Association of *CYP3A5*3, CYP3A4*18 & CYP2B6*6* polymorphisms with imatinib treatment outcome in Azerbaijani chronic myeloid leukaemia patients

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Background & objectives: Imatinib mesylate (IM) is a reliable first line treatment for chronic myeloid leukaemia (CML). Nevertheless, despite promising results, a considerable proportion of patients develop resistance to the drug. Cytochrome P450 (CYP) enzymes play a crucial role in IM metabolism. Thus, point mutations in *CYP* genes may modify IM enzyme activity resulting in insufficient treatment response. This investigation was aimed to identify the functional impact of *CYP3A5*3, CYP3A4*18* and *CYP2B6*6* polymorphisms on the IM response in patients with CML in Azerbaijan.

Methods: Genotyping of *CYP3A5*3, CYP3A4*18* and *CYP2B6*6* was performed in 153 patients (102 IM non-responders and 51 IM responders) with CML by the PCR-restriction fragment length polymorphism (RFLP) assays. The odds ratios (ORs) with 95 per cent confidence intervals (CIs) were applied to assess the association between allelic variants and IM therapy outcome. The results were validated by sequencing.

Results: The frequency of the *CYP3A4*18* allele was considerably lower in the responder's group (97.1 *vs.* 100%; *P*=0.036). For *CYP3A5*3,* the allelic frequency was slightly higher among the IM responders (100 *vs.* 99.02%) with no significant difference. Although patients heterozygous (TC) for *CYP2B6*6* demonstrated a higher risk of acquiring resistance (OR 1.04; 95% CI: 0.492-2.218), differences were not significant (*P*=0.909). In addition, the homozygous genotype (TT) demonstrated a lower risk of unresponsiveness (OR 0.72; 95% CI: 0.283-1.836), but associations were not significant (*P*=0.491).

Interpretation & conclusions: Our results demonstrated that *CYP3A4*18* was significantly associated with IM treatment response in patients with CML in Azerbaijan, whereas rather common *CYP3A5*3* was identified to have no such association.

Imatinib mesylate (IM) is a selective tyrosine kinase inhibitor (TKI) that has become a prototype in target treatment against haematological malignancies. The management of chronic myelogenous leukaemia

(CML) altered its natural course with IM introduction. It is specifically designed to block the expansion of cells expressing not only the BCR-ABL fusion gene but also the receptor of stem cell factor, c-kit tyrosine kinases and platelet-derived growth factor. In addition, IM was the first TKI approved by the Food and Drug Administration[1].

Being a frontline therapy, IM has demonstrated a promising cytogenetic, haematologic and molecular response and ensured a decrease in the time of response, toxicity and the patients' morbidity and mortality[2].

Nevertheless, even though imatinib is the gold standard for treatment of CML, some patients develop resistance, which poses a major therapeutic challenge. Resistance may be orchestrated by various mechanisms such as mutation or amplification of the BCR-ABL domain, upregulation of multidrug-resistance gene 1 or genetic alterations of enzymes engaged in IM metabolism, not to mention the famous family of cytochrome P450 (CYP). Recent studies show that more than 25 per cent of patients with CML may demonstrate resistance to IM at least once through their lifetime[3].

CYP complex plays a crucial part in the metabolism of various drugs and IM is not an exception. This sophisticated complex comprises a family of haemoproteins that catalyze drug metabolism reactions and the synthesis of cholesterol, steroids and other lipids. Three major *CYP* gene families (*CYP1*, *CYP2* and *CYP3*) perform xenobiotic metabolism. CYP3A enzyme is highly inducible and plays a major role in the metabolism of drugs in the liver and gastrointestinal tract. The subfamily includes several isoforms: CYP3A4, CYP3A5, CYP3A7 and CYP3A43. *In vitro* studies using recombinant enzymes indicate that N-demethylation of IM is performed by CYP3A4, CYP2C8 and CYP3A5, while other enzymes play little or no role[4,5].

