Investigation of cytochrome p450 CYP1A2, CYP2D6, CYP2E1 and CYP3A4 gene expressions and polymorphisms in alcohol withdrawal

Alkol yoksunluğunda sitokrom p450 CYP1A2, CYP2D6, CYP2E1 ve CYP3A4 gen ekspresyonları ve polimorfizmlerinin araştırılması

Nazife Taşçıoğlu[1], Çetin Saatçi[1], Rabia Emekli[1], Gulten Tuncel[2], Ertuğrul Eşel[3], Munis Dundar[4]
[1]M.D., [4]Prof., Erciyes University, Faculty of Medicine, Department of Medical Genetics, Kayseri, Turkey https://orcid.org/0000-0002-9151-815X-https://orcid.org/0000-0003-2217-1460-https://orcid.org/0000-0003-1946-0130-https://orcid.org/0000-0003-0969-4611
[2]M.D., Near East University, Institute of Research Center of Experimental Health Sciences, Nicosia, Cyprus https://orcid.org/0000-0002-3332-471X
[3]Prof., Erciyes University, Faculty of Medicine, Department of Mental Health and Diseases, Kayseri, Turkey https://orcid.org/0000-0002-7051-5371

SUMMARY

Objective: This study was designed to investigate the changes in expression levels of CYP1A2, CYP2D6, CYP2E1 and CYP3A4 genes in patients treated for alcohol dependence and a control group. The frequency of selected polymorphisms of these genes that might be a risk factor for alcohol-dependence and may affect the treatment success is also investigated. **Method:** Blood samples were collected in the beginning and at end of treatment from inpatients taking alcohol dependence treatment and from the control group. DNA and RNA isolation were performed. Gene expression was quantified by quantitative PCR (qPCR) and RFLP technique was used for polymorphism studies. **Results:** No significant difference in the expression levels of studied genes in the patients before and after the treatment and between the control group was detected. However, a significant difference between the CYP1A2*F allele frequency in control and patient groups was observed. For CYP2D6*4 polymorphism, heterozygous genotypes have been detected in both patients and controls, whereas no CYP2D6*4/*4 was detected in either groups, indicating expression of a functional mRNA without reducing enzyme activity. No significant difference was found between the patient and control groups in the CYP2E1 c1/c2 polymorphism. CYP3A4*V polymorphism was not detected in either groups. **Discussion:** No difference in expression levels of studied genes in patients before and after treatment and in the control group was detected. A significant difference in the frequency of CYP1A2*1F c.734C>A polymorphism was detected between patient and control groups indicating a possible role of this allele as a risk factor for alcohol dependence.

Key Words: Alcohol, addiction, CYP, gene expression, polymorphism

ÖZET

Amaç: Bu çalışma, alkol bağımlılığı tedavisi gören hastalarda ve sigara kullanmayan kontrol grubunda CYP1A2, CYP2D6, CYP2E1 ve CYP3A4 genlerinin ekspresyon düzeylerindeki değişiklikleri araştırmak için tasarlanmıştır. Ek olarak, alkol bağımlılığı için risk faktörü olabilecek ve tedavi başarısını etkileyebilecek CYP1A2, CYP2D6, CYP2E1 ve CYP3A4 gen polimorfizmlerinin allel frekanslarının karşılaştırılması hedeflenmiştir. **Yöntem:** Yatarak tedavi gören ve alkol bağımlılığı tedavisi alan hastalardan ve kontrol grubundan tedavi başlangıcında ve sonunda perifer kan örnekleri alınarak DNA ve RNA izolasyonu yapıldı. Gen ekspresyonu kantitatif PCR (qPCR) ile ölçüldü ve polimorfizm çalışmaları için RFLP tekniği kullanıldı. **Bulgular:** Hastalarda çalışılan genlerin ekspresyon düzeylerinde tedavi öncesi ve sonrası ve kontrol grubu arasında anlamlı bir farklılık saptanmadı. Diğer yandan, kontrol ve hasta gruplarında CYP1A2*F allel frekansı arasında anlamlı bir fark gözlenmiştir. CYP2D6*4 polimorfizmi için, hem hastalarda hem de kontrollerde heterozigot genotipler tespit edilirken, her iki grupta da enzim aktivitesini azaltmadan fonksiyonel bir mRNA ekspresyonunu gösteren CYP2D6*4/*4 varyasyonu tespit edilmemiştir. CYP2E1c1/c2 polimorfizminde hasta ve kontrol grupları arasında anlamlı bir fark bulunmadı. CYP3A4*V polimorfizmine ise her iki grupta da saptanmadı. **Sonuç:** Çalışılan genlerin tedavi öncesi ve sonrası ve kontrol grubunda ekspresyon düzeylerinde farklılık saptanmadı fakat hasta ve kontrol grupları arasında CYP1A2*1F c.734C>A polimorfizminin sıklığında anlamlı bir fark tespit edildi, bunun da bu allelin alkol bağımlılığı için bir risk faktörü olarak olası bir rolüne işaret ettiğini düşünmekteyiz.

