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

Madde Kullanım Bozukluğunda CYP3A4*1B Gen Varyantının Rolü

Gazi Çapar¹, Hayriye Şentürk Çiftçi², Sacide Pehlivan²

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 bozukluğu yatkınlığı arasındaki ilişkiyi bulmayı amaçlamaktadır.

Gereç ve Yöntem: Çalışmaya madde kullanım bozukluğu olan cinsiyet, yaş ve etnik köken açısından eşleştirilmiş 158 hasta ile 100 sağlıklı birey alınmıştır. Genotipleme, polimeraz zincir reaksiyonu restriksyon parçaya uzunluğunda polimorfizmi (PCR-RFLP) yöntemi MboII restriktil kromatografi enzimi kullanılarak gerçekleştirildi. CYP3A4*1B varyantı ile madde kullanım bozukluğu arasındaki ilişki SPSS 21 ve de-Finetti programı kullanılarak analiz edildi.

Bulgular: Madde kullanım bozukluğu hastalarının CYP3A4 geni (5' promotor bölgesi) MboII gen varyantı) genotip dağılımları, sağlıklı kontrollerden anlamlı olarak farklı değişildi. Allel sıkılığı açısından madde kullanım ve kontrol grupları karşılaştırıldığında, madde kullanım grubunda CYP3A4 varyantlarında G allele sıkılığı artış gözlendi (p: 0.042).

Sonuç: Bu çalışma literatürde CYP3A4 geni 5' promotor bölgesindeki MboII gen varyantı ile madde kullanım bozukluğu arasındaki iliği araştırılarak ilk çalışmamızdır. Türk popülasyonunda madde kullanım bozukluğu hastalarında artış bir G allele'nin mevcut olduğu gösterilmiştir. Gelecekte, CYP3A4 geninin diğer varyantlarının araştırılacağı çalışmalarla ihtiyaç vardır.

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

| CYP3A4 | Patient n=158 | Control n=100 | OR | CI 95% | p |
|--------|--------------|--------------|----|--------|---|
| Genotype 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 | 0.000 | 0.000 | |

| CYP3A4 | Normal (AA) n=115 | Mutant (AG+GG) n=43 | OR | CI 95% | p |
|--------|-------------------|---------------------|----|--------|---|
| Sex Man/Woman | 104/11 | 39/4 | 2.667 | 0.362-19.464 | 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. 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.
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