Effects of Manganase Superoxide Dismutase Ala-9Val Polymorphism on Prostate Cancer: A Case-control Study

H.ARZU ERGEN[1], FEHMI NARTER[2], ÖZLEM TIMIRCI[1] and TURGAY ISBIR[1]

[1]*Institute of Experimental Medicine, Department of Molecular Medicine, Istanbul University, Capa, Istanbul;*
[2]*Clinic of Urology, Uskudar State Hospital, Uskudar, Istanbul, Turkey*

Abstract. *Background: Manganese superoxide dismutase (MnSOD) is a major enzyme that is responsible for the detoxification of reactive oxygen species in the mitochondria. Mitochondrial DNA damage may contribute to carcinogenesis as an important risk factor. The aim of this study was to investigate the relationship between prostate cancer and MnSOD Ala-9Val polymorphism in Turkish men with prostate cancer. Patients and Methods: Fifty patients with prostate cancer and 50 healthy controls were included in this study. Gene polymorphism was determined using a PCR-RFLP method. Results: The Ala/Ala genotype and the Ala allele were found at statistically higher frequencies in patients with prostate cancer as compared to controls ($p<0.05$). The patients suffering from prostate cancer were divided into two groups according to Gleason score: aggressive prostate cancer and non-aggressive prostate cancer. It was observed that carrying the Ala/Ala genotype or the Ala allele resulted in an insignificant increase in the frequency of aggressive prostate cancer compared to nonaggressive prostate cancer. It was concluded that MnSOD Ala allele might be the cause of prostate cancer risk among alcohol users. Conclusion: The results of our study of Turkish prostate cancer patients suggest that mutation of the MnSOD gene may be an improtant risk factor for prostate cancer.*

Abbreviations: Ala, alanine; ROS, reactive oxygen species; MnSOD, manganese superoxide dismutase; PCR, polimerase chain reaction; RFLP, restriction fragment length polymorphism; BMI, body mass index; Val, valine; PSA, prostate specific antigen.

Correspondence to: Prof. Dr. Turgay Isbir, Istanbul University, Institute for Experimental Medical Research, Department of Molecular Medicine 34390 Capa Istanbul, Turkey. Tel/Fax: +90 212 635 19 59, e-mail: tisbir@superonline.com

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Oxidative stress can cause DNA damage, mitochondrial damage and lipid peroxidaton *via* reactive oxygen species (ROS). Some studies delineated the fact that ROS are associated with carcinogenesis (1). The cell maintains a balance between ROS and antioxidants. Manganase superoxide dismutase (MnSOD) is an antioxidant enzyme that, being localized in the mitochondria, plays a role in the detoxification of superoxide free radicals. Basically, it is known to convert ROS to oxygen and hydrogen peroxide (2). As it is known that the mitocondria are considered the first source of ROS, MnSOD is suggested to have an important role in protecting cells against oxidative damage.

Prostate cancer is the second leading cause of death among men. A series of studies suggest that oxidative damage plays a significant role in prostatic carcinogenesis (3). A common polymorphism in the MnSOD gene was described to result in a valine-to-alanine substitution at the -9' position of the gene (4). This polymorphism may alter the secondary structure of the protein and affect the mitocondrial transport of MnSOD. It has been suggested that the MnSOD Ala variant is a 30-40% more active protein than the Val variant in mitochondria. Sutton *et al.* suggested that *AA* homozygous carriers have higher MnSOD activity than *VV* carriers (5).

Several studies have suggested that the *A* allele or *AA* genotype is associated with increased cancer risk. Woodson K *et al.* showed that Finnish male heavy smokers who carried the *AA* genotype had a 70% increased prostate cancer risk and 3-fold increased risk of high grade tumor compared with patients who carried *VA* and *VV* genotypes (6). Ambrosone *et al.* found that premenopausal women who were homozygous for the *A* allele had a 4-fold increase in breast cancer risk as compared with those with *V* alleles (7).

It has been estabilshed that not only MnSOD genotype but also some other factors such as diet, smoking and radiation play a significant role in prostatic carcinoma. A Finnish study showed that there was a 32% reduction in prostate cancer incidence among men who received vitamin E (6). Rose *et al.*

Table I. *Demographic characteristics of the study population.*

Groups	Control (n:50)	Prostate cancer (n:50)
Age (years)	64.36±8.68	68.11±9.13
Body mass index (kg/m^2)	26.25±3.03	25.98±2.68
Smoking (%/n)	28/14	36/9
Alcohol consumption (%/n)	8/4	29.2/7*
Familial cancer (%/n)	6/3	46.2/6*
Total-PSA (mg/dl)	1.26±0.92	92.88±258.4*
Free-PSA (mg/dl)	0.30±0.29	10.05±31.33*
BUN (mg/dl)	18.81±4.97	25.05±15.66*
Creatinine (mg/dl)	0.91±0.19	1.04±0.31*

n: Number of individuals. The results are shown as mean±SD, *$p<0.05$.

observed that there was a high correlation between mortality from prostate cancer and dietary fat intake (8).

