Polymorphism of glutathione S-transferase M1 and T1 genes and susceptibility to psoriasis disease: A study from North India

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Abstract

Background: Increased oxidative stress and resulting inflammation has been emphasized as a factor in the pathogenesis of many diseases including psoriasis. Glutathione S-transferases (GSTs) protect against oxidative stress, inflammation, and genotoxicity. Polymorphisms in the GST genes may lead to an imbalance in pro- and antioxidant systems resulting in the increased production of reactive oxygen species that could influence the pathogenesis of psoriasis.

Aim: The aim of this study was to investigate the association between GSTs (GSTM1 and GSTT1) gene polymorphism in patients with chronic plaque psoriasis as a factor in the susceptibility and development of psoriasis.

Materials and Methods: We assessed 128 patients with psoriasis and 250 age- and sex-matched healthy controls. Genomic DNA was extracted from peripheral blood by the phenol chloroform method. The null GSTT1 and GSTM1 genotypes were identified by multiplex polymerase chain reaction (PCR) method.

Results: The null genotype of GSTM1 and GSTT1 was seen in 45.3% and 40.6% in psoriasis patients whereas in the controls it was 34.4% and 20.0%, respectively. A significant association was seen between the null alleles of the GSTT1 (OR = 2.74) and GSTM1 (OR = 1.58) alone or in combination with tobacco use (P < 0.001) and psoriasis risk. The presence of both null genotypes of GSTM1 and GSTT1 further increased the risk of psoriasis (OR = 3.52) when compared with the positive genotypes of GSTM1 and GSTT1.

Limitations: A major limitation of this study was the small sample size. A large epidemiological study is necessary to confirm these findings.

Conclusions: The null genotype of GSTT1 is a strong predisposing factor for psoriasis in North India.

Key words: Gene-environmental interaction, glutathione S-transferases, polymorphism, psoriasis

Introduction

Psoriasis is a chronic, inflammatory, hyper-proliferative cutaneous disorder affecting 2–3% of the world population. Although the exact pathogenesis of psoriasis is still unclear, there is evidence that oxidative stress, genetic predisposition, infections, physical trauma, medications, and environmental factors may influence psoriasis either individually or in concert. Environmental toxins such as polycyclic aromatic hydrocarbons (PAHs) and hydroxylated metabolites of benzo (a) pyrene (xenobiotics) may influence the development of psoriasis. These chemicals can generate reactive oxygen species leading to oxidative damage of skin cells.

Between 20–90% of the variability in disposition of xenobiotics has been attributed to genetic factors. The genotype-specific form of drug metabolizing enzymes play an important role in the biotransformation of endogenous or exogenous compounds and might be associated in psoriasis.

Published data suggest that increased activity of antioxidant enzymes have synergistic effects in the reduction of oxidative damage and have role in cell protection. Metabolism of xenobiotics

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Recent studies have shown that the presence of null genotypes of *GSTM1* and *GSTT1* enzymes are associated with increased susceptibility to several diseases including psoriasis and vitiligo. Although some studies of *GSTM1* and *GSTT1* gene polymorphism and susceptibility to psoriasis have been reported, there are no reports from India. The current study was undertaken to ascertain whether high-risk alleles of *GSTM1/GSTT1* could influence the susceptibility to psoriasis in the North Indian population. We also aimed to assess whether these alleles could affect the grade and duration of psoriasis, and their relationship with tobacco-use.

**Materials and Methods**

**Subjects**

Approval for the study was obtained from the institutional review board. The study group consisted of 128 psoriasis patients with a mean age 41.9 years (SE 1.48), and 250 age and sex-matched normal healthy individuals as controls with a mean age of 42.9 years (SE 0.57). The ethnic origin of the cases and controls were similar. Patients were selected based on the basis of a questionnaire administered in the Outpatient Department (OPD) of the Department Of Skin and VD, Pt. B.D. Sharma PGIMS, Rohtak, that included medical records, family history of disease, gender, and history of consumption of tobacco in any form (cigarette/bidi smoking, chewing tobacco in beetle leaf, pan masala/gutka, etc.). Only patients with uncomplicated chronic plaque psoriasis were selected. Psoriasis patients with associated conditions such as diabetes, bronchial asthma, cancer, or any other diseases were excluded from the study. Consent from the participants was obtained after explaining the aims of the study, and age- and sex-matched healthy individuals were selected as controls. The inclusion criteria for controls were the absence of any prior history of psoriasis lesions.

**DNA extraction and genotyping**

Five ml of blood was collected in EDTA vials from controls and patients. Genomic DNA was extracted from blood lymphocytes using the proteinase K and phenol chloroform extraction procedure. The multiplex PCR method was used to detect the presence or absence of *GSTT1* and *GSTM1* genes in the genomic DNA samples, simultaneously in the same tube, as described previously. Electrophoreses of PCR products were done in 2% agarose gels and visualized by ethidium bromide staining. DNA from samples positive for *GSTM1* and *GSTT1* genotypes yielded bands of 215 bp and 480 bp whereas internal positive control (*CYPIA1*) PCR product corresponded to 312 bp [Figure 1].

