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Distribution of genetic polymorphism of CAT C-262T in three Iranian populations

Abstract: Objective: Catalase (CAT) activity is likely to be affected by functional polymorphism of C-262T (rs1001179) in the CAT gene (OMIM: 115500). It is hypothesized that individuals with the lower expressing forms of the CAT polymorphism may be more susceptible to breast cancer. In order to find the allelic frequency of the C-262T polymorphism among Iranian populations, the present study was carried out.

Methods: The total study subjects consisted of 1057, 200, and 200 individuals from Shiraz (Fars province; belong to Persians), Abarku (Yazd province; belong to Persians), and Yasuj (Kohgiluyeh va Boyer-Ahmad province; belong to Lurs), respectively. Genotypic analysis for the CAT C-262T polymorphism was determined by PCR.

Results: The frequency of the T allele was 0.2044±0.0138, 0.1825±0.0193, and 0.1800±0.0192 in Shiraz, Abarku, and Yasuj, respectively. The genotypic frequencies of the control subjects did not show significant deviation from Hardy-Weinberg equilibrium. Statistical analysis indicated that there was no significant difference between these populations for the genotypic distributions of the CAT C-262T polymorphism (χ²=2.73, df=4, P=0.603).

Conclusion: The frequency of the T allele among Iranian populations was very similar to that reported for Caucasians and was higher than Asians and African-American populations.

Keywords: CAT; genetic polymorphism; Iran

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Üç İran popülasyonu için T tipinin dağılımı

Özet: Amaç: Katalaz aktivitesinin, CAT geni (OMIM: 115500) üzerindeki C-262T (rs1001179) polimorfizminin etkilendiği düşünülmektedir. CAT geninin değişik polimorfik türlerinin düşük ekspresyonu olan bireylerde meme kanserine katkıda bulunabileceği varsayılmaktadır. Bu çalışma İran popülasyonlarında C-262T aleli frekanslarını saptamak için yürütülmüştür.

Metod: Bu çalışmada Şiraz (Fars eyaleti, Pers kökenli) bölgesinde 1057, Abarku (Yezidi eyaleti, Pers kökenli) bölgesinde 200, Yasui (Kohgiluyeh va Boyer-Ahmad eyaleti, Lur kökenli) bölgesinde 200 kişi çalışılmıştır. CAT C-262T polimorfizminin genotipik analizleri PCR tekniği kullanılarak saptanmıştır.

Bulgular: Şiraz, Abarku ve Yasui bölgeleri için T aleli frekansı sırasıyla 0.2044±0.0138, 0.1825±0.0193, ve 0.1800±0.0192 olarak bulunmuştur. Kontrol bireylerin genotip frekansları ise Hardy-Weinberg dengesinden sapma göstermemiştir. Ek olarak, CAT C-262T polimorfizmi genotipik dağılımı bu popülasyonlar arasında istatistiksel olarak anlamlı bir fark göstermemiştir (χ²=2.73, df=4, P=0.603).

Sonuç: İran popülasyonlarında saptanan T aleli frekansı beyaz ırk popülasyonları için raporlanan sonuçlarla yakın ancak Asya ve Afrika – Amerikan popülasyonlarından yüksek bulunmuştur.

Anahtar Kelimeler: CAT; genetik polimorfizm; İran

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Introduction

It is well established that transient alterations in reactive oxygen species (ROS) have important regulatory functions, but at high and/or sustained levels, it can cause DNA damage [1,2]. Catalase (CAT; EC 1.11.1.6; OMIM: 115500) is an important enzyme metabolizing hydrogen peroxide (H$_2$O$_2$) and detoxifies H$_2$O$_2$ to oxygen and water. Catalase is a ubiquitous detoxifying enzyme in aerobic cells. The CAT gene had 34 kb long and split into 13 exons [3]. The CAT enzyme activity in human cells is likely to be affected by a functional common C to T substitution genetic polymorphism in the CAT promoter gene (C-262T, rs1001179). Several studies indicating that the T allele was found to be associated with reduced the CAT enzyme activity [4–6].

