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Genetic Polymorphism of C-262T Catalase and Susceptibility to Schizophrenia

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Abstract

Background: Catalase (CAT, OMIM: 115500) plays an integral role in the primary defence against oxidative stress. The T allele of the C-262T *CAT* polymorphism (rs1001179) is associated with lower activity of CAT. Here we investigated whether polymorphism of C-266T *CAT* was associated with susceptibility to schizophrenia.

Methods: The present study was performed on 363 (267 males, 96 females) in-patients with schizophrenia diagnosis, and a total of 363 (266 males, 97 females) healthy controls. The C-262T CAT genotypes were determined using RFLP-PCR method.

Results: Although the association between genotypes and susceptibility was not significant in both genders, there was significant interaction between gender and the TT genotype (P=0.035). The Log-rank test and the Kaplan-Meier survival analysis were used to evaluate the influence of C-262T genotypes on age at diagnosis (AAD) of schizophrenia. Mean AAD of the CC and CT+TT genotypes in males was 22.8 and 24.9 years, respectively. The difference was significant (χ^2=4.26, P=0.039). Difference of mean AAD of the CC (26.7 years) and CT+TT (27.3 years) genotypes among females was not significant (χ^2=0.02, P=0.896).

Conclusion: Different associations between gender groups might be at least in part interpreted by the effect of gender on the association between C-262T polymorphism and *CAT* gene expression.

Introduction

While reactive oxygen species (ROS) are generated under physiological conditions, excess generation of ROS has pathological consequences. Oxidative stress is the condition arising from imbalance between toxic ROS and antioxidant systems. Oxidative stress may play a significant role in the risk of chronic diseases such as schizophrenia [1, 2].

Catalase (EC 1.11.1.6, CAT, OMIM: 115500) catalyzes the decomposition of H_2O_2 to O_2 and H_2O. Working together with other antioxidant enzymes, CAT plays an integral role in the primary defense against oxidative stress. Thus it limits the deleterious effects of ROS. A C/T polymorphism located 262 bp upstream to the *CAT* transcription site (C-262T, dbSNP rs1001179) has been described [3]. The less common T allele is associated with lower levels of red blood cell CAT activity [3, 4] which may increase ROS formation and oxidative stress.

Several studies indicated that the polymorphism of C-262T *CAT* is associated with some multifactorial diseases [5-10]. At present we know that chronic exposure to excess ROS may contribute to brain damage and pathophysiology of schizophrenia [1, 2, 11, 12], CAT involved in detoxification of ROS [5-10], and C-262T polymorphism is associated with CAT activity [3, 4]. Taken together, these findings sufficiently provide us with a theoretical ration to investigate the association between *CAT* C-262T polymorphism and susceptibility to schizophrenia.

Materials and Methods

Study Subjects

This case-control study was performed in Shiraz, south of Iran. Three hundred and sixty three schizophrenia in-patients (267 males, 96 females) from Ibn-Sina and Razi Hospitals, Shiraz University of Medical Sciences, (mean age $\pm$ SD, 41.4 $\pm$ 13.1 years) participated in the study. The patients were chronic cases. Each face-to-face interview was

conducted by three psychiatrics. Inclusion criteria for patients were being aged between 16-65 years and having chronic schizophrenia. The patients were diagnosed as chronic schizophrenia according to structured clinical interview using SCID-I (clinician version) to confirm and document DSM-IV diagnosis. These patients had no other psychiatric disorder; including schizoaffective disorder, major depressive, episode with psychotic features, substance misuse, bipolar disorder, or mental retardation. A total of 363 (266 males, 97 females) healthy blood donors (with no history of psychiatric disorders, cancers, asthma, cataract, psychotic disorder including schizophrenia, bipolar disorder, major depressive) matched with the patients according to age and gender was also studied, as a control group (mean age $\pm$ SD, 39.7 $\pm$ 11.1 years). Table 1 shows the socio-demographic characteristics of the cases and controls.

Considering the high heterogeneity of Iranian population [13, 14], the participants (patients and controls) were selected from Persian Muslims (Caucasians) living in Fars province (southern Iran). At the time of blood donation, a brief questionnaire that ascertained smoking status, age, history of cancers and asthma, was completed. This study was approved by the local ethics committee. Informed consent was completed for each subject before the study.

