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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Adenosine Deaminase Gene G22a Polymorphism as a Risk Factor for Schizophrenia in Turkish Population

Türk Populasyonunda Şizofreni Hastalığı İçin Bir Risk Faktörü Olarak Adenozin Deaminaz Geni G22a Polimorfizmi

Aydın Rustemoglu[1], Birgul Elbozan Cumurcu[2], Ayse Feyda Nursal[3], Mahmut Balkan[4], Melike Uzun[1], Serbulent Yigit[1]

ABSTRACT

Objective: The aim of this study was to investigate whether the G22A polymorphism of adenosine deaminase (ADA) gene is associated with schizophrenia in Turkish population.

Methods: In this study, we evaluated 113 patients with schizophrenia and 121 individuals without the disease. The ADA G22A polymorphism was examined using allele specific-polymerase chain reaction (PCR).

Results: The ADA G22A genotype frequencies of GG, GA, and AA were 79.3% (96/121), 20.7% (25/121) and 0% (0) in the control group, while 87.6 % (99/113), 10.6% (12/113), 1.8% (2/113) in the patient group, respectively. There was a statistically significant difference in genotype distribution between patients and controls (p=0.017). Also the frequency of GA genotype was found significantly lower in patients compared with healthy controls (OR = 0.46, 95% CI 0.22-0.96, p =0.048). However, there was not any noticeable difference in allele distribution between the groups (p>0.05). In addition, the frequency of GG genotype in the male patient group significantly higher, and GA genotype significantly lower compared to the male control group, were found (p=0.036, p=0.005, respectively). No association was found for the female group.

Conclusion: Our results show that, the G22A polymorphism of ADA gene may be associated with schizophrenia in Turkish population. The ADA GA genotype is likely to reduce, whereas GG genotype increased genetic susceptibility to schizophrenia, especially in males. Further studies should be repeated with different study subjects and/or other ethnic subjects to generalize the conclusion of this study.

Key words: Schizophrenia, ADA gene, G22A polymorphism.

ÖZET

Amaç: Bu çalışmanın amacı, adenozin deaminaz (ADA) genindeki G22A polimorfizminin Türk popülasyonunda şizofreni ile ilişkisinin araştırılmasıdır.

Yöntemler: Bu çalışmada biz 113 şizofreni tanısı almış hasta ve 121 sağlıklı kontrol bireylerini inceledik. ADA geni G22A polimorfizmi allel spesifik-polimeraz zincir reaksiyonu (AS-PCR) yöntemi kullanılarak belirlendi.

Bulgular: ADA genindeki G22A polimorfizmi için GG, GA ve AA genotip sıklıkları sırası ile kontrol grubu için %79.3 (96/121), %20,3 (25/121), %0 (0) ve hasta grubu için %87,6 (99/113), %10,6 (12/113), %1,8 (2/113) olarak belirlendi. Genotip dağılım bakımından hasta ve kontrol grupları arasında anlamlı fark bulundu (p=0,017). Ayrıca GA genotipinin sıklığı hastalarda kontrole nazaran anlamlı düzeyde düşük bulundu (p=0,048, OR=0.46, 95%CI 0.22-0.96). Fakat allel sıklıkları bakımından iki grup arasında anlamlı fark bulunamadı. Cinsiyet bakımından karşılaştırma yaptığımız zaman, erkek hastalarda GG genotipinin anlamlı düzeyde yüksek, GA genotipinin ise düşük olduğu gözlendi (sırası ile p=0,036 ve p=0,005). Kadın hastalarda herhangi bir anlamlı fark gözlenmedi.

Sonuç: Bizim sonuçlar ADA genindeki G22A polimorfizminin Türk popülasyonunda şizofreni hastalığı ile ilişkili olabileceğini gösterdi. ADA genindeki GA genotipinin özellikle erkeklerde şizofreniye yatkınlığı düşürdüğü, buna karşın GG genotipinin yatkınlığı arttırdığı muhtemeldir. Farklı hasta ve etnik gruplar ile gelecekte yapılacak çalışmalar, elde ettiğimiz sonuçların kesinleşmesine yardımcı olur.

