Manganese-superoxide dismutase (MnSOD) polymorphisms

[Çeviri: 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

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

Metod: Bu çalışmada, toplam 440 Türk sağlıklı bireyler incelendi. Türk toplumunda bu polymorfizmin 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 polymorfizmallerinin allele ve genotip frekans dağılımları hakkında temel bilgiler sağlamıştır. Bu frekanslar gelecekte çeşitli hastalıklar ve kanser yanıklılığı çalışmalarında yararlı referans parametreler olabilir.

Anahtar Kelimeler: Mangan süperoksit dismutaz, SOD2, polymorfizm, Türk popülasyonu

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

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Registered: 06 August 2014; Accepted: 25 October 2014
[Kayıt Tarihi: 06 Ağustos 2014; Kabul Tarihi: 25 Ekim 2014]

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 (O2•-) to hydrogen peroxide (H2O2) [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 disease [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.

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 GCC CCG G-3’ and reverse primer 5’- GCG CAG CTG GCG CCG G-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 MgCl2, 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 PdiI 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 (X²) 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 X² test. Also, statistical evaluation of allele frequencies was

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the study population</th>
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</thead>
<tbody>
<tr>
<td>Sample size</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Age (year) Range</td>
</tr>
<tr>
<td>Means±SD</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
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</table>
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 H2O2 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 ($\chi^2=0.534$. ($p=0.465$) (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

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.

**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 H2O2 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].

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

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**Table 2. Genotype frequencies for SOD2 rs4880 polymorphism**

<table>
<thead>
<tr>
<th>SOD2</th>
<th>Sample size</th>
<th>Percentage</th>
<th>$p$</th>
<th>$\chi^2$</th>
</tr>
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<tr>
<td>Allele frequency</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C allele</td>
<td>377</td>
<td>0.43</td>
<td>0.001</td>
<td>18.04*</td>
</tr>
<tr>
<td>T allele</td>
<td>503</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>77</td>
<td>17.5</td>
<td>0.465</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>223</td>
<td>50.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>140</td>
<td>32.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT+TT</td>
<td>363</td>
<td>82.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>440</td>
<td>100</td>
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</table>

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

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**Figure 1.** PCR-RFLP patterns of polymorphisms of SOD2 rs4880

M: pUC19/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.
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 allele (Val) frequency found more than C allele (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 diseases 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. Geographic 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

<table>
<thead>
<tr>
<th>Turkish population</th>
<th>Year</th>
<th>Sample size</th>
<th>CC (%)</th>
<th>CT (%)</th>
<th>TT (%)</th>
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<td>Dalan et al.</td>
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<td>11.7</td>
<td>33.3</td>
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<td>Atilgan et al.</td>
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<td>43.8</td>
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<td>Kadioolu et al.</td>
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<td>23.5</td>
<td>42.8</td>
<td>34.2</td>
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<td>38.3</td>
<td>24.3</td>
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</tr>
<tr>
<td>This study</td>
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<td>440</td>
<td>17.5</td>
<td>50.5</td>
<td>32.0</td>
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<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample size</th>
<th>CC (%)</th>
<th>CT (%)</th>
<th>TT (%)</th>
<th>Ref</th>
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<tr>
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<td>Taiwan</td>
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<td>62.8</td>
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<td>[42]</td>
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<tr>
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<td>57.1</td>
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<td>25.2</td>
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**Table 3.** Distribution of polymorphisms of SOD2 rs4880 genotype frequencies in Turkish population

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

Acknowledgements
This work is supported by the Scientific Research Project Fund of Cumhuriyet University (CUBAP) under the project number T-403.

Conflict of Interest
There are no conflicts of interest among the authors.

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