Original Article

Are Genetic Polymorphisms of Glutathione S-Transferase P1 Gene Associated with Urothelial Carcinoma of the Urinary Bladder?

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Abstract

Background: Polymorphisms in genes encoding glutathione S-transferase (GST) may affect susceptibility to develop urothelial carcinomas (UCs). One of the extensively studied genes in this group is GST P1 (GSTP1), but studies of the relationship of polymorphisms of GSTP1 and UC of the bladder have been equivocal. Hence, we assessed the association between genetic polymorphism of GSTP1 gene and the development of UC of the urinary bladder. **Materials and Methods:** This prospective, case–control study was conducted in the departments of urology and clinical genetics at a tertiary care teaching hospital in South India, which included 52 patients with histopathologically confirmed UC bladder and matched with 46 controls from August 2012 to July 2013. The study participants provided a single venous blood sample for extraction of genomic DNA. Laboratory personnel was blinded to sample groups. The primary outcome of the study was to detect association of genetic polymorphism of GSTP1 gene with UC of the bladder. The secondary outcome was to assess if the risk of urothelial bladder cancer is increased in smokers with polymorphism of GSTP1 gene. **Statistical Analysis Used:** We used the Chi-square or Fisher's (F) exact test to compare discrete variables. Unconditional logistic regression was used to estimate adjusted odds ratios and 95% confidence intervals. **Results:** Although the heterozygous polymorphic genotype ile/val (AG) was seen more frequently in cancer group (34.6% vs. 23.9%), the difference was not statistically significant (P = 0.202). None of the smokers had homozygous polymorphic valine allele and GSTP1 did not add to the susceptibility of UC bladder even among smokers. **Conclusions:** A lack of association between GSTP1 313 G/G polymorphism and urothelial cancer of bladder was observed.

Keywords: *Glutathione S-transferase P1, smoking, urothelial carcinoma bladder*

Introduction

Urinary bladder cancer (BC) is one of the most common urological malignancies. As per the Indian cancer registry data in men, it is the ninth-most common cancer accounting for 3.9% of all cancer cases.[1] In middle-aged and elderly men, BC is the second-most prevalent malignancy after prostate cancer. It is well documented that tobacco smoke[2] and occupational carcinogens[3] promote epithelial cell dysplasia and they are the most common environmental cause of urothelial carcinoma (UC) of the bladder. Most of these carcinogens are detoxified by Phase II metabolic enzymes like Glutathione S-transferase (GST). It is hypothesized that polymorphisms in genes encoding these enzymes, affects susceptibility to develop UCs.

One of the extensively studied genes in this group is GST P1 (GSTP1). The GST polymorphism not only appears to influence susceptibility to disease but also influences the responsiveness to various carcinogens. Apart from urothelial cancers, the GST polymorphism has been associated with various cancers, including hepatocellular carcinoma, breast, prostate, renal, and testicular cancers.[4] Studies of the relationship of polymorphisms of GSTP1 and UC of the bladder have been equivocal, with some studies claiming positive associations with GSTP1 polymorphism, while the others claiming a negative association. This study assessed the association between genetic polymorphism of GSTP1 gene and the development of UC of the urinary bladder. The primary outcome of the study was to detect the association of genetic polymorphism of GSTP1 gene with urothelial cell carcinoma of the bladder. The secondary outcome

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was to assess if the risk of urothelial BC is increased in smokers with polymorphism of GSTP1 gene.

Materials and Methods

This was a prospective, case–control study conducted in the Departments of Urology and Clinical genetics in a tertiary care center from August 2012 to July 2013. After approval by the Institutional Review Board (No. 8063/2012), 52 patients with histopathologically confirmed UC bladder were matched with 46 controls for age, gender, ethnic origin, tobacco use, occupational exposure, and family history of UC. Patients with a prior history of radiotherapy, chemotherapy, or metastatic carcinoma from other sites to the bladder were excluded from the study. The controls were patients who had negative cystoscopic bladder evaluation for nonmalignant urological conditions.

After obtaining informed consent, a detailed history, examination, and results of evaluation and treatment were recorded. The study participants were then asked to provide a single venous blood sample for extraction of genomic DNA. Laboratory personnel were blinded to sample groups.

