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*CYP3A5*3, CYP3A4*18* AND *CYP2B6*6* GENOTYPES AND CHRONIC MYELOID LEUKEMIA DEVELOPMENT IN AZERBAIJAN

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Abstract. The management of chronic myeloid leukemia (CML) has been dramatically improved in recent years. The cytochromes P450 (CYP) family is a major drug-metabolizing enzyme complex coordinating most currently prescribed drugs. Thus, single nucleotide polymorphisms (SNPs) in these enzyme coding genes may negatively affect drugs biotransformation and xenobiotics detoxification. The given experiment aimed to assess the influence of common CYP SNPs on susceptibility to CML. The genotyping of 153 CML patients and 100 healthy sex and age-matched controls were performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. To validate the obtained results, samples were sequenced via next-generation sequencing. The frequencies of homozygous mutant genotype (GG) and mutant G (**3*) allele of *CYP3A5*3* were significantly elevated in subjects compared to the control group; differences were statistically significant ($p<0.05$). In this study, no association was found between *CYP3A4*18* and CML susceptibility. Although CML patients carrying GT and TT genotypes and T allele were associated with a significantly higher risk of chronic myeloid leukemia (OR 1.2019; 95% CI: 0.6921–2.0873, OR 1.8919 95% CI: 0.8405–4.2587 and OR 1.3573;95% CI: 0.9188–2.0053, respectively), the association was not statically significant. We found that the abundantly present polymorphism of *CYP3A5*3* is associated with susceptibility to develop CML, which raises suggestions of its role as a genetic marker of the risk to deliver CML.

Keywords: *Cytochrome P450, chronic myeloid leukemia, polymorphisms.*

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1. Introduction

Chronic myeloid leukemia (CML) is a blood cancer driven by the BCR-ABL1 fusion gene and described by elevated production of immature granulocytes, which accumulate in the bone marrow, disrupting normal blood cell production. According to statistics, this condition is responsible for 15-20% of all cases of leukemias in adults (Soverini *et al.*, 2016).

The essential part of the molecular pathogenesis of CML is a gradual accumulation of genetic modifications that may alter drug-metabolizing enzymes

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activity. These enzymes play a crucial role in xenobiotics detoxification and drugs biotransformation. The P450 cytochrome superfamily is the most important drug-metabolizing complex, which converts drugs from a hydrophobic form to a readily excretable hydrophilic state (Manikandan *et al*., 2018).

Most human CYP genes possess specific endogenous functions, including the biosynthesis of prostaglandins, steroid hormones, bile acids and others. Thus, alterations at the CYP loci may be associated with cancer development or, on the contrary, can act as a protector from it, as CYPs are involved in the metabolism of a variety of anticancer drugs (Manikandan *et al*., 2018).

Among CYP families, CYP3A members are abundantly present in the liver and small intestine. The metabolism of more than 50% of recently prescribed drugs, carcinogens and harmful chemicals is coordinated by the CYP3A metabolizers. In people expressing CYP3A5, 50% of total hepatic CYP3A content is represented by the CYP3A5 enzyme. CYP3A4, in turn, is responsible for 40% to 45% of all phase I metabolism and plays a vital role in gastrointestinal CYP activity (up to 70%). Thus, CYP3A5 and CYP3A4 isoforms are considered crucial genetic contributors to interindividual differences in CYP3A-dependent drug metabolism (Wright *et al*., 2019).

Recent literature reports a great number of functional polymorphisms of CYP3A4 and CYPA5 families. However, among SNPs, the *CYP3A5*3* variant in the intron 3 at position 6986 (A>G, rs776746) is known to be one of the most prevalent polymorphisms of CYP3A5. It creates a cryptic consensus splice site and is suggested to influence CYP3A5 protein production and enzyme activity. Recent investigations indicated that this polymorphism might play an essential role in determining an individual's susceptibility to various types of cancer (Dandara *et al.*, 2005; Vaarala *et al*., 2008; Liang *et al*., 2018). Interestingly, variant-disease associations vary in different ethnic populations.

*CYP3A4*18* (rs28371759, Leu293Pro) is known as the most common allelic variation of CYP3A4. It affects bidirectional enzyme activity. Surprisingly, despite its frequent identification in many ethnic populations, there are few studies of its clinical relevance. It is known to be associated with high levels of testosterone and chlorpyrifos but with a low turnover of midazolam and nifedipine (Lee *et al*., 2013).

