Marmara Medical Journal 2018; 31: 61-67
DOI: 10.5472/marumj.430790

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

CYP 2D6* 4 polymorphism in Polycythemia vera patients in Turkish population

Türk popülasyonunda Polisitemia vera hastalarında CYP 2D6*4 polimorfizmi

Zehra OKAT, Kezban UÇAR ÇİFTÇİ, Kübra YAMAN, Selina TOPLAYICI, Elif KURT, Yavuz TAGA

ABSTRACT

Objective: Many studies have shown the association of susceptibility to several cancers with gene encoding enzymes' polymorphisms which engage in xenobiotics' biotransformation. In this study, the main purpose is to search the relation between cytochrome P450 (CYP) 2D6* 4 polymorphisms and Polycythemia vera (PV) incidence in Turkish population.

Materials and Methods: In this research article, 80 Polycthemia vera (PV) cases and 76 control samples have been used for the analysis of CYP 2D6* 4 polymorphism. The research has been performed by the methods of polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP).

Results: As a result, when patients and controls were compared in terms of CYP 2D6* 4 poor metabolizer (PM) and heterozygous extensive metabolizer (HEM) genotype frequency, it has been found that the patients have 1.35% PM and 32.43% HEM frequency, and controls have 2.63% PM and 21.05% HEM frequency. The correlation between the results of CYP 2D6 genotype analysis and the risk of disease in patients and controls was examined. We found that PM, HEM and extensive metabolizer (EM) genotypes were unrelated with the risk of PV (OR 0.51, 95% CI 0.04-5.71, OR 1.80, 95% CI 0.86-3.75, $\chi 2$ 1.93, P: 0.164, OR 0.61, 95% CI 0.30-1.24, $\chi 2$ 1.40, P: 0.235).

Conclusion: Our results suggested that the CYP 2D6* 4 polymorphism, in the studied population, does not play an important role in PV etiology.

Keywords: Cytochrome P450 2D6, Genetic polymorphism, Polycythemia vera (PV).

ÖZ

Amaç: Birçok çalışma, ksenobiyotiklerin biyotransformasyonundan sorumlu enzimleri kodlayan gen polimorfizmleri ile çeşitli kanserlere olan duyarlılık arasında bir ilişkili olduğunu göstermiştir. Bu çalışmada temel amaç, Türk popülasyonunda sitokrom P450 (CYP) 2D6*4 polimorfizmleri ile PV insidansı arasındaki ilişkiyi saptamaktır.

Gereç ve Yöntem: Bu araştırma makalesinde, CYP 2D6* 4 polimorfizminin analizi için 80 (PV) hastası ve 76 kontrol örneği kullanılmıştır. Deney, polimerize zincir reaksiyonu (PZR)-restriksiyon parça uzunluğu polimorfizmi (RFLP) methodu kullanılarak gerçekleştirilmiştir.

Bulgular: Sonuç olarak, hastalar ve kontroller CYP 2D6* 4 yavaş metabolizör (YM) ve heterozigot hızlı metabolizör (HM) genotip sıklığına göre karşılaştırıldıklarında, hastalardaki YM frekansı %1.35, heterozigot HM frekansı %32.43, kontrol grubunda ise YM frekansı %2.63, heterozigot HM frekansı %21.05 olarak tespit edilmiştir. CYP 2D6 genotiplerinin analiz sonuçları ile hasta ve kontrollerdeki hastalık riski arasındaki korelasyon araştırılmıştır. YM, heterozigot HM ve HM genotiplerinin PV riski ile ilişkili olmadığı bulunmuştur (OR 0.51, 95% CI 0.04-5.71, OR 1.80, 95% CI 0.86-3.75, $\chi 2$ 1.93, P: 0.164, OR 0.61, 95% CI 0.30-1.24, $\chi 2$ 1.40, P: 0.235).

Sonuç: Elde ettiğimiz sonuçlar, çalışılan popülasyonda CYP 2D6 * 4 polimorfizminin PV etyolojisinde önemli bir rol oynamadığını göstermiştir.

Anahtar Kelimeler: Sitokrom P450 2D6, Genetik polimorfizm, Polisitemia vera (PV).

