İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü Sağlık Bilimlerinde İleri Araştırmalar Dergisi
Istanbul University Institute of Health Sciences Journal of Advanced Research in Health Sciences

Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2020, Cilt 3, Sayı 3
DOI: 10.26650/JARHS2020-789794 Research Article / Araştırma Makalesi

Role of *CYP3A4*1B* Gene Variant In Substance Use Disorder

*Madde Kullanım Bozukluğunda CYP3A4*1B Gen Varyantının Rolü*

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ABSTRACT

Objective: This study aims to find out the possible association between the *CYP3A4*1B* gene variant (rs2740574) and substance use disorder susceptibility in the Turkish population.

Materials and Methods: 158 patients with substance use disorder and 100 healthy individuals matched for gender, age, and ethnicity were enrolled in the study. Genotyping was analyzed with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using the MboII restriction endonuclease. The association between the *CYP3A4*1B* variant and substance use disorder was analyzed using SPSS 21 and de-Finetti program.

Results: The *CYP3A4* gene (MboII gene variant in 5' promoter region) genotype distributions of substance use disorder patients were not significantly different from the healthy controls. When the substance use and control groups were compared in terms of allele frequency, increased G allele frequency was observed in *CYP3A4* variants in the substance use group (p: 0,042).

Conclusion: This is the first study that investigates the association between the MboII gene variant in *CYP3A4* gene 5'promoter region and substance use disorder in the literature. It was demonstrated that an increased G allele existed in Turkish substance use disorder patients. Plans have been made to research the other variants of the *CYP3A4* gene in the future.

Keywords: *CYP3A4*, substance abuse, MboII endonuclease, drug metabolism, addiction

ÖZ

Amaç: Bu çalışma, Türk popülasyonunda *CYP3A4*1B* gen varyantı (rs2740574) ile madde kullanım bozukluğuna yatkınlık arasındaki olası ilişkiyi bulmayı amaçlamaktadır.

Gereç ve Yöntem: Çalışmaya madde kullanım bozukluğu olan cinsiyet, yaş ve etnik köken açısından eşleştirilmiş 158 hasta ile 100 sağlıklı birey alındı. Genotipleme, polimeraz zincir reaksiyonu restriksiyon parça uzunluğu polimorfizmi (PCR-RFLP) yöntemi MboII restriktif endonükleaz enzimi kullanılarak yapılmıştır. *CYP3A4*1B* varyantı ile madde kullanım bozukluğu arasındaki ilişki SPSS 21 ve de-Finetti programı kullanılarak analiz edilmiştir.

Bulgular: Madde kullanım bozukluğu hastalarının *CYP3A4* geni (5 'promoter bölgesindeki MboII gen varyantı) genotip dağılımları, sağlıklı kontrollerden anlamlı olarak farklı değildi. Allel sıklığı açısından madde kullanım ve kontrol grupları karşılaştırıldığında, madde kullanım grubunda *CYP3A4* varyantlarında G allel sıklığında artış gözlendi (p: 0,042).

Sonuç: Bu çalışma literatürde *CYP3A4* geni 5'promoter bölgesindeki MboII gen varyantı ile madde kullanım bozukluğu arasındaki ilişkiyi araştıran ilk çalışmadır. Türk popülasyonunda madde kullanım bozukluğu hastalarında artmış bir G allelinin mevcut olduğu gösterilmiştir. Gelecekte, *CYP3A4* geninin diğer varyantlarının araştırılacağı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *CYP3A4*1B*, madde bağımlılığı, MboII endonükleaz, ilaç metabolizması, bağımlılık

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Submitted/Geliş tarihi: 03.09.2020
Accepted/Kabul Tarihi: 08.10.2020

Citation/Atıf: Capar G, Senturk Ciftci H, Pehlivan S. Role of *CYP3A4*1B* gene variant in substance use disorder. Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2020; 3(3): 130-134. https://doi.org/10.26650/JARHS2020-789794