CYP2B6 is known as a less-characterized human isoform of the CYP P450 family. The gene is located on chromosome 19q13.2 and is involved in the metabolism of many xenobiotics. Although it was considered to play no or minor role in drug metabolism due to the lack of data regarding substrates and inhibitors, currently, it is understood that CYP2B6 enzyme is involved in the catalyzation of nearly 10-12% of drugs, and many chemical compounds (Langmia *et al*., 2021).

Among gene polymorphisms, *CYP2B6*6* (c.*516G>T*, rs3745274) is one of the most common exonic alterations, which is known to decrease hepatic expression of CYP2B6 mRNA and enzyme protein, and therefore CYP2B6 activity (Hofmann *et al*., 2008). This alteration is reported to have a relationship with cancer at various sites, such as breast cancer and acute leukemias (Justenhoven *et al.*, 2014; Yuan *et al*., 2011; Berkoz & Yalin, 2009).

Therefore, the inconsistency of reported data, caused by an insufficient number of studies on the frequency of these SNPs and their clinical relevance, demonstrates a high demand for further research in various ethnic populations. In this study, we have proposed to elucidate the possible relationship between common polymorphisms of CYP P450 and the risk of CML in Azerbaijan.

2. Materials and Methods

Subjects. The investigation was carried out according to the Declaration of Helsinki of 1964 and revised in 2013. The study was approved by the Ethical Board of the Institute of Hematology and Transfusiology, named after B.Eyvazov (IHT). All the patients signed the informed consent. The experiment comprised volunteers and patients from the Azerbaijani ethnic group who have resided in Azerbaijan for three consecutive generations. The patients who, along with CML, have a history or evidence of chronic or acute disorders, other types of cancer, hepatic or hematologic abnormalities, hepatitis B or C or HIV infection were not included in the investigation.

Due to established criteria, the study involved 153 unrelated CML patients (102 IM resistant and 51 IM good responders) treated in IHT between February, 2017 and December, 2019. The study cohort comprised 79 males and 74 females aged 19-80 (mean; 46.3; SD± 14.43). The diagnosis of CML was based on the standard clinical and hematological criteria and the presence of the Philadelphia chromosome and/or BCR-ABL fusion gene. All patients were confirmed with translocation t(9; 22). CML patients were in chronic (129), accelerated (13), or blast phase (11). In addition, patients were grouped into high, medium, and low-risk categories using the SOKAL scoring system. Distribution to risk groups depended on various criteria like spleen size, hemoglobin levels, platelets, white blood cells and red blood cell count, age, and disease phase (Table 1). The control group included 100 healthy volunteers admitted to IHT only for a check-up. Controls and patient groups were selected correspondingly in terms of similarity of age and sex distribution.

Table 1. Patients' characteristics

Parameters	**Patients (n=153), n (%)**	**Controls (n=100), n (%)**	**P-value (χ^2)**
Sex			
Male	79 (51.6)	46 (46)	**p=0.712**
Female	74 (48.4)	54 (54)	**χ^2=0.1358**
Age (years)			
<20	7 (4.7)	3 (3)	
20-40	54 (35.2)	37 (37)	
>40	92 (60.1)	60 (60)	**p=0.807**
Phase of CML			
Chronic	125 (81)		
Accelerated	24 (16)		
Blast Crisis	4 (3)		
SOKAL risk groups			
High	19 (12)		
Medium	87 (57)		
Low	47 (31)		

DNA extraction. According to the manufacturer's instructions, genomic DNA was isolated using a DNA extraction kit, QIAamp DNA Blood Mini kit (QIAGEN, Hilden,

Germany). DNA quality and quantity were measured by NanoDrop *2000c* Spectrophotometers (ThermoScientific).

Genotyping. We performed genotyping at the Institute of Genetic Resources of ANAS using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Gene-specific primers and PCR reaction conditions were previously reported by Maddin et al. (2016) and Berkoz and Yalin (2009).

The PCR reaction mixture (25ml) included 2.5 ml of 10x PCR buffer, 2.0 ml magnesium chloride (MgCl2), 0.25 ml dNTPs, 0.25 FIREPOL Taq Polymerase (Solis Biodyne, Estonia), 0.5 ml of each primer, 17 ml deionized water and 2 ml DNA. The amplification conditions were initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 30s, annealing (varied for each SNP) for 1 minute, 72°C for 2 minutes, and a final extension step at 72°C for 5 min. The electrophoresis of the products was performed on a 1.5% agarose gel at 120 V for 40 minutes.