Genotyping and method of estimation

The GSTP1 genotype was determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) technique. For the extraction of genomic DNA from the blood sample, the blood sample was centrifuged, red blood cells were lysed, and DNA extracted from the white blood cells in the form of pellets. Using the primer pair, a 177 bp (base pairs) fragment of the GSTP1 gene-containing Isoleucine (Ile) to Valine (Val) substitution in exon 5 (codon 105) was amplified.
Forward: 5'-ACC CCA GGG CTC TAT GGG AA-3' and
Reverse: 5'-TGA GGG CAC AAG AAG CCC CT-3'

The amplification cycles included an initial denaturing at 94°C for 30 s, followed by an annealing step at 55°C for 30 s and a final extension step at 72°C for 30 s, a slight modification from the method described by Safarinejad *et al*.[5]

The PCR products were then digested with Alw26I restriction enzyme. Dye was added to the restriction products, and they were analyzed by 2% agarose gel. Homozygous Ile/Ile individuals have a single fragment of 177-bp (base pairs) and it appears as a dark band and homozygous Val/Val individuals will have both 92- and 85-bp fragments – appearing as a faded band. The presence of all three fragments corresponds to heterozygous Ile/Val individuals. Quality control: to maintain good quality control, 10% of the duplicates for both the cases and the controls were genotyped with 100% concordance to the genotype by PCR-RFLP.

Statistical methods

The power of the study – 80% was determined considering of following variables:

1. Case–control study design
2. Significance level 5% (2 sided)
3. Expected frequency of risk allele in the control population = 0.28
4. The genetic effect for odds ratio (OR) 2.5 or greater.

Assuming log-additive model of inheritance and frequency of risk allele to be 0.28[6] a total sample size of 98 (52 cases/46 controls) would be required to detect an OR of 2.5 or more with the desired power of 80% and a significance level of 5%.

Results are expressed as a mean ± standard deviation. The Hardy–Weinberg equilibrium was examined using the goodness of fit Chi-square test. It is done to compare the observed allele frequencies in the study with the expected frequencies determined from control objects. To assess the homogeneity between cases and controls regarding their geographic origin, smoking awareness, cigarette smoking, disease pathological status, and genotypes, the Chi-square or Fisher's (F) exact test was used. To determine the association of polymorphisms with susceptibility to develop transitional cell carcinoma (TCC) and between the various clinic-pathological characteristics of BC, we used unconditional logistic regression to estimate adjusted ORs and 95% confidence intervals (CIs). The (SPSS Version 18.0. SPSS Inc., Chicago, USA) was used for the statistical analysis.

Results

The demographic and clinical characteristics of the study population are shown in Table 1. Both groups were well matched on the basis of age and gender. Seventy-two percent of all the smokers had TCC bladder. It was interesting to note that 66.6% of the cases were aware of the fact that smoking causes TCC bladder, but they still continued to smoke. Thirty-six percent of our cases and 17.3% of our controls were exposed to various forms of toxins due to their occupation and this difference was statistically significant. There was a statistically significant association between smoking and urothelial cancer in our study population (OR = 4.53, $P = 0.000429$).

Table 2 depicts the genotype distribution of the GSTP1 polymorphism between cases and controls. The GSTP1 genotype was in the Hardy–Weinberg equilibrium for the controls ($\chi^2 = 0.68$). The frequency of both the homozygous wild type and homozygous polymorphic allele was more in the control group. We noticed that the predominant allele in both the cases and controls were the wild type, i.e., isoleucine-isoleucine, i.e., 63.4% and 67.4%, respectively. Although the heterozygous polymorphic genotype ile/val (AG) was seen more frequently in cancer groups (34.6% vs. 23.9%), the difference was not statistically significant ($P = 0.202$). However, no statistically significant difference was seen in the allele distribution among cases and control, suggesting the lack

of association of the homozygous or heterozygous valine allele with TCC bladder.

Table 3 presents the subset analysis of the genotype frequencies for smoking. We did a subset analysis to look for genotype frequency among smokers. Majority (73.3%) of the smokers with TCC had the wild-type allele, a few (31.2%) had ile/val heterozygous genotype, and none of them had homozygous polymorphic valine allele. Even among the control group, majority (66.6%) of the smokers had TCC with wild-type homozygous allele and 25% had ile/val heterozygous allele and 0.08% had homozygous polymorphic allele. There was no statistically significant difference among the cases and controls, suggesting that the polymorphic GSTP1 does not add to the susceptibility of TCC even among smokers.

