Research Article

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Superoxide Dismutase 1 (*SOD 1*) *A251G* Polymorphism

Süperoksit Dismutaz 1 (*SOD 1*) *A251G* Polimorfizmi

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Abstract

Objective: A genetic polymorphism of *SOD1 A251G*(*rs2070424*) is in the 3rd intron region of the *SOD* gene. The aim of this study was to determine the frequencies of the polymorphisms of the *SOD1 A251G* in a Turkish population, including 494 healthy individuals.
Methods: The 494 Turkish individuals were genotyped for polymorphisms of *SOD*1 gene. The distribution of *SOD1 A251G* polymorphisms in this population was examined using a PCR-RFLP method. Genotype and allele frequencies were estimated by counting. Hardy–Weinberg equation between expected and observed genotype distributions was assessed using the X^2 test.
Results: In the present study, the distribution of *SOD1 A251G* polymorphisms in a Turkish population including 494 (females: 278, 56.3% and males: 216, 43.7%) healthy individuals was examined. The mean age of the study population was 38.4 ± 16.6 years (males, 39.8 ± 17.1; females, 37.3 ± 16.1). The observed genotype frequencies of *SOD1 A251G* were 86.2, 13.4 and 0.4% for *AA, AG* and *GG*, respectively.
Conclusions: This study provides basic information about the allele and genotype frequency distributions of polymorphisms in the *SOD1 A251G* gene studied. These frequencies may be useful parameters as a reference for future studies on genetic basis of various diseases and cancer susceptibility.

Keywords: Copper/zinc-superoxide dismutase; Superoxide dismutase 1; Genetic polymorphism; Turkish population; *SOD1 A251G*; rs2070424.

Özet

Amaç: *SOD1 A251G* (rs2070424) genetik polimorfizmi SOD geninin 3. intron bölgesinde lokalizedir. Bu çalışma 494 sağlıklı birey içeren bir Türk popülasyonunda *SOD1 A251G* polimorfiziminin frekanslarını belirlemeyi amaçlamıştır.
Yöntemler: Bu çalışmada, 494 sağlıklı Türk bireyin *SOD1* geninin polimorfizmi genotiplendi. Bu popülasyonda *SOD1 A251G* polimorfizmin dağılımı bir PCR-RFLP yöntemi kullanılarak incelenmiştir. Genotip ve alel frekansları sayarak elde edilmiştir. Beklenen ve gözlenen genotip dağılımları arasında Hardy–Weinberg dengesi X^2 testi kullanılarak değerlendirilmiştir.
Bulgular: Bu çalışmada, *SOD1* genindeki *A251G* polimorfizmi toplam 494 (females: 278, 56.3% and males: 216, 43.7%) Türk sağlıklı birey incelendi. Çalışma grubunun yaş ortalaması 38.4 ± 16.6 yıl (erkek, 39.8 ± 17.1; kadın, 37.3 ± 16.1) idi. *SOD1* gözlenen genotip frekansları sırasıyla *AA*: % 86.2 *AG*: % 13.4 ve *GG* için % 0.4 olarak tespit edildi.
Sonuç: Bu çalışma, Türk toplumunda *SOD1 A251G* gen polimorfizmlerinin alel ve genotip frekans dağılımları hakkında temel bilgiler sağlamıştır. Türk popülasyonunda *SOD1 A251G* polimorfizmi ile yapılan ilk çalışmadır ve önemlidir. Bu frekanslar gelecekte çeşitli hastalıklar ve kanser yatkınlığı çalışmalarında yararlı referans parametreler olabilir.

Anahtar Kelimeler: Bakır/Çinko-Süperoksit Dismutaz; Süperoksit Dismutaz 1; Genetik Polimorfizmi; Türk Popülasyon; *SOD1 A251G*; rs2070424

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Introduction

Oxidative stress is accepted as one of the main factors involved in the development and progression of many diseases [1, 2]. Many studies have shown the relationship between the genetic polymorphism of *SODs* genes and multifactorial diseases such as cancers and age-related macular degeneration [3–7]. Superoxide dismutases are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. They play an important role in the antioxidant mechanism in almost all cells exposed to oxygen [8]. Three isoforms of SOD [cytoplasmic superoxide dismutase (SOD1), mitochondrial superoxide dismutase (SOD2) and extracellular superoxide dismutase (SOD3)] have been identified in mammals [9–11]. SOD1 accounts for approximately 85% of the total cellular SOD activity of the most mammalian cells [12].