**Statistical analysis**

Statistical analysis was performed using SPSS software version 20.0 (Chicago). Descriptive measures such as mean and standard deviation were applied for normally distributed variables and *t*-test for comparison between groups. Binary logistic regression model (BLRM) assessed differences in genotype prevalence and association between cases and controls. Multivariate analysis, Chi-square test, correlation coefficient, odds ratio (OR), and its 95% confidence interval (CI) were used to describe the strength of association. A *P* value of <0.05 was considered to be statistically significant.

**Results**

During the course of the study, results of 5% of the samples were checked randomly. Data input and process was double tracked, adopting logic check. Table 1 presents the frequencies of *GSTM1* and *GSTT1* genotypes by case-control status for psoriasis risk. Of the 128 patients with psoriasis, frequency distribution of null genotype of *GSTM1* and *GSTT1* was 45.3% and 40.6%, respectively while in the 250 control samples, the frequency of null genotype of *GSTM1* and *GSTT1* was 34.4% and 20.0%, respectively. We observed a significantly higher risk for psoriasis in patients with the null genotype of *GSTT1* (OR = 2.74; 95% CI = 1.71–4.38) and *GSTM1* (OR = 1.58; 95% CI = 1.02–2.44) as compared to controls [Table 1]. This association was stronger in patients with the null alleles of *GSTT1* (*P* < 0.001) as compared to *GSTM1* null genotypes (*P* = 0.039).

A combination of the two high-risk genotypes (null genotypes of *GSTM1/GSTT1*) was also compared to the non-risk genotypes (positive genotypes of *GSTM1/GSTT1*) for the risk of psoriasis [Table 2]. The OR for development of psoriasis in the two high-risk genotypes was 3.52-fold higher than the non-risk genotypes (*P* < 0.001).

The association between tobacco use and null genotypes of *GSTT1* and *GSTM1* in patients and controls is summarized in Table 3. Our data indicate that OR for null genotypes of *GSTM1* (OR = 3.14) and *GSTT1* (OR = 4.71) was higher in tobacco users as compared to positive genotypes of nonusers (*P* < 0.001).

The association between gender and changes in null genotypes of *GSTM1* and *GSTT1* in patients and controls is summarized in Table 4. We demonstrated a higher risk in females (3.5-fold) than that in males for *GSTT1* null genotypes for susceptibility to psoriasis (OR = 3.53; *P* < 0.001); however, OR for null genotypes of *GSTM1* was statistically nonsignificant (*P* > 0.05).
The body surface area affected by psoriasis (grade of disease) was categorized into three groups: <20%, 21–30%, and >30%. There was no correlation between GSTs genotypes and grade or duration of the disease (\(P > 0.05\)) with respect to initiation and progression of psoriasis [Charts 1-4].

### Discussion

In the present study, we found a 34% frequency of null genotypes of \(GSTM1\). This frequency is similar to that reported in African and Southern Asian populations. The 20% frequency observed for null alleles of \(GSTT1\) among healthy individuals also lies within the range reported in Caucasian, European, and South Asian populations [Table 5].

Our study indicated that the null allele of \(GSTT1\) gene is associated with a 2.7-fold higher risk for psoriasis as compared to healthy controls, while the null allele of \(GSTM1\) gene is associated with a lower risk of 1.58 times. No association was observed between...
GST genes polymorphism and psoriasis risk

GSTs genotypes and the grade or duration of psoriasis. Our findings are in accordance with published reports of the association of GST genes with a number of dermatologic diseases such as psoriasis, solar keratoses, vitiligo, atopic dermatitis, and Behçet's disease. A pioneer study by Richter-Hintz et al. demonstrated that the null alleles of GSTM1 significantly correlated with psoriasis, but not GSTT1. However, a study by Solak et al. in a Turkish population showed no association between GSTM1 and GSTT1 null genotypes with chronic plaque psoriasis. Smith et al. showed that the null genotype of GSTM1 influences erythemal sensitivity to phototherapy in adult Caucasian patients with psoriasis but not the GSTT1 genotypes. Another study showed significant correlation with GSTT1 geno- and phenotypes in psoriasis patients treated with fumaric acid esters but it was not substantially different from healthy controls. Thus, the association of psoriasis risk with null alleles of GSTM1 or GSTT1 varies significantly among different populations.

Patients with both high-risk genotypes of GSTM1 and GSTT1 gene (GSTM1 null and GSTT1 null genotypes) had a 3.5-fold higher risk for psoriasis development compared to positive genotypes of GSTM1 and GSTT1 (P < 0.001, OR = 3.52). Studies have shown that patients with both null alleles of GSTT1/GSTM1 have a higher risk of diseases the urinary bladder, skin, head and neck cancer, type 1 diabetes, and vitiligo when compared to patients with positive alleles of GSTM1/GSTT1 gene.