The study is more than sufficiently powered with an *N*=726 to detect a small-medium effect in allelic frequency between the two groups. Using the GPOWER software (version 2.0), to detect a real difference in allelic frequency with a power of 0.95 (α=0.01), *df*=1, Lambda=17.84, and an effect size of 0.2; a minimum sample of 446 would be necessary.

Extraction of DNA and C-262T CAT genotyping

Blood samples were obtained from patient and control groups. Immediately after collection, whole blood was stored at -20ºC until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples. The C-262T *CAT* genotypes were determined by PCR-RFLP method [10]. To test for contamination, negative controls (tubes containing the PCR mixture, without the DNA template) were incubated in every run. Any sample with ambiguous result due to low yield was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

Statistical analysis

The comparison of genotypes of C-262T *CAT* polymorphisms between the gender groups was done by χ^2 test. Hardy-Weinberg equilibrium was assessed with the Chi-square test. The association between the C-262T *CAT* polymorphism and the development of schizophrenia was examined by use of the odds ratios (OR) and 95% of confidence intervals (CIs). The Cox regression analysis were used to evaluate the influence of C-262T *CAT* genotypes on age at diagnosis (AAD) of schizophrenia. Age at diagnosis was considered as time in the Cox regression analysis. Statistical analysis was performed using SPSS statistical software package (version 11.5). A probability of P<0.05 was considered statistically significant. All P values were two-tailed.

Results

Table 1 shows the socio-demographic characteristics of the cases and controls. There were significant differences between cases and controls for smoking status, education level and marital status. The patients were more smokers, single and have lower education.

Table 1: The socio-demographic characteristics of the case and control groups.

Variables	Cases	Controls	Results of comparisons	
Continuous variable				
Age	41.4 $\pm$ 13.1	39.7 $\pm$ 11.1	t= 1.89	P=0.1
Age of onset	24.3 $\pm$ 8.6	-	-	-
Discontinuous variables				
Sex				
Females	95	97		
Males	268	266	χ^2=0.03 (df=1)	P=0.866
Smoking Status				
Non-smokers	146	266		
Smokers	189	87	χ^2=81.1 (df=2)	P<0.001
Missing data	28	10		
Educational level				
Illiteracy	59	9		
Primary school	50	51		
High school	171	198		
College	31	80	χ^2=69.8 (df=4)	P<0.001
Missing data	52	50		
Marital status				
Single	232	58		
Married	122	289	χ^2=174.2 (df=2)	P<0.001
Missing data	9	16		

Table 2 shows the prevalence of genotypes of the C-262T *CAT* polymorphism in cases and controls. The prevalence of the genotypes among controls (For males: χ^2=0.30, df=1, P=0.584; For females χ^2=1.11, df=1, P=0.290) and patients (For males: χ^2=1.33, df=1, P=0.248; For females χ^2=0.67, df=1, P=0.412) were consistent with those expected from the Hardy-Weinberg equilibrium. Considering that there is significant difference between gender groups of the patients for the genotypes (χ^2=9.02, df=2, P=0.011), the gender groups were not pooled.

Pearson Chi-square test showed that there was no difference between cases and controls for distribution of *CAT* genotypes among males (χ^2=4.44, df=2, P=0.108) and females (χ^2=2.27, df=2, P=0.321). There was no significant association between

genotypes (CT and TT) and risk of schizophrenia among males subjects (For CT *vs* CC: OR=0.77, 95%CI: 0.54-1.11, P=0.171; For TT *vs* CC: OR=0.44, 95% CI: 0.17-1.12, P=0.086). There was significant linear trend for presence of 0, 1 and 2 the C allele and schizophrenia risk among males (χ^2=4.16, P=0.041). Among females, there was no association between genotypes and risk of schizophrenia (For CT *vs* CC: OR=0.90, 95%CI: 0.50-1.64, P=0.754; For TT *vs* CC: OR=2.33, 95% CI: 0.67-8.07, P=0.179) (Table 2).

Table 2: Genotypic frequency of C-262T *CAT* in schizophrenia patients and healthy subjects.

