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# *GSTM1*, *GSTM3* and *GSTT1* gene variants and risk of benign prostate hyperplasia in North India

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**Abstract**. Glutathione S-transferases (GSTs) play an important role in detoxification of various toxic compounds like carcinogens in cigarette smoke and tobacco by conjugating to toxic compounds and inactivating their hazardous effect. Variation in Glutathione S-Transferases (GSTs) genes may alter the catalytic efficiency of GST isoenzymes leading to potential increase in cancer susceptibility due to various carcinogens. We therefore, investigated association of *GSTM1*, *GSTM3* and *GSTT1* variants with susceptibility to benign prostate hyperplasia (BPH) and cigarette, tobacco chewing and alcohol consumption as confounding factors in 141 BPH and 184 healthy controls. Results showed increased risk for BPH susceptibility in patients with *GSTM1* null genotype (OR-2.03, $p = 0.013$) and smoking (OR-3.12, $p = 0.028$), tobacco chewing (OR-2.54, $p = 0.039$) and alcohol habits (OR-3.39, $p = 0.010$). Null genotype of *GSTM1* with cigarette, tobacco and alcohol habits predisposed increased risk for BPH.

Keywords: Glutathione S-transferases, benign prostate hyperplasia, genotype, polymorphism, smoking, multiplex PCR

## 1. Introduction

Benign prostatic hyperplasia (BPH) is most common encountered non-neoplastic prostatic disease. Despite its high prevalence and major impact on quality of life of the patient the etiopathogenesis and risk genes for BPH are not clear. The only definitive risk factors are an intact androgen system, and aging [1]. Its prevalence is age dependent with histological evidence of BPH rarely under 50 years of age, but by age 80 virtually all men have some histological evidence of the process.

Several studies provide support for a genetic component to the development of BPH. The concordance rate for BPH among dizygotic compared to monozygotic twins suggests a genetic component to BPH [2]. A family history of BPH has been associated with an increased risk of urologic measures of BPH or clinical BPH [3,4]. Men with a family history of BPH have been reported to have larger prostates compared to men with sporadic BPH [5]. These suggest that certain genes may influence the development of BPH. Exposure to various environmental carcinogens and reactive oxygen and nitrogen metabolites generated by physiological processes may be the most proximate cause of cell injury that secondarily results in genetic instability and ultimately BPH or carcinogenesis [6]. Therefore alterations in different cellular detoxification enzymes including Glutthione-S-transferases (GSTs) may have a definite role in development of benign prostate hyperplasia. Glutathione S-transferases (GST) are a family of enzymes whose major role is inactivation of xenobiotic agents. The *GST* gene superfamily consists of four gene classes (A, M, T, and P) encoding for enzymes which catalyze the conjugation of electrophilic compounds to glutathione [7]. GSTM1 and GSTT1 are important detoxifying enzymes which detoxify the reactive metabolites of cigarette smoke-derived chemicals

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such as benzo[a]pyrene and other polycyclic aromatic hydrocarbons [8]. Polymorphisms in *GST* genes, therefore, may influence the development of BPH. These include polymorphisms in *GSTM1*, *GSTM3* and *GSTT1*.

Although associations between few of the aforementioned polymorphisms in *GST* genes have been examined in prostate cancer (PCa) patients in different ethnic groups including our previous study, however, no prior studies have evaluated effects of these polymorphisms exclusively on patients with benign prostate hyperplasia which is more common than prostate cancer [9, 10]. These findings inspired us to determine the role of *GSTM1*, *GSTT1* and *GSTM3* polymorphism with risk of BPH and also to study the influence of these polymorphisms with other confounding factors like smoking, tobacco chewing and alcohol consumption habits among BPH patients. To the best of our knowledge, no association study has been yet reported on *GSTs* polymorphisms in BPH. So, this case-control study has been carried out to find the association of *GSTM1*, *GSTT1* and *GSTM3* polymorphisms to genetic predisposition of BPH in North Indian population.