Anahtar kelimeler: Şizofreni, ADA geni, G22A polimorfizmi

[1] Department of Medical Biology, Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey
[2] Department of Psychiatry, Faculty of Medicine, Inonu University, Malatya, Turkey
[3] Department of Medical Biology, Faculty of Medicine, Giresun University, Giresun, Turkey
[4] Department of Medical Biology, Faculty of Medicine, Dicle University, Diyarbakır, Turkey

***Yazışma Adresi /Correspondence:** Aydın Rustemoglu,*
Gaziosmanpasa University, Faculty of Medicine, Dept. Medical Biology, 60100, Tokat/Turkey Email: arustamov@yahoo.com

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INTRODUCTION

Schizophrenia, a severe mental disorder, has a high recurrence rate and prolonged course. It is characterized by delusion, hallucination and disturbance of thought, which may lead to regression of patients from the society and mental disability [1]. Lifetime incidence of schizophrenia is 1-2% [2]. Although pathology is not clearly known, family studies indicate that genetic factors play a very important role [1].

Adenosine enzyme, which is an important modulator of the nervous system, has been implicated in the pathophysiology of schizophrenia [3]. The enzyme adenosine deaminase (ADA; EC 3.5.4.4) catalyzes the deamination of adenosine and deoxyadenosine and plays role in DNA replication, methylation process, cell growth and differentiation, and immune functions [4]. Congenital adenosine deficiency is an inherited disorder damages the immune system and causes severe combined immunodeficiency syndrome [5]. ADA is encoded by the ADA gene, which is located on chromosome 20q13.11 and regulates intracellular and extracellular adenosine concentrations [6]. It is considered that adenosine is an endogenous anticonvulsant and neuroprotective agent [3].

The most frequent single nucleotide polymorphism (SNP) of ADA gene is 22G to A transition in exon 1 (ADA G22A; rs73598374), resulting in an aspartic acid (Asp) to asparagine (Asn) substitution in codon 8 of the protein. This transition leads to alterations in the expression of ADA levels [7]. It was reported that GA genotype led to 20-30% lower enzymatic activity than GG genotype [8]. The aim of this study was to evaluate the influence ADA G22A polymorphism on the risk of schizophrenia.

METHODS

Patients

The ADA gene polymorphism was analyzed in 113 unrelated Turkish patients with a diagnosis of schizophrenia, who were admitted to the Psychiatry Department of Gaziosmanpaşa University. The diagnosis was based on the criteria for schizophrenia available in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [9]. Patients selected were within the age range from 20 to 50. During the selection of cases, the gender criterion was not taken into consideration.

The control group comprised 121 unrelated healthy subjects without any other inflammatory or organic or psychiatric disorder. Written and informed consent was taken from healthy as well as individuals with schizophrenia included in the study, according to the Helsinki Declaration. This study was approved by the Clinical Research Ethical Committee of Gaziosmanpaşa University (approval # 10-BADK- 018).

Molecular Analysis

EDTA tubes were used for blood collecting from both the patients and controls. The genomic DNA was isolated from the blood by the standard method and then stored at -20°C. The ADA G22A polymorphism was analyzed using allele-specific polymerase chain reaction (PCR) method previously described by Dutra et al. [3]. PCR reaction was performed in a 25 µL final volume containing 1.0 pmol of each primer, 0.2 mM of dNTP, 2 µg of genomic DNA, 1.5 mM of MgCl, 2 and 2.5 µL of 10xPCR buffer and 1 unit of Taq DNA polymerase according to the following protocol: initial denaturation at 94ºC for 5 min; 30 cycles of denaturation at 94ºC for 30 s, annealing at 63ºC for 30 s, and extension at 72ºC for 30 s; and final extension at 72ºC for 5 min. ADA primers were as follows: forward-G, 5' -CCC AGA CGC CCG CCT TCG-3'; forward-A, 5' -CCC AGA CGC CCG CCT TCA- 3'; reverse, 5' -GAA CTC GCC TGC AGG AGC C- 3'. PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.

Statistical Analysis

Statistical analyses were performed using SPSS 16.0 and Arlequin 3.11 software. The genotype and allele distributions of ADA gene G22A polymorphism, were compared by using Fisher's exact chi-square ($\chi 2$) test and p-values smaller than 0.05 were considered significant. The odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine the association of ADA gene allelic and genotypic variants with the occurrence of patients.

