RESEARCH COMMUNICATION

Glutathione S-transferase Gene Variants and Risk of Benign Prostate Hyperplasia in a North Indian Population

Rituraj Konwar[1], Parmeet Kaur Manchanda[2], Preeti Chaudhary[1], V Lakshma Nayak[1], Vishwajeet Singh[3], Hemant Kumar Bid[1*]

Abstract

Glutagthione S-transferase (GST) is over-expressed in benign prostate hyperplasia (BPH) patients, but the significance of GST polymorphisms for susceptibility to diseases of the prostate is unclear. The objectives of this study were to determine relationships between polymorphisms in the GSTM1, T1 and P1 genes with risk of symptomatic BPH and influence on standard therapy. A gene polymorphism association study conducted with 160 symptomatic BPH patients with BPE (benign prostatic enlargement) and LUTS (lower urinary tract symptoms) and 200 age-matched controls. Patient inclusion criteria are age >50 years prostate size >30cm^3, AUA (American urological association) score >7 and PVR volume ≤200 ml. Patients were treated with α-adrenergic blockers and 5α-reductase inhibitors for 6 months and subdivided based on their significant improvement in parameters between pre and post 6 month combined therapy to study associations with the GST polymorphisms. The GSTT1 and GSTM1 variants genotyped with multiplex-PCR, whereas GSTP1 polymorphisms were determined with PCR-RFLP (polymerase chain reaction- restriction fragment length polymorphism). We observed a lack of any association with the GSTT1 (p=0.45, OR=2.25, 95% CI=1.71-2.22) and GSTP1 (p=0.92 and 0.99) genes. However, there was a significant link with the null alleles of the GSTM1 (p=0.000, OR=2.24, 95%CI=1.46-3.42) gene. The combined analysis of the three genotypes demonstrated further increase in the risk of symptomatic BPH (p= 0.009, OR= 8.31 95%CI=1.71-40.37). Polymorphisms of GST genes were not associated with responders or non-responders. Thus the GSTM1 deletion polymorphism is significantly associated with increased risk of symptomatic BPH, but none of the genes appearedto influence response to standard BPH therapy.

Keywords: Glutathione S-transferase -polymorphism - symptomatic benign prostatic hyperplasia

Asian Pacific J Cancer Prev, **11**, 365-370

Introduction

Benign prostate hyperplasia (BPH) is an independent disease with clinical symptoms similar to that of carcinoma of the prostrate (CaP). Although not a life-threatening condition, symptomatic BPH has a severe impact on quality of life and evidently requires immediate therapeutic interventions.

Glutathione S-transferases (GST) consist of a family of enzymes having major roles in the inactivation of toxic endogenous byproducts and xenobiotic agents and thus also involved in cellular resistance to oxidative stress (Nebert et al., 2004). Prostatic luminal epithelial cells of BPH express high levels of GST (Cookson, 1997; Di Ilio., 2004; Bostwick et al., 2007) as well as increased serum levels in BPH patients (Srivastava et al., 2005). Chronic insult of prostatic tissues by infection or toxic metabolites could result in the influx of inflammatory cells releasing reactive oxygen species (ROS) prompting increased expression of GST in luminal cells (Marzo et al., 1999; Palapattu et al., 2005). In contrast, normal secretory cells and high grade prostatic intraepithelial neoplastic cells do not express these enzymes (Marzo et al., 1998). Oxidative stress is known to induce cell proliferation and reduced apoptosis (Dragin et al., 1999). Recent evidences are indicating BPH as an immune inflammatory disease (Kramer et al., 2007) and chronic inflammation has been implicated (Sciarra et al., 2007). However, it is not known how and to what extent oxidative stress-induced over expression of GST promote proliferation in BPH at prostatic tissue level. Due to its presence in prostate gland, it is speculated that in addition to participation in elimination of toxic metabolites, GST has possible involvement in prostatic steroid metabolism. Local biotransformation enzymes and transporter proteins may exert a profound effect on drug pharmacokinetics (Cookson, 1997; Di Ilio., 2004). Besides, GSTs are also involved in other hepatic drug metabolizing functions and thus may influence drug response in BPH patients. There is a possibility that the administered drugs in BPH patient may be influenced by high level expression of drug metabolizing enzymes such as GST. However, there is no

*[1]Endocrinology Division, Central Drug Research Institute, [3]Chatrapati Sahuji Maharaj Medical University, Lucknow, India, [2]Division of Endocrinology, James Cancer Center, The Ohio State University, USA, *For Correspondence: hk_bid@cdri.res.in*

evidence on the influence of drug metabolizing enzymes in combination therapy of BPH.