It is important to note that IM is mainly metabolized by the CYP3A4 isoform, and to a lesser extent, by several other enzymes such as CYP3A5, CYP2C9, CYP2B6 and CYP1A2. Thus, genetic variability among the patients could explain the differences in IM bioavailability, affecting intracellular and plasma levels and eventually influencing the therapeutic response[6].

The *CYP3A5* gene is located on chromosome 7q21.1 position and indicates interindividual variations at expression levels. The most common isoform variant is *CYP3A5*3* (A6986G, rs776746), which involves splicing defects. This polymorphism that is situated in intron 3 may decrease the expression of *CYP3A5* to less than 1/1000 of what is found in carriers of the wild-type allele (*CYP3A5*1*)[7]. Moreover, several recent studies indicate its potential association with IM metabolism[8-11].

Another alteration of a crucial drug metabolism contributor, the *CYP3A4* gene, is *CYP3A4*18* (rs28371759). One of the most frequent coding variants is located on exon 10 and involves a substitution from thymine (T) to cytosine (C) at position 878, which is known to have an association with high levels of testosterone and chlorpyrifos. Moreover, it is also associated with low bone mineral density and elevated prostate cancer risk[12].

Being one of the most polymorphic *CYP* genes in humans, the *CYP2B6* gene is situated on chromosome 19q13.2 and metabolizes different types of drugs, including cyclophosphamide, isophamide, tamoxifen, ketamine, propofol and various environmental carcinogens. Its alterations affect transcriptional regulation, splicing, mRNA and protein expression and catalytic activity. *CYP2B6*6* (Q172H) is the most common variant leading to complex interactions between substrate-dependent and independent mechanisms. The frequencies of G15631T (rs3745274) vary from 15 to 60 per cent in different populations[13]. Recent investigations have shown this polymorphism to be associated with a genetic predisposition to acute leukaemia[14-16].

Despite the fact that a significant number of investigations of *CYP* single-nucleotide polymorphisms (SNPs) are involved in the pharmacogenetics and pharmacodynamics of imatinib, the contribution of these variants to patients' drug resistance remains unclear and requires further research.

This study was designed to estimate the frequency of *CYP3A5*3* (G6986A), *CYP3A4*18* (C878T) and *CYP2B6*6* (G15631T) in patients with CML in the Azerbaijani population undergoing IM therapy along with the assessment of their impact on imatinib treatment response.

Material & Methods

Study participants: This study was carried out by the department of Haematology, Institute of Haematology and Blood Transfusion (IHB; named after B. Eyvazov), Baku, Azerbaijan, from April 2017 to December 2019. The study was approved by the Institute's Ethical Board

and carried out according to the Declaration of Helsinki of 1964, as revised in 2020. All the patients included in the investigation signed the informed consent. The study involved individuals who belonged to the Azerbaijani ethnic group and resided in Azerbaijan for three consecutive generations. Individuals who along with CML, had a history or evidence of chronic or acute disorders, other types of cancer, hepatic or haematological abnormalities and hepatitis B or C or HIV infection were excluded from the study.

A total of 748 CML patients were admitted to the IHB during the study period, of whom 404 (54%) were female and 344 (46%) male (median age, 47.4 yr; standard deviation, 15 yr and range, 4-84 yr). The resistance to IM occurred in 155 participants; based on inclusion criteria, our study involved 153 unrelated patients (102 IM resistant and 51 IM good responders) who were treated in IHB between February 2017 and December 2019. The study cohort comprised 79 females (51.6%) and 74 males (48.4%) (mean age, 46.1 yr; standard deviation, 14.4 yr and range, 19-80 yr). Diagnosis of CML was based on the standard clinical and haematological criteria and the presence of the Philadelphia chromosome and/or BCR-ABL fusion gene. Participants with CML were in chronic (n=129), accelerated (n=13) and blast (n=11) phases, treated for at least 12 months with IM on frontline treatment. Each patient had started with a standard dose of 400 mg/day; subsequently, the dose was increased to 600 or 800 mg in the absence of major molecular response (MMR) at 12 months. Among the patients included in this investigation, 123 were treated with 400 mg, 27 participants with 600 mg and three patients with 800 mg.