Introduction

Within the group of myeloproliferative disorders (MPDs), we can include idiopathic myelofibrosis (IMF), Polycythemia vera (PV), essential thrombocythaemia (ET), and also chronic myeloid leukaemia (CML) [1]. All those mentioned disorders share many clinical features [2,3]. Their chronic development is caused by a single pluripotent haematopoietic stem cell mutation [4], which may transform

Zehra Okat (✉), Kezban Uçar Çiftçi, Kübra Yaman, Selina Toplayıcı, Elif Kurt, Yavuz Taga
Department of Biochemistry, School of Medicine, Marmara University, Maltepe, 34854 Istanbul, Turkey and Genetic and Metabolic Diseases Research Center (GEMHAM), Marmara University, Maltepe, 34854 Istanbul, Turkey
e-mail: zehraokat1980@gmail.com

Submitted / Gönderilme: 04.02.2018 **Accepted/Kabul:** 17.04.2018

into acute leukaemia. All of these four MPDs increase the production of haematopoietic elements. Erythrocytosis is the predominant clinical feature of PV which is defined by an increased red cell mass [5]. In PV, morbidity and mortality are basically initiated by thromboembolic complications. After diagnosis, life expectancy is reduced to a median of 15 years because of those complications. However, individual PV patients' clinical course is quite heterogeneous and prolonged [6].

Nowadays individuals are exposed to food additives, agrochemicals, environmental pollution, drugs, cosmetic products, and processed foods which are accepted as the source of most xenobiotics [7]. Generally, those lipophilic chemicals cannot be eliminated in an efficient way in the absence of metabolic reactions. This, in turn, would lead to their accumulation in the body, which then results in toxicity. Cytochrome P450 CYP-dependent monooxygenases catalyse the reactions involving the implication of a molecular oxygen atom into substrate. Phase I/II reactions participate in the biotransformation of both xenobiotics and endogenous compounds. Phase I reactions, including oxidations, hydrolysis and reductions are commonly referred to as "functionalization" reactions [8]. Typical functional groups, which are unmasked or proposed in phase I reactions, include carboxylic acid (-COOH), amino (-NH_2) and also hydroxyl (-OH) [9]. Generally, phase II reactions are called conjugation reactions, including acetylation, glycine/glutamine conjugation, glucuronidation, sulfonation, methylation and glutathione (GSH) conjunction [9, 10].In cancer formation cytochromes P450 (CYPs) play a crucial role by mediating the metabolic activation of various precarcinogens [8, 9]. And also in the treatment of cancer, these precarcinogens are responsible for anticancer drugs' activation and deactivation [8, 9]. Many studies have mostly focused on the association of the distribution of specific variant CYP alleles with the risk of various cancer types, because all xenobiotics metabolising CYPs are polymorphic. However, they have not revealed a consistent view.

Only particular CYPs have been examined for PV risk in terms of their genetic variants. As a polymorphic gene, cytochrome P450 2D6 (CYP 2D6) involves in phase I metabolism [11]. The CYP 2D6 enzyme is responsible for the metabolism of almost 100 drugs and substantial number of these drugs are clinically significant including neuroleptics, antidepressants, some antiarrhythmics, opioids and lipophilic β-adrenoceptor blockers [12]. Furthermore, CYP 2D6 activity is primarily identified by the presence of genetic polymorphisms [13]. And also, genetic polymorphisms in CYP 2D6 protein lead to 1,000-fold difference in CYP 2D6 metabolic ratio capacity [13]. CYP 2D6 gene having two non-functional alleles does not present CYP 2D6 enzyme activity, as a result of this it is classified as poor metabolizer (PM). On the other hand, individuals who own two functional alleles are called extensive metabolizers (EMs), indicating normal enzyme activity. And also, individuals who have one functional allele and one non-functional allele are generally characterized as intermediate metabolizers (IMs), resulting in damaged drug oxidation capacity [14, 15]. Ultrarapid metabolizers (UMs) arise from gain-of-function variant and require more than average doses of drugs which are catalysed by CYP 2D6 in order to attain targeted therapeutic plasma concentrations [15, 16].