Anahtar Sözcükler: Alkol, bağımlılık, CYP, gen ekspresyonu, polimorfizm

(Turkish J Clinical Psychiatry 2021;24:298-306)
DOI:10.5505/kpd.2021.60938
The arrival date of article: 03.12.2020, Acceptance date publication: 03.04.2021

INTRODUCTION

Alcoholism as a medical concept was described by Magnus Huss in 1849, whereas addiction or dependence as a biopsychosocial disease was explained by Jellinek in 1960. Psychoactive substances are listed as; alcohol, hemp (marijuana, cannabis, poppy), opiates (heroin, codeine, morphine), cocaine, amphetamines (speed, crystal), hallucinogens (acid, LSD, PCP), tranquilizers (xanax, valium, atarax, diazem), volatile substances (thinner, bali, sprays), steroids, nicotine and caffeine. They can be taken orally, by injection or by inhalation. Alcohol and nicotine are the most common psychoactive substances worldwide (1).

According to the World Health Organization (WHO), there are 2 billion alcohol users worldwide. Currently, understanding the genetic basis of alcoholism is an important step in developing adequate prevention strategies and personal treatments (2). Although genetic factors are clearly known to play a role in alcoholism, the specific genes involved are difficult to detect and it is genetically complex, showing no clear form of Mendelian inheritance (3). Metabolic gene variants, smoking and alcohol consumption are important upper digestive system cancer (UDTC) risk factors. However, gene-gene and gene-environment interactions still remain uncertain (4). Also; the pathogenesis of alcoholic liver disease depends not only on the toxic effects of alcohol, but also on the complex interaction of the host and environmental factors. Therefore, genetic predisposition, concomitant diseases and behavioral factors all play a role in individual variations in disease outcomes. Alcohol-related epigenetic factors are also important in pathogenesis as reversible but hereditary gene expression changes, histone modulation, DNA methylation, and micro RNAs, and may serve as diagnostic markers and therapeutic targets in the future. Early diagnosis and multidisciplinary interventions are essential to ensure long-term abstinence and prevent alcoholic cirrhosis (5).

Alcohol addiction manifests itself with increased tolerance (increased alcohol & substance consumption of the person over time) and withdrawal symptoms (physical symptoms such as tremor, nausea, vomiting, insomnia, and headaches if alcohol & substance are not taken). Loss of control, memory loss, behavioral changes and loss of functionality are also observed (2).

Cytochromes 450 (CYPs) are an enzyme superfamily that are involved in the metabolism of various compounds in the body, including alcohol. Therefore, changes in the expression levels or structural changes in the protein caused by gene polymorphisms might affect how the body metabolize alcohol, directly affecting an individual's tolerance. CYP superfamily is composed of gene families including CYP1, CYP2 and CYP3.

CYP1A2 is the only hepatic member of CYP1 family and is encoded on the 15q24.1 region of chromosome 15 in humans. CYP1A1 and CYP1B1 are other members of this gene family. CYP1A1 is the largest extrahepatic form of CYP enzymes in humans. Besides detoxification, members of the CYP1 family are often responsible for the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) and aromatic amines, which are associated with chemical carcinogenesis (6, 7).

The human CYP2 family is quite diverse and contains many important drug-metabolism CYPs. CYP2B6, CYP2D6 and CYP2E1 are the most functional enzymes in the family (8). There are some studies stating a possible relationship between CYP2D6 enzyme and the side effects of serious psychiatric diseases and antipsychotics. CYP2D6 is a 497 amino acid protein and is encoded by the approximately 4.5 kb long CYP2D6 gene located on the long arm of chromosome 22 (22q13.1) (9). Although CYP2E1 is one of the most common hepatic CYPs, only a few drugs are metabolised through this enzyme. But in terms of toxicology, CYP2E1 has an important role. It is encoded on the q terminal of chromosome 10 in position 10q24.3. CYP2E1 polymorphisms result in significant phenotypic differences as they affect expression and are also associated with alcohol and nicotine addiction (10). Cytochrome P450 (P450) enzymes play a role in the metabolism of carcinogens as well as drugs, steroids, vitamins and other chemical classes. They, especially P450 2E1, also oxidize ethanol to acetaldehyde and then to acetic

acid. The role of P450 2E1 in cancer is complex in that P450 2E1 is also induced by ethanol, P450 2E1 is involved in the bioactivation and detoxification of a number of chemical carcinogens, and ethanol is an inhibitor of P450 2E1 (11).