GST super family gene polymorphisms, largely polymorphism of GSTM1, GSTT1 and GSTP1 have been shown to be associated with increased susceptibility of several diseases (Engel et al., 2002; Vineis et al., 2002; Habdous et al., 2004; Egan et al., 2005; Lai et al., 2005; Ntais et al., 2005). GST hypermethylation is also known to be associated with CaP (Henrique et al., 2004). But none of the GST polymorphisms has been investigated for association with susceptibility of BPH as an independent entity and their influence on combination therapy of BPH. Therefore, we examined the possible relationship of genetic polymorphisms of three GST enzymes with the risk of BPH patient in North India along with their possible genetic influence on responsiveness to the therapy for BPH.

Materials and Methods

Selection of patients and controls

In the present study compromising of 160 symptomatic BPH patients out of 200 patients from the Department of Urology, CSMMU (Chatrapati Shahuji Maharaj Medical University), Lucknow, were enrolled during the period of July 2005 to July 2007. Patients were included in the study with inform consent prior to qualifying study inclusion criteria. Inclusion criteria for patient were BPE with LUTS of age >50 years, prostate size >30cm^3, AUA score >7 and PVR volume ≤200 ml.

Patients with PSA((prostate serum antigen) <4ng/ml were included in the study and patients with PSA within the range of 4-10ng/ml were included only after DRE (digital rectal examination) and true-cut biopsy for confirmation for free of Ca-P. Patients with PSA>10ng/ml were excluded for possible CaP. Other exclusion criteria were history of urinary tract infection (UTI), previous lower tract surgery or procedures that may alter prostate anatomy/architecture or contribute to LUTS. History of postural hypotension, dizziness, vertigo, orthostasis or any other signs and symptoms which are suspected to be exacerbated by α-blockers and result in putting the subject at risk of injury were excluded from the study.

A total of 200 normal healthy controls of age >50yrs were recruited from staff employee of the institute and patients visiting the hospital for minor medical or surgical problems after their inform consent. The range of age of this study was 50-70 years with mean age match of 62.6 + 9.4 years for control and patient. All were screened for normal PSA level and absence of LUTS. This study was conducted with previous clearance from the ethical committee of CSMMU, Lucknow.

Therapeutic schedule and response measures

Eligible subjects were treated with combined therapy of 0.5 mg α-adrenergic blockers (Dutasteride) and 0.4 mg of 5α-reductase inhibitors (Tamsulosin) once daily. Short-term combination therapy continued for a period of 6 month as per recommendation of EAU guideline (Madersbacher et al,. 2004). Use of phytotherapy, any other α-adrenoreceptor blockers or anticholinergics were prohibited during the study.

The efficacy endpoints included changes in the Prostate volume, AUA score and Qmax that were evaluated at baseline, after 3 weeks and 6 months post-treatment. Prostate volume was measured with transrectal ultrasonography. Based on the AUA guideline, a significant decrease in the matrices of AUA score by 3-6 units and/or 2-3 ml/s increase in Qmax and/or 20% reduction in prostate volume at 6 months from baseline were defined as "therapeutic response" for this study purpose. Patient during study period with adverse events related to alpha blockers and 5-alpha reductase inhibitors leading to intolerance or other reasons for discontinuity (n=40) were excluded and treated as per guideline. At the end of 6 months 160 patients were included for data analysis.

Genotyping of GSTM1, GSTT1 and GSTP1

Polymorphisms at GSTM1, GSTT1 and GSTP1 gene loci were determined using multiplex-PCR and restriction fragment length polymorphism (RFLP). Homozygous null deletion polymorphism in both GSTM1 and GSTT1 genes were determined by multiplex PCR using specific primers using CYP1A1 gene as an internal control (Arand et al., 1996). A total of 100ng DNA as a template with 10pmol of each primer and 1.5 units Taq DNA polymerase (MBI-Fermentas, Maryland) was used in a total volume of 25_l. The annealing temperature was 580C; PCR was carried out for 34 cycles. The PCR products were separated on 2% agarose gel.