The *SOD1* gene is localized in the chromosome 21 (region 21q22) in humans. It contains four introns and five exons. Possible associations of single nucleotide polymorphisms in *SOD1* gene in patients with diabetes mellitus, amyotrophic lateral sclerosis, encephalitis, and breast cancer have been reported [2, 13, 14]. A genetic polymorphism of *A251G* (rs2070424) is located in 3rd intron of the *SOD1* gene. This polymorphism plays important roles as a risk factor of many diseases, including Alzheimer's disease [15], type 1 diabetic nephropathy [16], in age-related cataract [4], noise-induced hearing loss [17], myelomeningocele [18], antituberculosis drugs-induced hepatitis [19], ulcerative colitis [20] and gastric cancer [21].

This study is the first study of the Turkish population in the *SOD1 A251G* polymorphism. The aim of this study was to determine the frequencies of *SOD1 A251G* gene polymorphisms in a Turkish population. Here, we describe a polymerase chain reaction (PCR) based method for identifying single nucleotide polymorphism.

Materials and methods

Subjects

The study protocol was approved by both scientific and ethics committees (Cumhuriyet University) and written informed consents were obtained from all participants. In the present study, a total of 494 (females: 278, 56.3%) and males: 216, 43.7%) Turkish healthy individuals were studied. The mean age of study population was 38.4 ± 16.6 year (males, 39.81 ± 17.10; females, 37.32 ± 16.14). Healthy controls were composed of 494 individuals who visited the outpatient department for physical examination, without tumors. All participants in the study gave informed consent, provided a blood sample, and completed a comprehensive epidemiologic questionnaire. A questionnaire given to each control collected information on demographic factors, such as age and sex.

DNA isolation

Two milliliters peripheral blood samples were collected into citrate containing tubes from all subjects. DNA was extracted from whole blood by salting out procedure as soon as the samples reached to laboratory [22].

Genotyping

SOD1 A251G in polymorphism in Turkish population was examined using a *MspI*-RFLP method. For amplifying this polymorphism, forward primer 5′-*AGTACTGTCAACCACTAGCA*-3′ and reverse primer 5′-*CCAGTGTGCGGCCAATGATG*-3′ were used. PCR reactions contained 1 µL (10 pmol/µL) of each primer, 1 µL dNTPs (1 mmol/L), 3 µL of 25 mmol/L $MgCI_2$, 5 µL buffer, 1 Units of Taq DNA polymerase and 37.5 µL sterile deionized water. 50–100 ng DNA in a total volume of 50 µL. PCR conditions were 94 °C for 4 min, followed by 35 cycles of 94 °C for 50 s, 63 °C for 50 s, 72 °C for 50 s, and a final extension step at 72 °C for 7 min. Amplified products were digested with *MspI* (MBI Fermentas, Burlington, CA) at 37 °C for 5 h and analyzed with 2% agarose gels. Genotypes were determined for the polymorphism as 570 bp *AA*; two fragments of 369 and 201 bp *GG*; and three fragments of 570, 369, and 201 bp *AG* [4] (Figure 1).

Statistical analysis

For all polymorphisms studied, all statistical analyses were performed using the Statistical Package for Social Sciences Program (SPSS, version 11). Genotype-related odds ratios (ORs), their corresponding 95% confidence intervals (CIs), and associated p-Values were estimated *via* unconditional logistic regression. The Chi-square (χ^2) was used to compare the gender distribution, test the association between the genotypes and alleles in relation to the controls, and test for deviation of the genotype distribution from Hardy–Weinberg equation (HWE).