## 2. Materials and methods

### 2.1. Study population

The details regarding selection of patients have been previously published [11]. The participants in this study (BPH patients and controls) were unrelated individuals of similar ethnicity from Lucknow and other adjoining cities, visiting department of Urology at the tertiary referral centre of North India during May 2005 to December 2007. All the patients visiting the department were asked about lower urinary tract symptoms (LUTS) and recorded as per AUA symptom score. Digital Rectal Examination (DRE) and Prostate Specific Antigen (PSA) screening was done in all patients with LUTS. Cases with PSA $\geqslant$ 4 ng/ml and/or abnormal DRE finding were advised for prostatic biopsy and histological examination. In 20 patients with transurethral resection of the prostate (TURP) followed by histological examination was done for bothersome LUTS. Cases having previous history of cancer were excluded. Only symptomatic and histologically proven BPH cases ($n = 141$) were inducted in the study. Healthy men ($n = 184$) of similar ethnicity and those attending regular health checkups camp/or participating in prostate cancer screening program were recruited as controls. None of the controls had any history for cancer. Controls were taken in age range 40–80 years to match the age of patients. The total PSA levels were determined in controls and BPH patients using CanAg PSA ELISA kits (Fugirebio, Sweden). Control individuals with PSA levels $>$ 4.0 ng/ml were excluded. The study was approved by ethical committee of the Institute. Participants were asked to report weekly frequency of alcohol consumption and were grouped in drinkers (alcohol consumption frequency $>$ 1/week) and non drinkers (alcohol consumption frequency $<$ 1/week). Participants were also questioned about their tobacco chewing habits and grouped into non tobacco users and tobacco users. Data on smoking habits were collected by asking whether they had smoked 20 packs of cigarettes or more in their lifetimes and, if yes, whether they currently smoke or had smoked in the past. A subject was considered as former smoker if he has not smoked for past one year.

### 2.2. Genotype determination

Standard venipuncture was used to collect blood samples from all study participants in tubes with EDTA as an anticoagulant. For genotype determination, genomic DNA was extracted by salting out method [12]. A 260/280 nm reading was taken with Nano Drop. DNA was used only if A260/280 nm quality of DNA was between 1.8–2.0.

The *GSTM1* and *GSTT1* polymorphisms, which results in the presence (non-deleted) or absence (null or deleted) of the enzyme, was detected by a PCR product co-amplified with $\beta$-globin as a positive internal control within a multiplex PCR as previously described [13]. The 3 bp deletion in intron 6 of *GSTM3* was determined using the previously described method and separated on 20% polyacrylamide gel [14]. To improve the quality and validation about 20% of the blinded and randomly selected samples were re-genotyped by a second investigator. Concordance in genotyping was greater than 99.0 %.

### 2.3. Statistical analysis

Statistical analysis was performed using the $\chi^2$ test to compare the genotype frequency distribution in patients and controls with SPSS software (version 11.5) using Pearson chi-square. Deviation from the Hardy-Weinberg equilibrium in controls was assessed using the $\chi^2$ test. Unconditional logistic regression analysis was used to fit the statistical models to predict the association of *GSTM1*, *GSTT1* and *GSTM3* with the

risk of susceptibility to benign prostate hyperplasia and different confounding factors such as smoking, tobacco chewing and alcohol consumption associated with BPH. Association was expressed as odds ratios (OR) as risk estimates with 95% confidence intervals (95% CI). ORs were adjusted for relevant biological variables (age, cigarette smoking, tobacco chewing and alcohol consumption). P value $< 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Demographic details and genotype distribution between cases and controls

There was no significant difference between mean age of controls ($61.88 \pm 7.53$ years) and patients ($62.89 \pm 9.74$ years). The mean PSA of cases was 9.75 ng/ml (range 3–25 ng/ml). None of the controls were having total PSA above 4 ng/ml. However number of former smokers was very low therefore we have made all analysis by grouping as non smokers and smokers. Patients consisted of higher number of cigarette smokers (50%) in comparison to controls (22.2%). As expected our results show that subjects with cigarette smoking habit are at 4-folds higher risk for susceptibility to BPH (OR 3.89, $p < 0.001$). The percentage of tobacco chewers was higher in cases (43.8%) as compared to controls (26.6%), although not statistically significant. The above results are presented in Table 1. The genotype frequency distribution of *GSTM1*, *GSTM3* and *GSTT1* in healthy controls followed Hardy-Weinberg equilibrium. The frequency of null deletion genotypes of *GSTM1* and *GSTT1* was 51.1% and 12.8% respectively in cases as compared to 40.2% and 20.1% respectively in controls. We observed a two fold risk for BPH associated with null genotype of *GSTM1* (OR-2.03, $p = 0.013$). However, no association was observed with variant forms of *GSTM3* and *GSTT1*.