The presence of the GSTP1 polymorphism was screened by PCR-RFLP analysis (Harries et al., 1997). A total of 100ng DNA was used as a template with 10pmol of each primer and 1.5U Taq DNA polymerase in a total volume of 25_l. The annealing temperature was 600C; 35 cycles were carried out for PCR. The 176-bp PCR product was digested with Alw261 (MBI-Fermentas, Maryland) overnight at 370C and electrophoresed on 10% polyacrylamide gel. The GSTP1 (Ile/Ile) genotype corresponded to a 176-bp band; the GSTP1 (Ile /Val) genotype showed 176-, 95- and 81-bp bands; and the GSTP1 (Val /Val) genotype bands corresponded to 81 and 95bp. To improve the genotyping quality and substantiation, 30% of samples were re-genotyped by independent laboratory personal and results were found to be reproducible with no discrepancy recorded in genotyping.

Statistical analysis

Differences in genotype prevalence between both the groups were assessed by the Chi-square test. A $p<0.05$ was considered as being statistically significant. Sample size was calculated and found to be adequate using QUANTO software version 1.0 (http://hydra.usc.edu/gxe) for each genetic marker, GST M1, T1 and P1 (Gauderman et al., 2002). All analyses were performed using the SPSS statistical analysis software, version 11.5 (SPSS, Chicago). To examine whether the genotype frequencies were in Hardy_Weinberg Equilibrium, Goodness of fit v2 test was used. Odds ratio (OR) at 95% confidence intervals (CI) was determined to describe the strength

of association by Logistic Regression Model. In order to carry out statistical analysis of association of GST polymorphism with therapeutic response of symptomatic BPH, all the patients were categorized in two groups as follows, Group A: Symptomatic BPH with BPE and LUTS patients showing "therapeutic response"; Group B: Symptomatic BPH with BPE and LUTS patients fail to show "therapeutic response" as described above.

Results and Discussion

Several polymorphisms, both functional and non-functional have already been reported to demonstrate positive associations with BPH, but still lack definitive and complete picture of these subtle genetic markers (Konwar et al., 2008). GST enzyme isoforms including GSTT1, GSTM1 and GSTP1 play an important role as a cellular guard against toxic metabolites. The GSTs are genotypically and phenotypically polymorphic with variable genotype frequencies in different ethnic groups (Mishra et al, 2004). Influence of individual variation in their genes in differential risk and susceptibility for BPH is not known.

Table 1 represents the frequency distribution of GSTM1, GSTT1 and GSTP1 alleles and genotypes between both the groups along with their double and triple combinations and the association of GST variants with BPH. In the control samples, frequency of GSTM1 null and GSTT1 null were 36.5% and 14.0% respectively. The GSTP1 was present in the homozygous Ile-allele state (Ile/Ile) in 59.5% while the homozygous Val-allele (Val/Val) was in 3.0% cases. The remaining 37.5% were heterozygous (Ile/Val). Genotype distributions in controls were in agreement with the Hardy-Weinberg equilibrium. We observed significant association with null genotype of GSTM1 (OR= 2.24, 95% CI= 1.46-3.42, p=0.00) and lack of association with null genotype of GSTT1 (OR= 1.25, 95% CI= 0.70-2.21, p=0.45). However, the Ile/Val genotype (OR= 0.98, 95% CI= 0.64-1.50, p=0.92) and Val/Val genotype (OR= 0.00, p=0.99) of the GSTP1 gene polymorphism were no association compared to the controls (Table 1). The combination of the two high-risk genotypes GSTM1 null and GSTT1 null or GSTP1 (Ile/Val or Val/Val) genotyped showed that the risk increased by up to 2.38 times (OR= 2.39, 95% CI: 1.38-4.12, p=0.002) for GSTM1 and GSTP1 and 4.44 times (OR= 4.44, 95% CI: 1.86-10.61, p=0.001) for GSTM1 and GSTT1 genotypes. However, risk increased 1.34 folds (OR= 1.34, 95% CI: 0.62-2.86, p=0.001) for GSTT1 and GSTP1 genotype when we compared with non-risk

