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RESEARCH

Associations Between SNPs Within Antioxidant Genes and the Risk of Prostate Cancer in the Siberian Region of Russia

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Abstract In the present study we investigated the association of a number of polymorphic changes in antioxidant system genes (SNPs rs1050450 in the *GPX1* gene, rs1695 and rs1138272 in the *GSTP1* gene and rs4880 in the *MnSOD* gene) with the risk of prostate cancer. The association of disease stage and PSA levels with specific genotypes was also analyzed. A study was conducted with the participation of 736 Russian men. We compared the frequency of occurrence of the studied alleles in patients with prostate cancer (392) to a control group (344) of men without a history of cancer. Genotyping was performed by real-time PCR. Comparison of frequencies of alleles and genotypes were performed using logistic regression analysis. No statistically significant association with the risk of prostate cancer was found for any of the SNPs studied ($p>0.05$). For SNP rs1695 in the *GSTP1* gene, a correlation with cancer disease stage was observed: a GG genotype is significantly more common in patients with PCa in the 3rd and 4th stage than 1st and 2nd (OR[95%CI]= 2.66[1.15–6.18], $p=0.02$). Both studied SNPs of *GSTP1* gene are associated with the level of PSA: the GG rs1695 and the TT rs1138272 genotypes are associated with higher PSA levels ($p=1.5*10^{-3}$).

Keywords *GPX1* · *GSTP1* · *MnSOD* · Prostate cancer · Single nucleotide polymorphisms (SNPs) · *SOD2*

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Introduction

Many issues remain in the etiology of prostate cancer (PCa) and studies are often contradictory. Proven risk factors include age, ethnicity and genetic background. It is believed that around 30 % of all human tumors are induced by inflammation [1]. With regard to prostate cancer, chronic prostatitis is classified as a probable risk factor as its role in malignancy is not confirmed by all studies.

One of the main mechanisms causing malignancy in inflammation is oxidative damage. A large body of evidence points to a role for DNA damage in carcinogenesis caused by reactive oxygen species (ROS) [2, 3]. Increased ROS can result from an increase in their concentration and/or a decrease in antioxidant defense mechanisms. Consequently, polymorphic variants in genes encoding antioxidant defense proteins may determine individual susceptibility to the effects of these factors: inflammation, lifestyle, diet and can be associated with the risk of cancer [4, 5].

The aim of our study is to investigate the influence of several polymorphic changes in the genes encoding the antioxidant system (rs1050450 in GPX1, rs1695, rs1138272 in GSTP1 and rs4880 in MnSOD) on the risk of prostate cancer in men living in the Siberian region of Russia.

MnSOD (manganese superoxide dismutase), also known as SOD2, is a mitochondrial enzyme and plays a key role in protecting cells from oxidative damage. The importance of this enzyme in normal function of tissues is confirmed by death of MnSOD gene knockout mouse lines in the first

10 days of life [6]. The MnSOD gene is localized on chromosome 6q25.3. SNP rs4880 in MnSOD leads to the replacement of amino acid valine (Val) with alanine (Ala) [7] altering the secondary structure of the protein. This change makes it difficult to transport the enzyme into mitochondria and to form the active MnSOD tetramer in the mitochondrial matrix [8]. Malignant or transformed cells of the prostate gland are characterized by decreased gene expression of MnSOD [9]. Therefore, polymorphic gene mutation, reducing the functional activity of the enzyme, can increase the likelihood of developing cancer. A meta-analysis report published in 2009 confirmed the association of SNP rs4880 of MnSOD with the risk of prostate cancer (OR=1.3; 95 % CI: 1.0–1.6) [10].

GPX1 is a selenium-dependent enzyme which reduces oxidant stress. The GPX1 gene is localized on chromosome 3p21.3 and contains two exons [11]. Published research on the association of SNP rs1050450 with the risk of prostate cancer is controversial. There is evidence of Pro/Leu or Leu/Leu genotype correlation with the risk of prostate cancer and with late stage disease [12]. However, a number of studies obtained a protective effect [13] or the lack of association [14].