Table 5: Worldwide comparative frequency of null genotypes of glutathione S-transferase M1 and glutathione S-transferases T1 in different ethnic population

| Ethnic origin           | Total number of sample for GSTM1 | GSTM1 null frequency (range in %) | Total number of sample for GSTT1 | GSTT1 null frequency (range in %) | Reference                  |
|-------------------------|----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|-----------------------------|
| North Indian            | 250                              | 0.340 (34.0)                      | 250                              | 0.200 (20.0)                      | Present study               |
| Indian                  | 5500                             | 0.302 (20-42)                     | 5428                             | 0.178 (12-35)                     | 19,29,31 and present study  |
| Southern Asian          | 6237                             | 0.329 (20-46)                     | 6195                             | 0.182 (12-38)                     | 29,30                       |
| Eastern and South Eastern Asian | 10597                         | 0.527 (42-65)                     | 8765                             | 0.463 (25-51)                     | 29,30                       |
| European                | 15126                            | 0.518 (46-58)                     | 11,682                           | 0.183 (10-26)                     | 29,30                       |
| Caucasian               | 2714                             | 0.529 (42-56)                     | 1223                             | 0.197 (12-27)                     | 29,31                       |
| African                 | 1291                             | 0.326 (11-55)                     | 1291                             | 0.363 (26-47)                     | 29                          |

GSTM1: Glutathione S-transferase M1, GSTT1: Glutathione S-transferases T1

Chart 1: Association of GSTM1 genotype with body surface area of disease. P value in between the strata of <20% and 20–30% = 0.892. P value in between the strata of <20% and >30% = 0.982

Chart 2: Association of GSTT1 genotype with body surface area of disease. P value in between the strata of <20% and 20–30% = 0.791. P value in between the strata of <20% and >30% = 0.402

Chart 3: Association of GSTM1 genotype with duration of the disease. P value in between the group = 0.850

Chart 4: Association of GSTT1 genotype with disease duration. P value in between the group = 0.154
We also observed a significant association of psoriasis in tobacco users with null genotypes of *GSTM1* (OR = 4.71, P < 0.001) and *GSTM1* (OR = 3.14, P > 0.001) in comparison to tobacco nonusers with positive genotypes. Tobacco smoke contains 10^12-10^17 free radicals and other highly reactive electrophiles such as PAH and hydroxylated metabolites of benzo (a) pyrene and GSTs are instrumental in the elimination of these other toxic metabolites thus protecting cells from oxidative stress.17 Inter-individual variability in the expression of the PAH and BaP metabolizing enzymes may be explained at least in part by genetic polymorphisms in the human genome. Published data suggest that psoriatic individuals with the null allele of *GSTM1* who smoke have higher PAH or hydroxylated benzo (a) pyrene genotoxicity and mutagenicity.4,5,8,9,11

Our data also showed a significant association (P < 0.001) of null genotypes of *GSTM1* in both males or females with psoriasis risk; however, no association was observed with gender and *GSTM1* null genotypes (P > 0.05). This suggests that females who possess null alleles of *GSTM1* have higher risk (OR = 3.53) for psoriasis as compared to males (OR = 2.57) in north Indian population [Table 4]. This finding is consistent with previous reports in Indian and Chinese populations.36,37

GSTs are phase II xenobiotic metabolizing enzymes active in detoxifying a wide variety of potentially toxic electrophiles by conjugating with glutathione and metabolizing them.2,3,5,8,9 A previous study purified and characterized the expression of glutathione transferase in psoriatic skin.18 GST enzymes play a crucial role in cell protection against oxidative damage which is probably associated with psoriasis.12-17 The association with null genotypes of GSTs observed in our study suggests that the inactive form of GSTs enzymes results in reduced detoxification of endogenous/or exogenous toxicants leading to the initiation and progression of psoriasis.

This is the first genetic study in the Indian population exploring the interaction between *GSTM1/GSTT1* genotype alone or in combination with tobacco use and susceptibility to psoriasis. To evaluate the interaction between genetic and environmental factors, adequately large sample size is needed. We have examined only two detoxifying genes and further study may be warranted to explore the involvement of other antioxidants and detoxification pathway genes that may be associated alone or in combined analysis in large epidemiological studies.

**Conclusion**

Our findings indicate that the null allele of *GSTM1/GSTT1* genotype alone or in combination with tobacco use are significantly associated with psoriasis risk; however, no association was observed between *GSTM1/GSTT1* genotypes and grade/or duration of the disease. Moreover, the presence of both high risk alleles of GST genotypes further augments the risk of psoriasis in the North Indian population.

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**Conflicts of Interest**

There is no conflicts of interest.

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