Cytochrome CYP2E1 gene is one of the candidate genes for alcohol dependence. Four single nucleotide polymorphisms of the CYP2E1 gene (CYP2E1*1D, *5B, *6 and *1B) have been previously associated with alcohol dependence in other ethnic populations (12). CYP3A4, localised on 7q22.1 region of chromosome 7, is the sixth most enzyme found in the human liver among other CYP proteins in human liver and small intestine at the mRNA level (13, 14). It is known to play a very important role in the metabolism of xenobiotics. It is estimated that it is responsible for metabolism of approximately 50% of the drugs used in the clinic (15).

Evaluation of genetic changes of drug metabolizing enzymes in the human genome contributes to the understanding of inter-individual and inter-ethnic variability for clinical response to potential toxic substances (16). Drugs and toxic substances that are accumulated in the blood are metabolized by CYP450 enzymes. Genes encoding these enzymes have single nucleotide variations and small deletions that affect the functionality of enzymes by increasing or decreasing their activity, which is very important in pharmacogenetics (17). In this context, this study was designed to investigate the changes in expression levels of CYP1A2, CYP2D6, CYP2E1 and CYP3A4 genes in patients treated for alcohol dependence and a control group. The frequency of selected polymorphisms of these genes that might be a risk factor for alcohol-dependence and may affect the treatment success is also investigated.

METHOD

Study Design and Sample Collection

This in vitro study was carried out between the years of 2008 and 2010 with two groups consisting of a patient group with 50 volunteers, who were admitted to the Department of Psychiatry in our institution for alcohol addiction, and a control group was used. The control group comprised 23 volunteers, who were selected from male smokers in the same age range as the patient group considering that alcohol dependence is almost always observed with smoking habit. The study protocol was approved by Institutional Ethics Review Board (Approval no 09/209). Informed consent forms were signed by all participants.

Blood samples (9ml) were collected into EDTA tubes from patients before treatment (the first day they were hospitalized) and after treatment (28th day). Likely, 9ml of blood samples were taken from the control group into EDTA tubes.

DNA and RNA Isolation

Red cell lysis (155mM ammonium chloride (Merck 101145), 10mM potassium bicarbonate (Merck 104852), 1mM EDTA (ThermoFischer 15575020)) followed by leukocyte isolation was performed. Then DNA isolation was done using MagnaPure LC device according to the manufacturer's instructions. Rest of the blood was used for total RNA isolation with Qiagen Mini Kit and cDNA was synthesized from 1µg RNA using QIAGEN QuantiTect Reverse Transcription kit (205311) according to the manufacturer's instructions.

Gene Expression Analysis

QIAGEN OneStep RT-PCR kit (210215) was used for quantitative PCR (qPCR) analysis for CYP1A2, CYP2D6, CYP2E1 and CYP3A4 genes in Corbett Real-Time PCR machine using gene-specific primers and probes, which are shown in Supplementary Figure 1. Specific Beta-actin was used as an internal control. Results were analysed using Corbett Software quantification analysis.

Genotyping

Polymerase chain reaction (PCR) (Perkin Elmer, 9700) with gene specific primers was performed to amplify the polymorphic regions of CYP1A2, CYP2D6, CYP2E1, CYP3A4.

ApaI restriction enzyme (Fermentas, ER411) was used to digest PCR products of CYP1A2 to detect alleles with CYP1A2*1F c.734C>A (rs762551) polymorphism. Restriction products were run on 2% agarose gel. Homozygote C genotype was observed at 518bp, heterozygote AC genotype had three bands at 518, 312 and 206bp and homozygote A genotype generated bands at 312 and 206bp.

BstnI restriction enzyme (Fermentas, ER0551) was used to digest PCR products of CYP2D6 to detect alleles with CYP2D6*4 c.1934G>A (rs3892097) and CYP2D6*6 c.1795delT (rs5030655) polymorphisms. Restriction products were run on 2% agarose gel. For CYP2D6*4 c.1934G>A; homozygote G genotype was observed as 190 and 163bp bands, homozygote A genotype generated bands at 353bp. For CYP2D6*6 c.1795delT; homozygous wild type genotype had 190 and 163bp long fragments and homozygous mutants generated bands on 190, 139 and 23bp.

Two different restriction enzymes were used to digest PCR products of CYP2E1 to study alleles having CYP2E1 c1/c2 (5B*) (Rsa+/Pst+) polymorphisms (rs2031920/rs3813867). RsaI enzyme was used for c1 genotype determination and PstI enzyme was used for c2 genotype determination. Restriction products were then run on 4% agarose gel. For CYP2E1 c1; homozygote wild type genotype generated bands at 360 and 50bp, heterozygotes had three bands at 412, 360 and 50bp and homozygote mutants generated a band at 412bp. For CYP2E1 c2; homozygote wild type genotype generated a band at 410bp, heterozygote genotype was observed as three bands at 410, 290 and 120bp and homozygote mutant genotype was observed as two bands at 290 and 120bp.

