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Manganese-superoxide dismutase (*MnSOD*) polymorphisms

[Manganez-süperoksit dismutaz (*MnSOD*) polimorfizmi]

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ABSTRACT

Objective: Manganese superoxide dismutase (*MnSOD,SOD2*), the only known superoxide scavenger in mitochondria, may be particularly important for antioxidant defense because mitochondria are the major sites for cellular metabolism and hence production of reactive oxygen species.

Methods: In this study, 440 Turkish individuals were genotyped for polymorphisms of SOD2 gene. The distribution of these polymorphisms in this population was examined using a PCR-RFLP method.

Results: In the present study, a total of 440(females: 201, 46% and males: 239, 54%) healthy individuals were studied. The mean age of the study population was 54,41±5,76 years (males, 55,34±5,76; females, 53,12±7,16). The observed genotype frequencies of SOD2 were 17.5, 50.5 and 32.0% for CC, CT and TT, respectively.

Conclusion: This study provides basic information about the allele and genotype frequency distributions of polymorphisms of rs4880 in the *SOD2* gene studied. These frequencies may be useful parameters as a reference for future studies on genetic basis of various diseases and cancer susceptibility.

Key Words: Manganese-superoxide dismutase, *SOD2*, polymorphisms

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Mangan süperoksit dismutaz (MnSOD, *SOD2*) hücresel metabolizmada reaktif oksijen türlerinin en çok oluştuğu mitokondride bilinen tek süperoksit süpürücü ve antioksidan savunma siteminin önemli bir enzimidir.

Metod: Bu çalışmada, toplam 440 Türk sağlıklı bireyler incelendi. Türk toplumunda bu polimorfizmin dağılımı bir PCR-RFLP yöntemi kullanılarak incelenmiştir.

Bulgular: Bu çalışmada, toplam 440 (kadın: 201,%46 ve erkek: 239, %54) Türk sağlıklı bireyler incelendi. Çalışma grubunun yaş ortalaması 54,41±5,76 yıl (erkek, 55,34±5,76; kadın, 53,12±7,16) idi. Mangan süperoksit dismutaz gözlenen genotip frekansları sırasıyla CC: %17,5 CT: %50,5 ve TT için %32,0 olarak tespit edildi.

Sonuç: Bu çalışma, Türk toplumunda *SOD2* geninde rs4880 polimorfizmlerinin allel ve genotip frekans dağılımları hakkında temel bilgiler sağlamıştır. Bu frekanslar gelecekte çeşitli hastalıklar ve kanser yatkınlığı çalışmalarında yararlı referans parametreler olabilir.

Anahtar Kelimeler: Mangan süperoksit dismutaz, *SOD2*, polimorfizmi, Türk popülasyonu

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Introduction

Oxidative stress has been linked to the progression of many diseases ranging from cancers [1], diabetes [2], cardiovascular [3] and chronic kidney disease [4], to neurodegenerative diseases such as Alzheimer's [5]. Reactive oxygen species (ROS) are unstable and cause damage by oxidizing macromolecules. Oxidative stress can occur due to an increased concentration of ROS and/or a reduction in antioxidant capacity. Exogenous antioxidants are consumed in the diet and consist mainly of carotenoids such as β-carotene, tocopherols such as vitamin E, and ascorbic acid (vitamin C). Endogenous antioxidants include thiols such as glutathione and the enzymes, superoxide dismutase (*SOD*), glutathione peroxidase (*GPx*) and catalase [6]. *SOD* is the key antioxidant enzyme involved in the detoxication of superoxide radicals [7]. *SOD* contains an active site that has transition metals for rapid electron exchange, converting the superoxide free radical (O_2•) to hydrogen peroxide (H_2O_2) [8]. Three isoforms of *SOD* have been identified. *SOD1* contains copper (Cu) and zinc (Zn) within the active site (also known as *CuZn SOD*), and is mainly found in cell cytoplasm. *SOD2* or manganese (*Mn*) *SOD* has an active site that contains manganese and is located in mitochondria. SOD3 or extracellular (EC) *SOD* also has Cu and Zn within the active site and is the least studied of the three *SOD* isoforms. *SOD2* is the only antioxidant enzyme known to be present within the mitochondria and this has important implications because this is a major site for the production of ROS during normal cellular metabolism [9,10]. There are several polymorphisms located in distinct regions of the *SOD2* gene. These polymorphisms have been associated with different diseases. The *SOD2* gene structure consists of five exons interrupted by four introns and the promoter, which control *SOD2* expression [11]. From the polymorphisms located in the coding sequence, the one called rs4880, located in exon 2 has been the most studied [12]. The C16T polymorphism is located in the mitochondrial targeting sequence, and is suggested to modify the peptide structure, affecting the protein translocation, and maturation into the mitochondrial matrix [7]. This polymorphism has been associated with different pathologies such as asthma [13], diabetes [14,15] aging [16] cardiomyopathy [17] and Parkinson's diseas [7] and cancer [18].