Table 1. Distribution of Single, Double and Triple GST Genotypes among BPH Patients and Controls

Genotypes	Control (n=200)	BPH Patients (n=160)	*p* value	OR (95% CI)
GSTM1				
Present	127 (63.5%)	70 (43.8%)		1.0 (Ref.)
Null	73 (36.5%)	90 (56.3%)	0.000	2.237 (1.463-3.421)
GSTT1				
Present	172 (86.0%)	133 (83.1%)		1.0 (Ref)
Null	28 (14.0%)	27 (16.9%)	0.452	1.247 (0.702-2.216)
GSTP1				
I/I	119 (59.5%)	99 (61.9%)		1.0 (Ref.)
I/V	75 (37.5%)	61 (38.1%)	0.918	0.978 (0.636-1.504)
V/V	6 (3.0%)	0 (0%)	0.999	0.00 (0.000)
Double				
GSTM1&GSTT1				
Both	110 (55%)	65 (40.6%)		1.0 (Ref.)
Either Null	82 (41.0%)	74 (46.3%)	0.059	1.527 (0.984-2.369)
Both null	8 (4.0%)	21 (13.1%)	0.001	4.442 (1.861-10.605)
GSTM1&GSTP1				
M1(+/+)&P1(I/I)	77 (38.5%)	43 (26.9%)		1.0 (Ref.)
M1(+/+)&P1(I/V)	50 (25.0%)	27 (16.9%)	0.912	0.967 (0.531-1.759)
M1(-/-)&P1(I/I)	42 (21.0%)	56 (35%)	0.002	2.388 (1.382-4.126)
M1(-/-)&P1(I/V)	31 (15.5%)	34 (21.3%)	0.031	1.964 (1.064-3.627)
GSTT1&GSTP1				
T1(+/+)&P1(I/I)	104 (52%)	83 (51.9%)		1.0 (Ref.)
T1(+/+)&P1(I/V)	68 (34%)	50 (31.3%)	0.730	0.921 (0.579-1.467)
T1(-/-)&P1(I/I)	15 (7.5%)	16 (10%)	0.455	1.337 (0.624-2.861)
T1(-/-)&P1(I/V)	13 (6.5%)	11 (6.9%)	0.893	1.060 (0.452-2.489)
Triple				
M1&T1(+/+)&P1(I/I)	72 (36.0%)	39 (24.4%)		1.0 (Ref.)
M1&T1(+/+)&P1(I/V)	41 (20.5%)	25 (15.6%)	0.713	1.126 (0.598-2.117)
M1(-/-),T1(+/+)&P1(I/I)	35 (17.5%)	44 (27.5%)	0.005	2.321 (1.286-4.190)
M1(-/-),T1(+/+)&P1(I/V)	27 (13.5%)	25 (15.6%)	0.116	1.709 (0.875-3.338)
M1(+/+),T1(-/-)&P1(I/I)	8 (4.0%)	4 (2.5%)	0.901	0.923 (0.261-3.260)
M1(+/+),T1(-/-)&P1(I/V)	9 (4.5%)	2 (1.3%)	0.269	0.410 (0.084-1.994)
M1(-/-),T1(-/-)&P1(I/I)	6 (3.0%)	12 (7.5%)	0.015	3.692 (1.286-10.600)
M1(-/-),T1(-/-)&P1(I/V)	2 (1%)	9 (5.6%)	0.009	8.308 (1.710-40.372)

I:isoleucine; V:valine

Table 2. Distribution of Single, Double and Triple GST Genotypes among Responder and Non-Responder Groups among BPH Patients

