



Molecular Genetics

Research Article – 70188

Ceren Acar*, Mustafa Mert Sözen, Harika Gözükara, Kübra Orman, Şükrü Kartalacı

Lack of association between catechol-O-methyltransferase and schizophrenia in a Turkish population

Türk popülasyonunda şizofreni ve catechol-O-methyltransferase arasındaki bağlantı çalışması

Abstract: Objective: Catechol-O-methyltransferase (COMT) is the key molecule in the catabolism of catecholamines like dopamine which is an important molecule in schizophrenia. Due to its function and location COMT gene is a strong candidate gene for schizophrenia. The aim of this study was to investigate the possible associations of 3 COMT single nucleotide polymorphisms (SNPs) and schizophrenia in our population. COMT enzyme activity is regulated by a widely known Val158Met polymorphism (rs4680), along with the variation of the SNPs rs737865 and rs165599.

Methods: Val158Met polymorphism (rs4680), the SNPs rs737865 and rs165599 were the targets of this study. The study was performed with 96 patients (66 male and 30 female) and 100 controls (47 male and 53 female) from Malatya region on eastern part of Turkey by using TaqMan genotyping assays.

Results: We couldn't find a significant difference between the schizophrenia patients and normal controls for any of the SNPs that were studied. The genotype frequencies in both the patient and control groups satisfied the Hardy-

Weinberg equilibrium. No significant gender differences were observed for the SNPs that were investigated. No significant difference was observed in the allele or genotype frequencies as well.

Conclusion: COMT gene doesn't appear to be a risk factor in this population of schizophrenia patients in Turkey.

Keywords: COMT, schizophrenia, association, Turkish population, polymorphism, SNP

Özet: Amaç: Katekol-o-metiltransferaz (Catechol-O-methyltransferase-COMT) şizofrenide önemli bir molekül olan dopamin gibi katekolaminlerin katabolizmasındaki anahtar moleküldür. İşlevi ve yerleşimi nedeniyle COMT geni, şizofreni için güçlü bir aday gendir. Bu çalışmanın amacı; popülasyonumuzdaki şizofreni ile 3 COMT tek nükleotit polimorfizminin (single nucleotide polymorphism-SNP) olası bağlantısını incelemektir. COMT enzim aktivitesi yaygın olarak bilinen Val158Met (rs4680) ve rs737865 ve rs165599 ile birlikte düzenlenmektedir.

Metod: Val158Met polimorfizmi (rs4680), rs737865 ve rs165599 bu çalışmanın hedef polimorfizmleridir. Çalışma, Türkiye'nin doğusunda yer alan Malatya ilinden 96 hasta (66 erkek ve 30 kadın) ve 100 sağlıklı kontrol (47 erkek ve 53 kadın) ile TaqMan genotipleme kitleri kullanılarak gerçekleştirilmiştir.

Bulgular: Çalışılan SNPlerden hiçbirisi ile şizofreni ve normal kontrol bireyleri arasında belirgin bağlantı bulunamamıştır. Hasta ve kontrol grupları Hardy-Weinberg dengesini sağlamaktadır. İncelenen SNPlerde cinsiyetlere yönelik bir farklılık görülmemiştir. Alel ve genotip frekansları arasında da belirgin bir farklılık bulunamamıştır.

*Corresponding author: Ceren Acar: Inonu University, Department of Molecular Biology and Genetics, Malatya, Turkey, e-mail: ceren.acar@inonu.edu.tr

Mustafa Mert Sözen: Inonu University, Department of Molecular Biology and Genetics, Malatya, Turkey, e-mail: rmhamms@hotmail.com

Harika Gözükara: Inonu University, Department of Biostatistics and Medical Informatics, Malatya, Turkey, e-mail: harika.gozukara@inonu.edu.tr

Kübra Orman: Inonu University, Department of Psychiatry, Malatya, Turkey, e-mail: kubradiltemiz@hotmail.com

Şükrü Kartalacı: Inonu University, Department of Psychiatry, Malatya, Turkey, e-mail: sukrü.kartalaci@inonu.edu.tr

Sonuç: COMT geni çalıştığımız Türk hastalardan oluşan popülasyonda risk faktörü olarak görünmemektedir.

Anahtar Kelimeler: COMT, şizofreni, bağlantı, Türk popülasyonu, polimorfizm, SNP

DOI 10.1515/tjb-2015-0002

Received July 1, 2014; accepted January 28, 2015

Introduction

Schizophrenia is a complex and debilitating psychiatric disorder affecting 1% of the population worldwide and it is characterized by negative and positive symptoms as well as cognitive disturbances. While reduced emotion, motivation and reduced hedonia are included in the negative symptoms, positive symptoms include hallucinations, delusion and paranoia. Failure to maintain attention and deficits in short-term memory are the part of cognitive symptoms. Genetics plays an important role in the etiology of the disease with a heritability rate of ~80% [1-4]. There are several studies about the molecular genetic data and functional candidate genes for schizophrenia have been published [3]. But still genomic architecture underlying the disease is not explained clearly.

