

An investigation of the relation between catalase C262T gene polymorphism and catalase enzyme activity in leukemia patients

Nazan Eras¹, Gozde Türkoz², Anil Tombak³, Naci Tiftik³, Serap Yalin⁴, Mehmet Berkoz⁵, Sema Erden⁶, Etem Akbas²

¹Department of Medical Genetics, Faculty of Medicine, Mersin University, Mersin, Turkey

²Department of Medical Biology, Faculty of Medicine, Mersin University, Mersin, Turkey

³Department of Hematology, Faculty of Medicine, Mersin University, Mersin, Turkey

⁴Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey

⁵Department of Pharmaceutical Technology, Faculty of Pharmacy, Yuzuncu Yil University, Van, Turkey

⁶Vocational School of Health Service, Mersin University, Mersin, Turkey

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Corresponding author:

Nazan Eras

Department of Medical

Genetics

Mersin University School

of Medicine

33343 Mersin, Turkey

Phone: +90 324 341 00 00

Fax: +90 324 361 00 15

E-mail: nazaneras@gmail.com

Abstract

Introduction: Catalase (CAT), an antioxidant enzyme, catalyzes conversion of hydrogen peroxide to water and molecular oxygen, protecting cells against oxidative stress. The aim of this study was to investigate the possible association between *CAT C262T* polymorphism in the promoter region of the *CAT* gene and leukemia risk and to determine the relationship between *CAT* genotypes and CAT enzyme activities.

Material and methods: Genotypes of 102 cases and 112 healthy controls' genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism methods. Catalase activity was measured with the method of Aebi.

Results: The frequencies of the T allele among the cases and controls were 28.4% and 25.9%, respectively ($p = 0.75$). The frequencies of CC, CT, and TT among cases were 57.8%, 27.4%, and 14.7%, respectively, while in controls, the frequencies of CC, CT, and TT were 54.4%, 39.3%, and 6.3%, respectively, which were not significantly different. Although CAT enzyme activity was lower in leukemia patients with TT genotypes than in controls, this did not reach statistical significance ($p = 0.37$).

Conclusions: This is the first report showing that *CAT C262T* polymorphism is not a genetic predisposing factor for the risk of leukemia in the Turkish population. However, additional research is needed to confirm these findings.

Key words: antioxidant, catalase, leukemia, oxidative stress, polymorphism.

Introduction

Oxygen is necessary for the survival of all aerobic organisms, and while it is greatly reduced to water, a small proportion of that oxygen is converted to reactive oxygen species (ROS) during aerobic metabolism [1]. Reactive oxygen species including the superoxide anion radical, the hydroxyl radical, hydrogen peroxide, and singlet oxygen are involved in many vital physiological processes. These processes include

various signal transduction pathways that modulate the growth, proliferation and differentiation of cells in low/moderate concentrations in hematopoietic stem cells [2–4]. However, high concentrations of ROS can lead to the impairment of cellular structures and functions or cell death [5]. Moreover, this can also cause damage to various cellular components including DNA, proteins, and lipids [6]. Hence, ROS-induced DNA damage can result in abnormal genetic alterations in hematopoietic stem cells that can lead to the initiation and progression of leukemia [4].

In order to avoid oxidative damage, cells biosynthesize several antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase, catalase (CAT), butyrylcholinesterase, and thioredoxin-peroxiredoxin [7, 8]. Superoxide dismutases convert the superoxide anion radical to hydrogen peroxide and molecular oxygen [9]. The hydrogen peroxide-scavenging enzymes such as glutathione peroxidase and CAT convert hydrogen peroxide to water and oxygen [10].

Catalase (EC 1.11.1.6), the most abundant protein in erythrocytes, liver, and the kidney, generally located within peroxisomes, is a tetrameric enzyme weighing 240 kDa that contains four porphyrin heme (iron) groups [11–13]. This enzyme is encoded by the *CAT* gene located in the 11p13 region, spanning 34 kb, and it is composed of 13 exons and 12 introns [14]. Several single nucleotide polymorphisms (SNPs) were identified in the 5'-untranslated region of the *CAT* gene such as *A21T* (rs794316), *C844T* (rs769214) and *C262T* (rs1001179) [15].