In this study, we investigated *SOD2* polymorphism. Here, we describe a polymerase chain reaction (PCR) based method for identifying single nucleotide polymorphism, rs4880.

Materials and Methods

Subject selection

The study protocol was approved by both scientific and ethics committees (Cumhuriyet University) and written informed consent was obtained from all participants. The study population included 440 (females: 201, 46% and males: 239, 54%) unrelated healthy volunteers from central Anatolia. There was no age and sex restriction for selection of the healthy volunteers, who were free of any chronic diseases, living in the same geographic area, and having no history of any cancer. We attempted to include all the control studies published to data on the *SOD2* rs4880 polymorphism.

Table 1. Characteristics of the study population

	Healthy individuals
Sample size	440
Gender	
Males	239 (54%)
Females	201 (46%)
Age (year)	
Range	14–80
Means±SD	54.41±5.76
Males	55.34±5.76
Females	53.12±7.16

DNA isolation

Two milliliters peripheral blood samples were collected in to citrate containing tubes from all subjects. DNA was extracted from whole blood by salting out procedure as soon as the samples reached to laboratory [19].

Genotyping

The distribution of these polymorphisms was examined using a PCR-RFLP method. For amplifying the *SOD2* rs4880 polymorphism, forward primer 5'-ACC AGC AGG CAG CTG GCG CCG G-3' and reverse primer 5'- GCG TTG ATG TGA GGT TCC AC-3'were used. PCR reactions contained 0,75 µL (25 pmol/µL) of each primer, 2µL dNTPs (1mmol/L), 1,5µL of 25 mmol/L MgCI2, 1.5 Units of Taq DNA polymerase and 16,2µL sterile deionized water. DNA 50-100 ng is in a total volume of 50 µL. The PCR program was initial denaturation at 95°C for 5 minutes followed by 35 cycles of 95°C for 1 minute, 61°C for 1 minute (annealing), and 72°C for 2 minutes (extension). The PCR was completed by a final extension cycle at 72°C for 7 minutes. Amplified product was digested overnight with *Pdi*I restriction enzyme at 37°C and electrophoresed on 3% agarose gel stained with ethidium bromide. Genotypes were determined for the polymorphism as TT (107 bp), CT (107, 89, 18 bp), or CC (89, 18 bp) [20].

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences Program (SPSS, version 11). The Chi-square (X^2) test was used to evaluate the association between healthy individuals and SOD genotypes. Genotype, allele frequencies was estimated by counting. Hardy–Weinberg equilibrium between expected and observed genotype distributions was assessed using the X^2 test. Also, statistical evaluation of allele frequencies was

Table 2. Genotype frequencies for *SOD2* rs4880 polymorphism

SOD2	Sample size	Percentage	*p*	χ^2
Allele frequency				
C allele	377	0.43	0.001	18.04[a]
T allele	503	0.57		
Genotype frequency				
CC	77	17.5	0.465	0.534[b]
CT	223	50.5		
TT	140	32.0		
CT+TT	363	82.5		
Total	440	100		

[a]For one-sample Chi-square (X^2) test with a critical alpha of 0.05 (i.e., p<0.05); [b]For Hardy-Weinberg equilibrium; (1 degree of freedom, p<0.05 if χ^2>3.84).

