

Investigation of *CYP1B1**3 and *CYP1B1**4 polymorphisms in a Turkish population

Fatma Özlem Kargın Solmaz¹Jale Akgöl²Merve Becit Kızılkaya¹Ahmet Oğuz Ada³

1 Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Afyonkarahisar Health Sciences University. Afyonkarahisar / Türkiye

2 Department of Medical Pharmacology, Faculty of Medicine, Afyonkarahisar Health Sciences University. Afyonkarahisar / Türkiye

3 Department of Toxicology, Faculty of Pharmacy, Ankara University. Ankara / Türkiye

Abstract

CYP1B1 is a P450 enzyme involved in activating pro-carcinogens to carcinogens as well as estrogen metabolism. In order to examine the effect of *CYP1B1* on cancer metabolism, it should be compared with healthy individuals and whether the polymorphism between healthy individuals and sick individuals is significant. This study aims to screen the *CYP1B1**3 and *CYP1B1**4 polymorphisms of a group of individuals who have not been diagnosed with cancer to examine the genetic differences of metabolic enzymes in the Turkish population. This study is a cross-sectional type descriptive study. The study included 295 patients without a cancer diagnosis. The research sample includes patients who applied to Ankara University Medical Faculty Hospital and Afyonkarahisar Health Sciences University Research and Application Hospital. The individuals signed voluntary consent forms before participation, and 3 ml blood samples were taken from each. DNA samples were obtained using a DNA isolation kit, and then polymorphism was determined by real-time PCR. The distribution of *CYP1B1**3 and *CYP1B1**4 polymorphism in healthy individuals was determined. The frequency of *CYP1B1**1/*1 (wild type), *CYP1B1**1/*3 (heterozygous) and, *CYP1B1**3/*3 (mutant) genotypes were found 39.33%, 50.67% and 10.0% respectively. The frequency of *CYP1B1**1/*1 (wild type), *CYP1B1**1/*4 (heterozygous) and, *CYP1B1**4/*4 (mutant) genotypes were found 39.31%, 60.69% and 0% respectively. No individuals with mutant genotype were detected in this genotype (*CYP1B1**4). The results show that the genotype frequencies of the *CYP1B1**3 gene polymorphism in a Turkish population are similar to other Caucasian populations. However, it was determined that the Turkish population did not show similarity with other races in terms of *CYP1B1**4 polymorphism.

Keywords: *CYP1B1*, polymorphism, rs1056836, rs1800440, Turkish population

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Corresponding Author:
Fatma Özlem Kargın Solmaz
Email: ozlem.solmaz@afsu.edu.tr



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Introduction

Cytochrome P450 (CYP) enzymes are membrane-bound hemoproteins that play a crucial role in xenobiotic detoxification, cellular metabolism, and homeostasis [1]. CYP1B1, an important isozyme of this enzyme family, is a crucial enzyme involved in the formation of drugs and reactive estrogen metabolites and the metabolism of environmental carcinogens such as polycyclic aromatic hydrocarbons [2].

The CYP1B1 enzyme system was first identified as a new enzyme when it was transcriptionally induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the keratinocyte cell line [3]. CYP1B1 is transcriptionally induced by 2,3,7,8-tetrachloro-hydro benzo-p-dioxin or dioxin and is regulated by specific transcriptional switches, including the estrogen receptor and aryl hydrocarbon receptor (AhR) [4]. Transcriptional regulation is important in treating CYP1B1-positive tumor cases [5]. In normal tissues, CYP1B1 binds to 2,3,7,8-tetrachlorobenzo-p-dioxin by cytoplasmic AhR and is activated by AhR, heat shock protein-90, XAP2, and p23 proteins. The elongation region (-5298 to -5110) of CYP1B1 contains several steroidogenic factors-1 that interact with two cAMP-sensitive elements (CRE1 and CRE2). The cAMP signal transduction pathway is critical in the adrenal glands, testes, and ovaries [6].