Following the PCR reaction, 2.5 ml of PCR products were resolved with 0.5 units of restriction enzyme (New England BioLabs, BioLabs) for 45 min at 37°C. The digested PCR products were analyzed by electrophoresis on a 3% agarose gel. Table 2 provides more detailed data on genotyping.

Table 2. Detailed data of the PCR-RFLP analysis

		RFLP conditions			PCR conditions		
SNP	SNP ID	Enzyme	Genotype	Fragment size (*bp*)	Primers	Product (*bp*)	Temperature (^{o}C)
*CYP3A5*3*	*rs776746*	SspI	AA AG GG	148, 125, 20 168, 148, 125, 20 168, 125	forward 5'-GGTCCAAACAGGGAAGAAATA-3' reverse 5'-CATGACTTAGTAGACAGATGAC-3'	293	50
*CYP3A4*18*	*rs28371759*	MspI	TT TC CC	450 168, 282, 450 168, 282	forward 5'CACATCAGAATGAAACCACC-3' reverse 5'-AGAGCCTTCCTACATAGAGTCA-3'	450	51
*CYP2B6*6*	*rs3745274*	BsrI	GG GT TT	60, 518 50, 518, 578 570	forward 5'-CTGTGTCCTTGACCTGCTGC-3' reverse 5'TCCAGGAGCAGAATAGACATGAAG-3'	570	56

***Sequencing*.** Following genotyping, 10% of all samples from each genotype were randomly selected for confirmation sequencing. Initially, the PCR products were purified using a QIAquick PCR and then sent to the INTERGEN Laboratory (Ankara, Turkey) for confirmation via next-generation sequencing (Illumina Miseq).

***Statistical analysis*.** The chi-square test (χ^2) and Fisher's exact test were applied in order to compare the frequencies among control and CML subjects. We calculated the odds ratios (ORs) and 95% confidence intervals (CIs) for the risk association estimation. All the statistical tests were two-sided; the significance level was $p<0.05$. The statistical analysis was carried out using the *SPSS* package (ver. 22, SPSS, Chicago, IL) and Social Science Statistics (http://www.socscistatistics.com/tests/chisquare2/Default2.aspx).

3. Results

One hundred fifty-three CML cases and one hundred healthy volunteers were successfully recruited for the experiment. Table 1 demonstrates the subjects' characteristics. A higher frequency of subjects was observed in the age group >40 (60%) compared to other age groups. Among the patients, the overwhelming majority were found to be in the chronic phase (81%) in comparison with accelerated (16%) and blast phases (3%). No statistically significant association was identified between demographical parameters (gender, age) and the origin of CML ($p>0.05$) (Table 1).

In this investigation, we did not reveal statically significant associations of the given SNPs with the demographical parameters ($p>0.05$). Moreover, no statistically significant association was found between any of the polymorphisms and the phase of the disease ($p>0.05$).

Analysis of the *CYP3A5*3* frequency showed no wild-type homozygotes (AA) for the control group, while one case of AA genotype (0,7%) was reported for CML subjects. The frequency of heterozygous genotype (AG) in controls was 5%, while no AG genotype carriers were found in the patients' group (0%). The frequencies of homozygous mutant genotype (GG) and mutant G (*3) allele were significantly elevated in patients (99.3% and 99.3%, respectively) compared to the control group's (95% and 97.5%, respectively); differences were statistically significant ($p<0.05$) (Table 3).

Table 3. Distribution of *CYP3A5*3* in CML patients with respect to demographic and clinical parameters