Genotype	Responder (n=115)	Non-responder (n=45)	*p* value	OR (95%CI)
GSTM1	65 (56.5%)	25 (55.6%)	ref	1.0 (ref)
Null	50 (43.5%)	20 (44.4%)	0.912	1.04 (0.52-2.08)
GSTT1	96 (83.5%)	37 (82.2%)	ref	1.0 (ref)
Null	19 (16.5%)	8 (17.8%)	0.849	1.09 (0.44-2.71)
GSTP1				
I/I	73 (63%)	26 (57.8%)	ref	1.0 (ref)
I/V	42 (37%)	19 (42.2%)	0.505	1.27 (.63-2.56)
V/V	0.0 (0%)	0.0 (0%)	0.0	
Double				
GSTM1&GSTT1				
Both	58 (50%)	22 (48.9%)	0.982	1.0 (ref)
Either Null	45 (39.1%)	18 (40.0%)	0.887	1.05 (0.51-2.19)
Both null	12 (10%)	5 (11.1%)	0.873	1.09 (0.35-3.48)
GSTM1&GSTP1				
M1(+/+)& P1(I/I)	40 (34.8%)	16(35.6%)	0.728	1.0 (ref)
M1(+/+)& P1(I/V)	24 (20.9%)	9 (20.0%)	0.895	0.94 (0.36-2.45)
M1(-/-)& P1(I/I)	33 (28.7%)	10 (22.2%)	0.552	0.76 (0.31-1.89)
M1(-/-)& P1(I/V)	18 (15.7%)	10 (22.2%)	0.505	1.39 (0.58-3.65)
GSTT1&GSTP1				
T1(+/+)& P1(I/I)	62 (53.9%)	21 (46.7%)	0.873	1.0 (ref)
T1(+/+)& P1(I/V)	34 (29.5%)	16 (35.6%)	0.429	1.37 (0.63-2.96)
T1(-/-)& P1(I/I)	11 (9.6%)	5 (11.1%)	0.641	1.32 (0.41-4.24)
T1(-/-)& P1(I/V)	8 (7.0 %)	3 (6.7%)	0.906	1.09 (0.26-4.49)
Triple				
M1&T1(+/+)&P1(I/I)	35 (30.4%)	14 (31.1%)	0.885	1.0 (ref)
M1&T1(+/+)&P1(I/V)	23 (20.0%)	8 (17.8%)	0.787	0.87 (0.32-2.40)
M1(-/-)T1(+/+)&P1(I/I)	27 (23.5%)	7 (15.6%)	0.412	0.65 (0.23-1.83)
M1(-/),T1(+/+)&P1(I/V)	11 (9.6%)	8 (17.8%)	0.287	1.82 (0.60-5.47)
M1(+/+),T1(-/-)&P1(I/I)	5 (4.4%)	2 (4.4%)	1.000	1.00 (0.17-5.77)
M1(+/+),T1(-/)&P1(I/V)	2 (1.7%)	1 (2.2%)	0.860	1.25 (0.11-14.91)
M1(-/-),T1(-/-)&P1(I/I)	6 (5.2%)	3 (6.7%)	0.773	1.25 (0.27-5.71)
M1(-/-),T1(-/-)&P1(I/V)	6 (5.2%)	2 (4.4%)	0.835	0.83 (0.15-4.65)

genotypes. (Table 1). Our study for the first time revealed associations of GST polymorphism with risk of BPH. In our control group, frequencies of the three genes were within the range already reported in other studies from India (Srivastava et al., 2005; Pandey et al 2006). Our results indicate that the null genotypes of GSTM1 and T1 are associated with a higher risk for BPH than in controls (Table 2). The combination of the two high-risk genotypes, GSTM1 null and GSTT1 null or GSTP1 (Ile/Val or Val/Val) genotypes increased the risk 4 times for GSTP1 and T1 null genotypes whereas 2.3 times for the GSTM1 null & GSTP1 (Ile/Ile) genotypes. When the three risk genotypes were combined the risk increases to eight times in case of null GSTM1, T1 and & GSTP1 (Ile/Val) genotypes. It seems that combinations of rare metabolic genotypes should be considered as more appropriate for risk assessment rather than individual genotypes and suggests that the gene-gene interaction may contribute to a causal propensity for developing BPH. However, as this is the first report on triple combinations, caution should be exercised while investigating other populations for these genes given their variable genotype frequencies in different ethnic groups.