Oxidative stress is believed to play an important role in the pathogenesis of considerable number of complex multifactorial diseases [7–12]. The CAT enzyme activity is an important component of cell defense against oxidative stress, and the C-262T polymorphism which regulates the expression level of CAT [4–6] may play a role in host response to oxidative stress and indeed variant T allele has been associated with increased risk of several multifactorial traits, including some types of cancers, asthma, glaucoma, and infertility [13–18].

As we mentioned in our previous studies, Iranian populations showed high level of heterogeneity for genetic polymorphisms [19–22]. Based on our knowledge, there are published data on distributions of the CAT C-262T polymorphism among two Iranian populations living in Rasht (Gilan province, north Iran) [18] and Zahedan (Sistanva-Baluchestan province, south east Iran) [23]. However, there have been no data in regard to CAT C-262T polymorphism in Iranian populations living in central and southern parts of Iran. Accordingly, we investigated the CAT C-262T polymorphism distribution in healthy individuals in three Iranian populations. Our study could, therefore, provide information that may be used in the future to assess the relationship between the prevalence of the CAT C-262T polymorphism and susceptibility to several diseases in our population, as well as we get more insight into genetic structure of the Iranian populations.

Materials and Methods

Subjects

The total study subjects consisted of 1057, 200, and 200 individuals from Shiraz (Fars province; belong to Persians), Abarku (Yazd province; belong to Persians), and Yasuj (Kohgiluyeh va Boyer-Ahmad province; belong to Lurs), respectively. All individuals were healthy as assessed by medical history. Data on ethnicity were collected using a simple questionnaire including simple questions like the parental and grandparental ethnicity of each participant. Participants that their mothers and fathers (and also their grandparental) did not belong to same ethnic groups were excluded.

Because the CAT C-262T polymorphism showed significant associations with several multifactorial diseases [13–18], we excluded the participants with positive history for diagnosed cancers, psychiatric disorders, asthma, cataract and cardiovascular diseases. This study was approved by the local ethics committee and informed consent was obtained from each subject before the study.

| Populations  | CC  | CT  | TT  | Total | T allele (%) | HWE ($\chi^2$) | P-value | df=1 |
|--------------|-----|-----|-----|-------|-------------|---------------|---------|------|
| Shiraz       | 671 | 340 | 46  | 1057  | 20.44       | 0.12          | 0.724   |      |
| Abarku       | 135 | 57  | 8   | 200   | 18.25       | 0.40          | 0.525   |      |
| Yasuj        | 133 | 62  | 5   | 200   | 18.00       | 0.50          | 0.478   |      |

Hardy-Weinberg equilibrium (HWE); Chi-square test ($\chi^2$); degree of freedom (df).

Table 1: Genotypic distribution of the CAT C-262T polymorphism among healthy blood donors in three Iranian populations.

| Country/ethnic | T allele (%) | Sample size | References |
|----------------|--------------|-------------|------------|
| African-American (USA) | 5.0 | 109 | 4 |
| China | 4.9 | 308 | 26 |
| Korea | 3.4 | 400 | 27 |
| Taiwan | 4.9 | 224 | 14 |
| Bangladesh | 16.8 | 104 | 28 |
| Saudi Arabia | 15.5 | 403 | 15 |
| Iran (Zahedan) | 15.0 | 140 | 23 |
| Iran (Rasht) | 17.6 | 190 | 18 |
| Iran (Persians, Shiraz) | 20.4 | 1057 | Present study |
| Iran (Persians, Abarku) | 18.2 | 200 | Present study |
| Iran (Lurs, Yasuj) | 18.0 | 200 | Present study |
| Turkey | 21.6 | 250 | 29 |
| Russia | 18.4 | 103 | 6 |
| Slovak Republic | 27.1 | 249 | 16 |
| Polish | 23.9 | 199 | 30 |
| Germany | 23.0 | 117 | 17 |
| England | 22.1 | 3836 | 31 |
| France | 23.2 | 196 | 5 |
| USA (Caucasians) | 21.5 | 974 | 13 |