BstNI restriction enzyme (Fermentas, ER0551) was used to digest PCR products of CYP3A4 to detect alleles with CYP3A4*5 c.653A>G (rs55901263) polymorphism. Restriction products were run on 4% agarose gel. Homozygote A genotype generated three bands with lengths 396, 121 and 75bp, heterozygote genotype was observed as four bands 517, 396, 121 and 75bp and homozygous mutant genotype generated bands at 517bp and 75bp. Restriction enzymes and band sizes of studied polymorphisms are given in Supplementary Table 2.

Statistical Analysis

In our study, two techniques were used to analyze the effect of the aforementioned CYP genes on alcohol addiction. For the analysis of polymorphism experiments, Chi-square test was carried out to determine the difference between allele frequencies of genes between the three groups (patients before treatment, patients after treatment, control). For gene expression analysis, on the other hand, logarithms were taken to ensure the normal distribution of the data. The geometric mean of the data was taken within the 95% confidence interval. In comparison of patient and control samples between groups, covariance analysis and independent samples T tests were applied. For the comparison of the expression data before and after the treatment of the patients, covariance analysis and paired sample T tests were used.

RESULTS

Gene expression

Covariance analysis and independent samples T tests were used to evaluate the expression levels of CYP1A2, CYP2D6, CYP2E1 and CYP3A4 genes between the patient and control groups. No statistically significant difference was detected in the expression levels of these genes ($p > 0.05$). Also, covariance analysis and dependent sample T tests were used to compare the expression levels before and after treatment in the patient group. There was no statistical difference in gene expression levels before and after treatment in the patients ($p > 0.05$).

*CYP1A2*1F c.734C>A (rs762551) polymorphism*

Chi-square test was used to compare the allele frequencies of CYP1A2 gene *1F polymorphism between alcohol addicted patient groups and control group. The difference between the patient and control groups was found to be significant ($p < 0.05$). Frequency of the CYP1A2*1F polymor-

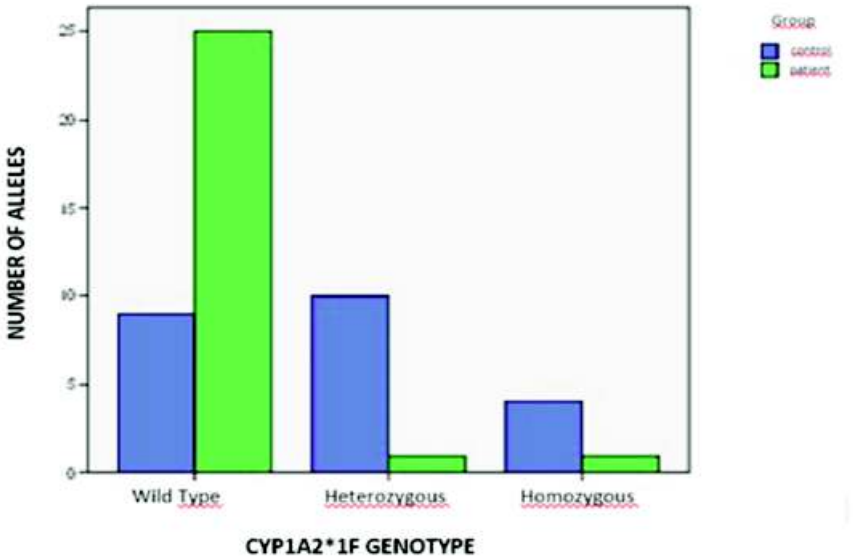

Figure 1. Frequency of the CYP1A2*1F allele in control and patient groups are shown in this graph.

phism increased significantly in the control group compared to the patient group, which is indicated in Figure 1.

*CYP2D6*4 c.1934G>A (rs3892097) and CYP2D6*6 c.1795delT (rs5030655) polymorphisms*

No significant difference between the patient and control groups was detected (p>0.05) for CYP2D6*4 c.1934G>A (rs3892097) and CYP2D6*6 c.1795delT (rs5030655) polymorphisms.

CYP2E1 c1/c2 (5B) (Rsa+/Pst+) (rs2031920/rs3813867) polymorphisms*

No significant difference between the patient and control groups was detected (p>0.05) for CYP2E1 c1/c2 (5B*) (Rsa+/Pst+) (rs2031920/rs3813867) polymorphisms.

*CYP3A4*5 c.653A>G (rs55901263) polymorphism*

CYP3A4 *5 polymorphism was not observed in alcohol-addicted patients and control groups so no statistical analysis was carried out for this polymorphism.