RESULTS

Two hundred and thirty-four Turkish individuals (113 controls and 121 patients) participated in the study. There were 43 female and 70 male participants in the patient group, 51 female and 70 male participants in the control group. The men constituted the majority of cases in both patient and control groups. The genotype and alleles distributions for ADA G22A Polymorphism among the patient and control groups are summarized in Table 1. Compared with the control group, the ADA G22A genotype distribution showed significant difference in the patient group (OR = 0.46, 95%Cl 0.22-0.96, p =0.048).

The ADA G22A Polymorphism was also analyzed between the female and male subgroups of schizophrenia patients. No significant differences were observed in the distribution of the ADA G22A Polymorphism genotype frequency (>0.05), or allele frequency (>0.05) between the female patients and female control group. Adversely, in the male subgroup, significant difference was observed in the distribution of the ADA G22A Polymorphism. The ADA G22A GG and GA genotype frequency was significantly higher in the male patient group compared with the control group (respectively, p=0.036, p=0.005). Table 2 shows the frequencies of genotypes and alleles of ADAG22A Polymorphism in female and male groups.

Table 1. ADA G22A allele and genotype distributions in the patient and control groups

Genotypes	Patients n (%)	Controls n (%)	p	OR (CI 95%)
GG	99 (87.6)	96 (79.3)	>0.05	NA
GA	12 (10.6)	25(20.7)	**0.048**	**0.46 (0.22-0.96)**
AA	2 (1.8)	0	>0.05	NA
Alleles				
G	210 (92.9)	217 (89.7)	>0.05	NA
A	16 (7.1)	25 (10.3)		

NA- Not Associated

Table 2. ADA G22A allele and genotype distributions between female/male patient group and control groups

Genotypes	Female group n (%)		p	Male group n (%)		p
	Patients	Controls		Patients	Controls	
GG	33 (76.7)	39 (76.5)	>0.05	66 (94.3)	57 (81.4)	**0.036**
GA	10 (23.3)	12 (23.5)	>0.05	2 (2.9)	13 (18.6)	**0.005**
AA	0	0	>0.05	2 (2.9)	0	>0.05
Alleles						
G	76 (88.3)	90 (88.2)	>0.05	134 (5.7)	127 (90.7)	>0.05
A	10 (11.6)	12 (11,7)		6 (4.3)	13 (9.3)	

DISCUSSION

Schizophrenia, a complex disease, have many negative effects on patients' lives. Although the specific cause of this disease has not been defined precisely yet, it is thought that genetic and environmental factors play important roles in its molecular pathogenesis [10]. Although the research on schizophrenia leads to significant developments in the field, the etiology of disease has still not been known clearly. A combination of environmental and genetic factors is likely to cause the disorder, and particularly genetic factors are considered to play a significant role in the etiology of disease [10]. A large number of genetic factors are likely to increase the risk of schizophrenia; according to genome-wide association studies (GWAS), over 100 common variants are associated with the development of schizophrenia [11]. SNPs within related genes are considered as risk factors for the disorder.

In present study we were analyzed and compared 113 schizophrenic patients, and 121 heathy controls for ADA gene G22A polymorphism. Our results show that, the frequency of ADA gene G22A polymorphism GA genotype statistically lower (p=0.048; OR, 95%CI= 0.46, 0.22-0.96) in patients (10.6%) than controls (20.7%). These results were consistent with the results obtained by Dutra et al. In this study, similar to our results, the frequency of GA genotype, which associated with low enzyme activity, was found less in patients than con-

trol group [3]. Previous studies have shown that the antipsychotic drugs were reduced adenosine level [12,13]. Therefore, new drugs used in therapy were targeted to reduce the ADA enzyme activity [14-16]. These data were explain why the frequency of GA genotype, which characterized by less enzyme activity, lower in the patients. But, we were obtained AA genotype in patient group (1.8%), which absent in controls, and the presence of the AA genotype in the patient group, it poses a question for to explain of this hypothesis.

Adenosine modulates most neurotransmitter systems and may play role in schizophrenia. Adenosine neurotransmission is modulated by four types of G protein activation, and these coupled adenosine receptors (A1R, A2AR, A2BR, and A3R) fulfill different functions in presynaptic and postsynaptic areas of the brain [17]. On the presynaptic area, adenosine regulates the release of both dopamine and glutamate, and whereas heterodimerization of ARs regulates the output of dopaminergic and glutamatergic neurotransmission with dopamine and glutamate receptors [18,19]. Therapeutic activation of A1Rs has some important antiepileptic and neuroprotective functions [20]. The studies, where a transgenic mouse model overexpressing adenosine kinase is used, indicate that the reduction of adenosine levels in the forebrain is likely to cause the emergence of behavioral endophenotypes seen in schizophrenia as well as abnormal response to psychostimulants [21]. Furthermore, A1R polymorphisms, also considered to be candidate markers in schizophrenia research, were found to play a role in the pathophysiological mechanisms of schizophrenia in the Japanese population [22].