We further investigated the risk associated with all the three high-risk GST genotypes compared to non-risk genotypes (non-deleted genotypes of GSTM1 and GSTT1 and GSTP1 Ile/Ile genotype were designated as the reference group). The OR for the three high-risk genotypes versus non-risk genotypes was 8.31 folds higher (p = 0.009, OR=8.31 95% CI = 1.71–40.37). (Table 1)

We also analyzed the association of these polymorphisms with the response of the drugs between two groups of patient. The patient groups categorized into two groups were also statistically analyzed for their association with GST polymorphisms to evaluate significant difference among responder and non-responder patients. However, we observed lack of association (p>0.05) for the GST genotypes between the two patient groups, responder (Group A) and non-responder (Group B) as shown in Table 2. Our results suggest the response of the combined therapy is not influenced by polymorphic variants of GST gene. GST enzymes play an important role in the metabolism of drugs and GST polymorphism in the form of deletion of genes modulate therapeutic response of drugs used in the treatment of several diseases (Ilio et al., 1990). Our preliminary study suggested that the GST polymorphism is associated with susceptibility of BPH, they however do not influence short-term combination therapy in BPH patients. However, this may be too early to speculate that more of an etiological role of GST in BPH rather than progression of pathogenesis. The association of genetic polymorphism in drug metabolism may differ

based on the nature and intensity of the treatment regimen that was not addressed in the present study.

Besides the role of GSTs in activation and inactivation of oxidative metabolites of carcinogenic compounds associated with cancer, they also detoxify a broad range of substances including carcinogens, environmental toxins, and drugs. In recent years, it has been investigated the possible effects of genetic variants of GSTM1, GSTT1, and GSTP1 genes in relation to various factors (Mittal et al., 2009). In contrast to the possible role of GST in environmental carcinogenesis, it has been suggested that GST genotypes conferring lower enzyme activity may be of advantage for the patients who are undergoing chemotherapeutic treatment for neoplastic disease because reduced detoxification potentially enhances effectiveness of cytotoxic drugs (Willett et al., 1989). Therefore, genetic differences can be important in drug response, and therapy regimens should ideally be adjusted accordingly. Therefore in the present study we tried to correlate the GST polymorphism with the drug response. We believe in the present study, allowing researchers of GST gene variants using large-scale studies will be of significance in both clinical and research prospective.

The potential limitations of the current findings are that we did not investigate other GST polymorphisms that could have provided further insights into a potential role of GST polymorphisms. In addition, sub categorization of treatment groups as Dutasteride with and without Tamsulosin or likewise would have provided settings for more specific therapeutic responsiveness of patients. It was also necessary to determine the transcript level, enzyme concentration and enzyme activity in the tissue lysate and serum to gain further insight in terms of the expressed phenotype of the genes that could not be performed due to financial limitations. Other limitation of this study is inability to encompass all theoretical categories of patients under BPH, for example asymptomatic BPE. Besides, there is also possibility that both the groups may have patients of early CaP as they are inadvertently included in most of similar case-control studies due unavailability of non-invasive micro-diagnostic modalities for very early CaP.

Finally, our results indicate that three GST M1 deletion polymorphism is associated with the susceptibility of BPH. GST polymorphisms are not associated with response of patients for standard BPH therapy, in particular the combined therapy of 5α-reductase inhibitors and α-adrenergic blockers. More detailed investigations would definitely help in future for rational genetic categorization for therapeutic responsiveness of patients.

Acknowledgements

The authors thank the Ministry of Health and Family Welfare, Govt. of India for financial support. CDRI communication no is 7438.

References

Arand M, Mühlbauer R, Hengstler J, et al (1996). A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase GSTM1 and GSTT1 Polymorphisms. *Anal Biochem*, **236**, 184-6.

Bostwick DG, Ie M, Shanks J (2007). Glutathione S-transferase: differential expression of α, μ, and π isoenzymes in benign prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma. *Hum Pathol*, **38**, 1394-401.

Cookson MS, Reuter VE, Linkov I, et al, (1997). Glutathione S-transferase PI (GST-pi) class expression by immunohistochemistry in benign and malignant prostate tissue. *J Urol*, **7**, 673-6.

De Marzo AM, Marchi VL, Epstein JI, et al (1999). Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol*, **155**, 1985-92.

De Marzo AM, Nelson WG, Meeker AK, et al (1998). Stem cell features of benign and malignant prostate epithelial cells. *J Urol*, **160**, 2381-92.