DISCUSSION

Alcohol addiction is a disease that develops approximately 5 years after the first alcohol use starts and it takes around 15-20 years for the alcohol addict to apply for clinical treatment (18, 19). Determining the reasons of patients for applying to treatment, determining the features in the clinical treatment process, monitoring the follow-up features after clinical treatment with controlled-prospective studies will provide great improvements in the treatment of addiction (20). Interpersonal genetic variations in drug metabolizing enzymes affect the effect and toxicity of many drugs. CYP1A2, CYP2C9, CYP2C19 and CYP2D6 gene polymorphisms were characterized using high-resolution melt analysis (HRMA) in psychiatric follow-up patients as a preliminary preparation for personalized medicine. Advances in pharmacogenomic knowledge and molecular genetics are increasing rapidly, which is expected to provide new methodologies for predicting activity in drug metabolizing enzymes in the future (21).

In our study, CC, heterozygous (CA) and AA genotype percentages of patients with CYP1A2*F were determined as 92.6%, 3.7%, 3.7%, respectively and 39.1%, 43.5%, 17.4% in control group. The difference in genotype frequencies between the groups was found statistically significant. The reason for the inclusion of this gene in the study is that both the patient and the control individuals consist of smokers and also diazepamine, which is among the substrates of the CYP1A2 protein, is used in the treatment process in alcohol patients.

In this study, it is considered as a positive result that the patients are mostly in wild type genotype

Table 1. Primer sequences used for gene expression analysis and RFLP

Gene Polymorphism	Sense (Forward)	Antisense (Reverse)
CYP1A2*1F	5 -AGA AGC TCT GTG GCC GAG AAG G-3	5 CAA CCC TGC CAA TCT CAA GCA C-3
CYP2D6*4 1934 (G/A) CYP2D6*6 1795 (1 bp del)	5 -CCT GGG CAA GAA GTC GTC GGA CCA G-3	5 - GAG ACT CCT CGG TCT CTC G-3
CYP2E1 (c1/c2)	5 -CCC GTC GAG TCT ACA TTG TCA -3	5 - TTC ATT CTG TCT TCT AAC TGG-3
CYP3A4*V(A/G)	5 -AAC AGG ACG TGG AAA CAC AAT -3	5 - CTT TCC TGC CCT GCA CAG -3

(CC) in terms of CYP1A2 *1F polymorphism as variant (A) is associated with increased enzyme induction and diazepamine given to alcohol addicts to reduce withdrawal symptoms may not have reached the therapeutic dose. CYP1A2 is also involved in the process of metabolic activation of chemical toxins (found in cigarette smoke) to carcinogens. Metabolic activity of CYP1A2 shows significant variation from genetic factors, environmental factors and drug-drug interactions (22,23).

In the literature, it has been reported that CYP1A2 can be induced or inhibited (brought to open or closed position) by many mediators or food-drug interactions (24,22).

In a study conducted by Herken et al. in psychiatric patients in our country, Turkey, the frequency of CYP2D6 *4 polymorphism was 8.1% and it was found to be 11% in another study conducted by Aynacıoğlu et al. in the Turkish population for screening purposes (23,25).

In our study, the frequency of CYP2D6 * 4 allele in the patient group was found to be 5.3% and 13.1% in the control group, which is compatible with previous findings in the literature (23, 25). The results of retrospective analysis in psychiatric patients treated with drugs metabolised by CYP2D6 show that genotyping improves treatment success, prevents adverse drug effects and reduces the cost of treatment. Therefore, it is very important to determine the CYP2D6 genotype and / or phenotype of the patient before the administration of drugs frequently used in psychiatry such as antidepressants or antipsychotics (23).

Pharmacogenomics represents a potentially strong increase in the current standard of care for psychiatric patients. However, several biological and technical challenges should be considered to provide adequate clinical decision support for personalized prescribing and dose adjustment based on genomic data. This is particularly true for CYP2D6, which encodes for an important drug metabolizing protein that not only contains a large number of genetic variants that are known to affect enzyme function, but also exhibits a wide range of copies and a wide range of hybrid alleles in various

Table 2. Restriction enzymes and band sizes of studied polymorphisms.

Gene polymorphism studied	Restriction enzyme used	PCR Products	Normal genotype	Heterozygous genotype products	Homozygous mutant genotype
CYP1A2*1F	*Apa I*	518 bp	518 bp	518 bp 312 bp 206 bp	312 bp 206 bp
CYP2D6*4(C1934A)	*BstN I*	353 bp	190 bp 163 bp	353 bp 190 bp 161 bp	353 bp
CYP2D6*6(delT1795)	*BstN I*	353 bp	190 bp 163 bp	190 bp 163 bp 139 bp	190 bp 139 bp 23 bp
CYP2E1 c1	*Rsal I+/Pst I-*	410 bp	360 bp 50 bp	410 bp 360 bp 50 bp	410 bp
CYP2E1 c2	*Rsal I-/Pst I+*	410 bp	410 bp	410 bp 290 bp 120 bp	290 bp 120 bp
CYP3A4*V	*BstNI*	592 bp	396 bp 121 bp 75 bp	517 bp 396 bp 121 bp 75 bp	517 bp 75 bp

patient populations. Here we describe various challenges in accurate measurement and interpretation of data from CYP2D6 analyses. In order to overcome these difficulties, research should be continued for future pharmacogenomic applications of CYP2D6 in psychiatry (26).