Parameters	Genotypes, n (%)			Allele		P-value
	AA	AG	GG	A	G	
Patients	1 (0. 7)	0 (0)	152 (99.3)	0.007	0.993	**p=0.015**
Controls	0 (0)	5 (5)	95 (95)	0.025	0.975	
Sex						
Male	1 (1.3)	0 (0)	78 (98.7)	0.02	0.98	
Female	0 (0)	0 (0)	74 (100)	0	1	**p=0.869**
Age (years)						
<20	0 (0)	0 (0)	7 (100)	0	1	**p=0.109**
20–40	1 (2)	0 (0)	53 (98)	0.02	0.98	
>40	0 (0)	0 (0)	92 (100)	0	1	
Phase of CML						
Chronic	1 (0.8)	0 (0)	128 (99.2)	0.08	0.92	**p=1**
Accelerated	0 (0)	0 (0)	13 (100)	0	1	
Blast Crisis	0 (0)	0 (0)	11 (100)	0	1	
SOKAL risk groups						
High	1 (5)	0 (0)	18 (95)	0.05	0.95	**p=0.035**
Medium	0 (0)	0 (0)	87 (100)	0	1	
Low	0 (0)	0 (0)	47 (100)	0	1	

In this study, for *CYP3A4*18*, all the subjects in the control group were wild-type homozygous (TT). The frequency of homozygous wild-type (TT) and heterozygous (TC) genotypes in the patients' group were 98% and 2%, respectively. The prevalence of T and C alleles in the patient's group was 99.9% and 1%, respectively. No statistically significant difference was observed between the *CYP3A4*18* and CML susceptibility (p=0.159) (Table 4).

Table 4. Distribution of *CYP3A4*18* in CML patients with respect to demographic and clinical parameters

Parameters	**Genotypes, n (%)**			**Allele**		**P-value (χ^2)**
	TT	**TC**	**CC**	**T**	**C**	
Patients	150 (98)	3 (2)	0 (0)	0.99	0.01	**p=0.159**
Controls	100 (100)	0 (0)	0 (0)	1	0	
Sex						
Male	77 (98)	2 (2)	0 (0)	0.98	0.02	**p=0.605**
Female	73 (99)	1 (1)	0 (0)	0.99	0.01	
Age (years)						
<20	7 (100)	0 (0)	0 (0)	1	0	
20–40	52 (97)	2 (3)	0 (0)	0.97	0.03	**p=0.109**
>40	91 (99)	1 (1)	0 (0)	0.99	0.01	
Phase of CML						
Chronic	126 (98)	3 (2)	0 (0)	0.98	0.02	
Accelerated	13 (100)	0 (0)	0 (0)	1	0	**p=1**
Blast Crisis	11 (100)	0 (0)	0 (0)	1	0	
SOKAL risk groups						
High	19 (100)	0 (0)	0 (0)	1	0	
Medium	87 (100)	0 (0)	0 (0)	1	0	**p=0.032**
Low	44 (94)	3 (6)	0 (0)	0.96	0.04	

In addition, *CYP3A5*3* and *CYP3A4*18* polymorphisms were found to have a statistically significant association with CML risk groups criteria (p=0.035 and p=0.032, respectively).

The frequencies of homozygous wild-type genotype (GG) of *CYP2B6*6* were 48.4% and 55% for patients and controls, respectively. Interestingly, results demonstrated relatively similar values for heterozygous genotype (GT) among patients and controls, 35,3% and 35%, respectively. Furthermore, the frequencies of *CYP2B6*6* homozygous mutant genotype (TT) and T allele (**6*) were notably elevated in CML subjects (16.3% and 34%, respectively) than that of the control group (10% and 27.5%, respectively). Nevertheless, the differences were not statistically significant (p=0.2910) (Table 5). When the genotype data were analyzed with respect to clinical parameters, none of the variables showed a significant association with *CYP2B6*6* (Table 5).

Individuals with mutant allele **3* had an estimated value of 4-fold (95% CI: 0.7683 to 20.8246) increased risk of CML, but the differences were not statistically significant (p=0.0996) (Table 6). Although CML patients carrying GT and TT

genotypes and T allele were associated with a significantly higher risk of chronic myeloid leukemia (OR 1.2019; 95% CI: 0.6921–2.0873, OR 1.8919 95% CI: 0.8405–4.2587 and OR 1.3573;95% CI: 0.9188–2.0053, respectively), the association was not statically significant (Table 6).

