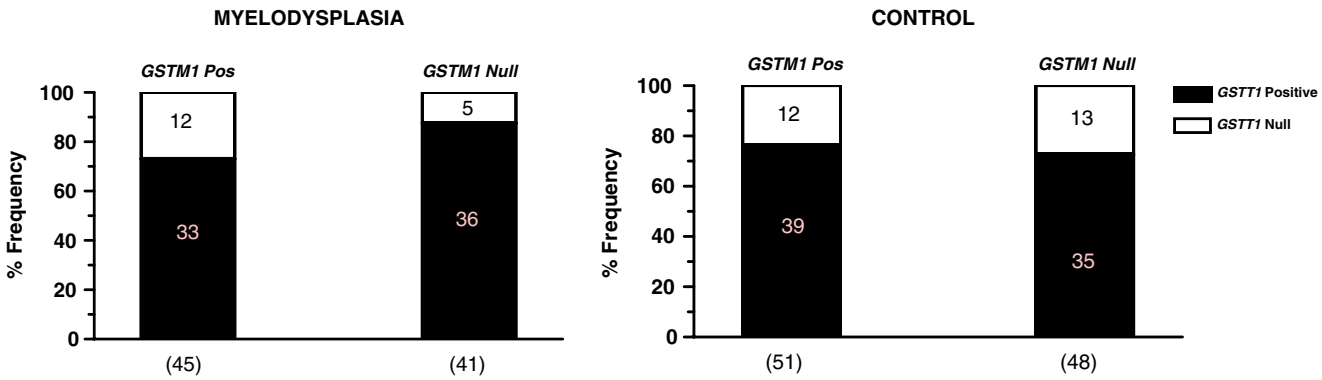


**Fig. 1** Frequency of combined *GSTM1* and *GSTT1* genotypes in the MDS and control groups. *Pos* gene present—wildtype homozygous and heterozygous, *null* homozygous gene deletion. *p* values of difference are >0.05 in the statistical comparisons between the corresponding genotype frequency distributions between cases and controls, and also within the case group and control group, respectively. Specifically, $p=0.11$ within the myelodysplasia group

environmental and life-style factors and occupational exposures. Many studies explore the influence of single genotypes on disease development. GST mu, theta and pi isoenzymes catalyse Phase II conjugation reactions, that lead mostly to the detoxification of a variety of reactive toxic and mutagenic compounds. It is reasonable to presume that polymorphic variants of the *GST* genes encoding these enzymes may have altered molecular detoxifying mechanisms, particularly with *GSTM1* and *GSTT1* homozygous gene deletions that do not express the enzymes and thereby modify the risk of the MDS as well. There can also be geographical and ethnic variation in the genotype frequencies of *GSTM1* and *GSTT1* [13, 14].

The various studies on the relationship between *GST* genetic polymorphisms and MDS produced contradictory results. In our study population, which represented primary MDS cases, we did not find relationship between *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* genotypes and MDS. Similar results were obtained by Atoyebi et al. who did not find significant difference in the genotype frequencies between MDS patients and controls for either *GSTM1* (odds ratio= 0.89 [95%CI=0.5–1.43]) or *GSTT1* (odds ratio=0.72 [95% CI=0.4–1.34]) [15]. Also, among eight cases of secondary MDS they found four subjects with *GSTM1* null genotype.

Several other studies, however, found that *GSTT1* null or *GSTM1* null genotypes might be predisposing factors for developing MDS. A 4.3-fold increased risk for MDS was found by Chen et al. in association with the *GSTT1* null genotype suggesting that the inherited absence of *GSTT1* contributes to the risk for the development of MDS [10].

In a study of 159 Japanese MDS patients in comparison with healthy controls Sasai et al. concluded, that higher percent of the patients had the *GSTT1* null genotype and the difference was significant ($p<0.01$). The odds ratio for disease risk in individuals with the *GSTT1* null genotype was elevated to 2.65 (95%CI=1.27–5.52) in the de novo MDS, 4.62 (1.48–14.4) in therapy-related AML, and 2.94 (1.07–8.07) in AML preceded by trilineage dysplasia [16].