There have been many theories of schizophrenia but the oldest and the most enduring one is the dopamine hypothesis which essentially states that certain dopamine neuronal pathways within the brain are over active in this illness. Dopamine dysregulation may account for the positive and negative symptoms of the disease [5].

Catechol-O-methyl transferase (COMT) is the key enzyme in the catabolism of neurotransmitter dopamine. The COMT gene has been mapped to 22q11.2 [6,7]. Both linkage and association studies implied that this locus is related with schizophrenia [8,9]. The microdeletions in this region are associated with a number of syndromes including Velocardiofacial syndrome (VCFS), Di George Syndrome (DGS) [7]. It has been reported that some schizophrenics carry the features of VCFS. VCFS patients have a variety of congenital abnormalities, learning difficulties and some of the patients have psychosis [8]. Due to its genomic location and its function in the catabolism of dopamine COMT is considered as a strong candidate gene for schizophrenia.

There are two forms of COMT- soluble form (S-COMT) and membrane bound form (MB-COMT). The latter is expressed in brain neurons and soluble form found in cytoplasm is mostly expressed in other tissues such as

liver and kidney [10]. MB-COMT has approximately 10 fold affinity to dopamine and noradrenaline than S-COMT [11]. A functionally common SNP *rs4680*, a G→A substitution in exon 4 causes a Val/Met conversion at codon 158 of MB-COMT has a functional effect on enzyme activity. Met allele causes low enzyme activity. In a detailed study association between schizophrenia and COMT 12 SNPs were studied by Shifman et al in Israel population and three SNPs (*rs4680*, *rs737865* and *rs165599*) were found to be associated significantly [12]. These SNPs were also screened in Irish population and *rs4680* showed an association even though other two SNPs did not [13]. In addition Li et al found haplotype association with two other SNPs (*rs740603* and *rs4118*) in Han Chinese population [14]. However the association of *rs4680* with schizophrenia is controversial. Several studies in different populations failed to find an association between *rs4680* of COMT and schizophrenia [1,2,7,15]. Since a number of studies including different populations found an association for the SNPs *rs4680*, *rs737865* and *rs165599*, we decide to study these that weren't studied in Turkish population earlier, to get more insightful knowledge about the COMT variations in our group of patients.

In the present work we aimed to study the association of schizophrenia and three COMT SNPs *rs737865*, *rs4680* and *rs165599* in 96 nonrelated schizophrenia patients - with a family history at least one family member was also affected- from Malatya region on the eastern part of Turkey. We had 100 normal control samples with no family history of schizophrenia.

Materials and Methods

Subjects

The present study was performed in the Department of Psychiatry of Inonu University Medical School and Molecular Biology and Genetics Department. Ninety-six patients diagnosed with schizophrenia according to the Turkish version of the Structured Clinical Interview for the DSM-IV (SCID-IV) [16] and 100 healthy control subjects were included in the present study. 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 disorder, schizoid

disorder, and paranoid personality disorder.

Samples from healthy Turkish individuals were collected from volunteers. 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. The present study was carried out in accordance with the Declaration of Helsinki guidelines and was approved by the Local Ethics Committee. All participants were informed about the study protocol and provided written informed consent prior to the study.

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. A

coding system was applied to our blood and DNA samples in order to protect the confidentiality of the subjects. Samples were genotyped by using TaqMan SNP genotyping assays of Applied Biosystems (C_25746809_50 for *rs4680*, C_2255420_10 for *rs737865* and C_2255335_10 for *rs165599*).

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 Pearson's chi-squared test or Likelihood Ratio test was used to compare genotype and allele frequencies between cases and control group, and between genders. The level of significance was set at $p < 0.05$.

Table 1: Genotypes, alleles, and frequency distributions of COMT polymorphism *rs737865* in control and patients with schizophrenia in a Turkish population.

		Genotypic association						χ^2	p	Allelic association				χ^2	p
		AA		AG		GG				A		G			
		n	%	n	%	n	%			n	%	n	%		
All	Case	34	35.4	48	50.0	14	14.6	0.960	0.619	116	60.4	76	39.6	0.395	0.530
	Control	37	37.0	53	53.0	10	10.0			127	63.5	73	36.5		
Male	Case	18	27.3	37	56.1	11	16.7	0.599	0.741	73	55.3	59	44.7	0.409	0.523
	Control	16	34.0	24	51.1	7	14.9			56	59.6	38	40.4		
Female	Case	16	53.3	11	36.7	3	10.0	2.602	0.272	43	71.7	17	28.3	0.391	0.532
	Control	21	39.6	29	54.7	3	5.7			71	67.0	35	33.0		

Table 2: Genotypes, alleles, and frequency distributions of COMT polymorphism *rs4680* in control and patients with schizophrenia in a Turkish population.

Genotypic association								χ2	p	Allelic association				χ2	p
AA		AG		GG		A				G					
n	%	n	%	n	%	n	%			n	%				
All	Case	17	17.7	53	55.2	26	27.1	2.734	0.255	87	45.3	105	54.7	2.316	0.128
	Control	27	27.0	52	52.0	21	21.0			106	53.0	94	47.0		
Male	Case	10	15.2	38	57.6	18	27.3	2.279	0.320	58	43.9	74	56.1	1.884	0.170
	Control	11	23.4	28	59.6	8	17.0			50	53.2	44	46.8		
Female	Case	7	23.3	15	50.0	8	26.7	0.450	0.798	29	48.3	31	51.7	0.310	0.578
	Control	16	30.2	24	45.3	13	24.5			56	52.8	50	47.2		

Table 3: Genotypes, alleles, and frequency distributions of COMT polymorphism *rs165599* in control and patients with schizophrenia in a Turkish population.