CYP450 enzymes are primarily found in the liver, while CYP1B1 enzymes are also found in extrahepatic tissues and cells. CYP1B1 must be found in the ovaries, testicles, adrenal glands, prostate, uterus, and breast tissue. Immunohistochemical studies have shown that the protein-synthesizing this enzyme can be isolated from these tissues in esophageal, brain, lung, and breast cancer [7]. Polymorphisms in the *CYP1B1* gene cause changes in enzyme activity. There are many studies regarding the relationship of these polymorphisms with glaucoma, obesity, cardiovascular diseases, and hormone-mediated cancers [8, 9]. In previous studies on the Turkish population, Ada et al. [10] investigated the *CYP1B1*4* polymorphism in coke oven workers with 49 people. In their study, Güler et al. [11] examined *CYP1B1*2* and *CYP1B1*3* polymorphisms in lung cancer patients. An insufficient number of patients in the

first study and polymorphism in only lung cancer patients in the second study made it necessary to carry out studies with a more significant number of patients and control groups and to conduct these studies with other cancer types. Ozbek et al. [12], on the other hand, investigated the *CYP1B1*3* polymorphism in breast cancer with a case-control study. As a result of this study, polymorphism frequencies were 9,56.2 and 34.8% for wild-type, heterozygous and mutant, respectively. In this study, the classical PCR method was used for genotype determination.

Increased expression in certain diseases makes CYP1B1 a therapeutic target, especially for cancer diseases. Polymorphisms in this gene may also affect pharmacokinetic parameters and lead to differences in drug responses [13].

Molecular epidemiological studies have become increasingly important in determining countries' health policies, revealing social differences and similarities, and guiding treatment protocols. This study aims to screen the *CYP1B1*3* and *CYP1B1*4* polymorphisms of a group of individuals who have not been diagnosed with cancer to examine the genetic differences of metabolic enzymes in the Turkish population. It is crucial to perform the *CYP1B1*3* and *CYP1B1*4* polymorphism with a more significant number of healthy individuals since the above studies are conducted with a limited number of people, performed with classical PCR, and used only in studies of determining drug resistance in lung cancer patients in Turkish society. This study gains importance as it is the first study to determine the *CYP1B1*4* polymorphism in the Turkish population. It is also an important study in determining the polymorphic distribution of breast, prostate, over, endometrium, and many other cancer types, which are determined to be effective, especially with CYP1B1, and providing the possibility of comparison with cancer patients in further studies.

Materials and Methods

This study is a cross-sectional type descriptive study. The design of the study was created by following The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Study population

While determining the study sample, these two articles were taken as reference, and the sample size was determined to be a minimum of 145 for both groups [14-15](Figure 1). The study sample consists of patients who applied to Ankara University Medical Faculty Hospital and Afyonkarahisar Health Sciences University Research and Application Hospital. The study, which was started after the relevant permissions from the Ethics Committee of Ankara University (Approval no: 12-222) and Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2011-KAEK-2-2021/385) were included in the study, which consisted of volunteer women (111) and men (184), aged 25-71, without a history of cancer and who applied to the hospital for different reasons other than cancer screening and treatment. While the *CYP1B1**3 polymorphism studies of the research were carried out with samples taken from Ankara University Medical Faculty Hospital, the determination of *CYP1B1**4 polymorphism was carried out with samples taken from Afyonkarahisar Health Sciences University Research and Application Hospital. *CYP1B1**3 polymorphism was investigated in 150 individuals; *CYP1B1**4 polymorphism was investigated in 145 individuals. Two polymorphisms could not be studied in the same group, as samples were taken at different places from the control groups included in the study at different times.

DNA isolation

The patients who accepted the study and gave consent were asked to donate 3 ccs of blood to the hemogram tube. The samples were anonymized by adding age information to the initials of the person's names and surnames to be blinded. Samples are stored at 20°C For genotyping of the samples, DNA isolation was performed using the kit prepared according to the method applied by Miller et al. (1988) [16].

Genotype analyzes

A real-time polymerase chain reaction (Real-time PCR method) was used to determine the relevant gene polymorphisms. The researchers disposed of the remaining samples as medical waste without storage.

The *CYP1B1**3 Leu432Val polymorphism was determined according to the Real-time PCR method of Brünning et al. [17]. Accordingly, 25 µl of the reaction mix, 1 µl of each hybridization probe, 1 µl of each primer, 20 µl of water, and 1 µl of DNA. PCR conditions are 15 minutes of initial denaturation at 95°C followed by 40 PCR cycles of melting (95°C for 15 seconds), adhesion (55°C for 30 seconds), and synthesis (72°C for 30 seconds). Genotype differentiation was made by using the fact that the probe-DNA hybrid of the wild-type allele gave a higher melting temperature (T_m) than the probe-DNA hybrid of the mutant allele. In Figure 2, the PCR results of some samples determined depending on the melting temperature are given.