Discussion

GSTP1 plays an important role in protecting cells from cytotoxic and carcinogenic agents. It forms the thioester bond between the sulfur atom of GSH and the substrate and then catalyzes the conjugation of reduced glutathione (GSH) with the compounds that contain an electrophilic substrate. It also detoxifies the organic hyper-oxides by the reduction reaction.[7] Till date, human cytosolic GST enzymes are subdivided into eight distinct subtypes: alpha, mu, kappa, omega, pi, sigma, theta, and zeta. These are encoded by the genes GSTA, GSTM, GSTK, GSTO, GSTP, GSTS, GSTT, and GSTZ, respectively. However among these, the functional polymorphism is only identified in the GSTM1, GSTT1, and GSTP1 gene.

Table 1: Demographic and clinical characteristics of the cases and controls

Characteristics	Cases (*n*=52)	Controls (*n*=46)	*P*
Mean age (years)	56.2±10.6	52.7±13.2	0.151
Gender (male:female)	45:7	40:6	0.950
Smoking status (yes:no)	32:20	12:34	0.000429
Awareness smoking causes bladder cancer (yes:no)	30:22	44:2	1.0000
Occupational exposure (yes:no)	19:33	8:38	0.0283

Table 2: Genotype distribution of the glutathione S-transferase P1 polymorphism between the cases and controls

Genotype GSTP1	Cases (*n*=52), *n* (%)	Controls (*n*=46), *n* (%)	*P*
Ile/ile (AA)	33 (63.4)	31 (67.4)	0.202
Ile/val (AG)	18 (34.6)	11 (23.9)	
Val/val (GG)	1 (1.9)	4 (8.7)	

Ile: Isoleucine, Val: Valine, GSTP1: Glutathione S-transferase P1

Table 3: Subset analysis of the genotype frequencies for smoking

Smokers	Genotype frequency			*P*
	Ile/ile (AA)	Ile/val (AG)	Val/val (GG)	
Subjects	*n*=30	*n*=13	*n*=1	0.38
Cases	22	10	0	
Controls	8	3	1	

Ile: Isoleucine, Val: Valine

GSTP1 is the most commonly studied enzyme among all these. It is located on chromosome 11q13. It is well recognized and proved in various studies that GSTP1 plays an important role in protecting cells from various carcinogenic as well as cytotoxic agents. GSTP1 DNA hypermethylation at the CpG island in the promoter-5' region can result in the altered or decreased GSTP1 activity. Altered GSTP1 activity and expression have been reported in many tumors and it is largely due to GSTP1 DNA hypermethylation at the CpG island in the promoter-5' region.[8] In the GSTP1 at 313 Adenosine to Guanine polymorphism at the nucleotide level leads to an amino acid variation of isoleucine/valine at codon 105 in the protein. This substitution leads to three GSTP1 genotypes: Homozygous wild-type isoleucine/isoleucine allele (ile/ile) (genotype AA), heterozygous isoleucine/valine allele (ile/val) (genotype AG), and homozygous polymorphic valine/valine allele (val/val) (genotype GG). Various biochemical studies indicate that GSTP1 polymorphic valine allele has a lower thermal stability than GSTP1 homozygous isoleucine wild allele. The valine allele has a lower conjugating activity and the heterozygous isoleucine/valine allele has an intermediate activity. The valine amino acid results in decreased enzyme activity and greater propensity to the development of carcinoma. Polymorphism at the GSTP1 locus is of particular importance since this gene is universally present in many cell types including types, including the lung,[9] colon,[10] and breast cancer.[11] Since this protein is often overexpressed in tumor cells, it can make them resistant to anti-cancer treatment.