Among white European population, G1934A (allele *4) is the most common substitution on the line between intron 3 and exon 4 of CYP 2D6 gene, and its presence causes inaccurate mRNA splicing, which leads to the change of reading frame, termination of translation and defective or truncated protein product generation without enzymatic activity [13, 14]. CYP 2D6 engages in metabolic activation of procarcinogens. For this reason, when compared to the groups with lower enzyme activity, patients with EM phenotype may develop larger quantities of reaction-active compounds, and this may cause malignant transformation process. In addition, there are inconsistent results in the comparative analysis of CYP 2D6 allele variants' role in carcinogenesis [17, 18]. In some studies, it is stated that EM genotype may cause tendency to various cancers, whereas in others it is claimed that PM genotype may cause the same. The relationship between PM and EM genotypes, and tendency to cancer is still unclear. It is considered that the individuals with PM genotype are less exposed to carcinogenic and genotoxic xenobiotic metabolites compared to those with EM genotype, but they are exposed to unmetabolized xenobiotics and toxic effects, which are stemmed from countless undetermined factors [19]. Furthermore, it is estimated that previously mentioned toxic effects might contribute to the development of carcinogenesis in PMs [20]. There are limited studies in the literature on molecular epidemiology in PV, therefore it is quite demanding to anticipate the effective role of these genotypes, which are at risk in PV. In this study, our aim is to describe CYP 2D6 gene's genetic

polymorphism in G1934A (allele *4) and to clarify the role of polymorphism in the development of increased risk of PV among Turkish population.

Materials and Methods

Our study group is based on hospital case-control and composed of Turkish population. This study contains 80 PV patients and 76 healthy controls (age/sex-matched). Studying group samples were gathered from a hospital in Istanbul according to PV diagnostic criteria between the years of 2012 and 2014. None of the patients showed other major diseases in the past. The control group is elected out of healthy volunteers who are not related with PV disorder and have no medical history. The project was approved by the Ethics Committee of Marmara University Faculty of Medicine.

Approval of all controls and patients were obtained after giving detailed information on the nature of procedures to be applied in the investigation.

DNA isolation method

Venous blood samples (5 ml) which are used for DNA isolation, were collected from vacuum tubes containing EDTA. One hundred fifty six genomic DNA were isolated by commercial DNA extraction kit using salting out DNA extraction method. Isolated DNA samples from patients and controls were stored at – 20 ^{o}C.

Genotyping of CYP 2D6 gene polymorphisms

One hundred fifty six genomic DNA were used for genotyping CYP 2D6 gene polymorphisms. But in patients, six sample genotype processes could not be realized due to inadequate amount of DNA. Examination of the intron 3 polymorphism indicated that, 355 bp region of the CYP 2D6 (CYP 2D6* 4) gene was amplified using primers PF: 5'-GCC TTC GCC AAC CAC TCC G-3', and PR: 5' AAA TCC TGC TCT TCC GAG GC-3'). PCR was performed in 25 µl via master mix. In our study, PCR program requires the following procedures: initial denaturation program at 94 °C for 5 minutes, 30 cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, extension at 72 °C for 1.5 minutes, and final extension at 72°C for 10 minutes [20]. Amplified sample was digested overnight with 10 U of the restriction endonuclease *Bst* NI (*Mva* I) at 60 °C. Figure 1 illustrates the restriction patterns and classification of the genotypes.

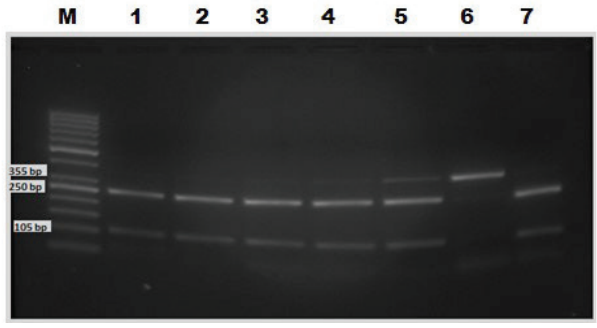

Figure 1. RFLP Patterns of CYP 2D6 Intron 3 Polymorphism

Marker: 50 bp DNA Ladder (1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 50 bp)

CYP 2D6* 4/*4 genotype; Poor metabolizer (PM); carries inactive in two alleles; 355 bp (Line 6)

CYP 2D6 wt/*4 genotype; Heterozygous extensive metabolizer (HEM); carries one functional allele; 355, 250,105 bp (Line 5)

CYP 2D6 wt/wt genotype; Homozygous extensive metabolizer (EM); carries two functional alleles; 250,105 bp (Line 1,2,3,4 and 7)

Statistical Analysis

To determine the importance of differences between genotype frequencies in case and control group, Chi-square test was performed in our study. By calculating the odds ratio (OR) with CI 95%, we evaluated the relative risk related with genotypes or certain alleles at 0.05 significance level. The chi-square test was applied only if at least 80% of the cells had an expected frequency of 5 or greater, and no cell had an expected frequency smaller than 1.0. If at least 20% of expected frequencies were less than 5, fisher exact test was performed. In this study for the statistical data analyses, we used version 14.0 of Statistical Package for the Social Sciences (SPSS).