performed by using one-sample Chi-square (X^2) test with a critical alpha of 0.05 (i.e., p<0.05)

Results

We confirmed the presence of the rs4880 single nucleotide polymorphism in human subjects. The distribution of these polymorphisms was examined using a *PdiI* -RFLP method. In this study, 440 (239 men and 201 women) Turkish individuals were genotyped for polymorphisms of *SOD2* gene. The demographic characteristics of the study population are listed in Table 1. The mean age of the study population was 54.41±5.76 years (males, 55.34±5.76; females, 53.12±7.16) in Table 1. Allele and genotype frequencies of the subjects for rs4880 polymorphism in the *SOD2* gene are shown in Table 2.

All genotypes and alleles in the healthy controls were in Hardy-Weinberg equilibrium (p>0.05). The frequencies of TT, CT, CC genotypes were found to be 32.5%, 50.5% and 17.5% in the controls respectively. The frequency distributions of C and T alleles were found to be respectively 43% and 57% in the controls (X^2=18.04, p=0.001) in Table 2. As shown in Figure 1, the analysis of the polymorphisms located at *SOD2* chromosome 6 (6q25) in the controls showed that TT (107 bp), CT (107, 89, 18 bp), CC (89,18 bp) genotypes.

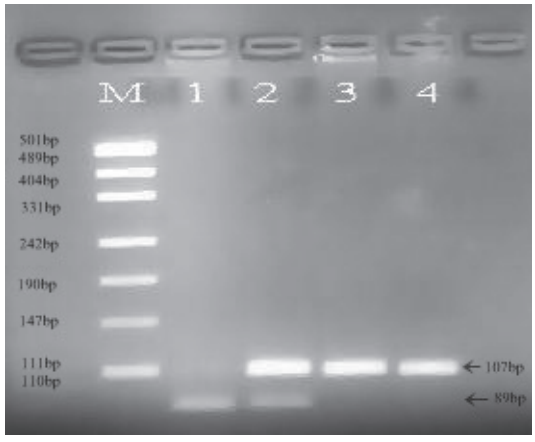


Figure 1. PCR-RFLP patterns of polymorphisms of *SOD2* rs4880 M: pUCI19/Msp I DNA ladder (501, 489, 404, 331, 242, 190, 147, 111, 110bp); 1, CC (89, 18 bp); 2, CT (107, 89, 18 bp); 3, TT (107 bp); 4, *SOD2* PCR product, 107 bp.

Discussion

Antioxidant enzymes constitute one of the major cellular protective mechanisms against oxidative stress in the human body. Many of the antioxidant genes are known to be polymorphic, which can lead to altered enzyme activity [21]. *SOD2* is a mitochondrial enzyme that catalyzes the formation of H_2O_2 from superoxide radicals. The variant allele of *SOD2* has been associated with elevated risk of breast [22], prostate [23], lung [24] and ovarian [25] cancers and non-Hodgkin's lymphoma [26].

There is a polymorphic site identified in the mitochondria targeting sequence of human *SOD2* gene. This C to T substitution results in an amino acid change from valine (Val) to alanine (Ala) at codon 16, which is predicted to form amphiphilic helix with higher enzyme activity [7]. These polymorphisms have variable allele and genotype frequencies among ethnic groups [7,27]. Our study aimed to determine the allelic and genotypic frequency distribution of polymorphisms of rs4880 in the *SOD2* gene and to compare findings with other ethnic groups. The frequencies of C and T alleles were 43% and 57% respectively, whereas the frequencies of the CC, CT and TT genotypes were 17.5%, 50.5%, and 32.0% respectively in the study population. The distribution of three genotypes fitted the Hardy-Weinberg equation χ^2=0.534. (P=0.465) (Table 2). There is wide range variation of CC genotype frequency ranging from 11.7% (28) to as high as 36.9% (34) (Table 3) [28-34]. C16T genotype frequency was found to be 17.5% in the present study. Our sample size was much larger than those of other studies in Turkish population, which is very important for the making of more precise estimations in epidemiological studies in Turkish population.