Ozbeck et al. found no association between the *GSTT1* gene deletion and acute leukemia but the *GSTM1* null variant seemed to contribute to the development of the disease (OR=2.1, 95%CI=1.0–4.2) in a study population of 155 (acute lymphocytic leukemia) ALL and 94 AML (acute myeloid leukemia) patients in comparison to 140 healthy controls [17]. Similar conclusion was obtained in the study by Tsabouri et al. on 54 MDS patients in which significantly increased frequency of *GSTM1* null genotype was found among MDS patients (57.4%) compared to controls (33.3%; $p<0.01$), whereas the frequency of *GSTT1* null genotype was not significantly higher in MDS patients (11.1% vs. 6. 66%) [18].

In a small study population comprising 49 MDS and 38 AML patients Arruda et al found a 4.7-fold and a 2.3-fold increased risk for AML related to the *GSTM1* and *GSTT1* null genotype, respectively, however no such effect was obtained among MDS patients [19]. Such results may suggest separate pathways in the development of de novo AML and MDS.

**Table 1** Frequency of *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* genotypes in a Hungarian study population of MDS patients ($n=86$) and controls ($n=99$)

| | Frequency of genotypes | | | |
|---|---|---|---|---|
| | MDS cases | | Controls | |
| | Number | % | Number | % |
| *GSTM1* positive | 45 | 52.3 | 51 | 51.5 |
| *GSTM1* null | 41 | 47.7 | 48 | 48.5 |
| *GSTT1* positive | 69 | 80.2 | 74 | 74.7 |
| *GSTT1* null | 17 | 19.8 | 25 | 25.3 |
| *GSTP1* Ile/Ile | 37 | 45.1 | 49 | 49.5 |
| *GSTP1* Ile/Val | 37 | 45.1 | 42 | 42.4 |
| *GSTP1* Val/Val | 8 | 9.8 | 8 | 8.1 |

For *GSTP1*, $n=82$.

