

Original article / Araştırma**An association study of D-amino acid oxidase and D-amino acid oxidase activator polymorphisms and schizophrenia in patients from Turkey****Şükrü KARTALCI,¹ Ceren ACAR²****ABSTRACT**

Objective: Schizophrenia is a highly heritable complex disease. The genetic interactions underlying this heritability is still needs to be understood. Linkage studies pointed out the D-amino acid oxidase (DAO) and D-amino acid oxidase activator (DAOA-G72) genes as candidate genes in susceptibility to schizophrenia. The association studies of interaction partners D-amino acid oxidase and D-amino acid oxidase activator gene polymorphisms and schizophrenia has been performed in different studies with different ethnic origins. However these studies showed conflicting results. In this study, it is aimed to examine the effects of several DAO and DAOA polymorphisms in Turkish schizophrenia patients. **Methods:** In the present study two single nucleotide polymorphisms (SNPs) within DAO gene (rs2111902 and rs3741775) and one in DAOA gene (rs2391191) were genotyped in individuals with Turkish origin from Malatya region on eastern part of Turkey. 96 schizophrenia patients and 100 mentally healthy individuals are the subjects of this study. **Results:** No significant difference was found in between the controls and schizophrenia sample for the SNPs rs2111902 and rs3741775 of DAO gene but a significant association was found on the allelic level of rs2391191 of DAOA gene. The relationship between aggressive behaviour, suicide attempts and genotypes were also evaluated in the patient group. **Discussion:** A significant association for the G allele of rs2391191 at the allelic level ($p=0.036$) was found, on the other hand no association could be shown between the examined rs2111902 and rs3741775 polymorphisms and the pathology of the disorder in the group of Turkish schizophrenia patients included in this study. (Anatolian Journal of Psychiatry 2016; 17(5):341-346)

Keywords: DAO, DAOA, G72, schizophrenia, Turkish population, SNP, genetics

Türk şizofreni hastalarında D-amino asit oksidaz ve D-amino asit oksidaz aktivatör polimorfizmleri arasındaki ilişki**Öz**

Amaç: Şizofreni yüksek derecede kalıtsal, karmaşık bir hastalıktır. Bu kalıtsallığın altında yatan genetik etkileşimler hala anlaşılmaya çalışılmaktadır. Bağlantı çalışmaları şizofreniye yatkınlıkta aday genler olarak D-amino asit oksidaz (DAO) ve D-amino asit oksidaz aktivatör (DAOA-G72) genlerini işaret etmiştir. Etkileşim ortakları DAO ve DAOA genlerinin polimorfizmleri ile şizofreni arasında, farklı etnik kökenler ile çeşitli ilişki çalışmaları yapılmıştır. Ancak bu çalışmalardan çelişkili sonuçlar alınmıştır. Bu çalışmada Türk şizofreni hastalarında bazı DAO ve DAOA polimorfizmlerinin etkilerinin incelenmesi amaçlanmıştır. **Yöntem:** Bu çalışmada Türkiye'nin doğusunda yer alan Malatya bölgesinden, Türk kökenli bireylerde, DAO geninde iki adet (rs2111902 and rs3741775) ve DAOA geninde bir adet (rs2391191) olacak şekilde, tek nükleotid polimorfizmleri (TNP) genotiplendirilmiştir. Bu çalışma 96 şizofreni hastası ve 100 ruh sağlığı yerinde olan katılımcı ile yapılmıştır. **Sonuçlar:** Kontrol grubu ve şizofrenili hastalar arasında DAO genine ait rs2111902 and rs3741775 TNP'leri açısından anlamlı farklılık bulunmamıştır; ancak DAOA

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geninde rs2391191 TNP'sinde alelik düzeyde anlamlı ilişki bulunmuştur. Hasta grubunda ayrıca şiddet davranışı, özkiyim girişimleri ve genotipler arasındaki ilişki de incelenmiştir. **Tartışma:** Bu çalışmada yer alan Türk şizofreni hasta grubunda bu hastalık patolojisi ile rs2391191'in G aleli için istatistiki olarak anlamlı bir bağlantı bulunurken, incelenen diğer TNPLer rs2111902 and rs3741775 arasında anlamlı bir ilişki gösterilememiştir. (*Anadolu Psikiyatri Derg* 2016; 17(5):341-346)

Anahtar sözcükler: DAO, DAOA, G72, şizofreni, Türk popülasyon, TNP, genetik

INTRODUCTION

Schizophrenia is a complex disease with an 81% heritability rate.¹ Several linkage studies had been performed on schizophrenia pedigrees and they pointed out different candidate locations on different chromosomes. These include 12q23-24 and 13q32-34 on which DAO and DAOA genes are located respectively. Studies reporting association between these two genes were in different populations including German, Chinese, French Canadians, Ashkenazi Jews and populations from US and South Africa.²⁻⁷ There has not been a study about schizophrenia and DAO-DAOA association reported from Turkish population to date.