Results

In our study, Chi-square Hardy-Weinberg equilibrium was calculated for CYP 2D6* 4 allele in controls and cases (χ2: 1.0620, P: 0.3028 for cases, χ2: 0.4718, P: 0.4922 for controls).

Totally 150 individuals (74 patients and 76 controls) were genotyped for CYP 2D6 gene. Individuals who are heterozygous for the CYP 2D6* 4 allele, expressed one normally active (250, 105 bp) and one mutated allele

(355 bp), however homozygous individuals for this gene showed 355 bp fragment. Beside this information, normal individuals showed only 250, 105 bp fragments.

CYP 2D6 intron 3 genotype distributions in the study group are illustrated in Table I In our analysis, it was found that while 58 out of 76 controls (76.32%) had EM genotype, 16 of them (21.05%) had HEM genotype, only 2 of them (2.63%) had PM genotype. When patients with PV were evaluated in terms of their genotypes, 66.22 % of the patients had EM genotype, 32.43 % of the patients had HEM genotype and only 1 of them (1.35%) had PM genotype. CYP 2D6* 4 WT allele frequency in 74 patients has been measured as 82.43%, and MUT allele frequency as 17.57% (Table I). WT allele frequency in the control group has been determined as 86.84% and the MUT frequency as 13.16%. When patients and controls were analysed for the frequency of allele, the mutant allele frequency of patients (17.57%) were higher than the controls (13.16%). When patients and controls were compared in terms of CYP 2D6* 4 (PM) + (HEM) genotype frequency, frequency of patients (33.78%) was higher than the controls (23.68%) (Table I). However, our results from the association analysis of CYP 2D6 genotypes vs risk of PV in cases and controls demonstrate that PM, HEM and EM genotypes were not found to be related with the risk of PV (OR 0.51, 95% CI 0.04-5.71, OR 1.80, 95% CI 0.86-3.75, $\chi 2$: 1.93, P: 0.164, OR 0.61, 95% CI 0.30-1.24, $\chi 2$: 1.40, P: 0.235) (Table I). In PM group, expected frequencies are less than 5 so that the chi-square test could not be realized.

Table I. Genotype and allele frequency of CYP 2D6* 4 among Turkish population

PV Genotype		**Cases (n=74)**	**Controls (n=76)**	**OR**	**95 %CI**	**Chi square $\chi 2$**	**P value**
PM		1 (1.35%)	2 (2.63%)	0.51	0.04-5.71	-	-
HEM		24 (32.43%)	16 (21.05%)	1.80	0.86-3.75	1.93	0.164
EM		49 (66.22%)	58 (76.32%)	0.61	0.30-1.24	1.40	0.235
Allel frequency	**WT**	82.43%	86.84%				
	MUT	17.57%	13.16%				
Hardy Weinberg Cases $\chi 2$: 1.06, P: 0.302*** Control $\chi 2$: 0.47, P: 0.492***							
Fisher exact; P: 0.313*,***							

* Fisher exact **P<0.05 significant difference ***P>0.05 insignificant difference (N; total number; WT, wild type allele; MUT, mutant allele; PM, homozygous mutant status; EM, refer to homozygous normal status; HEM, heterozygote $\chi 2$; $_{\text{Chi-square}}$.)

Discussion

In the literature, the information about the significant effect of environmental factors and genetic susceptibility on the improvement of PV is very limited [21]. Among the risk factors of PV, hair dyes, exposure to radiation and certain occupations can be listed, however there is not enough information in this regard [21, 22]. Including the metabolism of several potential carcinogens in the environment, various enzymes critically affect a broad-scale of xenobiotics. A great number of carcinogens necessitate the activation of genotoxic electrophilic intermediates before they can be ejected from the body [22]. This activity is generally affected by CYP family, and genetically determined polymorphisms. Generated genotoxic intermediaries level is specified by the activity level of those proteins, and those intermediaries are directed by their removal rate [22]. Various cancer susceptibilities have been determined to be caused by the genetically determined differences in metabolism, associated with CYP.