Glutathione S-Transferase P (GSTP1) gene is localized on chromosome 11q13 [15]. Somatic mutations in the gene GSTP1 leading to inactivation have been identified in transformed malignant prostate cells [16]. Hypermethylation of the regulatory sequences of GSTP1 is not found in normal tissue of the prostate or benign prostatic hyperplasia [17, 18]. Thus, in more than 90 % of adenocarcinomas, GSTP1 is not expressed [17].

Materials and Methods

Subjects

A study was conducted with the participation of 736 Russian men who lived in the Altai and Krasnoyarsk region of Russia. Case patients were men of European descent (by self-report) who underwent treatment for CPa at The N. N. Blokhin Russian Cancer Research Center RAMS and The A.I. Kryzhanivskyi Krasnoyarsk Regional Oncology Center from January 1, 2008, to December 31, 2010. The studied group consisted of 392 men with histologically verified prostate cancer. The study sample was presented mainly by sporadic forms of prostate cancer and familial history of prostate cancer was recorded in only six patients (1.5 %). The control group included 344 men older than 40 years, residing within the study area, with no history of CPa or other cancer. The average age was 69.2±8.4 years for the study sample and 64.3± 14.7 years for the control sample. All participants included in this study provided written informed consent. DNA was isolated from venous blood using a standard procedure involving the isolation and lysis of blood cells, protein hydrolysis with proteinase K, DNA cleaning by extraction of impurities with phenol-chloroform and ethanol precipitation of DNA. The distribution of patients with prostate cancer including study of clinical parameters is presented in Table 1.

Genotyping

Genotyping of allelic variants was performed by PCR using TaqMan probes of complementary polymorphic DNA sequence. Amplification was performed in a volume of 25 μl. The PCR mixture consisted of 300 nM primers, 100 nM TaqMan-probes, 65 mM TrisHCl (pH 8.9), 24 мМ $(NH_4)_2SO_4$, 3.0 мМ $MgCl_2$, 0.05 % Tween-20, 0.2 мМ dNTP, 0.5–10 ng DNA and 0.5 U Taq-DNA polymerase (hot-start, Biosan, IHBFM). The sequences of oligonucleotide primers and probes are shown in Table 2. PCR was carried out under the following conditions: initial denaturation of 3 min at 96 °C, then 48 cycles including denaturation at 96 °C for 5 s, annealing of primers and subsequent elongation (each cycle was accompanied by a recording of the fluorescent signal at the emission wavelength of fluorophores FAM and R6G).

Statistical Analysis

Accordance of frequencies of studied SNPs's genotypes to Hardy-Weinberg equilibrium was determined separately for the control group and the group of patients with prostate cancer using Fisher's exact test [19]. To determine the contribution of the test SNP to the change in risk of prostate cancer the odds ratio (OR) and its confidence interval (CI 95 %) were calculated. Comparison of frequencies of alleles and genotypes were performed using logistic regression analysis. The differences were considered statistically significant at $p<0.05$. As mean age was significantly different between the case and control groups the OR and confidence intervals (CI 95 %) were adjusted for age. Calculations were performed with the free software R, version 2.15.1, library «GenABEL». For the analysis of linkage disequilibrium parameters D' and r2 were calculated using CubeX software (http://www.oege.org/software/cubex/) [20]. Haplotype frequencies and the

Table 1 Characteristics of PCa patients

Stage	n (%)
1	18 (6.7)
2	144 (52.7)
3	94 (34.4)
4	17 (6.2)
PCA level (ng/ml)[a]	n (%)
≤4	135 (35.3)
4.1-10	141 (36.8)
10.1-20	45 (11.7)
>20	62 (16.2)