		Genotypic association						χ^2	p	Allelic association				χ^2	p
		AA		AG		GG				A		G			
		n	%	n	%	n	%			n	%	n	%		
All	Case	33	34.4	46	47.9	17	17.7	0.463	0.793	112	58.3	80	41.7	0.290	0.591
	Control	39	39.0	44	44.0	17	17.0			122	61.0	78	39.0		
Male	Case	23	34.8	29	43.9	14	21.2	0.696	0.706	75	56.8	57	43.2	0.541	0.462
	Control	20	42.6	18	38.3	9	19.1			58	61.7	36	38.3		
Female	Case	10	33.3	17	56.7	3	10.0	0.624	0.732	37	61.7	23	38.3	0.027	0.870
	Control	19	35.8	26	49.1	8	15.1			64	60.4	42	39.6		

Results

The distribution of allele and genotype frequencies of *rs737865*, *rs4680* and *rs165599* are shown in the Tables 1, 2 and 3 respectively. The genotype frequencies in both the patient and control groups satisfied the Hardy–Weinberg equilibrium. We couldn't find a significant difference between our patient and control groups. No association was observed in different genders for both alleles and genotypes. Our results indicate that three COMT polymorphisms we studied has no influence on the molecular pathology of schizophrenia in our patient group.

Discussion

In the present study we investigated the association of COMT SNPs *rs737865*, *rs4680* and *rs165599* with schizophrenia. To our knowledge this is the first report from our homogenous population from Malatya region that includes these three SNPs of COMT gene. We failed to find an association between these SNPs and schizophrenia.

Previous studies mostly focused on *rs4680*-the functional Val158Met polymorphism-in different populations, since it causes a significant reduction in COMT enzyme activity [1]. But the results from the association studies performed with the populations worldwide are inconsistent. The inconsistency of the results shows the need for the results to be replicated in different populations from different geographic parts of the world. The controversy may also be cleared by studying the SNPs located in 22q11 other than *rs4680*.

Schizophrenia is a multifactorial disease and there are several reports about the lack of association between COMT variants and schizophrenia and our results are consistent with these results [1,2,7,15]. The possibility of other genes that is modifying the COMT function or expression levels of the gene or the presence of other candidate genes should always be considered since schizophrenia is a multifactorial disease.

Previous studies targeted COMT as a candidate gene for schizophrenia and reported association between COMT polymorphisms and the negative symptoms of schizophrenia [3]. In a study performed by Li et al, they suggested that The COMT gene may be associated with negative symptoms in Han Chinese schizophrenic patients, especially females [14]. Sazci et al reported the association of *rs4680* with their patient group (297 patients and 341 control) from a particular hospital in Istanbul, Turkey [17], on the contrary we failed to find a significant association with neither of the SNPs we studied. Our patient population was established only from Malatya region of Turkey. Although Sazci et al studied Turkish population their group didn't include the other SNPs of COMT gene. So our study is the first one to report the relationship between Turkish schizophrenia patients and COMT SNPs *rs737865* and *rs165599* which were found to be associated with schizophrenia in other populations.

The studies reported by Shifman et al with a large case-control sample a highly significant association was shown between COMT and schizophrenia [12]. This study included two non coding SNPs (*rs737865* and *rs165599*) at either end of the COMT gene as well as *rs4680*. Along with Shifman et al results Bray et al also pointed the importance of SNPs in and around COMT gene other than *rs4680* should be considered to identify the risk variants in schizophrenia [18].

This study had some limitations one of which is the number of patients we recruited and the other is the number of SNPs we had tested. The number of polymorphisms in the 35 kb region, where COMT is located, is more than 50 [14]. There may be sexual dimorphism between COMT variants and genetic predisposition to schizophrenia and number of the female and male samples in our sample group may not be enough to see that difference significantly [12,17,19,20]. But on the other hand when the population size of Malatya is taken into account the size of our patient number maybe reasonable and the molecular pathology underlying the disease maybe related with other dopamine related pathways/molecules other than COMT or different molecules like adenosine, GABA, glutamate and related genes.

In conclusion we didn't find any association between three SNPs we tested and schizophrenia in our patients. The distributions of alleles and genotypes were similar and there weren't any significant gender differences unlike reported by others. However further studies including different SNPs with a large number of case-control groups are required to identify the relationship between the symptoms of the patients and COMT variants.

Ethical issues: Malatya Clinical Research Ethic Board approved our study. Ethical board report is given as an attached form.

Acknowledgements: Funding for this study was provided by İnönü University Scientific Research Projects Unit. The funding source had no further role in study design, collection, analysis and the interpretation of data, in the writing of the report and in the decision of paper to submit for publications to any journals.

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

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