### 3.2. Gene-gene interaction

As BPH is a multifactorial as well as polygenic disease therefore, we analysed combined effect of *GSTM1*-*GSTM3* and *GSTM1*-*GSTT1* genes. A seven fold risk for susceptibility to BPH (OR-7.2, $p = 0.002$) was observed in carriers of variant form of both *GSTM1* (null) and *GSTM3* (AB + BB) genotype (Table 2). We did not observe any association with combined effect of variant forms of *GSTM1* (null)-*GSTT1* (null) genotypes.

### 3.3. Genetic association with lifestyle related confounding factors

Further, we tried to analyse the possible role of *GST* gene polymorphisms related to various confounding factors such as cigarette smoking, tobacco chewing and alcohol consumption. The results are represented in Fig. 1. We categorised the subjects according to smoking status and observed significant difference in genotype distribution of *GSTM1* among cases and controls in non smokers (chi square $p = 0.034$) as well as in smokers category (chi square $p = 0.040$). We also observed that individuals with smoking habit and carrying null genotype of *GSTM1* were highly susceptible to BPH (OR 3.12, $p = 0.028$). Similarly, risk was also observed for null genotype carriers for *GSTM1* among tobacco users (OR 2.54, $p = 0.039$) and alcohol consumers (OR 3.39, p= 0.010). Surprisingly, we observed risk associated with non tobacco users and carriers of variant allele of *GSTM3* (OR 2.23, $p = 0.045$). In the present study no association of *GSTT1* polymorphism was observed with the above confounding factors.

## 4. Discussion

The absence of a single and reliable measure of BPH for research purposes complicates BPH research and may, in part, explain the lack of consistency in the associations across all measures of BPH. Though urological measures provide insight into the BPH phenotype yet no single measure is diagnostic for BPH, necessitating the need to evaluate association of polymorphisms with several outcomes of BPH.

The present study reports the preliminary evidence of association of genes encoding drug detoxifying enzyme GST and susceptibility to BPH. The study suggested significant influence of null genotype of *GSTM1* gene with the susceptibility to BPH either alone (OR 2.03, $p = 0.013$) or in combination with *GSTM3* (AB/BB) genotype (OR 7.2, $p = 0.002$). These findings can be supported by the fact that both *GSTM1* and *GSTM3* are in linkage disequilibrium [14]. The null allele of *GSTM1* has also been reported to be associated with prostate cancer in different ethnic groups like North Indians, Chilean population and Japanese [10,15,16]. *GSTM3* variant (B) allele is also known to be associated with prostate, larynx, colorectal, bladder and breast cancer [9,17–20]. It is well documented that *GSTM3**B allele induces YY1 transcription factor which in turn suppresses the expression of various cytokines or stress

Table 1
Demographic details and genotype frequency distribution between cases and controls

| | Cases | Controls | OR# (95% CI) |
|---|---|---|---|
| Age (years ± SD) | 62.89 ± 9.74 | 61.88 ± 7.53 | |
| Total PSA (mean ± SD) ng/ml | 9.75 ± 13.17 | 2.3 ± 1.2 | |
| Demographic Details | n (%) | n (%) | |
| *Cigarette/bidi smoking* | | | |
| Non smokers | 48 (50.0) | 123 (77.8) | Ref |
| Smokers | 48 (50.0) | 35 (22.2) | 3.89 (1.99–7.59)* |
| *Tobacco chewers* | | | |
| Non- tobacco chewers | 54 (56.2) | 116 (73.4) | Ref |
| Tobacco chewers | 42 (43.8) | 42 (26.6) | 1.55 (0.81–2.95) |
| *Alcohol consumption* | | | |
| Non drinkers | 63 (65.6) | 108 (68.4) | Ref |
| Drinkers | 33 (34.4) | 50 (31.6) | 0.55 (0.27–1.06) |
| Genotypes | n (%) | n (%) | |
| *GSTM1* | | | |
| Present | 69 (48.9) | 110 (59.8) | Ref |
| Null | 72 (51.1) | 74 (40.2) | 2.03 (1.16–3.56)** |
| *GSTM3* | | | |
| AA | 111 (78.7) | 158 (85.9) | Ref |
| AB + BB | 30 (21.3) | 26 (14.1) | 1.39 (0.67–2.85) |
| *GSTT1* | | | |
| Present | 123 (87.2) | 147 (79.9) | Ref |
| Null | 18 (12.8) | 37 (20.1) | 0.70 (0.33–1.49) |

*$p < 0.001$, **$p = 0.013$.
#Depicted odds ratio is corrected for age, smoking, alcohol consumption and tobacco chewing.