Table 5. Distribution of CYP2B6*6 in CML patients with respect to demographic and clinical parameters

Parameters	**Genotypes, n (%)**			**Allele**		**P-value (χ^2)**
	GG	**GT**	**TT**	**G**	**T**	
Patients	74 (48.4)	54 (35.3)	25 (16.3)	0.65	0.35	**p=0.291 χ^2=2.472,**
Controls	56 (56)	34 (34)	10 (10)	0.725	0.275	
Sex						
Male (79)	43 (54.5)	23 (29)	13 (16.5)	0.69	0.31	**p=0.257 χ^2=2.71,**
Female (74)	31 (41)	30 (40)	13 (19)	0.61	0.39	
Age (years)						
<20	4 (57)	2 (28)	1 (15)	0.71	0.29	**p=0.974**
20–40	29 (53)	15 (28)	10 (19)	0.68	0.32	
>40	45 (48)	28 (30)	19 (22)	0.63	0.37	
Phase of CML						
Chronic	1 (0.8)	0 (0)	128 (99.2)	0.08	0.992	**p=0.504**
Accelerated	0 (0)	0 (0)	13 (100)	0	1	
Blast Crisis	0 (0)	0 (0)	11 (100)	0	1	
SOKAL risk groups						
High	1 (5)	0 (0)	19 (95)	0.05	0.95	
Medium	0 (0)	0 (0)	88 (100)	0	1	**p=0.820**
Low	0 (0)	0 (0)	45 (100)	0	1	

Table 6. Genotype and allele frequencies of CYP SNPs in patients and controls

SNP, rs number	**Genotype**	**Frequency**		**Risks**	**P-value**	**Allele**	**Allele Frequency**		**Risks**	**P-value**
		Patients	*Controls*	*OR (95% CI)*			*Patients*	*Controls*	*OR (95% CI)*	
*CYP3A5*3 rs776746*	AA	0.007	0			A	0.07	0.025		
	AG	0	0.5	-		G	0.993	0.975	4 (0.7683–20.8246)	**0.0996**
	GG	0.993	0.95	-						
*CYP3A4*18 rs28371759*	TT	0.98	1			T	0.99	1		
	TC	0.2	0	-		C	0.01	0	--	
	CC	0	0	-						
*CYP2B6*6 rs3745274*	GG	48.4	0.55			G	0.65	0.725		
	GT	35.3	0.35	1.2019, (0.6921–2.0873)	**0.5137**	T	0.35	0.275	1.3573 (0.9188–2.005)	**0.1249**
	TT	16.3	0.1	1.8919 (0.8405–4.2587)	**0.1235**					

4. Discussion

The CYPs enzymes metabolize a great number of xenobiotics and endogenous compounds. Also, being a primary group of biotransformation enzymes, this large multi-gene family of hemoproteins plays a vital role in regulating the toxic, mutagenic and neoplastic effects of carcinogens (Kassogue *et al*., 2014). Thus, a combination of genetic polymorphism and carcinogen exposure may affect cancer susceptibility (Maddin et al., 2016). The metabolic activation of chemical carcinogens may trigger cancer initiation. The xenobiotic-metabolizing mechanism constitutes two major groups of enzymes: the phase-I CYPs P450 and phase-II conjugating enzymes. As plenty of them appear to be polymorphic, they demonstrate significant variation in the ability to detoxify xenobiotics and thereby raise suggestions related to their contribution to individual cancer susceptibility as genetic modifiers of malignant tumor risk (Bajpai *et al*., 2007; Bozina *et al*., 2009).

Though pathological and clinical aspects of chronic myeloid leukemia are well studied, the etiology of genes that affect susceptibility to this disorder remains unclear (Bruzzoni-Giovanelli, 2015; Malak *et al.*, 2012; Kim *et al.*, 2009). Nevertheless, over recent years the number of association studies with gene candidates increased, revealing single nucleotide polymorphisms (SNPs), which may be associated with the risk of CML development (Maddin *et al*., 2016; Bajpai *et al*., 2010; Ozten *et al*., 2012; Hishida *et al*., 2005).