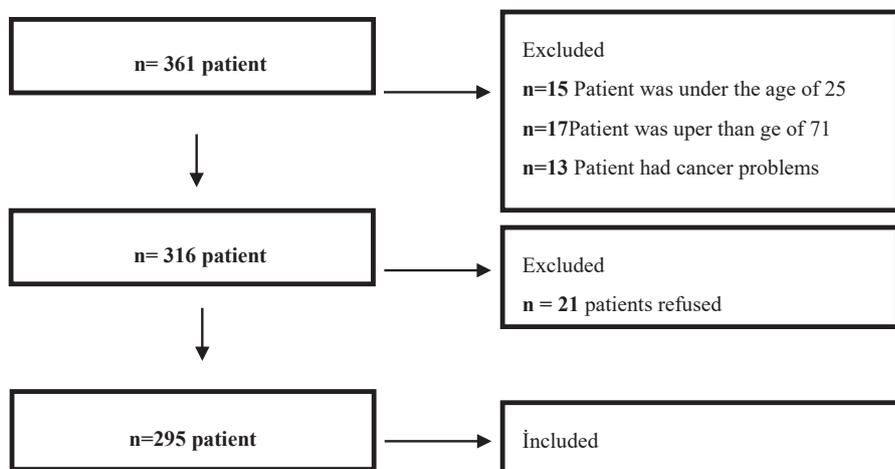


Figure 1. Included and excluded participants

The probes and primers used and their sequences are given in Table 1.

The *CYP1B1**4 Asn453Ser polymorphism was determined according to the Taqman Real-time PCR method. Accordingly, 10 μ l of the reaction mixture contains 0,4 μ l of each hybridization probe, 0,8 μ l of each primer, 2 μ l of DNA, and

5,6 μ l of water. PCR conditions are 5 minutes of initial denaturation at 95°C followed by 40 PCR cycles of melting (95°C for 15 seconds), adhesion (58°C for 30 seconds), and synthesis (72°C for 15 seconds). PCR results of some samples are given in Figure 3.

Table 1. Probes and primers and their sequences used in the detection of *CYP1B1**3 polymorphism

Primer/Prob	Primer/Probsequencing 5'-3'
Prob A	LCR-AACTTTGATCCAGCTCGATTCTTGGACAA-
Prob B	ATGACCCACTGAAGTGACCTAACCC-FL
Primer F	GAAATAAGAATTTTGCTCACTTGC
Primer R	CTTAGAAAGTTCTTCGCCAATG

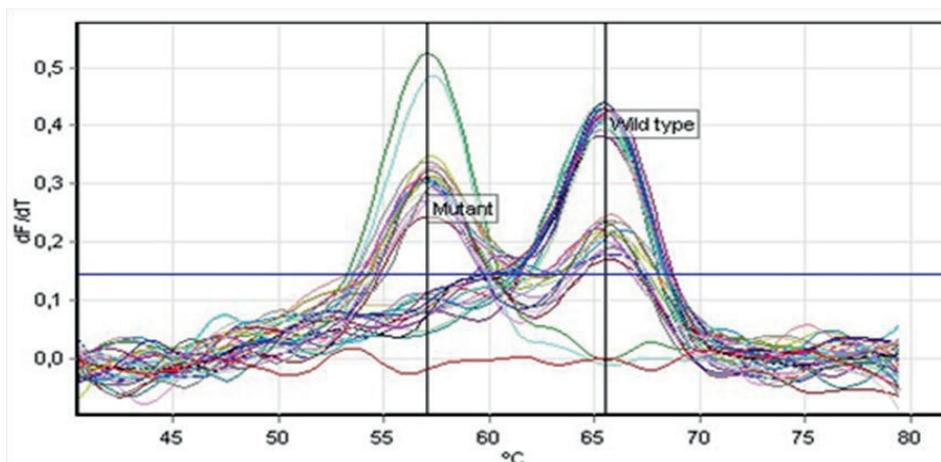


Figure 2. The results of some individuals belonging to the *CYP1B1**3 polymorphism with the melting temperature graph used for genotype discrimination by real-time PCR