Genetic polymorphism of CYP 2D6 enzyme affects individual susceptibility to various pathologies, including cancer [23]. CYP 2D6 EM phenotype is related to some malignant processes such as bladder and lung cancer [24, 25]. Moreover, CYP 2D6 PM phenotype predisposes for cancers such as breast, lung, oral, basal cell, prostate, bladder and it is also related with the diseases of the central nervous system such as Parkinson and Alzheimer [26-29]. CYP 2D6's role in lymphoma susceptibility has been studied in the literature, but the findings are conflicting in terms of PM and EM allele frequency and lymphoma risk [30]. According to some studies, the PM and/or HEM allele carriers have the potential of increased risk for leukaemia caused by insufficient detoxification [24]. Especially CYP 2D6 polymorphisms are susceptible to some haematological neoplasis. An increment in the chemical carcinogens' metabolic activation or connection to another cancer-causing gene may possibly cause this. Previous studies have reported that variation in CYP 2D6 enzyme activity contributes to cancer susceptibility especially in the case of haematological malignancies. CYP 2D6 poor metabolizer genotype (PM) and leukaemia have been discovered to have a significant association among Caucasian patients who have acute myeloid leukaemia (AML) or chronic myeloid leukaemia (CML) suggesting the role of CYP 2D6 in a leukaemogen detoxification [27]. A relation between elevated risk of developing AML and inheritance of the CYP 2D6 PM phenotype has been presented in a large

control study [27]. In addition, Aydın et al., have determined that CYP 2D6 null variants significantly contribute in the development of acute leukaemia [31]. In their study, Aydın et al., have noted that CYP 2D6* 4 homozygous and heterozygous variant – genotypes are not crucial neither for acute lymphocytic leukemia (ALL) nor AML patients, however the frequency of CYP 2D6* 3 HEM individuals show a decrease in all types of leukaemia [31]. Sailaja et al. have determined that CYP 2D6* 4 polymorphism does not show a significant association with CML [32].

Moreover, Lemos et al., have revealed that there is a compelling relation between the CYP 2D6 EM genotype and leukaemia [33]. Supporting these findings, Tayser et al., have also demonstrated a significant elevation of EM genotype in AML patients, but not in ALL patients [34]. In another study, Joseph et al. have reported that children with ALL show a higher frequency of CYP 2D6 EM genotype compared to control group [35]. The rising activation of procarcinogens through extensive metabolizers may lead to the risk of carcinogenesis. However, some studies failed to report the association of CYP 2D6 polymorphism with haematological malignancies [36, 37]. Although CYP 2D6 polymorphism has been associated with many types of cancer, this gene has an unknown role in the development of PV because of the limited molecular epidemiology studies in the literature. This study emphasizes the role and importance of phase I CYP 2D6 polymorphic variant in the risk of PV among Turkish population, for the first time. In our analyses, CYP 2D6* 4 poor PM and HEM frequencies (respectively 1.35% and 32.43%) increased in patients compared to control group (respectively 2.63%, 21.05%) which indicated that impaired detoxification of environmental and endogenous substrates in PM might confer risk to develop PV (Table I). Moreover, the substrates metabolized by CYP 2D6 may involve in the pathogenesis of PV as major carcinogens. But in this present study, PM, HEM and EM genotypes were not detected to be related with the risk of PV (OR 0.51, 95% CI 0.04-5.71, OR 1.80, 95% CI 0.86-3.75, $\chi 2$: 1.93, P: 0.164, OR 0.61, 95% CI 0.30-1.24, $\chi 2$: 1.40, P: 0.235).

In our study group CYP 2D6 allele frequencies were crosschecked with frequencies found in different ethnic populations. Researches about this topic up to now have shown that, CYP 2D6* 4 is the most important defective allele in Caucasian population (21%), 18% in Americans and also it is rare in the Chinese, Japanese (1%), Korean, and (8%) African Americans [38-40]. In a different study conducted in Turkey reported that CYP 2D6* 4 allele frequency as 15.4% [40]. Another study which is implemented in Turkey, homozygote mutation rate of CYP 2D6* 4 was found as 4% and its allele frequency as 0.21 [41]. In the present study, CYP 2D6* 4 allele frequency was divided between controls and patients as 0.13 (13.16%) and 0.18 (17.57%), respectively. As we compared our own results with the data of Aynacıoğlu et al., (404 patients), Aydın et al., (140 patients), and Sahin et al., (249 patients); we determined that the values of allele frequencies and homozygous mutations were nearly close [40, 42, 43]. A possible genetic drift depending on the limited population size may account for the discrepancies in the allele distributions of CYP 2D6. Consequently, certain allele's frequencies increase or decrease by coincidence. On the other hand, non-random marriage may cause the occurrence of this genetic drift, which in turn may trigger altered genotypic frequencies. Among other possible causes, we may list mutation, natural selection and migration of genetically distinct population as the most critical ones [44, 45].