In addition, as risk group types define treatment strategy, patients were grouped into high, medium and low risk categories according to the Sokal scoring system. The distribution to risk groups depended on various criteria such as white blood cells and platelets counts, age, baseline spleen size, basophils, eosinophils and myeloblasts in peripheral blood (Table I).

Imatinib mesylate (IM) response assessment: Patient inclusion was performed according to the criteria defined by European Leukaemia Net recommendations for the management of chronic myeloid leukaemia[17]. The criteria for complete haematological response were defined on the basis of the total leucocyte, platelet and white blood cell counts, absence of immature forms and disappearance of splenomegaly[18]. In the given

cohort, 146 (96%) patients with CML reached complete haematological response after seven weeks of therapy.

The molecular response assessment was defined, referring to the international scale (IS) as the ratio of BCR-ABL1 transcripts to ABL1 transcripts[17]. A BCR-ABL1 transcript level of ≤0.1 per cent corresponds with the MMR.

A complete molecular remission was attained by 107 (70%) patients after 12 months of treatment.

The BCR-ABL1 (p210) expression was evaluated using the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) technique for initial diagnosis and subsequent monitoring of the efficacy of TKI therapy. The analysis of BCR-ABL1 (p210) e13a2 and e14a2 fusion transcripts in peripheral blood and bone marrow samples was performed by using Mbcr IS-MMR kit (Qiagen, Hilden, Germany) on Rotor-Gene Q (Qiagen).

Thus, IM good responders group included patients who fulfilled optimal response criteria. IM resistant group consisted of the patient who failed to achieve MMR at 12, 18 or 24 months. Treatment options for patients with inadequate IM response included a second generation TKI (nilotinib and dasatinib) or allogeneic haematopoietic stem cell transplantation (HSCT).

DNA extraction: Two millilitres of peripheral blood samples in EDTA tubes were collected from the study participants. The isolation of genomic DNA was performed with QIAamp DNA Blood Mini kit (Qiagen), according to the manufacturer's protocol. DNA quality and quantity were analyzed using NanoDrop 2000c Spectrophotometers (Thermo Scientific, Waltham, MA, USA).

Genotyping: Genotyping was carried out at the Institute of Genetic Resources of Azerbaijan National Academy of Sciences (ANAS) by the PCR-restriction fragment length polymorphism (RFLP). Gene specific primers and PCR reaction conditions were previously published by Maddin *et al*[11] and Berköz and Yalin[14] for *CYP3A5*3*, *CYP3A4*18* and *CYP2B6*6,* respectively.

The PCR reaction mixture (25 µl) included 2 µl DNA, 2.0 µl magnesium chloride ($MgCl_2$), 0.25 µl dNTPs, 0.25 FIREPOL Taq Polymerase (Solis Biodyne, Estonia), 0.5 µl of each primer, 17 µl deionized water and 2.5 µl of ×10 PCR buffer. The electrophoresis of