The common polymorphism in the promoter region of the *CAT* gene (*CAT C262T*, rs1001179) consists of a C to T substitution at position –262 in the 5' untranslated region [16]. It has been shown that this polymorphic variation within the promoter region affects the transcriptional factor binding. Therefore, this leads to changes in transcription and subsequent expression of the *CAT* gene [17]. Individuals carrying the C allele compared to the T allele have been found to have altered CAT serum concentrations and increased CAT activity [18]. Thus, increased ROS levels may influence the response to oxidative stress, leading to increased disease risk. Recently, a series of studies has demonstrated the associations between the *CAT C262T* polymorphism and risk for various diseases, such as hepatocellular carcinoma [19], prostate cancer [20], invasive cervical cancer [21], endometriosis [22], ulcerative colitis [23], etc. However, no significant association was found between *CAT C262T* polymorphism and susceptibility to myeloid leukemia [24, 25].

Thus, further detailed studies are necessary to demonstrate the association between *CAT C262T*

polymorphism and leukemia risk. For this reason, the purpose of our study was to investigate the possible association between *CAT C262T* polymorphism and its enzyme activity in leukemia.

Material and methods

Patients and tissue samples

The sample consisted of 102 patients (32 ALL (acute lymphoblastic leukemia), 32 acute myeloid leukemia (AML), 17 chronic lymphocytic leukemia (CLL) and 21 chronic myeloid leukemia (CML)) recently diagnosed and under treatment at the Department of Hematology. Healthy volunteers were randomly chosen from the general population of the same area. The general inclusion criterion for controls was no evidence of cancer or history of cancer. The general exclusion criteria for controls were: any intake of regular medication, active smoking, regular high-dose vitamin supplementation during 3 months prior to the study. These individuals participated in a previously published case-control study. The cases and controls have been described previously in terms of data collection and study procedures [26]. This study has the approval of the Ethics Committee, Faculty of Medicine, The University of Mersin. All participants were informed about the aim and design of the study.

Genotype analysis

Blood samples from all study participants were collected into the tubes containing 50 mmol/l disodium-EDTA. Genomic DNA was extracted from peripheral whole blood with the standard phenol/chloroform-based method. All extracted DNA samples were stored at 4°C until further analysis. All DNA samples from cases and controls were genotyped by polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP) analysis. In order to avoid potential contamination, the PCR assays were performed with at least one known DNA genotype (positive control) and one negative control (without DNA template). *CAT* –262 C/T polymorphism was determined using an antisense primer 5'-AGAGCCTCGCCCGCCGACCG-3' and sense primer 5'-TAGAGCTGAGAAAGCATAGCT-3' [27]. Polymerase chain reaction was performed in a 50 µl volume with 50 ng of genomic DNA, 100 µM dNTPs, 20 pmol of each primer, 2 mM MgCl₂, 1 × PCR buffer with (NH₄)₂SO₄ and 2 U Taq polymerase. Amplification was carried out in a TC-512 Thermal Cycler (Techne), and the cycling conditions were: 95°C for 15 min, 35 cycles of 94°C for 30 s, 60°C for 45 s, 72°C for 30 s, and a final extension at 72°C for 10 min. Amplicons (185 bp) were digested with 10 U of *Sma*I (Promega, Southampton, UK) at 37°C for

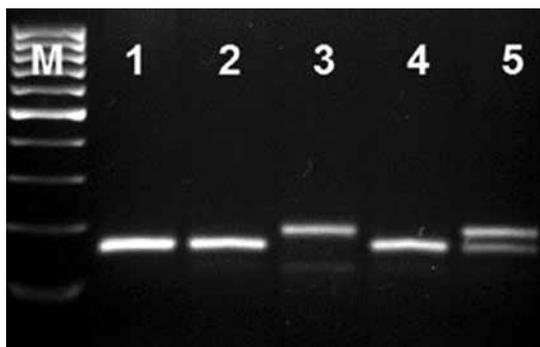


Figure 1. *CAT-262 C/T* genotyping by PCR-RFLP. M: 100-bp ladder; Lanes 1, 2, 4: C/C (155 bp); 3: T/T (185 bp); 5: C/T (185, 155 bp)