Table 2
Risk for BPH associated with different gene combinations

| | | Cases n (%) | Control n (%) | OR# | 95% CI |
|---|---|---|---|---|---|
| *GSTM1* | *GSTM3* | | | | |
| Present | AA | 54 (38.4) | 89 (48.4) | Ref | |
| Present | AB + BB | 15 (10.6) | 21 (11.4) | 0.96 | 0.38–2.41 |
| Null | AA | 57 (40.4) | 69 (37.5) | 1.54 | 0.85–2.78 |
| Null | AB + BB | 15 (10.6) | 5 (2.7) | 7.21 | 2.04–25.41* |
| *GSTM1* | *GSTT1* | | | | |
| Present | Present | 61 (43.2) | 74 (40.2) | Ref | |
| Present | Null | 6 (4.3) | 14 (7.6) | 0.99 | 0.33–2.98 |
| Null | Present | 62 (44.0) | 72 (39.2) | 1.35 | 0.75–2.46 |
| Null | Null | 12 (8.5) | 24 (13.0) | 0.62 | 0.22–1.74 |

*$p = 0.0023$.
#Depicted odds ratio is corrected for age, smoking, alcohol consumption and tobacco chewing.

response genes like IFN-c [21]. Recently, Bostwivk et al demonstrated consistent reduction or loss of expression of all subclasses of GST with progression of prostate neoplasia from benign epithelium to high grade prostatic intra-epithelial neoplasia and carcinoma [22].

Furthermore, while studying the role of *GST* gene polymorphisms in association with confounding factors in BPH progression, we observed 2–3 folds increased risk in individuals with either smoking habit (OR 3.12, $p = 0.028$), tobacco chewing (OR 2.54, $p = 0.039$) or alcohol consumption (OR-3.39, $p = 0.010$) and carrying a null genotype of *GSTM1* gene. These results strongly suggest significant modulation of risk for BPH with respect to *GSTM1* null genotype among cigarette smokers, tobacco chewers and alcohol consumers. In support of above findings, Platz et al reported cigarette smoking to be a risk factor for BPH [23]. Smoking has been shown to affect the immune system. Studies have reported that smokers have decreased natural killer cell activity and impaired T lymphocyte suppressor cell function compared with nonsmokers, while smoking cessation has been associated with increased natural killer cell activity and decreased white blood cell counts [24–26]. Smoking/ tobacco chewing may act by increasing inflammation in prostatic tissue, so that smokers are more prone to prostatic diseases in-

