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Lack of Association between Glutathione Peroxidase 1 (pro197leu) Polymorphism and Cchizophrenia in a Turkish Population

Şizofreni ve Glutatyon Peroksidaz 1 (pro197leu) Polimorfizmi Arasındaki İlişkinin bir Türk Hasta Grubunda Araştırılması

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ABSTRACT

Introduction: Schizophrenia is a complex disease characterized with psychosis, cognitive dysfunction and negative symptoms. The etiology is unknown in most cases. Free radicals and antioxidant system balance shifting in favor of oxidants resulted from oxidative stress has been accounted for in the pathogenesis of schizophrenia. In this study, we aimed to elucidate the role of the pro197leu polymorphism of glutathione peroxidase 1 (*GPX1*) enzyme, the major subunit of glutathione peroxidase, in the etiopathogenesis of schizophrenia.
Materials and Methods: The study group was composed of 100 patients (54 males and 46 females) and 100 healthy volunteers (66 males and 34 females). The study was conducted using the PCR\RFLP method.
Results: The allele frequencies of pro197 and leu197 variants of *GPX1* were similar in the control group and schizophrenic patients (p=0.318). Distributions of the genotype frequencies were similar between the controls and patients (p=0.402), as well as males and females in patient group (p=0.225).
Conclusion: The pro197leu polymorphism of *GPX1* gene may not constitute any susceptibility for schizophrenia in Turkish population studied.
Key Words: Glutathione Peroxidase 1, Polymorphism, Schizophrenia

ÖZET

Amaç: Şizofreni, psikoz, bilişsel fonksiyon bozuklukları ve negatif semptomlarla seyreden kompleks bir hastalıktır. Etyoloji, bir çok vakada bilinmemektedir. Şizofreni patogenezinde serbest radikal-antioksidan sisteminde dengenin oksidanların lehine bozulması suçlanmaktadır. Bu çalışmamızda glutatyon peroksidazın en önemli alt grubu olan glutatyon peroksidaz 1 (GPX1) enzimini kodlayan gendeki pro197leu polimorfizminin şizofreni etyopatogenezindeki rolünü aydınlatmayı hedefledik.
Gereç ve yöntem: Çalışmamıza 100 hasta (54 erkek ve 46 kadin) ve 100 sağlıklı gönüllü dahil edildi. PCR/RFLP yöntemi çalışma metodu olarak seçildi.
Bulgular: GPX1 genindeki pro197 ve leu197 allellerinin sıklıkları (p=0,318) ve pro/pro, pro/leu, leu/leu genotiplerinin dağılımları (p=0.402) kontrol ve şizofreni gruplarında benzer bulundu. Ayrıca genotip dağılımları açısından kadınlar ve erkekler arasında anlamlı bir ilişki bulunamadı (p=0,225).
Sonuç: GPX1 genindeki pro197leu polimorfizminin çalışılan Türk toplumunda şizofreniye yatkınlık oluşturmadığı tespit edildi.
Anahtar Kelimeler: Glutatyon Peroksidaz 1, Polimorfizm, Şizofreni

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INTRODUCTION

Schizophrenia is a complex disorder, characterized by psychosis, cognitive dysfunction and negative symptoms (1). It is a rather common disorder affecting approximately 1% of population with 10-15/100.000 of incidence (2). It influences both gender equally. But the age of onset and prognosis show differences in each gender (3). The etiology is unknown in most cases (4). The interactions of genetic and environmental factors have been implicated in the development of the disease (1). Although several environmental factors such as geography (5), race (6), infection agents (7), and gestational complications (8) have been investigated, no evidence has been found that none of them is responsible for its etiology alone.