This study aimed to investigate the relationship between prostate cancer and MnSOD Ala-9Val polymorphism in Turkish men.

Patients and Methods

Patient selection and clinical investigation. This study included 50 prostate cancer patients who were treated in the Uskudar State Hospital and the Haydarpasa Numune Research and Educational Hospital. Between 2004 and 2006, individuals with prostate cancer, being histologically confirmed for adenocarcinoma of the prostate, were enrolled in the study. All subjects and controls were born in Turkey. Age-matched controls were recruited from hospitals. The tumor differentiation was evaluated using Gleason score criteria. There were three patients who had unknown tumor status. Clinical parameters (body mass index, total-PSA, free-PSA, BUN, creatinine) were collected from the hospital records.

The control group was selected from the patients of the urology clinics of the same hospitals who were not diagnosed positive for prostate cancer.

DNA isolation. Blood specimens were collected in tubes containing EDTA and DNA samples were extracted from whole blood with a salting-out procedure (9).

MnSOD Ala-9Val genotyping. For amplification of the MnSOD Ala-9Val polymorphism, the following primers (MBI Fermentas, Lithuania) were used: 5'-ACC AGC AGG CAG CTG GC GCC GG-3 '; 5'- GCG TTG ATG TGA GGT TCC AG-3'.

For detection of the MnSOD Ala-9Val, 50-100 ng genomic DNA was amplified with 1x reaction buffer, 3 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM each primer and Taq polymerase (MBI Fermentas) in a 25 μl reaction volume. The PCR conditions were: initial denaturation step at 95˚C for 5 min followed by 35 cycles at 95˚C for 1 min, 61˚C for 1 min, 72˚C for 2 min and 72˚C for 7 min. PCR products were digested with PdiI restriction enzyme (MBI Fermentas) at 37˚C overnight and electrophoresed on 3% agarose gels stained with ethidium bromide. Genotypes were determined as Val/Val (107 bp), Val/Ala (107, 89, 18 bp) or Ala/Ala (89, 18 bp) for the polymorphism (10).

Table II. *Distribution of MnSOD genotypes and alleles in study groups.*

Groups	Control (n:50)	Prostate cancer (n:50)
MnSOD genotypes	n (%)	n (%)
Ala/Ala	0	6 (12)
Val/Val	32 (64)	19 (38)
Ala/Val	18 (36)	25 (50)
MnSOD alleles		
Ala	18 (18)	37 (37)
Val	82 (82)	63 (63)

n: Number of individuals.

Statistical analyses. Statistical analyses, using SPSS version 7.5 for Windows (SPSS Inc), included the χ^2 test for genotype and allele frequency comparison. Whenever an expected cell value of less than 5 was obtained, Fisher's exact test was used. The Odds Ratios and the confidence intervals were calculated as an estimate of the relative risk. Clinical characteristics were compared using Student's *t*-test. A *p*-value of less than 0.05 was regarded as being statistically significant.

Results

Clinical investigation. Clinical characteristics of study groups are shown in Table I. There were significant differences in total-PSA, BUN ($p<0.01$), free-PSA and creatinine ($p<0.05$) levels between subjects and controls. Furthermore, alcohol consumption ($p=0.032$, OR=4.73, 95% Cl=1.22-18.24) and a family history of cancer ($p=0.002$, OR=13.42, 95% Cl=2.71-66.33) had a higher frequency in patients than in controls.

Frequencies of MnSOD Ala-9Val polymorphism. According to the distribution of MnSOD Ala-9Val genotypes, Ala/Ala genotype ($p=0.012$, $\chi^2=6.383$, OR=1.136, 95% Cl=1.02-1.25) and Ala allele ($p=0.009$, $\chi^2=6.763$, OR=2.901, 95% Cl=1.28-6.63) were shown to increase significantly in prostate cancer patients compared to healthy controls. However, the Ala/Ala genotype was not observed in controls (Table II).

According to the Gleason Score, the prostate cancer patients were divided into two groups: aggressive prostate cancer (Gleason Score ≥7) and non-aggressive prostate cancer (Gleason Score<7). It was found that carrying the Ala/Ala genotype or the Ala allele resulted in an insignificant increase in the frequency of aggressive prostate cancer compared to non-aggressive prostate cancer (Table III).