Many patients give up taking antidepressant drugs because of their side effects. Genetic factors and psychological factors, including current state or trait anxiety, can explain the differences in side effect results. The aim of this study is to examine the relative contribution of genetic and psychological factors in people with antidepressant side effects. A low compliance was observed between the participants' CYP2D6, CYP2C19 and CYP2C9 phenotypes and antidepressant tolerability history. As a result; for this patient cohort, it was found that history of tolerance was not associated with changes in pharmacogenes, health anxiety or neuroticism in the treatment of serotonin reuptake inhibitor (SSRI) or seratonin / norepinephrine reuptake inhibitor (SNRI) (27).

CYP2D6 expression was analyzed in both patient and control groups in our study, and no statistical difference was found between the groups in terms of expression levels; however, we think that this gene may be important for alcoholism when the number of patients and controls is increased. As a result of CYP2D6 *4 polymorphism, a stop codon is formed and no functional CYP2D6 protein can be produced. In our study, heterozygous genotypes were detected in both patients and controls in terms of CYP2D6 *4 polymorphism and no CYP2D6 *4 homozygotes were present. Accordingly, a functional allele appears to continue mRNA expression without reducing enzyme activity.

When we evaluate the literature data and our results together, we think that the determination of cytochrome P450-2D6 enzyme activity, which is responsible for the metabolism of most of this group of drugs, before use of antipsychotic and antidepressants, can be beneficial in terms of predicting effects and side effects.

Among the various P450s, CYP2E1 attracts a lot of attention due to its role in the bioactivation and metabolism of many low molecular weight compounds such as ethanol, acetone, acetaminophen, isoniazid, and many procarcinogens, benzene, N-nitrosodimethylamine (NMDA) and citerin. Like other xenobiotic-metabolism enzymes, CYP2E1 polymorphisms also vary among different ethnic and social groups. In this study, when the groups were evaluated in terms of CYP2E1 *1A / *5B polymorphism, 90.9% (*1A / *1A), 9.1% (*1A / *5B) and 0% (*5B / *5B) in the patient group; In the control group, 95.5% (*1A / *1A), 4.3% (*1A / *5B) and 0% (*5B / *5B) genotype were detected. The rare *5B / *5B allele has not been detected among our participants. There is no statistical difference between the groups. Our results were found to be compatible with the literature (28). In a study investigating the frequency of CYP2E1 polymorphisms and gene profile in the Chinese Uighur population, 100 healthy volunteers' DNA were screened by PCR and sequencing for mutated alleles and genotypes and results revealed that there are important clinical results for the use of metabolized drugs in this population (16).

Our findings in the CYP2E1 expression levels showed no statistically significant difference when expression in the patient group was compared to the expression in the control group in the 95% confidence interval. However, although non-significant, increased expression values have been determined and we think that the difference may be significant if the number of patients are increased. The activity of CYP2E1 is altered by ethanol as well as by various physiological markers such as obesity, hunger and liver dysfunction. In the literature, studies related to alcohol consumption and expression of CYP2E1 in the blood indicated that heavy alcohol consumption is correlated with CYP2E1 expression (29). CYP2E1 is an effective enzyme for reactive oxygen production due to altered NADPH oxidase activity and high production of O2 and H2O2 radicals even in the absence of substrate (30). In another study, genetic relationship of CYP2E1 with alcohol dependence was examined in Taiwan population. 319 healthy individuals as control and 340 patients were compared and genotyping was performed for CYP2E1 gene SNPs and no genetic relationship has been found between CYP2E1 and alcohol dependence (12).

No statistically significant difference was found in this study when CYP2E1 gene expression was compared in alcohol addicts before and after treatment. This may be due to the lack of specific elements sensitive to ethanol in the CYP2E1 gene, as noted above. However, it is not clear why ethanol can induce CYP2E1 expression, but the absence of ethanol does not reduce the expression of this gene. Possible explanations for this may be that the duration of of alcohol dependence treatment (about 28 days) is not sufficient to reduce the level of CYP2E1 mRNA, or that patients who are in remission after this treatment have a high rate of about 88.2%.

In our study, CYP3A4*5 polymorphism was not detected in either the alcohol-dependent patient group or the control group. No epidemiological studies about CYP3A4 gene polymorphisms in the Turkish population is present in the literature. In other populations, frequency of CYP3A4*5 allele was found to be very low. So, the reason that were not able to detect this allele in our study is probably the low frequency of this allele and relatively small size of our study groups. CYP3A4 plays a very important role in the metabolism of xenobiotics. It is estimated that it is responsible for the metabolism of approximately 50% of the drugs used in the clinic. Since the active region of CYP3A4 is very wide and flexible, it enables many small molecules to be bound to the active area at the same time (4, 18).