Being the most common polymorphism in the CYP3A subfamily, *CYP3A5*3* demonstrates significant variation in protein expression between different ethnic groups; also, it is known to have a functional effect on environmental pollutants activation (Bajpai et al., 2010). It is known to create a cryptic splice site mutation, which results in the non-expression of CYP3A5. In this study, we revealed a statistically significant association between *CYP3A5*3* and the risk of CML development. Elevation in the frequency of polymorphic *3 allele with lower CYP expression might cause disruption in the xenobiotics' detoxification mechanism, which in turn might be responsible for disease progression. Furthermore, this may result in resistance to therapy, drug toxicity and poor survival among CML patients. Our study is supported by Rao et al. and Sailaja et al., where results showed that *CYP3A5*3* is significantly associated with the risk of acute lymphocytic leukemia (ALL) or acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) (Rao *et al*., 2011; Sailaja *et al*., 2010). Abd El Wahab et al. (2017) reported similar results in the Egyptian population, suggesting that *CYP3A5*3* carriers might have an increased risk of AML. A study in the Nordic population showed an elevated risk of developing childhood acute lymphoblastic leukemia (ALL) for CYP3A5 expressors compared to nonexpressors (Borst *et al.*, 2011). In contrast, several recent studies in Indian and Chinese populations demonstrated no significant association between SNP and CML susceptibility (Bajpai *et al.*, 2010; Shen *et al*., 2008; Liu *et al*., 2002). In addition, recent metanalysis on the relationship between *CYP3A5*3* and cancer risk provided evidence that the given polymorphism plays an important role in the development of blood cancer, especially among Asian and Caucasian populations (Wang *et al*., 2013).

Due to emerging evidence that *CYP3A5*3* might play an essential role in the etiology of carcinogenesis, there are a number of studies on its possible role as a risk factor for different cancers; nevertheless, the results varied. For instance, studies on *CYP3A5*3* were positively associated with non-small cell lung and prostate cancers

(Vaarala *et al*., 2008; Nogal *et al.,* 2007). In African populations, *CYP3A5*3* was suggested to be associated with an increased risk of prostate cancer (Liang *et al*., 2018) but no significant association was found between the given SNP and the risk of developing prostate cancer in the Finnish population (Markku *et al.*, 2008). In addition, an investigation in the Bulgarian population with sporadic colorectal cancer failed to demonstrate an association with CYP3A5 variants (Petrova *et al.*, 2007).

The analysis of the *CYP3A4*18* allele in 270 CML patients in the Malaysian population revealed an absence of a homozygous mutant genotype despite its high prevalence in different ethnic populations, especially in Asians (Berkoz & Yalin, 2009). In this study, we revealed no association of *CYP3A4*18* with the risk of CML.

Stratifying patients into risk groups is an essential clinical variable that bears a prognostic role in estimating disease progression and possible treatment outcomes. In this investigation, *CYP3A5*3* and *CYP3A4*18* polymorphisms were found to have a statistically significant association with the SOKAL risks group parameter. Interestingly, the frequency of **3* was higher in the low-risk group than in the high-risk group, while the frequency of **18* was elevated in high-risk carriers compared to low-risk subjects. Thus, it raises suggestions regarding *CYP3A4*18* as a possible therapeutic predictor.

*CYP2B6*6* is known to be a common exonic SNP, which plays an important role in the metabolism of a significant number of xenobiotics (Berkoz & Yalin, 2009). In addition, this SNP was suggested to have an influence on various types of blood cancer and solid tumors and even affect anticancer therapy outcomes (Justenhoven *et al*., 2014; Berkoz & Yalin, 2009, Kassogue *et al*., 2014). In this experiment, although the frequency of mutant genotype (TT) was significantly elevated in patients compared to controls, the differences were not statistically significant. Our results are in agreement with Kassogue et al. findings (Maddin *et al*., 2016). In contrast, a study in the Turkish population demonstrated 2.48-fold and 1.92-fold elevated risks of developing acute leukemia for subjects with GT genotype and T allele carriers, respectively (Berkoz & Yalin, 2009). In addition, another association study of CYP2B6 and blood cancer susceptibility in the Chinese population revealed the *6 allele as one of the risk factors predisposing to acute leukemia (Yu *et al*., 2020).

5. Conclusions

To the best of our knowledge, this is the first study to reveal the involvement of common *CYP3A5*3*, *CYP3A4*18*, and *CYP2B6*6* polymorphisms in the origin of CML in the Azerbaijani population. However, study limitations should be acknowledged. First, as this investigation is classified as small sample-sized, there might be an overestimation of associations with susceptibility of CML or treatment response. Thus, there is a necessity for further research in larger cohorts to unveil the contribution of the given SNPs. Second, the lack of related data in different ethnic populations limits the confirmation of the obtained findings.

In conclusion, we found that abundantly present polymorphism of *CYP3A5*3* significantly influences susceptibility to develop CML, which raises suggestions of its role as a genetic marker of the risk to delivering CML.

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