Moreover, it was shown that the frequency of carrying the Ala allele was higher than that for the Val allele in patients who used alcohol ($p=0.352$, OR: 4.20, 95% Cl=0.41-43.03) (Table IV).

Table III. *Distribution of MnSOD genotypes and alleles in aggressive and non-aggressive prostate cancer.*

Groups	Aggressive prostate cancer (n:27)	Non-aggressive prostate cancer (n:20)
MnSOD genotypes	n (%)	n (%)
Ala/Ala	4 (14.8)	2 (10)
Val/Val	9 (34)	8 (40)
Ala/Val	14 (51.2)	10 (50)
MnSOD alleles		
Ala	22 (40.7)	14 (35)
Val	32 (59.3)	26 (65)

n: number of individuals, aggressive prostate cancer: Gleason Score ≥7, non-aggressive prostate cancer: Gleason Score <7.

Table IV. *According to MnSOD alleles, alcohol consumption, smoking and familial cancer in patient group.*

Patients	Ala Allele	Val Allele
Alcohol consumption (%/n)	37.5/6*	22.7/5
Smoking (%/n)	31.3 /5	30.4 /7
Familial cancer (%/n)	37.5/3	50/6

*p=0.352, OR=4.20, 95% Cl=0.41-43.03, n: number of individuals.

Discussion

MnSOD, as an endogenous antioxidant enzyme in the mitochondria, may play a role in preventing prostate cancer. Li *et al.* suggested that overexpression of MnSOD in the prostate inhibits cancer cell growth *in vivo* (11).

Previous studies have examined the role of MnSOD Ala-9Val polymorphism and risk of cancers. In another study on bladder cancer, it was found that than Val/Val genotype increased the risk of bladder cancer (12). It was also concluded that the polymorphism might alter the secondary structure of the protein and affect the mitochondrial transport of MnSOD. However, it was not clear how polymorphism was more effective in the mitochondrial transport of MnSOD.

In this study, we investigated the relationship between MnSOD polymorphism and prostate cancer risk in Turkish prostate cancer patients. This is the first study that evaluated the effects of MnSOD on prostate cancer risk in Turkey. We found the frequencies of the Ala/Ala genotype and the Ala allele to be higher in patients than controls. Our findings demonstrated that the Ala genotype for the MnSOD Ala-9Val polymorphism conferred an increased risk of prostate cancer. Li *et al.* found that the *AA* genotype for MnSOD had a 5-fold higher risk of aggressive prostate cancer (13).

Dividing the prostate cancer patients into two groups: aggressive prostate cancer (Gleason Score ≥7) and non-aggressive prostate cancer (Gleason Score<7), we showed that carrying the Ala/Ala (OR: 1.56) genotype or the Ala (OR: 1.33) allele resulted in an insignificant increase in the frequency of aggressive prostate cancer compared to non-aggressive prostate cancer. This finding was in agreement with that by Woodson *et al.* who observed that men homozygous for MnSOD Ala showed a 3-fold increased risk of high-grade tumors (6).

Some studies have even implicated alcohol consumption in cancer risk. Hayes *et al.* found an elevated risk for men who had 22-56 drinks per week compared with non-users (14). In our study, we found an association between alcohol consumption and prostate cancer risk (p=0.032, OR=4.73). We also found that the frequency of carrying the Ala allele was higher than that for the Val allele in patients who used alcohol (p=0.352, OR: 4.20, 95% Cl=0.41-43.03). Mitrunen *et al.* showed that the risk of breast cancer was 2.2-fold higher among women who used alcohol and had Ala/Ala and Val/Ala genotypes compared to Val/Val (10).

Furthermore, in the current study, prostate cancer patients showed an increased risk as compared to controls (p=0.42, OR=1.44, 95% Cl=0.52-4.02) with smoking. Cigarette smoke is a source carcinogens. Sharpe *et al.* suggested that tobacco smoking might be a risk factor for prostate cancer in a population case-control study (15). Plaskon *et al.* indicated that smoking was associated with a moderately increased relative risk of prostate cancer (16). However, there was no significant interaction between MnSOD genotype and smoking in our study.

Our study showed an effect of the Ala/Ala genotype and the Ala allele in Turkish prostate cancer patients. Moreover, this result was associated with aggressive prostate cancer. The MnSOD Ala allele may be a risk factor for prostate cancer among alcohol users. Additional studies will be helpful in determining the role of MnSOD in the progression of prostate cancer.

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