[a] At diagnosis

Table 2 Sequences of primers and TaqMan probes used in this study

SNP	Primers	TaqMan probes
GPX1	U 5′-GCTTCCAGACCATTGACATC-3′	5′R6G-CTCAAGGGCTCAGCTGTGC-BHQ-3′
rs1050450	R 5′-CGAGGTGGTATTTTCTGTAAGATC-3′	5′FAM- CTCAAGGGCCCAGCTGTGC-BHQ-3′
GSTP1	U 5′-GATGCTCACATAGTTGGTGTAG-3′	5′R6G-CTGCAAATACGTCTCCCTCAT-BHQ-3′
rs1695	R 5′-GGTGGACATGGTGAATGAC-3′	5′FAM- CTGCAAATACATCTCCCTCAT-BHQ-3′
GSTP1	U 5′- GGAGCAAGCAGAGGAGAATC-3′	5′R6G- CTTGCCCGCCTCCTGC -BHQ-3′
rs1138272	R 5′-CAGCAGGGTCTCAAAAGGC-3′	5′FAM- CCTTGCCCACCTCCTGC -BHQ-3′
MnSOD	U 5′-CTGTGCTTTCTCGTCTTCAG-3′	5′R6G- CTGGCTCCGGTTTTGGGG -BHQ-3′
rs4880	R 5′-CGTTGATGTGAGGTTCCAG-3′	5′FAM- CTGGCTCCGGCTTTGGGG-BHQ-3′

corresponding values were calculated using the OR function «haplo.score» and «haplo.glm» from the «haplo.stats» package of the R 2.13.2 software [21].

Results

This study defines the frequencies of alleles and genotypes of polymorphic loci studied in the control group and the group of patients with prostate cancer. In both samples, the frequencies of genotypes are consistent with the Hardy-Weinberg equilibrium (Table 3). Our study found no statistically significant association of studied polymorphic changes to the risk of prostate cancer. Results of the comparison of frequencies of the studied SNPs alleles and genotypes in the analyzed groups are presented in Table 3. As studied polymorphic loci of GSTP1 gene are linked (D'=0.87; r2=0.18), we estimated haplotype frequencies for rs1695 and rs1138272 in both study and control groups (Table 4). No statistically significant difference has been observed for any haplotype. However, there is a correlation of studied SNPs with clinical parameters. An association with the stage of the disease was found for the GSTP1 SNP rs1695: a GG genotype is associated with advanced forms of the disease, namely stages 3 and 4 (OR [95 % CI]=2.66 [1.15–6.18], p=0.02). For both investigated SNPs in GSTP1, we found an association to the level of prostate-specific antigen (PSA) in the blood: a GG rs1695 genotype and a TT rs1138272 genotype are associated with higher rates of PSA (Figs. 1 and 2).

Discussion

No sufficient data are available on the specific inflammatory response in prostate tissue. Conducting epidemiological studies on the role of inflammation in the development of prostate cancer is difficult due to the lack of real data on the prevalence of chronic prostatitis, as it is often asymptomatic. In addition, men with symptoms of prostatitis see urologists more often, thus they are more prone to urological examination and a prostate cancer diagnosis. This can lead to a shift in the results of epidemiological studies due to a false correlation between the development of chronic inflammation and prostate cancer. Experimental evidence for the contribution of inflammatory cytokines in prostate carcinogenesis are obtained mainly in cell lines and cannot be directly extrapolated to the processes occurring in the body. If inflammation contributes to the development of prostate cancer, the polymorphic variants in genes responsible for the inflammatory response may contribute to the genetic predisposition to disease development.

ROS are products of normal cellular metabolism and play an important role in various signaling pathways. However, chronically elevated ROS leads to DNA damage over time, lipid peroxidation, protein modification, membrane damage and mitochondria damage [22, 23]. The role of oxidative stress in the development of prostate cancer is consistent with the free-radical theory of aging, as age is a proven risk factor for the disease. Decline in intracellular antioxidant defense during aging may be one of the factors that contributes to development of prostate cancer in men of age over 55. The positive correlation between the consumption of large amounts of animal fat and risk of prostate cancer [24, 25] may also be due to increased oxidative stress and lipid peroxidation [26]. Namely, it is believed that the increased incidence of prostate cancer among men in the U.S. population compared to other countries is to some extent due to specific dietary habits. ROS cause oxidative damage to DNA leading to mutations and changes in gene function and can also induce the expression of a set of transcription factors involved in neoplastic transformation of normal cells [27]. The antioxidant system is an important component of the anti-tumor protection, therefore polymorphic variants in genes of the antioxidant system may determine individual susceptibility to the development of prostate cancer. ROS can alter the conformational structure of the tumor suppressor protein p53, thus inhibiting its binding to DNA [28]. Inhibition of the p53 protein is associated with the progression of multiple tumors including prostate cancer [29]. Evidence that the progression of prostate cancer is associated with high levels of oxidative stress and increased lipid peroxidation was not