Moreover, contradictions among the published results on the relation between leukaemia or PV risk and gene polymorphisms may be attributed to the different features of the patient populations, and the polymorphic variants associated with the certain characteristics of the patients. In addition, the risk of adult PV may be elevated by polymorphic variants in xenobiotic metabolism genes, particularly when they are combined. Genetic risk factors associated with this disease should be better determined by conducting studies on all xenobiotic-metabolizing enzymes. In addition to that, these studies should be conducted on other environmental factors such as; nutrition, pesticides, consumption of GDO products, perfume and detergent usage, smoking, alcohol drinking, exposure to radiation, air pollution in that area, occupational exposure to carcinogens and also hereditary transmission as risk factors for the susceptibility to PV [46]. CYP 2D6 metabolic status should be evaluated before starting the therapy, this may be fruitful in identifying the patients with the risk to fail to reply to the therapy or toxic drug effects, and it may also be useful in optimal dosing recommendations in medicine [47]. Because in PMs, more drugs which are metabolized with this enzyme are slowly metabolized. Therefore, the duration is prolonged and toxic effects can occur. Also for UMs patients, when metabolized drugs are administered at therapeutic doses, it can be ineffective and for effective treatment , higher doses are required. For example, Tamoxifen is an important selective estrogen receptor (ER) modulator and is frequently used in

the treatment of breast cancer in postmenopausal women. CYP 2D6 is liable for the metabolism of tamoxifen to create its major active metabolite, endoxifen. CYP 2D6*4 polymorphisms play a crucial role in the breast cancer etiology and might help in planning hormonal therapy where tamoxifen is used because CYP 2D6 polymorphism may contribute variations in treatment efficiency [48].

CYP 2D6 is a potential useful guide in the diagnosis of PV. However, according to our knowledge, this study shows the genotype relationship in PV patients among Turkish population, for the first time. Finally, the results of our study demonstrate that genetic polymorphisms which take place in xenobiotic metabolizing enzymes may critically contribute to environmental carcinogen-induced carcinogenesis mechanism. We can say as a suggestion that the detection of CYP 2D6 gene mutations may be useful in the determination of risk. There is a need for large-scale studies about the effect of the CYP 2D6 genotype on clinical outcomes.