16 h and analyzed following the electrophoresis in 3% agarose gel stained with ethidium bromide (0.5 µg/ml). The T allele was not digested, giving a 185-bp fragment; the C allele was digested, showing two fragments of 155 and 30 bp (due to limitation of agarose gel in the detection of fragments that are smaller than 50 bp, the 30 bp fragment was invisible) (Figure 1). Genotyping was performed blindly with respect to case/control status and repeated twice for all subjects, but no discordant genotype classifications were identified.

Plasma samples

Ten milliliters of fresh venous blood in lithium heparin were taken from each subject and immediately centrifuged at $1000 \times g$ for 10 min at 4°C. The supernatant plasma was aspirated, transferred to a polypropylene tube and fresh plasma samples subsequently were stored at -80°C until further analysis.

Analysis of catalase activity

Catalase activity was measured by the method of Aebi [28]. This method is based on the principle that spectrophotometric recording of the initial rate of hydrogen peroxide disappearance (0–60 s) is at a wavelength of 240 nm. Catalase enzyme activity is expressed as U/ml of plasma.

Statistical analysis

Allele and genotype frequencies among cases and controls were calculated by logistic regression and the χ^2 test. The deviation from Hardy-Weinberg equilibrium was examined by χ^2 . Mann-Whitney *U* test, Kolmogorov-Smirnov, and independent *t* tests were used to assess differences in CAT activities according to the genotypes between the patient and control groups. Results were reported as the mean \pm SD. The analysis of data was performed using SPSS software package version 11.5 for Windows. *P*-values less than 0.05 were

accepted as statistically significant. Post hoc power analysis with pre-established effect size, error probability, and sample size was carried out using the G*Power version 3.1.3 program.

Results

Table I shows the demographic characteristics of the case and control groups. The mean age of the 2 groups was similar: 51.3 years in the case group and 49.3 years in the control group. Distribution of gender was similar in both groups ($p = 0.81$). We included AML, CML and CLL patients (except ALL) to make a comparison among age groups, because ALL is commonly seen in young people. We observed that the patients between 41 and 50 years had 4.4-fold ($p = 0.002$) and the patients 51 years and older had 4.8-fold ($p = 0.001$) higher leukemia risk compared to the patients 30 years and younger.

As shown in Table II, the frequencies of C and T alleles were 71.6% and 28.4% in the cases and 74.1% and 25.9% in the controls, respectively. These differences were not significant ($p = 0.75$). The frequencies of the CC, CT and TT genotypes in cases were 57.8%, 27.4% and 14.7%, respectively and 54.4%, 39.3% and 6.3% in controls, respectively. Compared with the CC genotype, OR for the CT and TT genotypes were 0.65 (95% CI: 0.36–1.19) and 2.21 (95% CI: 0.84–5.82), respectively. In addition, the χ^2 test was used to determine whether there was a relationship between groups and genotypes ($\chi^2 = 6.044$, $p = 0.049$). This relationship at borderline significance might become clearer by increasing the sample size. *CAT* genotype frequencies were in Hardy-Weinberg equilibrium in the control group ($p = 0.802$), but not in the patient group ($p = 0.002$). Using power calculation we demonstrated that the study had 97.5% power in detecting associations of *CAT-262 C/T* polymorphism with risk of leukemia, at a significance level of 0.30 ($df = 2$).