Table 3 Analysis of association of SNPs rs1050450 in *GPX1*, rs1695 and rs1138272 in *GSTP1* and rs4880 in *MnSOD* with risk of prostate cancer development

dbSNP_rs[a]		Control (n)	PCa (n)	OR[95 % CI]	*p*	H-W[b] P(exact) control	H-W[b] P(exact) case
rs1138272	Genotype					0.24	1.0
GSTP1	CC	277	305	Reference genotype			
	CT	60	66	1.03 (0.69–1.54)	0.87		
	TT	6	3	0.47 (0.11–1.94)	0.29		
Call rate	Allele	n (%)	n (%)				
97 %	C	614 (89.5)	676 (90.4)	Reference allele			
	T	72 (10.5)	72 (9.6)	0.94 (0.66–1.33)	0.72		
rs1695	Genotype					1.0	0.49
GSTP1	AA	151	158	Reference genotype			
	AG	149	157	0.94 (0.68–1.31)	0.73		
	GG	36	46	1.24 (0.80–2.06)	0.41		
Call rate	Allele	n (%)	n (%)				
95 %	A	451 (67.1)	473 (65.5)	Reference allele			
	G	221 (32.9)	249 (34.5)	1.05 (0.84–1.33)	0.64		
rs1050450	Genotype					0.13	0.7
GPX1	CC	153	183	Reference genotype			
	CT	132	146	0.94 (0.68–1.31)	0.73		
	TT	41	32	0.68 (0.40–1.16)	0.16		
Call rate	Allele	n (%)	n (%)				
93 %	C	438 (67.2)	512 (70.9)	Reference allele			
	T	214 (32.8)	210 (29.1)	0.87 (0.69–1.09)	0.22		
rs4880	Genotype					0.08	0.76
MnSOD	CC	99	94	Reference genotype			
	CT	152	194	1.43 (0.97–2.07)	0.05		
	TT	86	92	1.17 (0.77–1.78)	0.47		
Call rate	Allele	n (%)	n (%)				
97 %	C	350 (51.9)	382 (50.3)	Reference allele			
	T	324 (48.1)	378 (49.7)	1.09 (0.88–1.34)	0.45		

[a] ID number of SNP in the international database NCBI dbSNP http://www.ncbi.nlm.nih.gov/snp/

[b] X H–W P (exact): significance of the group genotype distribution disagreement with the Hardy–Weinberg equilibrium

observed with localized forms of prostate cancer [30]. Thus, long-term chronic inflammation and/or lack of antioxidant system may encourage progression of the disease.

In the conducted association studies, a statistically significant association between studied SNPs in the antioxidant system genes and prostate cancer has not been found. However, we identified a correlation between SNPs studied and the stage of disease: a GG rs1695 genotype of GSTP1 is associated with advanced forms of prostate cancer (stages 3 and 4).

The most generally recognized tumor marker in the diagnosis of prostate cancer is prostate specific antigen (PSA), which is widely used as a screening test. It should be noted that the PSA is not a tumor specific marker, rather its level

Table 4 Frequencies and association of rs1695 and rs1138272 haplotypes in *GSTP1* with prostate cancer

Haplotype		Frequency		OR [95 % CI]	*p*
rs1695	rs1138272	Control group	Study group		
A	C	0.66	0.65	Reference haplotype	
G	T	0.1	0.09	0.95 [0.65–1.38]	0.78
G	C	0.23	0.26	1.12 [0.88–1.43]	0.37
A	T	0.01	0.008	0.83 [0.25–2.77]	0.77