DAO gene is involved in the oxidation of D-amino acids and its major substrate in brain is D-serine, the gene is located on 12q24 and it consists of 11 exons. The coded protein consists of 347 amino acids and 39 kDa. DAOA gene is located on 13q34 where on complementary strand G30 is expressed.⁴ DAOA has shown to be interacting partner of DAO by Chumakov et al. Both DAO and DAOA are thought to play a role in the N-methyl-D-aspartate receptor (NMDAR) regulation pathway.⁸⁻⁹ Glutamatergic neurotransmission abnormalities were shown to be involved in schizophrenia pathogenesis. The hypofunction of N-methyl-D-aspartate glutamatergic neurotransmission has an important effect in the pathophysiology of the disease.¹⁰ Several studies showed that D-serine facilitates the NMDAR function and DAO activity is increased in the case of schizophrenia.¹¹ D-serine is a coagonist for NMDAR and DAO gene product –D-amino acid oxidase enzyme- degrades D-serine. On the other hand DAOA gene product D-amino acid oxidase activator-activates DAO enzyme.¹²

Both by being located on the candidate chromosomal regions for schizophrenia and because of their functions in the brain we aimed to investigate the influence of some of DAO and DAOA SNPs on 96 Turkish schizophrenia patients. The relationship of genotypes and schizophrenia was examined using a case control association design and relationship of genotypes and alleles and suicidal attempts and aggressive

behavior in the patients were also examined.

METHODS

Subjects

The present study was performed in the Department of Psychiatry and Department of Molecular Biology and Genetics of Inonu University and was carried out in accordance with the Declaration of Helsinki guidelines and was approved by the Local Ethics Committee. All subjects were recruited from the same geographical location. Written informed consent was obtained from all patients and controls prior to the study. Ninety six patients- who have a family history of at least one family member affected- diagnosed with schizophrenia according to the Structured Clinical Interview for the DSM-IV (SCID-I) and 100 healthy control subjects were included in the present study.¹³ The inclusion criteria specified that subjects had to be diagnosed with one of the schizophrenia subtypes that all four grandparents of each subject were of Turkish ethnic origin. Exclusion criteria included the presence of psychotic disorder due to a general medical condition, substance-induced psychotic disorder, mood disorder with psychotic features, schizoaffective disorder, schizophreniform disorder, schizotypal, schizoid, and paranoid personality disorder.

Samples from healthy Turkish individuals were collected from volunteers. Samples from Turkish relatives of patients with other diseases, which served as additional controls in the present study, were also collected in Turkey under standards similar to those used for the patients with schizophrenia. The control group subjects were evaluated by a senior psychiatrist to confirm that they did not have any Axis I psychotic disorder or first-degree relatives with a psychotic disorder.

DNA isolation and genotyping

Genomic DNA was extracted from anticoagulated venous blood by using a commercial kit (QIAamp DNA Blood Mini Kit) according to the manufacturer's protocol. All blood and DNA samples were coded using a coding system in order to protect the confidentiality of the sub-

jects. Samples were genotyped by using TaqMan genotyping assays of Applied Biosystems. Two SNPs were chosen from DAO gene-rs2111902 and rs3741775- and one SNP was chosen from DAOA gene-rs2391191- which had been reported to be associated with schizophrenia in the previous studies.

Statistical analysis

The distribution of the allele and genotype frequencies are represented by count and percentage. Hardy-Weinberg equilibrium was tested using Pearson's goodness-of-fit chi-squared test. The statistical significance of HWE analysis was defined at $p < 0.01$. The Pearson's chi-squared test or Likelihood Ratio test was used to compare genotype and allele frequencies between cases and control group, and aggressive behavior (+) and (-) groups, and suicidal attempt (+) and (-) groups. The level of significance was set at $p < 0.05$.

RESULTS

All the results obtained from that study were found to be in Hardy Weinberg equilibrium. The

distribution of allele and genotype frequencies of rs2111902, rs3741775 (DAO) and rs2391191 (DAOA) are shown in the Table 1. We couldn't find a significant association between our patient group and control group for DAO SNPs that we have tested. Results are given in Table 2 and 3 for rs2111902 and rs3741775 respectively. No association was observed in aggressive behavior or tendency for suicide for both alleles and genotypes of DAO SNPs. Although we couldn't find a significant association in the genotype distribution for rs2391191 the G allele of rs2391191 has a higher ratio in schizophrenia patients with a statistically significant difference ($p = 0.036$). On the other hand we grouped our patients depending on their tendency to violence or suicidal attempts by looking at their life stories. Heterozygous genotype of rs2391191 showed a higher tendency to violence in our group of schizophrenia patients shown in Table 4.

