RESEARCH ARTICLE

# GSTT1 Null Genotype Distribution in the Kumaun Region of Northern India

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## Abstract

**Glutathione S-transferases (GSTs) constitute a multigene family of multifunctional phase II metabolic enzymes. GSTT1, an important member of this group has a wide range of substrates including carcinogens. Total homozygous deletion or null genotype resulting in total lack of enzyme activity exists in populations for this enzyme. Since the null genotype may contribute to lower detoxification of carcinogens, this genotype is expected to increase cancer risk. The frequency of the *GSTT1* null genotype is known to vary significantly among populations. However, little is known about its distribution in the hilly Kumaun region of northern India. Therefore, in this study, we determined the prevalence of the *GSTT1* null polymorphism in the Kumaun popilation by conducting duplex PCR in 365 voluntary healthy individuals. The *GSTT1* null genotype was detected in 18.4% of the individuals. Since GSTs play significant role in xenobiotic metabolism, the present data on *GSTT1* genotype distribution should contribute in understanding genetic association with cancer risk in this understudied population.**

**Keywords:** GSTT1 - null genotype - Kumaun region - Northern India

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## Introduction

Genetic polymorphisms of metabolic enzymes are able to modify normal metabolic pathways for xenobiotics in our body. Thus they are often associated with disease susceptibility. Glutathione S- transferases (GSTs) constitute a multigene family of phase II metabolizing enzymes, which catalyze conjugation of tripeptide glutathione (GSH) to a wide variety of compounds including those with electrophilic functional groups and render them more water soluble (Hayes and Pulford, 1995). Thus they play a major role in phase II cellular detoxification, and protect cellular components from reactive electrophiles including environmental carcinogens. Genetic polymorphisms that decrease or abolish their detoxifying ability of carcinogens are thus expected to play important role in cancer development.

Human GST genes are polymorphic, either due to presence of single nucleotide polymorphisms (SNPs) or due to deletions (Ekhart et al., 2009). Total gene deletion or null polymorphism that leads to no functional enzymatic activity, and frequently affects both alleles has been reported to be present in populations for GSTT1 and GSTM1. GSTT1 contributes significantly in detoxification process and is included in GSTθ class, the most ancient of GSTs (Di Pietro et al., 2010). Enzymes of this group have a wide range of substrates including carcinogens in food, air or medications, tobacco smoke, combustion products (Meyer et al., 1991). Frequency of *GSTT1* null polymorphism differs largely among different populations (Spurdle et al., 2001). On the whole, it has highest frequency in Asians (50%) followed by African Americans (25%) and Caucasians (20%) (Landi, 2000).

Distribution of *GSTT1* genotypes have been studied in different parts of India (Roy et al., 2001; Mishra et al., 2004; Naveen et al., 2004; Thoudam et al., 2010) also, however, no such study has yet been reported from Kumaun region of Uttarakhand, a hilly state of northern India. Therefore, this study was designed to determine the distribution of *GSTT1* genotypes in this geographic region.

## Materials and Methods

*Subjects*

In total of 365 unrelated healthy volunteers (blood donors) coming at Susheela Tiwari Govt. Hospital, Haldwani with no history of cancer, blood pressure,

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diabetes and related diseases were recruited as study subjects. All the study participants were informed and signed consent was taken from each of them. A questionnaire form consisting of questions including age, sex, geographic localities, individual and family health history were collected from the participants. Ethical approval for the study was obtained from Institutional Ethics Committee of Govt. Medical College, Haldwani. Since there was a very little number of female donors, they were excluded from the final study and data for only male donors were taken (n=365).

*Genotype detection*

5 ml of peripheral blood was collected in sterile EDTA tubes. DNA was extracted from whole blood following standard phenol- chloroform method (Sambrook et al., 1989). The homozygous gene deletion polymorphism (-/-) for *GSTT1* was detected by performing duplex polymerase chain reaction (PCR). Primer set for *GSTT1* genotypes was lying within the *GSTT1* gene (Spurdle et al., 2001) and another set of primer was selected from human mitochondrial manganese superoxide dismutase (MnSOD) as positive control of PCR. Primer sequences are presented in Table 1.

PCR reaction was performed in a 25 µl of reaction volume using 10 pmol of each forward and reverse primer (Eurofins, Bangalore), dNTP mix (200 µM of each dNTP), and 0.5 U of Taq polymerase (Promega, USA) and PCR buffer provided along with the enzyme. The PCR condition followed an initial denaturation at 95$^0$C for 1 min followed by 30 cycles of 30 sec denaturation at 95$^0$C, 30 sec annealing at 56$^0$C, 30 sec extension at 72$^0$C, and final extension at 72$^0$C for 5 mins. PCR products were checked in polyacrylamide gels stained with ethidium bromide under UV transilluminator. Negative control without template was set each time. The absence of a 131 bp band indicated homozygous deletion (null; -/-) for *GSTT1*. Presence of this band indicated homozygous or heterozygous genotypes (+/+ or +/-, respectively). A 175 bp band of positive control indicated successful PCR. This protocol did not differentiate between genotypes either with one or both copies of the gene. Duplex PCR were repeated for all samples showing null genotype.