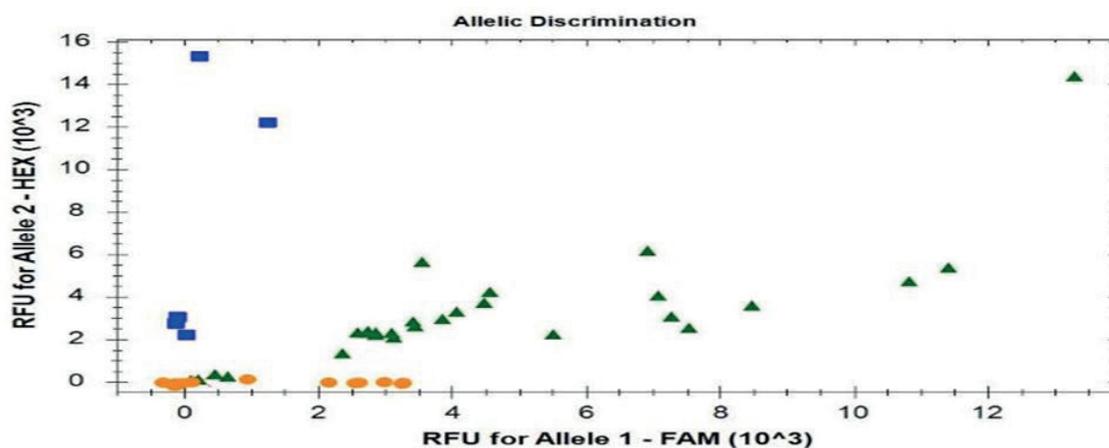


Figure 3. The plot of allelic discrimination from the qPCR reaction of *CYP1B1**4

The probes and primers used and their sequences are given in Table 2.

In the *CYP1B1*3* polymorphism, the hybridization probe was used with the Brünning method, while the Taqman probe was used in the determination of the *CYP1B1*4* polymorphism. Different graphics were obtained by using different probes.

Statistical Package for the Social Sciences, SPSS 26.0 (IBM Corp. 2019 IBM SPSS Statistics for Windows, version 26.0.) was used to analyze the data on alleles whose numbers and frequencies were specified according to the Hardy-Weinberg equation. Categorical variables were presented as percentages and frequencies.

Results

90% of the individuals participating in the determination of *CYP1B1*3* polymorphism were male, and 10% were female. The mean age of the participants in this polymorphism was 46 (min-max; 21-71). On the other hand, 66.21% of the individuals who determined *CYP1B1*4* polymorphism were female, and 33.79% were male. The mean age of the individuals participating in this polymorphism was 45.08 (min-max 23-63 years) (Table 3).

In *CYP1B1*3* polymorphism, out of 150 individuals, 59 (39.33%) had wild type (*CYP1B1*1/*1*) genotype, 76 (50.67%) had heterozygous (*CYP1B1*1/*3*) genotype, 15

(10%) were found to have homozygous mutant (*CYP1B1*3/*3*) genotype. The genotype frequencies we determined were compatible with the expected genotype frequency according to the Hardy-Weinberg equation ($\chi^2 = 1.794$). When the genotypes are analyzed by gender, in males, 52 individuals (38.52%) have wild type (*CYP1B1*1/*1*) genotype, 70 individuals (51.85%) have heterozygous (*CYP1B1*1/*3*) genotype, and 13 individuals (9.63%) have genotypes. Homozygous mutant (*CYP1B1*3/*3*) genotype was determined. In females, 7 individuals (46.66%) had wild type (*CYP1B1*1/*1*) genotype, 6 individuals (40%) had heterozygous (*CYP1B1*1/*3*) genotype and 2 subjects (13.34%) had homozygous mutant (*CYP1B1*3/*3*) genotype was determined (Table 4).

Table 4 shows genotype frequencies and expected frequency values of *CYP1B1*3* Leu423Val polymorphism.