References

1. Tefferi A, Elliott M. Thrombosis in myeloproliferative disorders: Prevalence, prognostic factors, and the role of leukocytes and JAK2V617F. Semin Thromb Hemost 2007; 33:313-20. doi: 10.1055/s-2007-976165.
2. Spivak JL, Barosi G, Tognoni G, et al. Chronic myeloproliferative disorders. Hematology (Am Soc Hematol Educ Program) 2003; 200-24. doi: 10.1182/asheducation-2003.1.200.
3. Tefferi A. Pathogenetic mechanisms in chronic myeloproliferative disorders: polycythemia vera, essential thrombocythemia, agnogenic myeloid metaplasia, and chronic myelogenous leukemia. Seminars in Hematology 1999; 36:3–8.
4. Bilgrami S, Greenberg, B R. Polycythemia rubra vera. Semin Oncol 1995; 22:307-26.
5. Samuelsson J. Survival in a patient with polycthemia vera for over thirty years: implications for treatment decisions in younger patients. Leukemia and Lymphoma 1998; 32:195-8. doi: 10.3109/10428199809059262.
6. Gonzalez FJ, Tukey RH, (editors). Drug Metabolism: How Humans Cope with Exposure to Xenobiotics." Goodman and Gilman's the Pharmacological Basis of Therapeutics. New York, NY: McGraw-Hill, 2012.
7. Guengerich FP. Cytochrome p450 and chemical toxicology. Chem Res Toxicol 2008; 21:70-83. doi: 10.1021/tx700079z.
8. Bock KW, Lilienblum W, Fischer G, et al. The role of conjugation reactions in detoxication. Arch Toxicol 1987; 60:22-29.
9. Jancova P, Anzenbacher P, Anzenbacherova E. Phase II drug metabolizing enzymes. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2010; 154:103-16.
10. Abraham JE, Maranian MJ, Driver KE, et al. CYP 2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. Breast Cancer Res 2010;12:R64. doi: 10.1186/bcr2629.
11. Cholerton S, Daly AK, Idle JR. The role of individual human cytochromes P450 in drug metabolism and clinical response. Trends Pharmacol Sci 1992; 13:434-9.
12. Bertilsson L, Dahl ML, Dalén P and Al-Shurbaji A. Molecular genetics of CYP 2D6: Clinical relevance with focus on psychotropic drugs. Br J Clin Pharmacol 2002; 53:111-22. doi: 10.1046/j.0306-5251.2001.01548.x.
13. Sachse C, Brockmoller J, Bauer S, et al. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1997; 60:284-95.
14. Hanioka N, Kimura S, Meyer UA, et al. The human CYP2D locus associated with a common genetic defect in drug oxidation: a G1934----A base change in intron 3 of a mutant CYP 2D6 allele results in an aberrant 3' splice recognition site. Am J Hum Genet 1990; 47:994-1001.
15. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression,enzyme activities, and impact of genetic variation. Pharmacology & Therapeutics 2013; 138:103-41. doi: 10.1016/j.pharmthera.2012.12.007.
16. Bertilsson L, Dahl ML, Sjo¨qvist F, et al. Molecular basis for rational megaprescribing inultrarapid hydroxylators of debrisoquine. Lancet 1993; 341:63.
17. Christensen PM, Gotzsche PC, Brosen K. The sparteine/debrisoquine (CYP 2D6) oxidation polymorphism and the risk of lung cancer: a metaanalysis. Eur J Clin Pharmacol 1997; 51:389-93. doi: 10.1007/s002280050219.
18. Taninghera M, Malacarnea D, Ugolinia A, et al. Drug metabolism polymorphisms as modulators of cancer susceptibility. Mutation Research/Reviews in Mutation Research 1999; 436:227–61.
19. Preston-Martin S, Pike MC, Ross RK, et al. Increased cell division as a cause of human cancer. Cancer Res 1990; 50:7415-21.
20. Schur BC, Bjerke J, Nuwayhid N, et al. Genotyping of cytochrome P450 2D6*3 and *4 mutations using conventional PCR*. Clinica Chimica Acta 2001; 308:25-31. doi: 10.1016/S0009-8981(01)00422-3.
21. Kim JW, Lee CG, Park YG, et al. Combined analysis of germline polymorphisms of p53, GSTM1, GSTT1, CYP1A1, and CYP2E1: relation to the incidence rate of cervical carcinoma. Cancer 2000; 88:2082-91. doi: 10.1002/(SICI)1097-0142(20000501)88:9<2082::AID-CNCR14>3.0.CO;2-D.
22. Morgan GJ, Smith MT. Metabolic enzyme polymorphisms and susceptibility to acute leukemia in adults. Am J Pharmacogenomics 2002; 2(2).
23. Hatagima, A. Genetic polymorphisms and metabolism of endocrine disruptors in cancer susceptibility. Cad. Saúde Pública Rio de Janeiro 2002; 18:357-77.