We also investigated the relation between *CAT* genotypes and *CAT* enzyme activities. Catalase enzyme activity of the individuals with TT genotypes belonging to *CAT C262T* polymorphism was found to be 143.75 ± 105.81 U/ml in the control group, and decreased to 101.79 ± 70.15 U/ml in leukemic patients, but this did not reach statistical significance ($p = 0.37$). The individuals with CT genotypes had lower *CAT* enzyme activities in patients with leukemia (132.81 ± 86.86 U/ml) compared to the control group (188.63 ± 119.41 U/ml) ($p = 0.03$) (Table III).

Discussion

Extrinsic factors such as ROS or ionizing radiation accumulate over time and can result in DNA mutations, deletions or translocations by induc-

Table I. Characteristics of patients and controls

Factor	Patients, n (%)	Controls, n (%)	P-value	OR (95%)
Gender:				
Male	52 (50.9)	60 (53.6)	0.81	0.9 (0.52–1.54)
Female	50 (49.1)	52 (46.4)	–	1.0
Age:				
≤ 30	8 (11.6)	14 (12.5)	–	1.0
31–40	7 (10.1)	24 (21.4)	0.099	2.28 (0.85–6.07)
41–50	11 (15.9)	41 (36.6)	0.002	4.46 (1.71–11.62)
≥ 51	43 (62.3)	33 (29.5)	0.001	4.85 (2.17–10.86)
Subtype:				
ALL	32 (31.4)	–	–	–
AML	32 (31.4)	–	–	–
CLL	17 (16.6)	–	–	–
CML	21 (20.6)	–	–	–

ALL – acute lymphoblastic leukemia, AML – acute myeloid leukemia, CLL – chronic lymphocytic leukemia, CML – chronic myeloid leukemia.

Table II. Genotype and allele distribution of the CAT C262T polymorphism in cases and controls

Variable	Cases, n (%)	Controls, n (%)	P-value	OR (95% CI)
Genotype frequencies:				
CC	59 (57.8)	61 (54.4)	–	1.0
CT	28 (27.4)	44 (39.3)	0.17	0.65 (0.36–1.19)
TT	15 (14.7)	7 (6.3)	0.11	2.21 (0.84–5.82)
Allele frequencies:				
C	73 (71.6)	83 (74.1)	–	1.0
T	29 (28.4)	29 (25.9)	0.75	0.88 (0.48–1.60)

Post hoc power analysis: $df = 2, n = 224$. Effect size $n = 0.30$; power $(1 - \beta \text{ err prob}) = 0.975$

Table III. Findings of CAT C262T genotypes and catalase activity (U/ml) in patients and controls

Genotype	Patients		Controls		P-value
	n	Mean ± SD	n	Mean ± SD	
CC	59	164.08 ±148.23	61	174.98 ±138.57	0.67
CT	28	132.81 ±86.86	44	188.63 ±119.41	0.03
TT	15	101.79 ±70.15	7	143.75 ±105.81	0.37

ing double strand breaks in hematopoietic stem cells [29]. Under normal conditions, autophagy prevents ROS accumulation through elimination of damaged mitochondria. However, dysregulation of autophagy may lead to development of tumorigenesis through the accumulation of oncogenic mutations and malignant transformation [30]. Autophagy eliminates damaged or harmful cel-

lular components, whereas catalase plays an important protective role by detoxifying ROS [31]. In this population-based case-control study, we examined the association of C262T polymorphism in the promoter region of the CAT gene with the risk of leukemia in a Turkish population. Furthermore, we investigated the effect of genotype on CAT enzyme activity in Turkish leukemia patients.

We analyzed the relation between leukemia and age, because advanced age is the most important risk factor for cancer overall. Within this context, increased risk was observed for advanced age in the case group excluding ALL.

In our study, the TT genotype incidence of the *CAT C262T* polymorphism in the control group was found to be 6% in Mersin province located in the southern part of Turkey. Suzen *et al.* [32] also found TT genotype frequency of the healthy individuals living in Ankara, in the central region of Turkey, as 6%. Tefik *et al.* [33] reported TT genotype frequency of healthy individuals as 10.2% in Istanbul, which is slightly higher than our genotype frequencies. Moreover, the TT genotype frequency of healthy individuals was 9% and 6% in Swedish [27] and German populations [34], respectively. This distribution of genotypes is similar to that seen in our control group. However, no healthy individual with TT genotype was found in a Chinese population [35].