Reactive oxygen species (ROS) are generated in human body in many pathways and neutralized by antioxidant substances and enzymes in the body. Glutathione peroxidase (GPX) is one of the most important antioxidant enzymes converting H_2O_2 to H_2O and O_2. Glutathione peroxidase 1 (*GPX1*) is the most important one in four isoenzymes of GPX. Although it is expressed ubiquitously, it shows the highest activity in the liver. *GPX1* is located on 3p21.3 (9) and has a polymorphism on the position 593 resulting in the substitution of proline to leucine which has been shown to be associated with the increased risk of vascular disease in patients with type 2 diabetes (10), lung (11) and breast cancer (12).

Brain contains a high proportion of polyunsaturated fatty acids which are the targets for oxidative stress damage. Accumulating evidences point to many interrelated mechanisms that increase production of reactive oxygen or decrease antioxidant protection in schizophrenic patients (13). In the present study, we aimed at investigating the role of pro197leu polymorphism of *GPX1* gene in the etiopathogenesis of schizophrenia in a group of Turkish patients

MATERIALS and METHODS

The study was conducted at Firat University, Faculty of Medicine, Department of Medical Biology & Genetics and Elazig Mental Health Hospital. The study was approved by Firat University Ethical Committee. A total of 100 patients and 100 healthy volunteers were included into the study after giving their informed consents. The patients with epilepsy, mental retardation and other neuropsychiatric disorders were excluded. Patient group was composed of 54 males and 46 females whereas control group was consisted of 66 males and 34 females. Mean age of patients was 38.9 ± 10.8 yrs. whereas that of control group was 38.5 ± 11.7 yr.

DNA was isolated using Promega kit (Promega, Madison, WI) according to the manufacturer's recommendations from the venous blood of patients and controls. Genotyping for pro197leu was conducted according to a PCR/RFLP method described by Forsberg et al. with some minor modifications on annealing temperature (14).

The PCR products were digested by the restriction enzyme, Dde I (Promega, Madison, WI) and visualized under UV after running on a 2% agarose gel.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 12.0 for Windows (SPSS, Chicago, IL).

Chi-square or Fisher's exact test were used to compare the allele and genotype frequencies between the patient and control groups as well as between males and females.

RESULTS

The genotype and allele frequencies of the subjects for the *GPX1* pro197leu polymorphism are summarized in Tables 1 and 2. The frequencies of pro/pro, pro/leu, leu/leu genotypes were 48%, 43%, 9%, respectively, in schizophrenia group and 57%, 34%, 9%, respectively, in control group (Table 1). There was no statistically significant difference in the genotype distribution between schizophrenia and control groups (p=0.402). Allele frequencies of pro197 and leu197 variants of *GPX1* were 69.5% and 30.5%, respectively, in schizophrenia group and 74% and 26%, respectively, in control group (Table 1). The difference between the two groups was statistically insignificant (p=0.318). Since there are some difference between male and female schizophrenic patients regarding the age of onset and prognosis, we also evaluated the *GPX1* genotype and allele frequencies in male and female schizophrenic patients. We found the frequencies of pro/pro, pro/leu, leu/leu genotypes 53.7%, 38.9%, 7.4%, respectively, in males and 41.3%, 47.8%, 10.9%, respectively, in females (Table 2). There was no statistically significant difference between male and female genotype frequencies in schizophrenic group (p=0.452).

Groups	***GPX1* genotypes [n (f)]**[a]				***GPX1* allele frequencies**[b]		
	n	Pro/Pro	Pro/Leu	Leu/Leu	n	Pro197	Leu197
Schizophrenia patients	100	48 (0.48)	43 (0.43)	9 (0.09)	200	0.695	0.305
95% CI[c]		(0.382-0.578)	(0.333-0.527)	(0.034-0.146)		(0.631-0.759)	(0.241-0.369)
Control	100	57 (0.57)	34 (0.34)	9 (0.09)	200	0.740	0.260
95% CI		(0.473-0.667)	(0.247-0.433)	(0.034-0.146)		(0.679-0.801)	(0.199-0.321)

[a] *A statistically insignificant relationship between patient and control group in terms of GPX1 genotypes.*(X^2 *= 1.823, df= 2, p= 0.402).*
[b] *A statistically insignificant relationship between patient and control group in terms of allele frequencies.* (X^2 *= 0.999, df= 1, p= 0.318).*
[c] *95% Confidence Interval*

Table 1. Pro197Leu polymorphism of *GPX1*, genotypes and allele frequencies.

