Null Results in Brief

No Association between the I105V Polymorphism of the Glutathione *S*-Transferase P1 Gene (*GSTP1*) and Prostate Cancer Risk: A Prospective Study[1]

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Introduction

GSTs[3] conjugate glutathione to harmful substances facilitating their removal. Allelic variants of the *GSTP1* gene have been identified, one isoleucine (GSTP1-1/I-105) and one valine (GSTP1-1/V-105) at codon 105. The allele frequency varies between ethnic groups. The isozymes have activities that differ by substrate. GSTs noncovalently bind and sequester steroid hormones. Smaller epidemiologic studies have reported an association between the GSTP1-1/Val-105 allele and higher risk of CaP.

We sought to test the hypothesis that men homozygous for GSTP1-1/Val-105 would be at higher risk for developing CaP than men who are GSTP1-1/Ile-105 homozygotes or heterozygotes and to determine whether smoking alters any such association. We also hypothesized that circulating levels of androgens would be higher in men homozygous for GSTP1-1/Val-105.

Materials and Methods

Study Population. Blood was obtained in 1982 from 14,916 men enrolled in The Physicians' Health Study and men were followed until 1995. Study characteristics including blood collection and processing, stage classification, clinical follow-up and documentation of prostate cancer were described previously.

Genotyping. Genomic DNA was isolated from whole blood using a Qiamp DNA extraction kit (Qiagen, Chatsworth, CA) with investigators blinded to status. Samples were diluted to 20 ng/μl and stored at −20°C. Twenty ng of DNA were added to the PCR reaction mixture: 0.3 mM primers P105F and P105R, 1.5 mM $MgCl_2$, and 1.5 units of Qiagen Taq in a final volume of 22 μl. Cycling conditions were 94°C for 2 min, 35 cycles of 94°C for 30 s, 60°C for 1 min, 72°C for 30 s, and 72°C for 8 s in a Perkin-Elmer GeneAmp PCR System 9600 or an MJ Research PTC-200 (MJ Research, Waltham, MA). Digestion was with 2.5 units of *Bsm*A1 (New England Biolabs, Beverly, MA), separated on a 2% agarose gel stained with ethidium bromide. The genotypes were successfully determined for 590 cases and 803 controls.

Hormone Levels. Plasma hormone concentrations were measured as described by Gann *et al.* (5).

Statistical Analysis. We used the χ^2 test to evaluate whether the distribution of *GSTP1* alleles or genotypes varied among cases and controls. We calculated ORs as an estimate of relative risk and 95% CIs from logistic regression models. To increase power in the analysis of genotype by stage and grade, we included all controls while controlling for the matching factors of baseline age (5-year intervals) and smoking status. We used stratified analysis to evaluate whether CaP risk associated with genotype varied by smoking status and assessed interaction by use of a cross-product term. From a previous analysis, plasma steroid hormone concentrations were available for 389 controls. We calculated mean levels of testosterone, dihydrotestosterone, and androstanediol glucuronide by genotype and assessed whether hormone levels varied by genotype using the Kruskal-Wallis test.

Results

The valine frequencies did not differ between CaP cases and controls (Table 1). Neither *val/val* nor *ile/val* genotype was associated with CaP overall or with high or low stage/grade disease (Table 1). No difference (*P*-interaction = 0.4) was found in the OR for developing CaP by *GSTP1* genotype between 761 ever-smokers (*ile/ile* referent, *ile/val* OR, 0.84; 95% CI, 0.62–1.13; *val/val* OR, 0.80; 95% CI, 0.47–1.36) and 632 never-smokers (*ile/ile* referent; *ile/val* OR, 0.91; 95% CI, 0.65–1.28; *val/val* OR, 0.87; 95% CI, 0.52–1.48). We saw no important differences by stage/grade of CaP when considering smoking status either. Levels of testosterone, dihydrotestosterone and androstanediol glucuronide did not differ by *GSTP1* genotype (all $P \geq 0.08$).

Statistical Power

We had 80% power to detect as statistically significant an OR of 1.4 when comparing the *val/val* and *ile/val* genotypes to the *ile/ile* genotype.

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[3] The abbreviations used are: GST, glutathione *S*-transferase; CaP, prostate cancer; OR, odds ratio; CI, confidence interval.

Table 1 GSTP1-1 allele and genotype frequencies and prostate cancer

GSTP1-1 allele and genotype frequencies and OR[a] and 95% CI for prostate cancer by *GSTP1-1* allele and genotype, Physicians' Health Study.

	Cases *n* (%)		Controls *n* (%)
Allele frequencies			
ile	824 (69.8)		1084 (67.5)
val	356 (30.2)		522 (32.5)
Total	1180		1606
P (χ^2 test of independence)		0.2	
Genotype frequencies			
ile/ile	290 (49.1)		365 (45.4)
ile/val	244 (41.4)		354 (44.1)
val/val	56 (9.5)		84 (10.5)
Total	590		803
P (χ^2 test of independence)		0.4	

	Prostate cancer cases		
	Total (OR) [CI]	High grade/stage[b] (OR) [CI]	Low grade/stage[b] (OR) [CI]
Genotype			
ile/ile	290 (1.00 ref)	131 (1.00 ref)	157 (1.00 ref)
ile/val	244 (0.87)	117 (0.93)	122 (0.80)
	[0.69–1.09]	[0.70–1.25]	[0.60–1.06]
val/val	56 (0.84)	22 (0.74)	32 (0.87)
	[0.58–1.22]	[0.44–1.24]	[0.56–1.37]
P-trend	0.2	0.3	0.2
val/val	300 (0.86)	139 (0.90)	154 (0.81)
+ *ile/val*	[0.70–1.07]	[0.68–1.18]	[0.63–1.06]

[a] Estimated from a logistic regression model controlling for the matching factors smoking status (never, former, current) and age.
[b] Does not sum to total because of missing histological grade and stage at diagnosis for some cases.

Limitations

The population examined in this study was predominantly United States Caucasian. Allele and genotype frequencies vary at this locus between ethnic groups; the impact it may have on CaP occurrence in other groups requires further study. Smoking status was assigned at baseline in 1982. Heavy, recent smoking may be a risk factor for developing highly aggressive CaP.

Conclusion

In this large, nested case-control study, we found no evidence of a differential risk for CaP among primarily Caucasian, United States men possessing the isoleucine or valine variants of codon 105 of *GSTP1* in any genotypic combination. Our large sample size resulted in narrow CIs and allowed us to examine high and low stage/grade CaP while maintaining adequate statistical power. The upper CI limit of 1.22 excludes a large risk associated with the *val/val* genotype. Selection or survival bias was not an issue because of the prospective design and the high follow-up response rate.

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