INTRODUCTION

Substance use refers to harmful or dangerous uses of alcohol and psychoactive substances that includes illicit drugs (1). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria; substance use disorder causes important clinical and functional disorders such as health problems, disability and failure to fulfill the main responsibilities at school or at home (2). In the World Drug Report (2018) it is stated that approximately 275 million people in the world had used drugs at least once in 2016, corresponding to about 5.6 percent of the population aged 15-64. 31 million people who use drugs have substance use disorder (3). Substance use disorder (addiction) is a complex process with physical, spiritual, and social dimensions. It can be said the factors that affect the structure of addiction are genetic and environmental factors in both genders. Factors such as the rate and sensitivity of metabolism in the body are genetic factors, but factors such as the desire for love and respect in society constitute environmental factors (4).

Drug metabolism concerns many parts of the body, including the liver, intestinal wall, lungs, kidneys, and plasma. As the main site of drug metabolism, the liver enzymatically converts fat-soluble compounds into more water-soluble compounds to remove and detoxify xenobiotics (foreign drugs or chemicals). Drug metabolism is achieved by phase I reactions, phase II reactions, or both. The most common phase I reaction, oxidation, is catalyzed by the CYP system (5). Cytochrome P450 (CYP) enzymes metabolize about 70% of drugs in clinical use (4). There are many different subfamilies in the cytochrome P450 family. One of them is the CYP3A subfamily with isoenzymes CYP3A4, CYP3A5, CYP3A7, and CYP3A43 (6). CYP3A4 is the most common P450 enzyme in the human liver (6,7). It constitutes 30% of the total P450 protein content and is also expressed in the prostate, breast, intestine, colon, small intestine, and brain (7,8). It shows a broad substrate specificity and is responsible for the oxidation of various non-structurally related compounds, including many therapeutic drugs, steroids, fatty acids, and xenobiotics (8). The *CYP3A4* gene on chromosome 7q21.3-q22.1 has 27.592 base pairs and 13 exons (9). The promoter region includes a basal transcription element (-35 to -50). Also, there is an AP-3 binding site in the 5 'untranslated region (UTR), a p53 binding motif (a specific DNA sequence in which protein p53 can bind), a hepatocyte nuclear factor-4 element, two hepatocyte nuclear factors- 5 elements, a glucocorticoid which is associated with the response element and an estrogen response element (8). Changes in the activity of the CYP3A4 enzyme alter the blood concentrations of the drugs which are metabolized and affect the form of treatment and toxicity of the drug that is taken to the body. Genetic polymorphisms that occur or exist in the gene encoding the CYP3A4 enzyme affect the expression of the gene (10). The Adenine-Guanine (A -> G) transition in the 5 'promoter of the *CYP3A4* gene was reported by Rebbeck et al in 1998 (10). This functional variant, also known as *CYP3A4*1B* (rs2740574), is known to alter the gene's transcriptional activity and thus the overall activity of CYP3A4. It has been reported to reduce its activity (11). It has been reported that this activity change can lead to serious toxicity or therapeutic failure by altering the relationship between dose and blood concentration of the pharmacologically active drug by affecting the metabolic rate of drugs (10).

The aim of this study is to determine whether there is a relationship between the *CYP3A4* gene (MboII gene variant in 5'promotor region) and substance use disorder sensitivity in the Turkish population.