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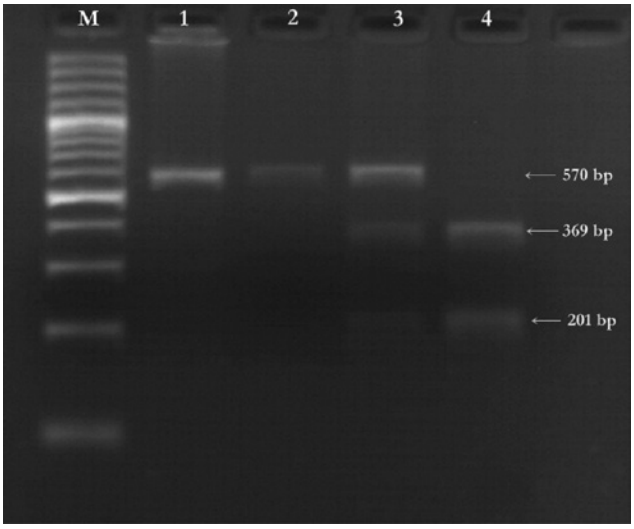


Figure 1: PCR-RFLP patterns of polymorphisms of *SOD1 A251G*, *SOD1* PCR product (570 bp); 2, AA (570 bp); 3, AG (570, 369, 201 bp); 4, GG (369, 201) bp; Marker GeneRuler 100bp Plus DNA Ladder; 3000, 2000, 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100. It contains two reference bands (1000 and 500 bp) for easy orientation.

Results

In this study, *SOD1 A251G* single nucleotide polymorphism was determined in human subjects. The distribution of these polymorphisms in Turkish population was examined using a *MspI*-RFLP method. The principal characteristics of the study population are listed in Table 1. There were no significant differences for sex, age, and ethnicity when compared to the controls. *SOD1 A251G*, 494 (216 men and 278 women) Turkish individuals were genotyped for polymorphisms of *SOD1* gene. The demographic characteristics of the study population are listed in Table 1. The mean age of the study population was 38.41 ± 16.59 years (males, 39.81 ± 17.10; females, 37.32 ± 16.14). Allele and genotype frequencies of the subjects for *SOD1 A251G* polymorphism in the *SOD1* gene are shown in Table 2. The distributions of the *SOD1 A251G* genotype were in accordance with the Hardy–Weinberg Equilibrium among the study population ($p > 0.05$). The frequencies of *AA*, *AG* and *GG* genotypes were found to be % 86.2, % 13.4 and % 0.4 respectively in the controls. The frequency distributions of *A* and *G* alleles were found to be % 92.9 and % 7.1 respectively in the controls.

Table 1: Characteristics of the study population.

	Healthy individuals
Sample size	494
Gender	
Males	216 (43.7%)
Females	278 (56.3%)
Age (year)	
Range	7–88
Means ± SD	38.41 ± 16.59
Males	39.81 ± 17.10
Females	37.32 ± 16.14

Discussion

Genetic variations in the antioxidant genes coding for the SOD, CAT, and GPX enzymes may lead to decreased or impaired regulation of their enzymatic activity and changed ROS detoxification. Hence, genetic variations among enzymes that protect the cell against ROS may modulate the disease risk [23]. Due to the high interaction opportunity of ROS with genetic material, polymorphisms in genes coding for antioxidant enzymes may play an important role for inter-individual differences in maintaining the human genome's integrity. Genetic polymorphisms in *SOD*, *CAT*, and *GPX* enzymes have been suggested to involve in the tendency to cancer and other diseases [24–26]. *SOD1* plays important roles in diseases like heart failure [27], cancer [28], diabetes [25], Down's syndrome [29], and amyotrophic lateral sclerosis [30]. In fact, the first paper about Down's syndrome was published in 1984 [31], and the first paper on *SOD1* gene mutations associated with familial amyotrophic lateral sclerosis were described in 1993 [30]. These possible significant genetic variants related to the oxidative stress have already been studied extensively, including SNP *A251G* of the *SOD1* gene. *SOD1 A251G* polymorphisms are

Table 2: Genotype frequencies for *SOD1 A251G* (rs2070424) polymorphism.