DISCUSSION

It is clear that schizophrenia is a complex mental disorder that includes complex genetic contributions. Several mechanisms including dopamin, γ -

Table 1. Genotype and allele distributions of the polymorphisms in control and schizophrenia patients in Turkish population from Malatya region

SNP	hw	Distribution of genotypes (%)			p	Distribution of alleles (%)		p
rs2111902(DAO)		GG	GT	TT	0.585	G	T	0.382
Controls	0.306	21	44	35		43	57	
Patients	0.859	22.9	49	28.1		47.4	52.6	
rs3741775(DAO)		AA	AC	CC	0.232	A	C	0.579
Controls	0.017	40	37	23		58.5	41.5	
Patients	0.939	31.3	48.9	19.8		55.7	44.3	
rs2391191(DAOA)		AA	AG	GG	0.088	A	G	0.036
Controls	0.239	22.2	43.4	34.3		43.9	56.1	
Patients	0.012	17	33	50		33.5	66.5	

Table 2. Genotype and allele distributions of the DAO rs2111902 in schizophrenia patients with or without aggressive behavior and suicidal attempts

SNP	Distribution of genotypes (%)			p	Distribution of alleles (%)		p
rs2111902(DAO)	GG	GT	TT		G	T	
Aggressive behavior (-)	21.3	46.7	32	0.276	44.7	55.3	0.152
Aggressive behavior (+)	28.6	57.1	14.3		57.1	42.9	
Suicidal attempt (-)	26	46.8	27.3	0.355	49.4	50.6	0.275
Suicidal attempt (+)	10.5	57.9	31.6		39.5	60.5	

Table 3. Genotype and allele distributions of the DAO rs3741775 in schizophrenia patients with or without aggressive behavior and suicidal attempts

SNP	Distribution of genotypes (%)			p	Distribution of alleles (%)		p
rs3741775(DAO)	AA	AC	CC		A	C	
Agressive behavior (-)	30.7	49.3	20	0.973	55.3	44.7	0.835
Agressive behavior (+)	33.3	47.6	19		57.1	42.9	
Suicidal attempt (-)	31.2	49.4	19.5	0.984	55.8	44.2	0.949
Suicidal attempt (+)	31.6	47.4	21.1		55.3	44.7	

Table 4. Genotype and allele distributions of the DAOA rs2391141 in schizophrenia patients with or without aggressive behavior and suicidal attempts

SNP	Distribution of genotypes (%)			p	Distribution of alleles (%)		p
rs2391141 (DAOA)	AA	AG	GG		A	G	
Agressive behavior (-)	20.6	26	53.4	0.019	33.6	66.4	0.978
Agressive behavior (+)	4.8	57.1	38.1		33.3	66.7	
Suicidal attempt (-)	17.3	33.3	49.3	0.966	34	66	0.778
Suicidal attempt (+)	15.8	31.6	52.6		31.6	68.4	

aminobutyric acid or glutamatergic neurotransmission are known to contribute to the pathophysiology of schizophrenia and several genes have been found as candidate genes for the susceptibility to this disease. Genomewide screenings pointed different regions on chromosomes 5q, 3p, 11q, 12q, 13q, 6p, 1q, 22q, 8p, 20q, and 14p.⁸ Previous studies showed that N-methyl D-aspartate receptor (NMDAR) hypofunction has a role in schizophrenia pathogenesis. Endogenous D-amino acids like D-serine or D-alanine act as coagonists of NMDAR at the glycine site of NMDAR.^{10,12} NMDAR is an ionotropic glutamate receptor and DAO regulates its function by degrading D-serine so it might contribute to NMDAR hypofunction.⁹ D-amino acid oxidase and D-amino acid oxidase activator plays a role in this hypofunction. DAO degrades D-serine and its interaction partner DAOA has been shown to be involved in schizophrenia.⁴ In vitro studies showed that the increased levels of G72 protein stimulated the D-serine oxidation.^{4,14} Different groups also detected reductions of D-serine in serum.¹⁵ These two proteins -DAO and DAOA- are located on the genomic regions (12q24 and 13q32-33) that showed a linkage to schizophrenia.

Several association studies have been done with

schizophrenia patients from different populations. But the studies performed with these genes displayed conflicting results. On the other hand few association studies were performed with DAO gene SNPs and schizophrenia. Chumakov and colleagues were one of the frontiers who showed association between DAO SNPs and schizophrenia.⁴ They reported the interaction between DAO and DAOA (G72) and the association of four DAOA SNPs with schizophrenia in Canadian samples.