**Table 1. Primer Sequences and Product Size for PCR**

| Gene | Primer Sequences | Product Size |
|---|---|---|
| GSTT1 | Forward 5 GGTCATTCTGAAGGCCAAGG 3 | 131bp |
| | Reverse 5 TTTGTGGACTGCTGAGGACG 3 | |
| MnSOD | Forward 5 AGCACCAGCAGGCAGCTGGCTCCG 3 | 175bp |
| | Reverse 5 CGGTGACGTTCAGGTTGTTCACG 3 | |

**Table 2. Frequency Distribution of GSTT1 Alleles in Kumaun Region**

| GSTT1 (n=365) | | Age Group* | |
|---|---|---|---|
| | | 15-35 (n= 297) | 36-55 (n= 59) |
| Presence | 298 (81.64%) | 246 (82.82%) | 45 (76.27%) |
| Null | 67 (18.35%) | 51 (17.17%) | 14 (23.72%) |

*Age was not known for nine individuals; two of them were null for GSTT1. P value: Non significant

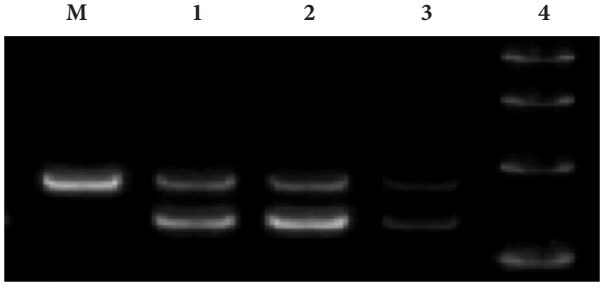


**Figure 1. Duplex PCR for GSTT1 Genotypes.** Lane M show 100 bp DNA ladder, GSTT1 gene is present in lanes 1-3 and lane 4 represents null (-/-) genotype

## Results

The individuals were aged between 18-55 years and their mean age was 28.79±7.02. They were mainly from hills, foothills and planes of Kumaun region, and 6% of them were from adjoining areas of neighboring Uttar Pradesh and Nepal. *GSTT1* null genotype was observed in 18.35% of the individuals. Age- wise distribution of null genotypes is represented in Table 2. Nine individuals did not mention their age, two of whom were null for *GSTT1*. Higher frequency of null genotype was found in older age group (36-55 years), although the difference with younger group (15-35 years) was non-significant as found on the basis of odds ratio with 95%CI value. Figure 1 represents GSTT1 genotypes.

## Discussion

Apart from their classical role in metabolism, GSTs are now being assessed for their involvement in cancer susceptibility and therapeutic outcome, and more recently for drug discovery (Di Pietro et al., 2010). Recent studies have recorded ethnic differences in the distribution of genetic polymorphisms of GST enzymes. Frequencies of *GSTT1* null genotypes have been found to vary drastically among populations (0-64.4) (Di Pietro et al., 2010). It is recorded particularly high among Asians from China, Japan, Korea and Singapore, and in some populations of Brazilians and in Somalians. Our result showing 18.35% of null genotypes corroborates with the previous studies on Indian populations, e.g., from North India (18.4%) (Mishra et al., 2004) and from South India (17.6% by Vettriselvi et al., 2006 and 16.8% by Naveen et al., 2004). No difference in allele frequency according to age group in present population matches with earlier finding on this polymorphism by Garte et al. (2001).

This study presents first report on *GSTT1* genotype distribution from Kumaun region. Further, strength of this study lies in the fact that it involves large number of individuals (n=365) from a small geographic part (Kumaun region). This is particularly important as it has been found that in India, genetic diversity is present not only in intrapopulational level but also within smaller geographic regions (Kashyap et al., 2004). Limitation of the study is that this data is collected purely from male population due to lack of enough female donors. However, this may not be a serious departure from a generalized data as no significant difference in *GSTT1* genotype

distribution by sex was observed for Asians in earlier studies (Garte et al., 2001).

Allele frequency at a genetic locus can influence ethnic variation in cancer predisposition and drug response. Considering the central role played by GSTs in metabolism of carcinogens and drugs, the present study is expected to provide important baseline information for future studies on genetic association with cancer and with treatment outcome in this population of northern part of India.

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