Sutton *et al.* found that the C allele (Ala) form of *SOD2*

Table 3. Distribution of polymorphisms of *SOD2* rs4880 genotype frequencies in Turkish population

Turkish population	Year	Sample size	*SOD2* (%)			Ref.
			CC	*CT*	*TT*	
Dalan *et al.*	2008	51	11.7	33.3	54.9	[28]
Atilgan *et al.*	2014	50	16.0	38.0	46.0	[29]
Gurel *et al.*	2004	105	16.2	43.8	40.0	[30]
Kadioglu *et al.*	2010	40	20.0	57.5	22.5	[31]
Akyol *et al.*	2005	196	23.5	42.8	34.2	[32]
Erbil *et al.*	2008	35	25.7	51.4	22.9	[33]
Kocabas *et al.*	2005	103	36.9	38.3	24.3	[34]
This study	2014	440	17.5	50.5	32.0	—

Table 4. Distribution of polymorphisms of *SOD2* rs4880 genotype frequencies in different populations

Country	Year	Sample size	*SOD2* (%)			Ref
			CC	CT	TT	
African, American	2008	331	25.2	53.7	21.1	[35]
Finn	2002	63	14.7	57.9	27.4	[36]
Italian	2006	275	24.2	48.8	27.0	[37]
Korean	2006	106	20.3	47.9	31.7	[38]
Japan	2007	107	0.0	35.8	64.2	[39]
Chinese	2010	1065	24.6	50.8	24.7	[40]
Taiwan	2007	115	14.4	62.8	22.9	[41]
American	2007	395	20.0	49.1	30.9	[23]
Polish	2005	361	32.0	50.0	18.0	[42]
Danish	2009	1650	19.0	57.1	23.9	[43]
German	2009	603	17.9	60.4	21.7	[44]
Russian	2001	88	1.9	24.2	73.9	[45]
This study	2014	440	25.0	49.8	25.2	—

is targeted into the mitochondria, whereas the T allele (Val) form is partially arrested in the inner mitochondrial membrane. Their study exposed that Ala form of *SOD2* was 30% to 40% more efficiently localized to the mitochondria than the Val form. Based on these findings, it is expected that the Val form is likely to be associated with higher levels of ROS and thus predisposes to a greater risk of cancer. On the other hand, various experiments aiming to study association between this polymorphism and different carcinoma reveal a controversial picture. Although few reports find associations between Val form and higher cancer risk, major studies have shown the Ala form to be associated with the risk of different types of cancer (7). Our study, T allel (Val) frequency found more than C allel (Ala) frequency ($\chi 2$=18.04, P=0.001) (Table 2). In view of this finding, *SOD2* rs4880 polymorphism in Turkish population may pose a risk for some dieseases such as cancer. We compared the distribution of three genotypes in different ethnic groups in the world in Table 4. Data presented here show that the Turkish population has the similar CC genotype frequency as seen in African-American [35], Finn [36], and Italian populations [37]. We detected considerably lower CC genotype frequency than those found in Korean [38], Japan [39], Chinese [40] and Taiwan [41] populations and higher frequencies than those found in American [23], Polish [42], Danish [43], German [44] and Russian [45] populations. Eventually, CC genotype frequency in populations of the eastern countries, were lower (between 0-17%) found than the western countries (between 42-67%) (Table 4). CC genotype frequency found in the present study was consistent with the above mentioned situation that the value (17.7%) was between the values of eastern and western populations. CC genotype frequency is between west and east in the Turkish population (17.5%) were identified. Geographical situation confirms this result.

This study provides basic information about the allele and genotype frequency distributions of polymorphisms of rs4880 in the *SOD2* gene studied. Our sample size was much larger than those of other studies (in Turkey), which

is very important for the making of more precise estimations in epidemiological studies. The results of the present study, in conjunction with the results regarding *SOD2* polymorphisms in a Turkish population, provide a framework for further studies concerning the role of this enzyme as a susceptibility many diseases, including certain cancers.

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Conflict of Interest

There are no conflicts of interest among the authors.

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