In *CYP1B1*4* polymorphism, out of 145 individuals, 57 (39.31%) had wild-type (*CYP1B1*1/*1*) genotype, and 88 (60.69%) had heterozygous (*CYP1B1*1/*4*) genotype determined. No individuals with mutant genotype were detected in this genotype. According to the Hardy-Weinberg equation, we determined that the genotype frequencies were incompatible with the expected genotype frequency ($\chi^2 = 27,58$).

Table 2. Probes and primers and their sequences used in the detection of *CYP1B1*4* polymorphism

Primer/Probe	Primer/Prob sequencing 5'-3'
Prob A	5FAM/ ATC AAC AAG GAC CTG ACC AGC A /BHQ-1/
Prob B	5HEX/ ATC AAC AAG GGC CTG ACC AGC A /BHQ-1/
Primer F	GGA TGG AGA TGA AGA GAA
Primer R	GAT TCT TGG ACA AGG ATG

Table 3. Gender and age averages of the individuals participating in the study

Genotype	Sample (n)	Female (n-%)	Male (n-%)	Mean Age (Standard Deviation)
<i>CYP1B1*3</i>	150	15 -10%	135-90%	46(13,13)
<i>CYP1B1*4</i>	145	96- 66.2%	49-33.8%	45,08(10,81)

Considering the genotypes by gender, it was determined that 17 individuals (34.69%) had wild type (*CYP1B1**1/*1) genotype and 32 individuals (65.39%) had heterozygous (*CYP1B1**1/*4) genotype in males. It was determined that 40 individuals (41.67%) had wild type (*CYP1B1**1/*1) genotype and 56 individuals (58.33%) had heterozygous (*CYP1B1**1/*4) genotype in female individuals.

Discussion

No study looks at both polymorphisms in the Turkish population. Ada et al. [10], while looking at only *CYP1B1**4 in people working in a coke oven, Bilgin et al. [18] looked at NSCLC (Non-Small Cell Lung Cancer) patients and *CYP1B1**4. Güler et al. [11] examined *CYP1B1**2 and *CYP1B1**3 polymorphisms in NSCLC patients.

A study examining the relationship between material or breast cancer treatment and *CYP1B1* 4326 C>G polymorphism showed that TNBC (Triple Negative Breast Cancer) patients (37.0%) who were carriers of the *CYP1B1* 4326 GG variant genotypes had significantly lower disease-free rates than TNBC patients who were carriers of the *CYP1B1* 4326 CC/CG genotypes (71.0%) [1]. In several gene polymorphisms addressed with

breast cancer, including the *CYP1B1* Leu432Val polymorphism in Mexican women, no significant association was found between the Leu432Val gene polymorphism and breast cancer [19].

In a case-control study performed on 1000 control groups and 911 individuals diagnosed with breast cancer, 11 single-nucleotide polymorphisms were examined. As a result of the examination, it was determined that the rate of developing breast cancer was higher in heterogeneous and homozygous mutant individuals with *CYP1B1* Leu432Val and Asn453Ser polymorphisms [20]. It was studied in 200 lung cancer patients and an equal number of controls. A significant difference was observed in the distribution of variant genotypes of *CYP1B1*Arg48Gly and Ala119Ser polymorphisms (*CYP1B1**2) in cases compared to controls. No significant difference was observed in the distribution of variant genotypes of the *CYP1B1*Leu432Val (*CYP1B1**3) and *CYP1B1*Asn453Ser (*CYP1B1**4) polymorphisms [21].

As it can be understood from the examples above, although both polymorphisms were examined in limited numbers in other societies, no study in the Turkish population looked at both at the same time.

Table 4. Distribution of *CYP1B1**3 genotypes

Genotype	n (150)	Observed frequency (%)	Expected frequency (%) (Hardy-Weinberg)
<i>CYP1B1</i> *1/*1	59	39,33	41,8
<i>CYP1B1</i> *1/*3	76	50,67	45,7
<i>CYP1B1</i> *3/*3	15	10	12,5

Table 5. Distribution of *CYP1B1**4 genotypes

Genotype	n (150)	Observed frequency (%)	Expected frequency (%) (Hardy Weinberg)
<i>CYP1B1</i> *1/*1	57	39,31	70.4
<i>CYP1B1</i> *1/*4	88	60,69	61.3
<i>CYP1B1</i> *4/*4	-	-	13.4