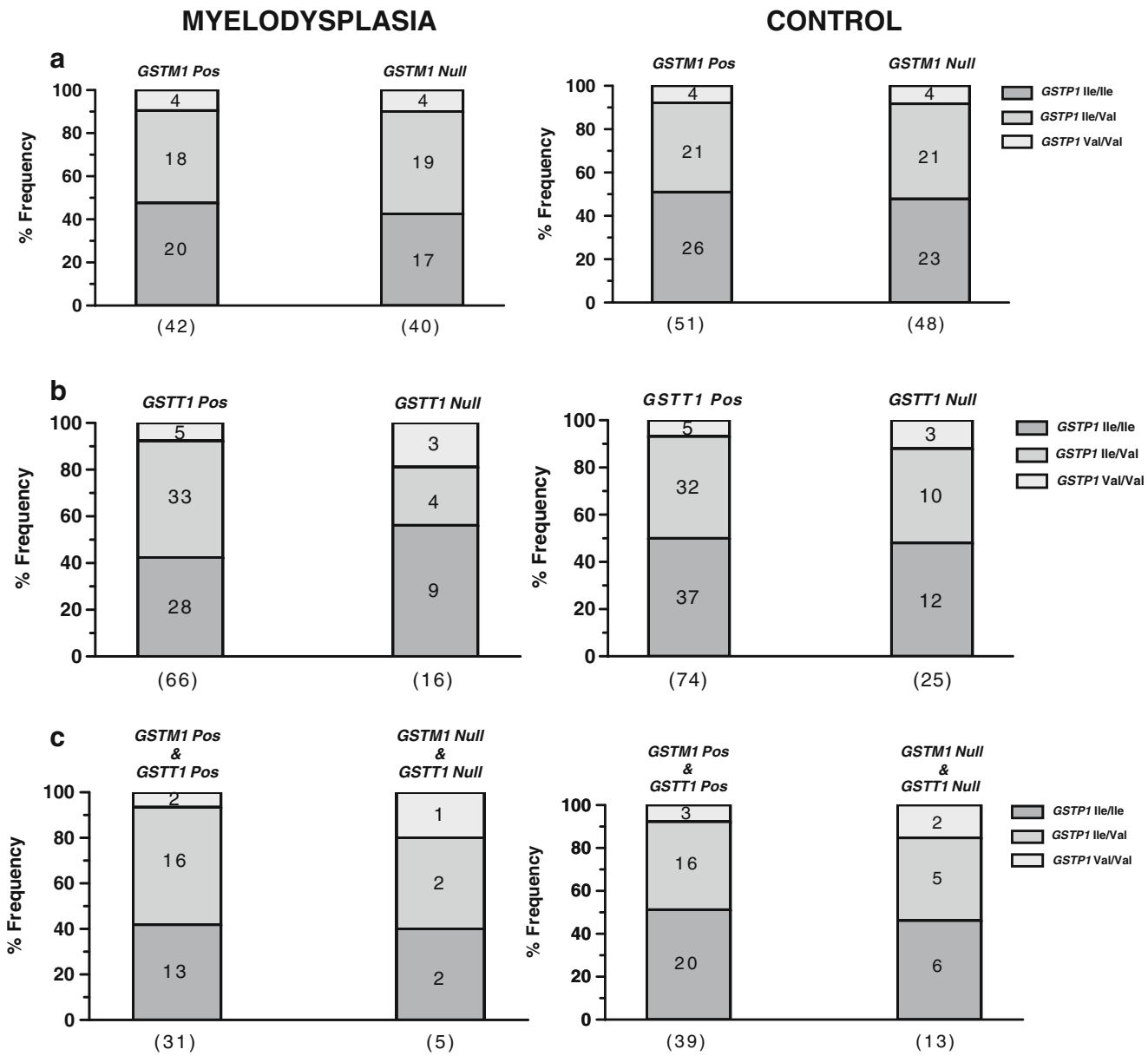


**Fig. 2** *GSTP1 Ile105Val* genotype frequencies in combination with **a** *GSTM1*, **b** with *GSTT1*, and **c** with *GSTM1* and *GSTT1* combined genetic polymorphisms in the MDS and control groups. *Pos* gene present—wildtype homozygous and heterozygous, *null* homozygous gene deletion. *p* values of difference are >0.05 in the statistical comparisons between the corresponding genotype frequency distributions between cases and controls, and also within the case group and control group, respectively. Specifically, in **b**, $p=0.14$ within the myelodysplasia group

In a group of 292 AML/MDS patients, mostly of Caucasian origin, there was a significant increase in the frequency of *GSTM1* null genotype as compared to the control group (64% vs. 47% OR=2.0, 95%CI=1.3–3.0, $p=0.001$), whereas there was no such association for the *GSTT1* polymorphism [20].

Haase et al. demonstrated an increase of risk for development of MDS/AML in 439 breast cancer patients with double deletion of *GSTT1* and *GSTM1*. The substances had been used where shown efficient in clinical trials as alkylating agents, antimetabolites, anthracyclines, topoisomerase inhibitors, platinum derivatives and taxans. The highest risk for secondary leukemias has been attributed to concurrent radiochemotherapy [21].

## Conclusions

Our results suggest on the basis of this relatively small Hungarian MDS study population that *GSTM1*, *GSTT1* and *GSTP1 Ile105Val* genetic polymorphisms may not influence the risk for de novo MDS significantly. Literature data

are controversial, both positive and negative findings have been reported. The controversial results might be related to the small size of the study populations and that MDS itself represents a heterogeneous group of diseases. In view of the literature data it is possible that the pathomechanism of secondary MDS/AML differs significantly from that of de novo MDS/AML in the absence of GSTT1 and GSTM1 metabolizing enzymes.

The influence of *GSTT1*, *GSTM1* and *GSTP1* genetic polymorphisms to the risk for the development of de novo MDS may also vary in different geographic and ethnic populations. Exploration of the underlying molecular mechanisms is a huge challenge of research, and current studies are overwhelmingly observational ones. Creation of an international database including 'null results' to avoid bias, on genetic susceptibility–disease associations, and the statistical evaluation of the data in meta- and pooled analyses would be desirable to fill the gaps of our current knowledge of MDS development. That necessitates further investigations and collaboration between research groups in the field.

**Acknowledgements** This study was supported by the "Judy Rák Alapítvány" foundation and ETT 279/2003. The technical assistance by Ms. Lívia Anna and Gizella Papp are gratefully acknowledged.

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