In the other hand, schizophrenia is characterized by major sleep/wake disturbances including increased vigilance and arousal, decreased slow wave sleep, and increased REM sleep drive [23;24]. Previously studies shows that, ADA enzyme activities and adenosine levels have important role in human sleep quality, and GA genotype of G22A polymorphism associated with better sleep [25,26]. This data is further evidence to support our findings. We obtained results that GA genotype, which is relation to good sleep and may be create resistance to the schizophrenia, were significantly higher in the control group than in the patient group (OR, 95%CI= 0.46, 0.22-0.96).

A study by Stubbs et al. showed that ADA serum activity decreased in children with autism compared to normal controls as well as individuals with cerebral palsy and with intellectual impairment [27]. However, Zoruglu et al. found no difference in ADA activity between children with autism and sex- and age-matched controls [28]. In different studies, it was found that the serum levels of ADA were higher in patients with both panic disorder [29, 30] and major depression [31] compared to control groups. On the contrary, it was also reported that ADA enzyme activities decreased in major depressives compared to controls in a study by Elgun et al. [32]. In addition, it was demonstrated that schizophrenic patients treated with either typical antipsychotics or clozapine had increased serum ADA activity compared to controls [33].

Carriers of the ADA A allele have lower enzymatic activity and thus higher circulating and intracellular adenosine, and this is likely to cause a number of consequences [34]. In a study, it was reported that A allele frequency of ADA gene was higher in 118 Italian autistic children compared with 126 healthy controls [35]. However, another study provided no significant increase in the frequency of the ADA A allele in autistic cases from North America [5]. Thus, the role of ADA gene in autism has still been debatable.

Furthermore, we were investigated association ADA gene G22A polymorphism and schizophrenia by gender status. We found that GG genotype was significantly higher in the male patient group than in the female patient group (p=0.008), and GA genotype was significantly lower in the man patient group than in the female patient group (p=0.001) (Data not shown). In the other hand, male patient group have significantly difference from male control group for GG (p=0.036) and GA (p=0.006) genotypes (Table 2). Because of Dutra et al is not given gender distribution data, we could not compare in terms of gender.

In a study that examines the plasma total ADA, ADA1, ADA2 and ADA1/ADA2 ratio in the first 12 hours following the start of an attack in acute ischemic stroke patients, it was found that there was no

significant difference in total ADA and ADA2 activities between stroke patients and control groups, but there were significant differences in ADA1 activity and ADA1/ADA2 ratio between male and female stroke patients. Women had significantly higher ADA1 activity and ADA1/ADA2 ratio than men in the group of stroke patients. This suggests that the primary mechanism in men with ischemic stroke is likely to reduce ADA1 activity or ADA1 inactivation by some inhibitors [36]. It has been known that there are differences between men and women with regard to the risk, onset and severity of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and stroke [37]. There may be several reasons that cause lower stroke outcomes in women, including the decrease in estrogen levels after menopause [37]. One of these reasons may be partly due to higher activity of AD1 in women when compared to men which subsequently reduces adenosine in the site of damage and thus protects brain against ischemic injury. Therefore together with higher ADA1 activity in women compared to men, this change possibly plays a role in the poor stroke outcomes seen in women with ischemic stroke.

The previous studies which have shown differences between ADA activities on the basis of gender, are supporting our achieved results. Thus, the differences between males and females in terms of association between ADA gene polymorphism and schizophrenia, it is more understandable. The results of this study are important in that they show the difference between sexes, and may play a guiding role for further studies.

But we have a limitation in this study for selecting samples, because of during sample collection we were not applicated specific symptom assessments (PANSS, CGI, etc) for the classification of disease violence in schizophrenic patients.

In conclusion, we have demonstrated that there is an association between ADA gene G22A polymorphism and schizophrenia. Our results shows that, the GA genotype may be a protective factor for schizophrenia, especially in males. For the generalization of the conclusions of this study, however, further studies should be repeated with larger study subjects and/or other ethnic groups, are needed.

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Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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