When the *CYP1B1*3* polymorphism is compared with some countries, and the results of the *CYP1B1*3* polymorphism in this study and in other countries are examined; *CYP1B1*3* allele frequency was found 40.07% in the Czech Republic, 17.27% in China, 40.67% in Germans, 21.67 in Indians, 54.45% in Americans and 35.34% in our study (Table 6). As can be seen from the table, the *CYP1B1*3* allele frequency is similar to the populations of Germany and the Czech Republic. However, it differs from studies conducted with Chinese, Indian, and American societies. This shows that the incidence of *CYP1B1*3* polymorphism in the Turkish population is similar to that of European Caucasian populations. We can explain the differences in these findings with racial diversity, including Chinese, Indian-Asian race, and American - African American race.

When *CYP1B1*4* polymorphism is compared with other countries, In studies conducted in Spain, England, and America, the frequency of alleles was found to be 21, 18, and 15%, respectively. In Türkiye, the allele frequency of *CYP1B1*4* polymorphism was 30% (Table 7). Therefore, it can be said that the Turkish population does not show similarities with European white races and America in terms of this allele. Apart from this, we also do not show similarity with Japanese, that is, with Asian races, because no heterozygous and homozygous mutant individuals were found in studies conducted with Japanese.

The *CYP1B1* polymorphism is significant in understanding the mechanism of many hormone-mediated cancer types and disease types such as glaucoma.

Table 6. Comparison of *CYP1B1*3* polymorphism results with other countries

Country	n	Leu432Val (<i>CYP1B1*1/1</i>) (%)	Leu432Val (<i>CYP1B1*1/3</i>) (%)	Leu432Val (<i>CYP1B1*3/3</i>) (%)	Reference
Czech	122	30,83	58,20	10,97	[22]
Chinese	278	69,78	25,90	4,32	[23]
German	300	36,33	46	17,67	[24]
India	150	60,67	35,33	4	[25]
USA	1226	20,88	49,34	29,78	[26]
Turkey	150	39,33	50,67	10	This study

Table 7. Comparison of *CYP1B1*4* polymorphism results with other countries

Country	n	Asn453Ser (<i>CYP1B1*1/1</i>) (%)	Asn453Ser (<i>CYP1B1*1/4</i>) (%)	Asn453Ser (<i>CYP1B1*4/4</i>)(%)	Reference
Spain	297	63,97	30,30	5,73	[27]
England	2694	66,44	30,73	2,83	[28]
England	296	64,19	32,77	3,04	[29]
USA	182	72,52	25,27	2,21	[30]
Japan	200	100	-	-	[31]
Turkey	145	39,31	60,69	-	This study

4326C>G TNP leads to the amino acid substitution Leu432Val (L432V).

This change results in the *CYP1B1**3 allele. This polymorphism is important in the catalytic activity of CYP1B1. This activity causes the formation of 4-OHE2(4-Hydroxyestradiol) and thus increases estrogen carcinogenicity. It is also responsible for the AhR-mediated CYP1B1 gene expression increase. 4390A>G TNP leads to amino acid substitution Asn453Ser (N453S). This change results in the *CYP1B1**4 allele. The amino acid change caused by the 4390 A> G Asn453Ser (N453S) polymorphism does not affect the catalytic properties that play a role in protein production [32]. This polymorphism causes an increase in protein degradation. Therefore it is associated with a decrease in protein expression. In particular, a significant relationship was found between MI (myocardial infarction) and *CYP1B1**4 and smoking. Polymorphisms in the CYP1B1 gene, particularly the *CYP1B1**3 allele, are considered essential determinants of estrogen-mediated cancers [33]. While investigating the estrogen activity of CYP1B1 in women, they should be differentiated according to whether they are in the premenopausal or postmenopausal period. These periods are important because the level of estrogen in both circulation and tissues varies. Since the CYP1B1 enzyme is known to have polymorphic properties, its relationship with various diseases has been investigated. The effect of CYP1B1 on breast cancer is thought to be through the conversion of estradiol to 4-OHE2 [34]. Among the theories, the carcinogenic intermediate metabolites that occur with excessive expression of CYP are oxidized, react with DNA and tubulin, and cause toxicity. Overexpression in cancer types makes CYP1B1 valuable for its potential as a biomarker and a new therapeutic target [35].