SOD1 A251G	Sample size	Percentage	p-Values	X^2
Allele frequency				
A allele	918	92.9	0.001	60.5
G allele	70	7.1		
Genotype frequency				
A A	426	86.2	0.743	0.107
AG	66	13.4		
GG	2	0.4		
AG + AA	492	99.59		
Total	494			

[a]For Hardy–Weinberg equilibrium; Hardy–Weinberg equilibrium (1 degree of freedom $p > 0.05$ if $\chi^2 < 3.84$).

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the most common and important of antioxidant enzymes. Most of these polymorphisms result in changes either in the level or in the activity of this enzyme, which can lead to reduced protection against oxidative stress [23–26]. Our study aimed to determine the allelic and genotypic frequency distribution of polymorphisms of *A251G* in the *SOD1* gene and to compare findings with other ethnic groups. The frequencies of *A* and *G* alleles were 92.9% and 7.1% respectively, and the frequencies of the *AA, AG* and *GG* genotypes were 86.2%, 13.4%, and 0.4% respectively in the study population. The distribution of three genotypes fitted the Hardy–Weinberg equation ($\chi^2 = 0.107$, $p = 0.743$) (Table 2). There is wide range variation of *AA* genotype frequency ranging from 23.4% [17] to as high as 88.3% [6] (Table 3). *AA* genotype frequency was found to be 86.2% in the present study in which the sample size was much larger than those of other studies performed in Turkish population which is very important for the making of more precise estimations in epidemiological studies in a such population.

SOD1 is an intracellular enzyme that primarily protects cells against cytosolic-generated superoxide [36]. SOD1 was found to have a widespread distribution in a variety of cells [37]. The expression of cytoplasmic SOD1 is stable and its activity is often considered as an internal control for *SOD1* gene expression. *SOD1 A251G* polymorphisms result in changes in the levels or the activities of SOD1 enzyme, which can lead to reduced protection against oxidative stress [23–25]. In the present study, *A* allele frequency was found more than *G* allele frequency ($\chi^2 = 60.5$, $p = 0.001$) (Table 2). This finding could suggest that *SOD1 A251G* polymorphism in Turkish population may pose a risk for some diseases such as cancer. Several studies have reported that the *A* allele of *SOD1* contributed to a high risk of cancer in various populations [21].

Table 3: Distribution of polymorphisms of *SOD1 A251G* (rs2070424) genotype frequencies in different populations.

Country	Year	Sample size	*SOD1 A251G* (%)			References
			AA	AG	GG	
China	2007	1369	87.3	12.4	0.3	[3]
USA	2008	469	88.3	11.7	0.0	[6]
China	2010	201	23.4	41.8	34.8	[17]
China	2011	386	44.8	41.5	13.7	[4]
Korea	2012	300	30.3	43.0	26.7	[32]
Iran	2014	241	83.9	15.6	0.41	[33]
Iran	2014	112	81.3	17.8	0.9	[34]
Polond	2014	368	77.7	21.5	0.8	[15]
India	2015	100	84.0	14.0	2.0	[35]
Turkey	2016	494	86.2	13.4	0.4	This study

In this study, we compared the distribution of three genotypes in different ethnic groups of the world in Table 3. Data presented here show that the Turkish population has the similar *GG* genotype frequency as seen in Chinese [3], Iranian [33], and Polish populations [15]. We detected higher GG genotype frequency than those found in American populations [6] and fairly lower frequencies than those found in Chinese [4, 17], Korean [32], Indian [35], Iranian [34] populations. Eventually, GG genotype frequency in populations of the eastern countries have been found higher (between 0.3 and 34.8%) than the western countries (between 0.0 and 0.82%) (Table 3). *GG* genotype frequency found in the present study was consistent with the above mentioned situation that the frequency value (0.4%) was between the values of eastern and western populations. Geographical situation confirms this result.

This study provides basic information about the allele and genotype frequency distributions of polymorphisms of *A251G* in the *SOD1* gene studied. Our sample size was much larger than those of other studies, 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 *SOD1* polymorphisms in a Turkish population, provide a framework for further studies concerning the role of this enzyme as a susceptibility of many diseases, including certain cancers.

Conflict of interest statement: There are no conflicts of interest among the authors.

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