Table 2: Distribution of the T allele of CAT C-262T polymorphism in several populations.
Genotyping

Blood samples were obtained from the participants. Immediately after collection, whole blood was stored at −20°C until use. Genomic DNA for PCR was extracted from whole blood [24]. Genotypic analysis for the CAT C-262T polymorphism was determined using the specific primers: 5’-CTG ATA ACC GGG AGC CCC CTG GTG CAT AF3’ and 5’-CTA GGC AGG CCA AGA TTG GAA GCC CAA TGG-3’ as described previously [25]. PCR products were digested by restriction endonuclease EcoR V for 16 h at 37°C. The products were electrophoresed on 1.5% agarose gel. The gels were stained with ethidium bromide and visualized by ultraviolet light. The C allele produced a 190 bp band, whereas the T allele digested by EcoR V and produced 157 and 33 bp bands.

Statistical Analysis

Allelic frequencies were calculated by genotype counting method. For each group, the observed frequencies of the CAT genotypes were assessed for Hardy-Weinberg equilibrium using the χ² statistic. The difference in genotype frequencies between populations was determined using the Chi-square test of goodness of fit. Data analysis was performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of P<0.05 was considered statistically significant.

Results

The genotypic frequency of the CAT C-262T polymorphism in Shiraz, Abarku, and Yasuj was shown in Table 1. The frequency of the T allele was 0.2044±0.0138, 0.1825±0.0193, and 0.1800±0.0192 in Shiraz, Abarku, and Yasuj, respectively. Among study ethnic groups, Lurs showed lower frequency of the T allele (18.0%).

The genotypic frequencies of the control subjects did not show significant deviation from Hardy-Weinberg equilibrium (For Shiraz: χ²=0.12, df=1, P=0.724; For Abarku: χ²=0.40, df=1, P=0.525; For Yasuj: χ²=0.50, df=1, P=0.478). Statistical analysis indicated that there was no significant difference between these populations for the genotypic (χ²=2.73, df=4, P=0.603) and allelic (χ²=1.95, df=2, P=0.376) distributions of the CAT C-262T polymorphism. Therefore, there was no genetic differentiation between three studied populations (Fst=0.006).

Discussion

It should be noted that the frequency of the T allele was reported 47.6% and 15.0% from Rasht (north Iran) and Zahedan (south east Iran), respectively [18,23]. There is remark difference between Rasht and other reports from both Iranian and other Caucasian gene pools [5,6,13,16,17,23]. At present we have no explanation for this difference.

A comparison of the prevalence of the T allele in our samples with published data from other populations [4–6,13–18,23,26–31] is presented in Table 2. When compared to Asians [14,26,27] and African-Americans [4], the T allelic frequency of Iranian gene pools showed marked differences. The frequency of the T allele in the Iranian populations varied from 18.0% (in Yasuj) to 20.4% (in Shiraz), whereas it was reported to be very low in Korean, Chinese, Taiwanese and African-American populations [4,14,26,27]. The frequency of the T allele among Iranian populations was very similar to that reported for Caucasian populations.

Based on data presented in Table 2, the T allele showed a specific geographical distribution. In overall, the frequency of the T allele revealed distinct differences between Caucasians, Asians and Africans. It has low prevalence in Asian and African [4,14,26,27], whereas the T allele shows higher frequency among Caucasians [5,6,13,16,17,29–31] gene pools. Therefore, it is suggested that the T allele increased from east to west.

Previous reports on other genetic polymorphisms, such as GSTM1, GSTT1, GSTO2, GSTZ1, XRCC1, XRCC4, XRCC7, and ACE, indicated that Iranian gene pools showed heterogeneity with each other and in overall they revealed intermediate frequency in comparison with Caucasians and Asians [19–21,32–34]. However the present study although showed that the T allele is more similar to Caucasians than Asians, there was homogeneity between the study gene pools.

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

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