It is not fully known whether CYP1B1 polymorphisms act alone or in combination with other polymorphisms. Many studies have been conducted on the effect of CYP1B1 polymorphism on breast cancer. There are many studies on the effect of CYP1B1_L432V polymorphism in particular. In this polymorphism, Leu-Val amino acid change occurs with the C-G change occurring

in both linker regions at codon 432 of CYP1B1. This change has been determined to increase the catalytic efficiency in 4-hydroxylation [36].

According to the results of a meta-analysis study in which 52 articles were analyzed, the association between CYP1B1 and breast and prostate cancer was significantly increased in the Asian population [37]. Studies have shown that it is also associated with colorectal and ovarian cancer [8].

Polymorphisms in CYP1B1 enzymes may lead to different results in oncology practices regarding drug safety profile and efficacy [38]. In a study of 95 breast cancers, the presence of the *CYP1B1**3 allele was significantly associated with groups with less hypersensitivity reaction to Taxane treatment [39]. This situation can be an example of genetic factors' effect on drug safety. Studies also show that paclitaxel resistance is more common in breast cancer patients with the *CYP1B1**3 allele [40]. In the study of Sissung et al., it is stated that the presence of the *CYP1B1**3 allele can be considered an important marker to predict the efficacy of docetaxel used in the treatment of 52 prostate cancer patients [41]. Anticancer drugs such as flutamide and mitoxantrone are valuable in terms of the effect of pharmacokinetic differences on the therapeutic process, leading to drug resistance by inhibiting Cyp1b1 metabolism [42]. In the study of Xie et al. involving 64 lung cancer patients, CYP1B1 expression was significantly higher in the group with resistance to cisplatin used in the treatment [43].

CYP1B1 rs1056836 was associated with increased CYP1B1 catalytic activity, while CYP1B1 rs1800440 was associated with a decrease in protein expression due to degradation [44]. *CYP1B1**4 (rs1800440) determined that women with the GG genotype were 3 times more likely to experience hot flashes for ≥ 1 year compared to the AA genotype [45].

In addition to many studies in the literature on the CYP-cancer relationship, some studies do not support this. It appears that having the *CYP1B1**4 (Asn453Ser) allele does not affect survival in patients with NSCLC [46]. It was also

concluded that the presence of the *CYP1B1**4 (Asn453Ser) mutant allele reduces enzyme activity and has a protective role for endometrial cancer carrying the *CYP1B1**4 (Asn453Ser) allele. Findings from case-control studies in lung and endometrial cancers, including breast cancer, do not confirm this information [47]. Therefore, there is no definitive judgment on this issue. Therefore, there is no definitive judgment on this issue. In the Copenhagen City Heart Study, which lasted 30 years and included more than 10 thousand people, no significant relationship was found between *CYP1B1**3 and *CYP1B1**4 genotypes and oncological and cardiovascular diseases [48].

Despite known dose-limiting side effects, many cancer drugs are used in treatment. Cardiotoxicity is an important problem in cancer diseases. Cardioprotection-based therapies can reduce anticancer drug effects and block chemotherapy [49]. The association between cardiovascular diseases and CYP1B1 has made CYP inhibitors a therapeutic target [50]. In vitro studies have also shown in various animal models that cyp1 inhibitors inhibit the cardiomyopathy-producing effect of doxorubicin [51]. Natural flavonoids such as Quercetin, chrysin, alpha-naphthoflavone, and 7,8-dehydrorutecarpy that inhibit Cyp1b1 and quinazoline derivatives are under investigation for their therapeutic value [52]. We think that research on this enzyme will contribute to drug development processes.

Conclusion

This study reveals the frequency of *CYP1B1**3 and *CYP1B1**4 alleles in a Turkish population in comparison with other populations and studies on the determination of individual susceptibility to various diseases and cancer that may be associated with CYP1B1 polymorphisms, as well as explains the metabolic differences between individuals of endogenous substances and therapeutics metabolized by CYP1B1 contributes to the literature for future studies on.

CYP1B1 needs to be investigated in larger populations to determine the effects of diagnosis, treatment process, and survival in many diseases, especially cancer diseases, and to provide primary data in determining the effect

size in the Turkish population.

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Conflict of interest

No conflict of interest was declared by the authors.

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