In the present study based on their function and genomic locations pointed by linkage studies we aimed to extend previous association studies of DAO-DAOA gene and schizophrenia in Turkish patients to see if we could replicate the data by our single-ethnicity samples. DAO and DAOA genes were not studied in Turkish schizophrenia population before. In order to examine the relationship between DAO and DAOA genes and schizophrenia we genotyped 96 schizophrenia patients. Of the two DAO SNPs we evaluated we did not find a statistically significant relationship between the genotypes or alleles and schizophrenia phenotype. On the other hand for rs2391191 allelic distribution showed statistically significant difference in the case-control comparisons with a p value of 0.036 by having the G

allele higher percentage in the patients. Since NMDAR regulates the behavior against emotional and environmental stimuli, we examined the relationship in between genotypes or allelic distribution and aggressive behavior and suicidal attempts in our patient groups and we failed to find an association in DAO SNPs again.¹⁶ On the other hand DAOA SNP rs2391191 gave statistically significant results for genotype and aggressive behavior relation. AG genotype was found to be higher in the patients who tend to have aggressive behavior (57.1% in aggressive behavior (+) and 26% aggressive behavior (-), $p = 0.019$). GG genotype was higher in aggressive behavior (-) patients (53.4%). The percentages were so close in between the patients who attempted suicide and who did not, so for this genotype DAOA SNP was not the risk factor in our patient group.

rs2111902 and rs3741775 had been previously examined by Chumakov et al., Schumacher et al. and Yamada et al. and these groups found association in their group of patients with different ethnic origins.^{2,4,17}

In a study that included 299 schizophrenia and 300 bipolar patients Schumacher et al found that DAO was associated with schizophrenia but not with bipolar disorder in their samples and they also found an evidence that variability in G72 contributes to the development of bipolar disorder. Their results showed the G72 contribution to the risk of schizophrenia.² Their study covers the SNPs that we included in our study. Unlike their results we couldn't find an association between DAO SNPs and schizophrenia neither at the genotype nor allelic level.

Yang et al. reported that DAO plays an important role in the genetic associations and interactions for schizophrenia. They worked with 912 schizophrenic patients from Taiwan Han Chinese population. They found the significant association of schizophrenia with SNP triplet rsDAO7-rsDAO8-rsDAO13 in their study.¹²

On the contrary a study reported by Chung et al found no association between polymorphisms of DAO and DAOA genes and aggressive behavior in Korean schizophrenic patients.¹⁸ They included rs 2111902 and rs 3741775 which were also the subjects of the present study. They didn't include rs2391191 of DAOA gene so we couldn't make a comparison with this study. Unlike their results we found a significant association between the aggressive behavior and AG genotype. Aggressive behavior is seen in the patients who carry the AG genotype (57.1%, p

0019). On the other hand GG genotype was seen higher (53.4%) in the patients who don't have aggressive behavior. These results were shown in Table 4. But for this SNP we couldn't get a significant difference between the patients who attempted suicide and who did not.

There are other studies that support our DAO SNPs' negative results. In a study that was performed with 431 patients with schizophrenia, Bass et al couldn't find an association of DAO markers with schizophrenia, but they detected an allelic association between the marker rs31983423, 3' to the DAOA gene and schizophrenia.¹⁹ On the other side in their schizophrenia patients they couldn't find a significant association with rs2391191 (p value is 0.470) which we have examined in our samples.

Sacchetti et al. found no association with the SNPs of DAO and DAOA in their case control study that includes 879 patients from Northern Italy and 32 tagSNPs (SNPs of DAO, DAOA, PPP3CC, and DTNBP1 genes).²⁰

Depending on the population and the sample size there are numerous groups reported significant associations with DAOA gene at the allelic, genotypic or haplotypic level. However there are also groups that reported the lack of association between schizophrenia and DAOA gene.²¹

There weren't many studies that examined DAO SNPs in psychiatric samples. DAO gene product has a remarkable role in NMDAR function which is important in pathophysiology of schizophrenia. In summary we tried to find an association between DAO and DAOA genes and schizophrenia. The underlying reason of the pathology in our patients was not related with DAO gene polymorphism we examined. Only DAOA SNP rs 2391191 showed an allelic association in our group of patients.

Our study had some limitations. Comparing with other studies our patient numbers were relatively low. We believe that data about the candidate genes and their relationship with schizophrenia should be expanded by doing more population studies from different sides of the world with bigger subject numbers so that the analysis can find more clues about the responsible genes and related SNPs. Also by having bigger numbers of samples it is possible to group the patients depending on the subgroups of schizophrenia.

Although our main limitation was the patient number that was included to the study, still this is normal when this is considered as a regional

study and all the patients were from Malatya and neighbouring cities. Besides while doing an association study we believe it would be better to include more SNPs in the analysis, so that it can give more data about the related gene. It should

be noted that schizophrenia is complex disease so that the interactions between the gene products and environmental conditions are taking important roles in the pathophysiology of the disease.

Authors' contributions: Ş.K.: Finding topic, planning, application, writing manuscript; C.A.: Literature searching, statistics, writing manuscript.

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