Polymorphism/ Gender	Cases N (%)	Control N (%)	OR	95% CI	P-Value
Males					
CC	172 (64.2)	151 (56.8)	1.0	-	-
CT	89 (33.2)	101 (38.0)	0.77	0.54-1.10	0.161
TT	7 (2.6)	14 (5.2)	0.43	0.17-1.11	0.084
CT+TT	96 (35.8)	115 (43.2)	0.73	0.51-1.03	0.080
Females					
CC	51 (53.7)	53 (54.1)	1.0	-	-
CT	35 (36.8)	40 (40.8)	0.90	0.50-1.64	0.754
TT	9 (9.5)	4 (4.1)	2.33	0.67-8.07	0.179
CT+TT	44 (46.3)	44 (44.9)	1.03	0.58-1.83	0.894
Total					
CC	223 (61.4)	204 (56.2)	1.0	-	-
CT	124 (34.2)	141 (38.8)	0.80	0.59-1.09	0.165
TT	16 (4.4)	18 (5.0)	0.81	0.40-1.63	0.562
CT+TT	140 (38.6)	159 (43.8)	0.80	0.59-1.08	0.152

It should be noted that although the association between genotypes and susceptibility was significant neither in males nor in females, there was significant interaction between gender and the TT genotype (P=0.035).

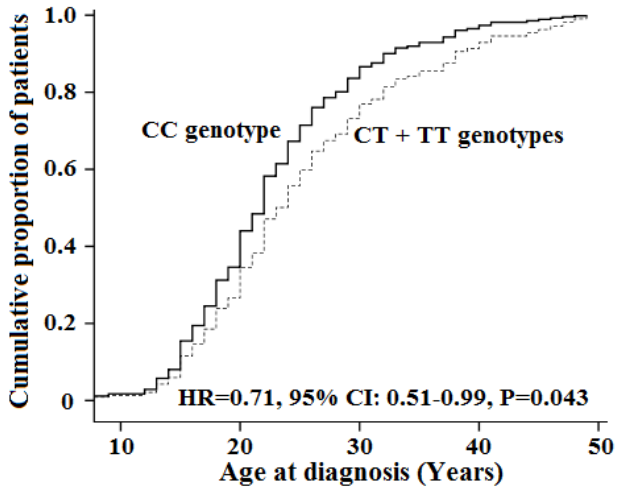

Figure 1: Association between genotypes of C-262T CAT polymorphism and age at diagnosis of schizophrenia among male patients.

The Cox regression analysis were used to evaluate the influence of C-262T *CAT* genotypes on age at diagnosis (AAD) of schizophrenia. The AAD was considered as time in the Cox regression analysis. Among male subjects mean AAD of the CC and CT+TT genotypes were 22.8 and 24.9 years, respectively. Using Cox proportional hazards regression model, there were significant association between the *CAT* polymorphism and AAD of schizophrenia, after adjusted for smoking status of participants. The CC genotype versus to the CT + TT genotypes showed lower age at diagnosis of schizophrenia (HR=0.71, 95% CI: 0.51-0.99, P=0.043). Figure 1 showed the difference of age at diagnosis of schizophrenia between genotypes of the C-262T *CAT* polymorphism, among males. Difference of mean AAD of the CC (26.7 years) and CT+TT (27.3 years) genotypes among females was not significant (HR=0.99, 95% CI: 0.55-1.78, P=0.989).

Discussion

It is suggested that oxidative stress may have a role in etiopathogenesis of schizophrenia [1, 2]. It has been shown that the polymorphism of C-262T *CAT* was associated with the expression and activity of CAT [3]. The less common T allele is associated with lower level of CAT activity [3, 4] which may increase ROS formation and oxidative stress. Therefore, we hypothesized that the TT genotype compared with the CC genotype increased the risk of schizophrenia. However, in the present study we found that there was no significant association between the *CAT* genotypes and risk of schizophrenia among subjects (Table 2). This finding is not consistent with the CAT activity in TT genotype compared with the CC genotype [3, 4].

Although the association between genotypes and susceptibility was not significant neither in males nor in females, there was significant interaction between gender and the TT genotype (P=0.035). Different associations between gender groups might be at least in part interpreted by the effect of gender on the association between C-262T polymorphism and *CAT* gene expression [15].

The key finding of the present study is the significant difference between CC and CT+TT genotypes for age at diagnosis (AAD) of schizophrenia among male patients. AAD was lower in the CC genotype compared with the CT+TT genotypes. On the other hand, there was no difference between *CAT* genotypes for AAD among female patients. This difference at least in part might be interpreted with the effect of gender on the association between C-262T polymorphism and *CAT* gene expression [15].

Considering that the magnitude of the alteration of *CAT* activity may depend on several factors such as race and diet [15-17], and the fact that ethnicity may influence the associations in multifactorial disease [18-21], replication of this study (with larger sample size) in other countries is recommended.

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