Even though a relatively higher expression of CYP3A4 was detected in patients and control subjects compared to the expression levels of other genes (CYP1A2, CYP2D6 and CYP2E1) studied in the study; no statistically significant difference was found between the patient and control groups. Despite the lack of psychological or neurological studies regarding the CYP3A4 gene, many studies are available in the literature investigating its relationship with diseases such as cancer, hypertension and diabetes (31, 32).

Considering the drug metabolism rates of CYP1A2 (11%), CYP2D6 (19%), CYP2E1 (4%), CYP2A4 (36%), this study presents valuable data about the polymorphisms of these genes and the effect of these polymorphisms on gene expression levels in the studied groups. All pharmacogenetic changes occur in different ethnic groups and among their subpopulations at different frequencies. Due to these differences, it is very important to consider ethnic origin in both pharmacogenetic studies and pharmacotherapy. The importance of such pharmacogenetic approaches for the treatment of alcohol-addicted patients included in the study is clear. We think that cross-interactions of alcohol and drugs used for therapeutic purposes are important points to be considered in the treatment of such patients. The fact that the patients experience remission and are hospitalized at least a few times for treatment cause doubts about the success of these treatments. Today, with the development of pharmacogenetic science, the patient's genotype is determined and tailor-made treatment options are offered to the patient. In this context, polymorphisms of genes that show wide variation such as CYP1A2 and CYP2D6 should be screened for allele frequencies and risk calculations should be made for drug side effects in our society.

Further studies can be planned to include a larger cohort. Also, only alcohol-dependent patients were included in the study and patients using both alcohol and other addictive substances were excluded. A further study can be planned to include patients using other addictive substances alongside alcohol to evaluate the effect of polymorphisms and expression levels of these genes.

Correspondence address: Prof., Münis Dündar, Erciyes University, Faculty Of Medicine, Department Of Medical Genetics, Kayseri, Turkey dundar@erciyes.edu.tr

REFERENCES

1. Yenigün M. Alkol Tüketimi ve Tıp (Alcohol Consumption and Medicine) Haseki Dergisi, 2006 :3-1.

2. World Health Organization Regianol Office in Europe. Handbook foraction to reduce alcohol-related harm. Erişim: [www.euro.who.int/data/assets/pdf_file/0012/43320/E92820.pdf] , Erişim Tarihi:10 Aralık 2010.

3. Guilherme P, Homero P. The Role of Genetics in Alcohol Dependence. Rev. Bras. Psiquiatr., May 2004: vol.26 suppl.1, 54-58.

4. Zhao F, Su JF, Lun SM, Hou YJ, Duan LJ, Wang NC, Shen FF, Zhang YW, Gao ZW, Li J, Du XJ, Zhou FY. Association between polymorphisms in the CYP1A1, CYP2E1 and GSTM1 genes, and smoking, alcohol and upper digestivetract carcinomas in a high-incidence area of northern China. 2019;18:1267-1277.

5. Pár A, Pár G. Alcoholic liver disease: theroles of genetic-epigenetic factors and the effect of abstinence. 2019;160:524-532.

6. Ioannides C, Lewis DF Cytochromes P450 in thebioactivation of chemicals. Curr Top MedChem 2004:4;1767–1788.

7. Corchero J, Pimprale S, Kimura S, Gonzalez FJ. Organization of the CYP1A cluster on human chromosome 15: implications for gene regulation. Pharmacogenetics. 2001;11:1-6.

8. Karabulut HG. Farmakogenetik: Laboratuvardan Klinik Uygulamaya Türkiye Klinikleri J Med Sci 2010;30:5-15.

9. Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. Annu Rev Med 2006:57, 119–137.

10. Cai L, Yu SZ, Zhang ZF. Cytochrome P450 2E1 genetic polymorphism and gastric cancer in Changle, FujianProvince World J Gastroenterol 2001;7:792-795.

11. Peter Guengerich F, Avadhani NG. Roles of Cytochrome P450 in Metabolism of Ethanol and Carcinogens. 2018; 1032:15-35.

12. Huang CY, Kao CF, Chen CC, Kuo PH, Huang MC. No association of CYP2E1 genetic polymorphisms with alcohol dependence in Han Taiwanese population. 2018 Jul;117: 646-649.

13. Scott EE, Halpert JR. Structures of cytochrome P450 3A4. Trends Biochem Sci 2005: 30: 5–7.

14. Inoue K, Inazawa J, Nakagawa H, Shimada T, Yazamaki H, Guengerich FP, Abe T. Assignment of the human cytochrome P-450 nifedipine oxidase gene (CYP3A4) to chromosome 7 at band q22.1 by fluorescence insitu hybridization. Jpn J Hum Genet. 1992;37:133-138.