Some SNPs in the *CAT* gene lead to amino acid alterations at a specific location of the gene. One of these SNPs is *CAT C262T* polymorphism, which is located in the promoter region [20]. Several studies were performed in order to investigate the correlation between *CAT* genotype and cancer risk [20, 21, 36, 37]. A meta-analysis of 5 case-control studies including 3865 cases and 28 224 controls demonstrated that *C262T* polymorphism was correlated with increased prostate cancer risk (OR = 1.094, 95% CI: 1.015–1.178, $p = 0.018$) [20]. Another meta-analysis conducted in 2015 with 22 case-control studies involving 9777 cancer patients and 12 223 controls also indicated a significant association between prostate cancer risk and *C262T* polymorphism (TT vs. CC: OR = 1.81, 95% CI: 1.07–3.04, $p = 0.03$; TT vs. CT + CC: OR = 1.61, 95% CI: 1.17–2.22, $p = 0.004$). However, such an association was not significant for breast cancer and non-Hodgkin lymphoma (all $p > 0.05$) [36]. Castaldo *et al.* [21] reported that individuals with TT genotype had increased risk for invasive cervical cancer (OR = 3.034, 95% CI: 1.462–6.298, $p = 0.003$). Quick *et al.* [37] found that CT or TT genotype carriers using hormone replacement therapy were at increased breast cancer risk (OR = 1.88; 95% CI: 1.29–2.75). In contrast, no correlation was observed between *CAT C262T* polymorphism and myeloid leukemias in two different studies ($p = 0.17$, $p = 0.98$) [24, 25], which is consistent with our results.

Endogenous antioxidant enzymes are designed to destroy ROS. Analysis of allelic variants of the *CAT* gene and measurements of CAT activities can give a more complete view of CAT status, because they reflect the underlying genetic backgrounds and environmental effects of CAT. Our data showed that the TT variant had significantly lower

CAT activity than CC and CT variants in our study group. One study reported that CAT levels were significantly higher in donors carrying the T allele in comparison to donors homozygous for the C allele ($p < 0.03$) [27]. In contrast, another study showed that *CAT TT* homozygotes had significantly lower CAT activity than did CT and CC genotypes in Caucasians and African Americans [18]. This finding is consistent with our results.

In this study, we investigated whether the activity of CAT has a protective effect against the oxidative stress conditions in individuals carrying the TT polymorphic genotype. There was no relationship between *CAT C262T* polymorphism and CAT enzyme activity in leukemia patients. One study suggested that the *-262TT* genotype of the *CAT* gene was significantly associated with higher erythrocyte CAT activity in blood of diabetic neuropathy patients compared to the *-262CC* genotype ($17.8 \pm 2.7 \times 10^4$ IU/g Hb vs. $13.5 \pm 3.2 \times 10^4$ IU/g Hb, $p = 0.0022$) [38]. Ho *et al.* [39] observed that patients with adenocarcinoma had significantly lower levels of CAT activity compared with healthy controls in those with CC genotype of the *CAT* gene ($p < 0.05$).

In conclusion, to the best of our knowledge, this is the first case-control study in the Turkish population which investigates the relation not only between *CAT C262T* polymorphism and leukemia, but also between its enzyme activity and leukemia. We found no significant differences in allele and genotype frequencies of *CAT* polymorphism between healthy controls and patients with leukemia. On the other hand, TT genotype of *C262T* polymorphism led to decreased CAT enzyme activity in leukemia patients, although this did not reach statistical significance. The reason could be the small sample size of patients with TT genotype. However, leukemia can be considered as an age-related disease, because it is clearly shown that the incidence of leukemia patients increases at older ages. Further studies are needed to assess the relationship between the *CAT C262T* polymorphism and its enzyme activity in leukemia with larger sample size.

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Conflict of interest

The authors declare no conflict of interest.

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