MATERIALS AND METHODS

A total of 158 patients with substance use disorder and 100 people as a healthy control group were included in the study. The individuals in the control group were chosen from those who had a similar ethnic origin and had no relationship with each other in the same geographical region. In addition, people who had substance use disorders in family history and/or personal history were excluded. This study was approved by Istanbul University Local Ethics Committee (2015/1374). Blood samples were taken into EDTA tubes and stored at -20 ° C. DNAs were isolated from the collected blood by using the Genemark isolation kit. The forward and reverse primers (rs2740574) were prepared for PCR

amplification of the *CYP3A4* gene 5' promoter region. Then the *CYP3A4* genes were amplified with PCR from isolated genomic DNAs. The replication material (PCR product) contained the polymorphic region for Mboll (270 bp). Cutting products were analyzed by using uncut PCR products and DNA ladder in 3% agarose gel electrophoresis. Homozygous wild type DNA (genotype: M+/M+) produced 175 bp and 169 bp alleles, the homozygous variant type (genotype: M-/M-) produced 210 bp and 175 bp alleles and heterozygote genotype (genotype M+/M-) was recognized by showing 210 bp, 175 bp, 169 bp fragments in electrophoresis. The comparisons between the control and patient groups were performed using the chi-square test. The deviation of the groups from Hardy-Weinberg equilibrium and the results of the substance use disorder group were analyzed in terms of clinical parameters (12).

RESULTS

The *CYP3A4* genotypes and allele frequencies of 158 patients and 100 healthy control subjects were determined with the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method (Table 1). There was no significant difference in genotype frequencies between the control and substance use disorder groups, but there was a significant difference in allele frequency (p=0.042) (Table 1). While there was a deviation in both patient and healthy controls according to Hardy Weinberg equilibrium, no significant difference was found between the clinical parameters and genotypes in the substance use disorder group (Table 2).

DISCUSSION

It is necessary to concentrate on the field of scientific studies related to substance use and substance use disorder, which is an increasing problem nowadays. Factors that affect the process, which leads to substance use and substance use disorder, can be classified in many titles. CYP3A4, one of the P450 enzymes, is quantitatively the most important. (4,6,7). The expression and function of CYP3A4 vary greatly both among different individuals and within individuals. There are numerous environmental, genetic, and physiological factors that affect CYP3A4 expression and activity. (13). Functional variants of the gene encoding the CYP3A4 protein alter enzyme activity by affecting the expression at the gene level (10). Genetic variations of drug-metabolizing enzymes can significantly change the pharmacokinetic features of a drug. Thus, these

Table 1. *CYP3A4* gene Mboll variant results

CYP3A4	**Patient**	**Control**	**OR**	**CI 95%**	**p**
Genotype	**n=158**	**n=100**			
AA	(72.9%) 115	(83.0%) 83	0.547	0.292-1.027	0.081
AG	(19.6%) 31	(13.0%) 13	1.634	0.808-3.299	0.227
GG	(7.6%) 12	(4.0%) 4	1.973	0.617-6.298	0.367
Allel					
A	(82.6%) 261	(89.5%) 179	0.556	0.325-0.953	0.042
G	(17.4%) 55	(10.5%) 21			
HWEp	0.000	0.002			

HWEp: Hardy Weinberg Equilibrium; OR: Odds ratio; Fisher's exact test P value is significant p>0.05

Table 2. Comparison of genotypes in Substance Use Disorder and control group with clinical parameters

CYP3A4	**Normal (AA)**	**Mutant (AG+GG)**	**OR**	**CI 95%**	**p**
	n=115	**n=43**			
Sex Man/Woman	104/11	39/4	2.667	0.362-19.646	0.311
Age	29.31±7.9	28.09±6.8	-	-	0.433
Synthetic use	72/43	25/18	0.766	0.361-1.628	0.488
Psychotic condition	70/45	26/17	0.926	0.436-1.970	0.843
Smoke Yes/No	100/15	36/2	0.833	0.681-1.109	0.243