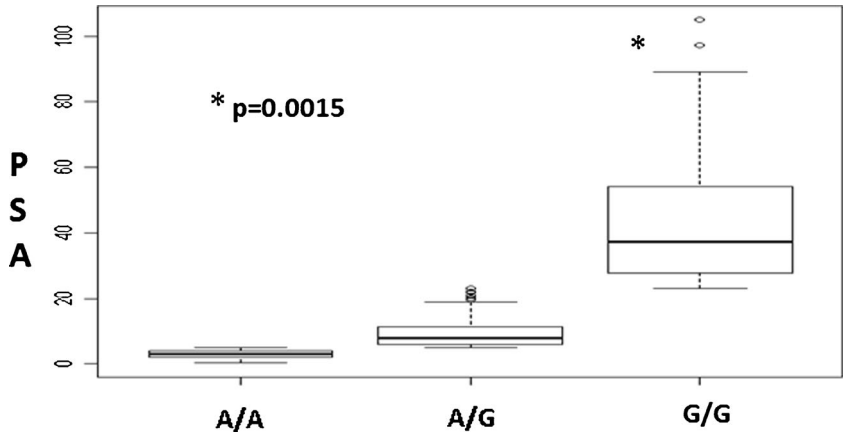

Fig 1 Dependence of PSA level from rs1695 genotype in the *GSTP1* gene

reflects the volume of total prostate tissue. There is no PSA concentration at which one can with a 100 % probability claim that the patient does or does not have prostate cancer. In our sample, 35 % of patients with prostate cancer PSA level was below the discriminatory value, 4 ng/ml, at the moment of diagnosis (Table 1). In our study, for both investigated SNPs in GSTP1, an association with PSA levels in the blood was found: a GG rs1695 genotype and a TT rs1138272 genotype are associated with higher rates of PSA (p=1,5*10^{-3}) (Figs. 1 and 2). PSA level is possibly determined, aside from hyperplastic and inflammatory processes in the prostate tissue, genetically. In addition, this result may be obtained due to severe inflammation of the prostate tissue in light of inadequate antioxidant system in carriers of minor alleles of studied SNPs.

Conclusion

No statistically significant association with the risk of prostate cancer was found for any of the SNPs studied in our work (p>0.05). For the rs1695 SNP of GSTP1 the correlation with disease stage was obtained: the GG genotype is significantly more common in patients with prostate cancer of stage 3 or 4 (OR[95%CI]=2.66[1.15–6.18], p=0.02). For both investigated SNPs in GSTP1 an association with the level of prostate-specific antigen (PSA) in the blood was found: the GG rs1695 genotype and the TT rs1138272 genotype are associated with higher rates of PSA (p=1,5*10^{-3}).

It is important to note that at the stated sample size of our study with 80 % level of statistical power it is possible to detect OR values of at least 1.48 for the rs1138272 in GSTP, 1.32 for rs1695 in GSTP1, 1.32 for rs1050450 in GPX1, and 1.3 for rs4880 in MnSOD. In other words, if we assume on the example of rs1138272 in GSTP1 that the SNP alters the risk of prostate cancer 1.48 times or less with a probability of more than 20 %, we do not find a statistically significant association in our sample. Therefore, if the effect of the studied SNPs on the risk of prostate cancer in this study is still present, but it is less than the above OR values, it can only be detected in the study of a larger sample.

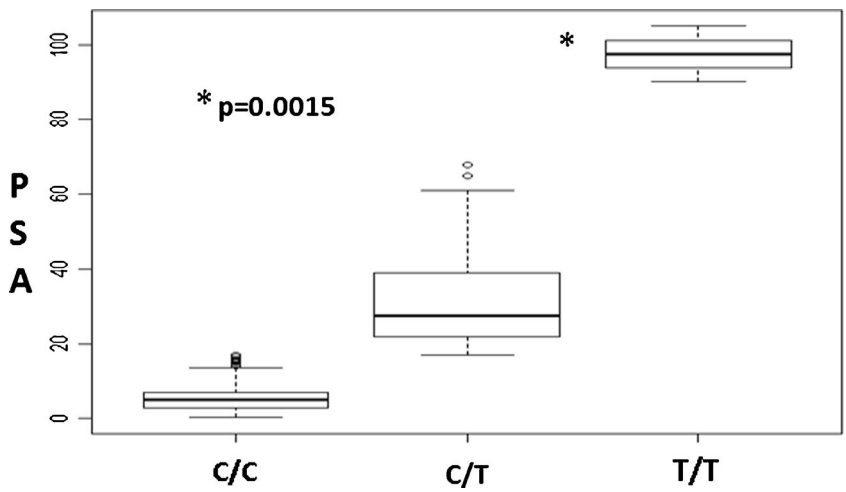

Fig 2 Dependence of PSA level from rs1138272 genotype in the *GSTP1* gene

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