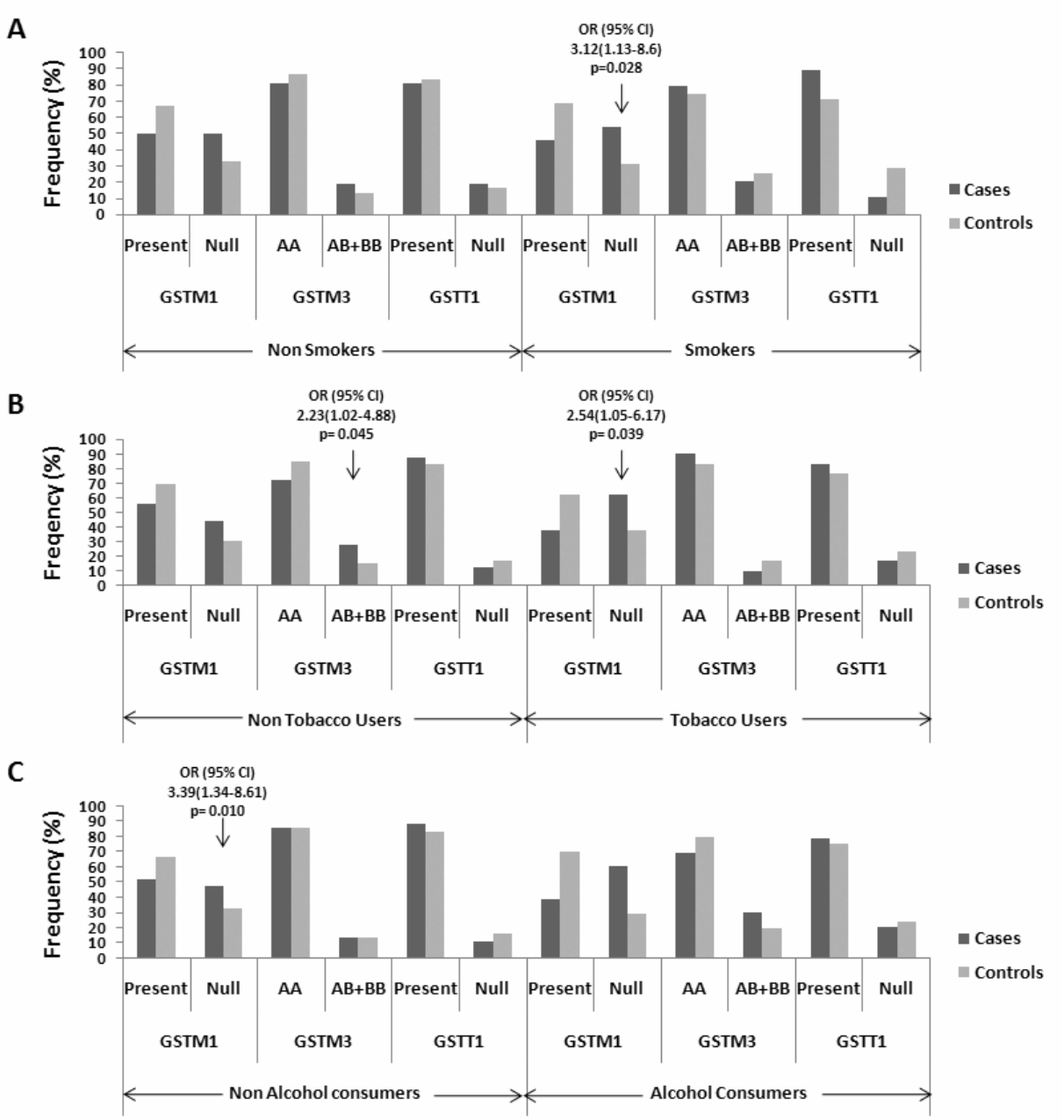


Fig. 1. Association of lifestyle related risk factors; smoking status (A), tobacco use (B) and alcohol consumption (C) with *GSTM1*, *GSTM3* and *GSTT1* genotypes.

cluding BPH or prostate cancer, than in nonsmokers. Androgens play a role in the development and maintenance of BPH particularly in the prostatic epithelium, which is supported by androgen receptor expression in prostatic epithelium [27]. Alcohol consumption is suggested to decrease testosterone concentrations and in some cases increase estrogens concentration hence higher susceptibility to BPH [28]. Our study suggested no influence of *GSTT1* gene polymorphism and susceptibility to BPH.

Few studies have investigated the association of *GST* gene polymorphisms with PCa using BPH patients as controls, however, none of them found association of *GSTM1* or *GSTT1* polymorphisms with risk for

PCa [29,30]. The reason for no association of *GST* gene with PCa in these studies could be possibly due to association of *GST* gene polymorphism with BPH risk itself.It is for this reason that BPH patients are no longer considered as good controls as some percentage of the BPH patients may in later years develop prostate cancer A meta-analysis investigating results from 14 studies from Caucasian populations and 3 studies from Japanese population reporting association of *GST* gene polymorphisms with PCa,could not validate their findings [31]. Recently, few studies have investigated the association of BPH and polymorphisms of genes involved in sex hormone metabolism, growth factors, cytokine and vitamin D receptors, however, the research in this field is still in its infancy and investigations are still underway. The present study relates the importance of association of *GST* gene polymorphism in BPH progression [32,33].

Although, we have analyzed that *GSTM1* gene may play an important role in BPH disease progression, we cannot rule out the possibility that other polymorphisms in potentially other important members of this family may also influence the risk associated with BPH. Furthermore, we have not categorized the smokers into current and former which may further validate these results. Due to the small sample size, these results should be interpreted with caution, as chance findings cannot be ruled out.

In conclusion, this study suggests significant influence of null genotype of drug metabolizing gene *GSTM1* and association of BPH. In gene-gene interaction the null genotype of *GSTM1* and AB/BB genotype of *GSTM3* posed a 7-folds increased risk for BPH. Further, the null genotype of *GSTM1* was also associated with increased risk of BPH in patients with smoking, tobacco chewing and alcohol drinking habits.

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