HWEp: Hardy Weinberg Equilibrium; OR: Odds ratio; Fisher's exact test P value is significant p>0.05

genetic variants are considered to be the main source of drug metabolism and drug response (efficacy and/or safety) differences between individuals (14 The A -> G transition in the 5 'promoter of the *CYP3A4* gene, one of the phenotypes affected by genetic variants, was reported by Rebbeck et al in 1998 and it is known that it changes the transcriptional activity and therefore the overall activity of CYP3A4 (9,10). This difference changes the drug metabolism, drug's blood levels, and drug dose adjustments that are used during treatment. It also changes the duration and effect of the drugs in the body, thus it widely affects the process that leads to substance use disorder in individuals (10). As of today, the *CYP3A4* gene (10 January 2019) is placed in the 13690 Pubmed article. There are only 44 articles that investigate the association between addiction and CYP3A4. These were published between 1999-2018. In previous studies, the association of the *CYP3A4*1B* variant with prostate cancer and ovarian cancer was investigated (15,16). It has been found that this functional variant may be associated with high tumor degrees in prostate cancer (13). There are studies about the *CYP3A4*1B* variant in atorvastatin users and tacrolimus users in the literature. (17,18). However, there has been no large-scale research on cannabinoid and synthetic cannabinoid users in the literature. We performed this study with 258 individuals, 158 of them had substance use disorder and 100 of them were healthy controls. For the first time, this study showed that there is a significant relationship in allele frequency and substance use disorder. In this study, the genetic differences of individuals with and without substance use disorder were investigated and it was shown whether or not there was a significant relationship between genotypes and alleles compared with clinical parameters (age, gender, marital status, cigarette smoking and quantity, psychotic state, history of the prison, etc).

CONCLUSION

The relation of the *CYP3A4* gene with the MboII gene variant was investigated in the literature for the first time in this study and it was shown for the first time that the frequency of G allele in individuals with Substance Use Disorder may have a predisposition role in Substance Use Disorder. Plans have been made to study the functional variants of the *CYP3A4* gene, which may be clinically relevant to diseases and which may guide the development of new diagnostic and therapeutic principles.

Hakem Değerlendirmesi: Dış bağımsız.

Peer Review: Externally peer-reviewed.

Etik Komite Onayı: Bu calışma icin etik komite onayı İstanbul Universitesi İstanbul Tıp Fakultesi Etik Kurulu'ndan alınmıştır. (2015/1374)

Ethics Committee Approval: This study was approved by the Ethical Committee of the Istanbul University Istanbul Faculty of Medicine. (2015/1374)

Bilgilendirilmiş Onam: Katılımcılardan bilgilendirilmiş onam alınmıştır.

Informed Consent: Written consent was obtained from the participants.

Yazar Katkıları: Çalışma Konsepti/Tasarım- G.Ç., S.P.; Veri Toplama-S.P.; Veri Analizi/Yorumlama-G.Ç., H.Ş.Ç., S.P.; Yazı Taslağı- G.Ç., H.Ş.Ç.; İçeriğin Eleştirel İncelemesi- S.P., H.Ş.Ç.; Son Onay ve Sorumluluk- G.Ç., H.Ş.Ç., S.P.; Malzeme ve Teknik Destek- S.P.; Süpervizyon- G.Ç., H.Ş.Ç., S.P.

Author Contributions: Conception/Design of Study- G.Ç., S.P.; Data Acquisition- S.P.; Data Analysis/Interpretation- G.Ç., H.Ş.Ç., S.P.; Drafting Manuscript- G.Ç., H.Ş.Ç.; Critical Revision of Manuscript-S.P., H.Ş.Ç.; Final Approval and Accountability- G.Ç., H.Ş.Ç., S.P.; Technical or Material Support-S.P.; Supervision- G.Ç., H.Ş.Ç., S.P.

Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir

Conflict of Interest: Authors declared no conflict of interest.