Gender	*GPX1* genotypes [n (f)][a]				*GPX1* allele frequencies[b]		
	n	Pro/Pro	Pro/Leu	Leu/Leu	n	Pro197	Leu197
Male	54	29 (0.537)	21 (0.389)	4 (0.074)	108	0.731	0.269
95% CI[c]		(0.404-0.670)	(0.259-0.519)	(0.004- 0.144)		(0.648-0.815)	(0.185-0.352)
Female	46	19 (0.413)	22 (0.478)	5 (0.109)	92	0.652	0.348
95% CI		(0.271-0.555)	(0.334-0.623)	(0.019- 0.199)		(0.555-0.749)	(0.251-0.445)

[a] *A statistically insignificant relationship in terms of GPX1 genotypes between males and females. (X^2= 1.588, df= 2, p= 0.452).*
[b] *A statistically insignificant relationship in terms of allele frequencies between males and females. (X^2= 1.474, df=1, p= 0.225).*
[c] *95% Confidence Interval*

Table 2. Pro197Leu polymorphism of *GPX1* based on gender, genotypes and allele frequencies.

DISCUSSION

Schizophrenia is a relatively common disease compared to other neuropsychiatric disorders and its etiology still remains unknown. The imbalance of the oxidant\antioxidant system may be responsible for the etiology. Since brain tissue consumes one fifth of the oxygen taken into the body, produces large amount of ROS, and contains abundant amount of unsaturated fatty acid, it is an open target for ROS mediated damage. In addition, its antioxidant capacity is also relatively low (15). It has been reported in schizophrenic patients that antioxidant system was weak and the oxidative stress has increased in biological materials such as blood, erythrocyte, and brain tissue (16). It is well-known that the increased ROS levels negatively affect the cellular components such as proteins, fatty acids and the DNA. ROS are, in part, neutralized by GPX1 activity. The functional consequences of Pro197Leu polymorphism are contradicting. Ravn-Haren et al. have shown that the Leu197 allele had 5% lower erythrocyte GPX activity (12), on the other hand, some other groups have shown that the pro197leu genetic polymorphism of *GPX1* had no effect on enzyme activity (14,17). Cominetti et al. and Hu et al. reported that there was no difference in enzyme activity among the *GPX1* genotypes but the subjects with at least one "leu" allele showed altered response to dietary selenium, they also observed higher DNA damage in subjects with leu/leu genotype after selenium supplementation suggesting possible effects on chronic diseases associated with ROS (17, 18). Although functional consequences of this polymorphism are considerable, it has been reported the association with the increased risk of lung (11), breast cancer (12) and recurrence risk of bladder cancer (19). However, in the present study, we couldn't find any association between *GPX1* polymorphism and schizophrenia. Our findings are in agreement with the results of the only study in the literature (20). They found the allele frequencies as 0.718 for pro197 and 0.282 for leu197 allele in schizophrenic patients which is very similar to what we found (0.695 and 0.305, respectively).

The genotype frequencies of our healthy individuals were 57%, 34% and 9% for pro/pro, pro/leu, leu/leu genotypes respectively, whereas they were 58%, 35%, 7% in UK and 49%, 41% and 10% in USA (21) which were very similar to the data in our study.

It is well known that the disease shows some differences in the onset and prognosis between males and females (3). No statistically significant differences were found between genders and neither *GPX1* genotypes nor allele frequencies in patient group. The *GPX1* polymorphism may not contribute to the differences between males and females.

In conclusion, our results indicate that pro197leu polymorphism of *GPX1* gene may not constitute susceptibility for schizophrenia, at least in our population. Further studies with larger population are required to elucidate the role of *GPX1* polymorphism in the etiopathogenesis of schizophrenia.

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