24. Anwar WA, Abdel-Rahman SZ, El-Zein RA, et al. Genetic polymorphism of GSTM1, CYP2E1 and CYP 2D6 in Egyptian bladder cancer patients. Carcinogenesis 1996; 17: 1923-29.
25. Caporaso NE, Tucker MA, Hoover RN, et al. Lung cancer and the debrisoquine metabolic phenotype. J Natl Cancer Inst 1990; 82:1264-72.
26. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P4502D6 (CYP 2D6): clinical consequences, evolutionary aspects and functional diversity, The Pharmacogenomics J 2005; 5:6 – 13. doi:10.1038/sj.tpj.6500285.
27. Roddam PL, Rollinson S, Kane E, et al. Poor metabolizers at the cytochrome P450 2D6 and 2C19 loci are at increased risk of developing adult acute leukaemia, Pharmacogenetics 2000; 10:605-15.
28. Worrall SF, Corrigan M, High A, et al. Susceptibility and outcome in oral cancer: preliminary data showing an association with polymorphism in cytochrome P450 CYP 2D6. Pharmacogenetics 1998; 8:433-9.
29. Smith CA, Gough AC, Leigh PN, et al. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. Lancet 1992; 339:1375–7.
30. Sarmanova J, Benesova K, Gut I, et al. Genetic polymorphisms of biotransformation enzymes in patients with Hodgkin's and non-Hodgkin's lymphomas. Hum Mol Genet 2001;10:1265-73.
31. Aydin-Sayitoglu M, Hatirnaz O, Erensoy N, et al. Role of CYP 2D6, CYP1A1, CYP2E1, GSTT1, and GSTM1 genes in the susceptibility to acute leukemias. Am J Hematol 2006;81:162-70. doi: 10.1002/ajh.20434.
32. Sailaja K, Vishnupriya S, Surekha D, et al. Association of CYP 2D6* 4 Polymorphism with Chronic Myeloid Leukemia. Journal of Medical Sciences Research 2007; 1(1).
33. Lemos MC, Cabrita FJ, Silva HA, et al. Genetic polymorphism of CYP 2D6, GSTM1 and NAT2 and susceptibility to haematological neoplasias. Carcinogenesis 1999; 20: 1225-9.
34. Tayser KE, Ehsan G, Ghonemy EE, et al. Study of genetic polymorphism of xenobiotic enzymes in acute leukemia. Blood Coagulation and Fibrionolysis 2007; 18:489-95. doi: 10.1097/MBC.0b013e3281eec930.
35. Joseph T, Kusumakkumary P, Chacko P, et al. Genetic polymorphism of CYP1A1, CYP 2D6, GSTM1and GSTT1 and susceptibility to acute lymphoblasticleukaemia in Indian children. Pediatric Blood Cancer 2004; 43:560 – 67. doi: 10.1002/pbc.20074.
36. Krajinovic M, Labuda D, Richer C, et al. Susceptibility to childhood acute lymphoblastic leukemia: ınfluence of CYP1A1, CYP2D6, GSTM1, and GSTT1 genetic polymorphisms. Blood 1999; 93: 1496-501.
37. Marsh JCW, Choudry J, Parry-Jones N, et al. Study of the association between cytochromes P450 2D6 and 2E1 genotypes and the risk of drug and chemical induced idiosyncratic aplastic anaemia. Br J Haematol 1999; 104:266-70.
38. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemisry. Naunyn Schmiedebergs Arch Pharmacol 2004; 369:23-37. doi: 10.1007/s00210-003-0832-2.
39. Alvan G, Bechtel P, Iselius L, et al. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. Eur J Clin Pharmacol 1990; 39:533-7.
40. Aydin M, Hatirnaz O, Erensoy N, et al. CYP 2D6 and CYP1A1 mutations in the Turkish population.Cell Biochem Funct 2005; 23:133-5. doi: 10.1002/cbf.1222.
41. Koseler A, Ilcol YO, Ulus IH. Frequency of mutated allele CYP 2D6* 4 in the Turkish population. Pharmacology 2007; 79:203-06. doi: 10.1159/000100959.
42. Aynacıoğlu AS, Sachse C, Bozkurt A. Low frequency of defective alleles of cytochrome P450 enzymes 2C19 and 2D6 in the Turkish population. Clin Pharmacol Ther 1999; 66: 185-92. doi: 10.1053/cp.1999.v66.100072001.
43. Sahin S, Aydogan L, Benli I, et al. Distribution of HLA-B27 and CYP 2D6* 4 mutations in the middle Black Sea area (Tokat) of Turkey. Genetics and Molecular Research 2011 10:3987-91. doi: 10.4238/2011.December.2.3
44. Hardy GH. Mendelian proportions in a mixed population. Science 1908; 28:49-50. doi: 10.1126/science.28.706.49
45. Rodriguez S, Gaunt TR, Day IN. Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009; 169:505-14. doi: 10.1093/aje/kwn359.
46. Anderson LA, Duncombe AS, Hughes M, et al. Environmental, lifestyle, and familial/ethnic factors associated with myeloproliferative neoplasms. Am J Hematol 2012 ;87:175-82. doi: 10.1002/ajh.22212.
47. Ozawa S. Drug-Drug Interactions with consideration of pharmacogenetics. Yakugaku zasshi Journal of the Pharmaceutical Society of Japan. 2018;138:365-71. doi: 10.1248/yakushi.17-00191-5.
48. Thota K, Prasad K, Basaveswara Rao MV. Detection of cytochrome P450 polymorphisms in breast cancer patients may ımpact on tamoxifen therapy. Asian Pac J Cancer Prev 2018 26;19:343-50. doi: 10.22034/APJCP.2018.19.2.343.