Di Ilio C, Aceto A, Bucciarelli T, et al (1990). Glutathione transferase isoenzymes from human prostate. *Biochem J*, **271**, 481-5.

Dragin N, Smani M, Arnaud-Dabernat S, et al (2006). Acute oxidative stress is associated with cell proliferation in the mouse liver. *FEBS let*, **580**, 3845-52.

Egan KM, Cai Q, Shu XO, et al (2004). Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai breast cancer study and meta-analysis. *Cancer Epidemiol Biomarkers Prev*, **13**, 197-204.

Engel LS, Taioli E, Pfeiffer R, et al (2002). Pooled analysis and meta-analysis of glutathione S-transferase M1 and bladder cancer: a HuGE review. *Am J Epidemiol*, **156**, 95-109.

Gauderman WJ (2002). Sample size requirements for association studies of gene-gene interaction. *Am J Epidemiol*, **155**, 478-84.

Habdous M, Siest G, Herbeth B et al (2004). Glutathione S-transferases genetic polymorphisms and human diseases: overview of epidemiological studies. *Ann Biol Clin (Paris)*, **62**, 15-24.

Harries LW, Stubbins MJ, Forman D, et al (1997). Identification of genetic polymorphisms at the glutathione S transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis*, **18**, 641-4.

Henrique R, Jeronimo C (2004). Molecular detection of prostate cancer: A role for GSTP1 hypermethylation. *Euro Urol*, **46**, 660-9.

Kramer G, Mitteregger D, Marberger M (2007). Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? *Euro Urol*, **51**, 1202-16.

Konwar R, Chattopadhyay N, Bid HK (2008). Genetic polymorphism and pathogenesis of benign prostatic hyperplasia. *BJU Int*, **102**, 536-44.

Lai R, Crevier L, Thabane L (2005). Genetic polymorphisms of glutathione s-transferases and the risk of adult brain tumors: A meta-analysis. *Cancer Epidemiol Biomarkers Prev*, **14**, 784-90.

Madersbacher S, Alivizatos G, Nordling J, et al (2004). EAU 2004 guidelines on assessment, therapy and follow-up of men with lower urinary tract symptoms suggestive of benign prostatic obstruction (BPH guidelines). *Euro Urol*, **46**, 547-54.

Mishra DK, Kumar A, Srivastava DS (2004). Allelic variation of GSTT1, GSTM1 and GSTP1 genes in North Indian population. *Asian Pac J Cancer Prev*, **5**, 362-5.

Mittal RD, Kesarwani P, Singh R, et al (2009). GSTM1, GSTM3

and GSTT1 gene variants and risk of benign prostate hyperplasia in North India. *Dis Markers,* **26**, 85-91.

Ntais C, Polycarpou A, Ioannidis JP (2005). Association of GSTM1, GSTT1, and GSTP1 gene polymorphisms with the risk of prostate cancer: A meta-analysis. *Cancer Epidemiol Biomarkers Prev*, **14**, 176-81.

Nebert DW, Vasiliou V (2004). Analysis of the glutathione S-transferase (GST) gene family. *Hum Genomics*, **1**, 460-4.

Palapattu GS, Sutcliffe S, Bastian PJ, et al (2005). Prostate carcinogenesis and inflammation: emerging insights. *Carcinogenesis*, **26**, 1170-81.

Pandey SN, Jain M, Nigam P (2006). Genetic polymorphisms in GSTM1, GSTT1, GSTP1, GSTM3 and the susceptibility to gallbladder cancer in North India. *Biomarkers*, **11**, 250-61.

Sciarra A, Di Silverio F, Salciccia S, et al,(2007). Inflammation and chronic prostatic diseases: Evidence for a link? *Euro Urol*, **52**, 964-72.

Srivastava DSL, Mittal RD (2005). Free radical injury and antioxidant status in patients with benign prostate hyperplasia and prostate cancer. *Indian J Clin Biochem*, **20**, 162-5.

Vineis P (2002). The relationship between polymorphisms of xenobiotic metabolizing enzymes and susceptibility to cancer. *Toxicol*, **181-182**, 457-62.

Willett W (1989). The search for the causes of breast and colon cancer. *Nature*, **338**, 389-94.