The role of genetic polymorphism in BC has been evaluated in various studies done at different centers with heterogeneous ethnic background. Hence, we conducted a case–control study to assess the role of GST GSTP1 polymorphism in urothelial cancers of the bladder in our patient population. The frequencies of the A/A, A/G, and G/G genotypes in our cases, i. e., TCC group were 63.4%, 34.6%, and 1.9%, respectively, whereas the frequency in the control group were 67.4%, 23.9%, and 8.7%, respectively. The genotypes were in the Hardy–Weinberg equilibrium among both the cases and the controls. Our demographic variables were similar to other studies done concerning TCC bladder and GSTP1 polymorphism. The genotype frequency of our control population was similar to that observed in other Indian studies by Vetriselvi *et al*.[12] and Pandith *et al*.[13]

We observed no association of GSTP1 A/G or G/G polymorphism with urothelial cancers of the bladder

among both the groups in our study ($P = 0.202$). Pandith *et al*. also found no statistically significant difference in the genotype frequencies between cancer and the control population in Kashmiri Indians. They reported a significant risk of more than 2.5 times for the polymorphic allele (AG + GG) with smokers in cases as compared to controls ($P < 0.05$).[13] Steinhoff *et al*. also suggested no correlation between GSTP1 polymorphism and UC of the bladder in the German population.[14] Similar observations were recorded by Katoh *et al*. in the Japanese population. They reported no association between valine polymorphism in control group or cancer population. They also found no association of polymorphism with smoking in any of the cancer group, oral, lung, gastric, or urothelial cancers.[15] Goerlitz *et al*.(2011) did not find any association of TCC bladder with GSTP1 polymorphism. They suggested no association with genotypes and smoking, environmental toxins, or infection.[16] A study from Morocco showed that GSTP1 expression is not associated with the development of BC and GSTP1 expression should not be used as a biomarker for BC management in Morocco.[17]

In contrast to our results, a study conducted to find the association of different environmental factors and GSTM1 and GSTT1 gene polymorphisms with susceptibility to BC in the Pakistani population suggested that GSTM1 and GSTT1 gene polymorphisms may be associated with increased susceptibility toward BC in the Pakistani population.[18] Our results were also contrary to other studies by Safarinejad *et al*.,[5] Harries *et al*.,[6] Cao *et al*.[19] showed a significant association of the polymorphic valine allele with the increased risk for urothelial cancer of the bladder. All these studies had a much higher number of cases as well as controls compared to our study. According to a meta-analysis (2007), GSTP1 Ile 105Val was associated with a modest increase in the risk of BC. In this meta-analysis (16 studies, 4273 cases, and 5081 controls), the unadjusted summary ORs for GSTP1 Ile/Val and Val/Val compared with GSTP1 Ile/Ile were 1.54 (95% CI: 1.21, 1.99; $P < 0.001$) and 2.17 (95% CI: 1.27, 3.71; $P = 0.005$). The association was the strongest in Asian countries, but the summary OR decreased when the analysis was limited to European descendents (OR = 1.24, 95% CI: 1.00, 1.52).[20]

Smoking is a well-known risk factor for TCC of the bladder.[15] A higher number of smokers in our study population developed UC with an OR of 4.53. We performed a subset analysis comparing all the smokers with TCC bladder and the smokers seen in the control group. Majority of the smokers with TCC had the wild-type allele; a few had ile/val heterozygous genotype. None of them had homozygous polymorphic valine allele. We noticed no statistically significant difference among the cases and controls, suggesting that the polymorphic GSTP1 does not add to the susceptibility of TCC among smokers in our patient population.

The lack of association noticed in our study can be attributed to small sample size or complex interaction between polymorphisms of other glutathione enzymes (possibilities of epistasis), for example, GSTM1 and GSTT1 genotypes as observed by Safarinejad *et al*.[5] with GSTP1. This needs further exploration by studying a larger sample size as well as looking into the polymorphisms of the other GST enzymes. The elucidation of complex interactions among different enzymes of glutathione enzymes in the xenobiotic pathways can be attempted by performing epistasis analysis once all the polymorphism frequencies are studied.

Conclusions

Although lack of association between GSTP1313 A/G polymorphism and urothelial cancer of bladder was seen in our study, this might be due to small sample size or the effect of complex interaction between polymorphisms of other glutathione enzymes. It is also possible that the polymorphism is not associated with UC of the bladder in our population. For further confirmation, large scale population-based studies are required.

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Conflicts of interest

There are no conflicts of interest.

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