Finansal Destek: Bu çalışma, İstanbul Üniversitesi Bilimsel Araştırma Projeleri Birimi (BAP) tarafından desteklenmiştir. (Proje No: TLLO-2018-28001)

Financial Disclosure: This study was supported by Istanbul University Scientific Research Projects Unit (BAP). (Project No: TLLO-2018-28001)

REFERENCES

1. Substance abuse. World Health Organization. https://www.who.int/topics/substance_abuse/en/ (Accessed January 17, 2019)
2. Volkow ND, Koob GF, Mclellan AT. Neurobiologic Advances from the Brain Disease Model of Addiction. N Engl J Med 2016;374(4):363-71.
3. World Drug Report 2018 http://www.unodc.org/wdr2018/prelaunch/WDR18_Booklet_1_EXSUM.pdf (Accessed January 17, 2019)
4. Werk AN, Cascorbi I. Functional Gene Variants of *CYP3A4*. Clin Pharmacol Ther 2014;96(3):340-8.
5. McDonnell AM, Dang CH. Basic review of the cytochrome p450 system. J Adv Pract Oncol 2013;4(4):263-8.
6. Chen L, Prasad GVR. *CYP3A5* polymorphisms in renal transplant recipients: influence on tacrolimus treatment. Curr Pharmacogenomics Person Med 2018;11:23-33.
7. Qin S, Liu D, Kohli M, Wang L, Vedell PT et al. TSPYL Family Regulates *CYP17A1* and *CYP3A4* Expression: Potential Mechanism Contributing to Abiraterone Response in Metastatic Castration-Resistant Prostate Cancer. Clin Pharmacol Ther. 2017;104(1):201-10.
8. Keshava C. *CYP3A4* Polymorphisms--Potential Risk Factors for Breast and Prostate Cancer: A HuGE Review. Am J Epidemiol. 2004;160(9):825-41.
9. Uçkun Z, Baskak B, Özdemir H, Özel-Kızıl E, Devrimci-Özgüven H et al. Genotype and Allele Frequency of CYP3A4 -392A>G in Turkish Patients with Major Depressive Disorder. Turk J of Pharm Sci 2018;15(2):200-06.
10. Cavalli SA, Hirata MH, Hirata RD. Detection of MboII polymorphism at the 5' promoter region of *CYP3A4*. Clin Chem 2001;47:348-51.
11. Veiga MG, Felizi RT, Reis DG, Carelli Filho I, Fernandes CE et al. The Influence of *CYP3A4* Polymorphism in Sex Steroids as a Risk Factor for Breast Cancer. Rev Bras Ginecol Obstet 2018;40(11):699-704.
12. Mitoprot. https://ihg.gsf.de/cgi-bin/hw/hwa1.pl (Accessed January 17, 2019)
13. Klein K, Zanger UM. Pharmacogenomics of Cytochrome P450 3A4: Recent Progress Toward the "Missing Heritability" Problem. Front Genet 2013;4:12.
14. Kiss Á, Menus Á, Tóth K, Déri M, Sirok D et al. Phenoconversion of CYP2D6 by inhibitors modifies aripiprazole exposure. Eur Arch Psychiatry Clin Neurosci 2019. 2020;270(1):71-82.
15. Pearce CL, Near AM, Van Den Berg DJ, Ramus SJ, Gentry-Maharaj A et al. Validating genetic risk associations for ovarian cancer through the international Ovarian Cancer Association Consortium. Br J Cancer. 2009;100(2):412-20.
16. Bangsi D, Zhou J, Sun Y, Patel NP, Darga LL et al. Impact of a genetic variant in *CYP3A4* on risk and clinical presentation of prostate cancer among white and African-American men. Urol Oncol 2006;24(1):21-7.
17. Rosales A, Alvear M, Cuevas A, Saavedra N, Zambrano T et al. Identification of pharmacogenetic predictors of lipid-lowering response to atorvastatin in Chilean subjects with hypercholesterolemia. Clinica Chimica Acta. 2012;413(3-4):495-501.
18. Tavira B, Coto E, Díaz-Corte C, Ortega F, Arias M et al. Pharmacogenetics of tacrolimus after renal transplantation: analysis of polymorphisms in genes encoding 16 drug metabolizing enzymes. Clin Chem Lab Med 2011;49(5):825-33.