15. Özsoy SD. Erken ve Geç Alkol Yoksunluğunda HPA Eksen Aktivitesi, Nörosteroidler ve Bunların Agresyon Eğilimi ile İlişkisi, Tıpta Uzmanlık Tezi, Erciyes Üniversitesi Tıp Fakültesi Psikiyatri Anabilim Dalı, Kayseri 2005, 5-6.

16. Zhu L, He Y, Niu F, Yan M, Li J, Yuan D, Jin T. Polymorphisms of drug-metabolizingenzyme CYP2E1 in Chinese Uygur population. 2018;97: e9970.

17. SkadrićI., Tojković O. Defining Screening Panel Of Functional Variants of CYP1A1, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 Genes in Serbian Population. 20 December 2019.

18. Sılvestrı L, Sonzognı L, De Sılvestrı A. CYP Enzyme Polymorphısms and Susceptıbılıty to HCV-Related Chronıc Liver Disease and Liver Cancer Int. J. Cancer, 2003: 104, 310–317.

19. Lieber CS. The Discovery of the Microsomal Ethanol Oxidizing System and Its Physiologic and Pathologic Role Drug Metabolism Reviews 2004: Vol. 36, No. 3-4, 511-529.

20. Mırsal H, Kalyoncu A, Pektaş Ö. İlk Kez Tedavi Gören Alkol Bağımlılarının Sosyodemografik Özellikleri ve Erken Dönem Tedavi Sonuçları Yeni Sempozyum: 2000;38:112-115.

21. Yenilmez ED, Tamam L, Karaytug O, Tuli A. Characterization CYP1A2, CYP2C9, CYP2C19 and CYP2D6 Polymorphisms Using HRMA in Psychiatry Patients with Schizophrenia and Bipolar Disease for Personalized Medicine. 2018;21:374-380.

22. Finnström N, Ask B, Dahl ML. Intra-individual variation and sex differences in gene expression of cytochromes P450 in circulating leukocytes. The Pharmacogenomics Journal. 2002: 2, 111–116.

23. Herken H, Aynacıoğlu Ş, Esgi K, Vırıt O. Psikiyatri Hastalarında Sitokrom P450 2D6 Yavaş ve Ultra Hızlı Metabolizör Sıklıkları Türk Psikiyatri Dergisi 2001:12;83-88.

24. Torres FC, Cant´u AM, Berm´udez de Le´on M, Cisneros B, Zapata-Perez O, Lopez-Carillo L, Salinas JE, Albores A. CYP1A2 Phenotype and Genotype in a Population From the Carbon if erous Region of Coahuila, Mexico Toxicology Letters 2005: 156, 331–339.

25. Aynacıoğlu AŞ. Psikiyatrik Hastalıkların İlaçlarla Tedavisinde Farmakogenetiğin Önemi Klinik Psikiyatri Dergisi, 2001;4:249-252.

26. Jarvis JP, Peter AP, Shaman JA. Consequences of CYP2D6 Copy-Number Variation for Pharmacogenomics in Psychiatry. 2019;10:432.

27. Maggo S, Kennedy MA, Barczyk ZA, Miller AL, Rucklidge JJ, Mulder RT, Foulds JA. Common CYP2D6, CYP2C9, and CYP2C19 Gene Variants, HealthAnxiety, and Neuroticism Are Not AssociatedWith Self-Reported Antidepressant Side Effects. 2019;10:1199.

28. Ulusoy G. Genetıc polymorphısms of alcohol inducible CYP2E1 in Turkish popülation.M.Sc., Department of Biochemistry 2004, 123.

29. Liangpunsakul S, Kolwankar D, Pinto et al. Activity of CYP2E1 and CYP3A enzymes in adults with moderate alcohol consumption: A comparison with nonalcoholics Hepatology, May 2005:41;1144–1150.

30. Lu Y, Cederbaum AI. Cisplatin-Induced Hepatotoxicity Is Enhanced by Elevated Expression of Cytochrome P450 2E1 Toxıcologıcal Scıences. 2006;89:515–523.

31. Zhang F, Wang F, Chen C, Wang T, Hu J, Su R, Li X, Gu B, Tang S, Chen H, Li Y. Prediction of progression of chronic atrophic gastritis with Helicobacter pylori and poor prognosis of gastric cancer by CYP3A4. J Gastroentrol Hepatol 2020:35;425-432

32. JuanWang, HongliangJi, HeleiJia and DongshengGuan. Association between CYP3A4 gene rs4646437 polymorphism and the risk of hypertension in Chinese population: a case–control study. Biosci Rep 2019:39(4);BSR20190296.