Table I. Patient's characteristics				
Parameters	All patients (n=153), n (%)	IM resistant (n=102), n (%)	IM good response (n=51), n (%)	P (χ^2)
Gender				
Male	74 (100)	52 (70.2)	22 (29.8)	0.36 (0.837)
Female	79 (100)	50 (63.2)	29 (36.8)	
Age (yr)				
<50	102 (100)	67 (65.7)	35 (34.3)	0.716 (0.132)
≥50	51 (100)	35 (68.6)	16 (31.4)	
CML stages				
Chronic	129 (100)	78 (60.5)	51 (39.5)	0.04[δ]
Accelerated	13 (100)	13 (100)	0	
Blast crisis	11 (100)	11 (100)	0	
Risk groups				
High	19 (100)	14 (73.7)	5 (26.3)	0.785 (0.483)
Medium	87 (100)	57 (65.5)	30 (34.5)	
Low	47 (100)	31 (66)	16 (34)	
Spleen				
Normal	21 (100)	16 (76.1)	5 (23.9)	0.009 (9.243)
Medium (growth up to 8 cm)	88 (100)	50 (56.8)	38 (43.2)	
Massive (>8 cm)	44 (100)	36 (81.8)	8 (18.2)	
HB (g/l)				
Normal (120-160 g/l)	13 (100)	8 (61.5)	5 (38.5)	0.288 (3.758)
Mild anaemia (100-119 g/l)	44 (100)	30 (68.1)	14 (31.9)	
Mean anaemia (80-99 g/l)	53 (100)	31 (58.4)	22 (41.6)	
Severe anaemia (65-79 g/l)	43 (100)	33 (75)	10 (25)	
WBC ($\times 10^9$/l)				
Normal (3.9-9)	1 (100)	1 (100)	0	0.781[δ]
Leucopenia (<3.9)	0	0	0	
Leucocytosis (>9)	152 (100)	101 (66.4)	51 (33.6)	
PLT ($\times 10^9$/l)				
Normal (180-400)	92 (100)	64 (69.5)	28 (30.5)	0.354 (2.074)
Thrombocytopenia (<180)	21 (100)	15 (71.4)	6 (28.6)	
Thrombocytosis (>400)	40 (100)	23 (57.5)	17 (42.5)	
Blast cell (0%)				
0	68 (100)	41 (60.2)	27 (39.8)	0.323[δ]
1-10	81 (100)	58 (71.6)	23 (28.4)	
>10	4 (100)	3 (75)	1 (25)	
$P<0.05$ was considered as significant. [δ]Test statistic not reported for Fisher's exact test. PLT, platelet; WBC, white blood cell; HB, haemoglobin; CML, chronic myeloid leukaemia; IM, imatinib mesylate				

the products was performed on a 1.5 per cent agarose gel at 120 V for 40 min.

PCR amplification products (2.5 µl) were subjected to digestion with 0.5 units of restriction enzyme (New England BioLabs, BioLabs). The digested PCR products were run on a three per cent agarose gel for visualisation.

Sequencing: We randomly selected 10 per cent of all samples from each genotype for confirmation sequencing. The amplified DNA was purified using

a QIAquick PCR (Qiagen) and sent to the Intergen Laboratory (Ankara, Türkiye) for confirmation *via* next generation sequencing. In addition, 10 per cent of total *CYP3A5*3* allele samples were sequenced at the Genetic Resources Institute of Azerbaijan National Academy of Sciences (ANAS) *via* 3130XL Sanger Sequencing (Applied Biosystems, MA, USA) to validate previously obtained results.

Statistical analysis: Chi-square test (χ^2) and Fisher's exact test were applied to compare the frequencies of polymorphic genotypes between good and poor responders. Fisher's exact test for contingency tables more than 2 × 2 was performed with Social Science Statistics (*http://www.socscistatistics.com/tests/chisquare2/Default2.aspx*). Binary logistic regression was carried out to calculate the odds ratios (ORs) with 95 per cent confidence intervals (CIs). All the statistical tests were two-sided; the significance level was *P*<0.05. Statistical analysis was performed using the SPSS package ver. 22 (Statistical Package for the Social Sciences, Chicago, IL, USA).

Results

Table I summarizes patients' demographic profiles and clinical variables. In this study, we did not find any association between IM response and demographic parameters such as gender and age. Among clinical parameters, Sokal risk groups, white blood cells and platelets counts, haemoglobin levels and blast cells range were not associated with IM response. However, the disease phase and spleen size were considerably associated with the IM response (*P*=0.04 and *P*=0.009, respectively).

Our data revealed a significant association between *CYP3A4*18* polymorphism and IM treatment response (*P*=0.013).

In this investigation, the frequencies of *CYP3A5*3*, *CYP3A4*18* and *CYP2B6*6 alleles were 99.3, one and 34 per cent, respectively. Between the two groups of CML participants, the frequency of the *CYP3A5*3* allele was not significantly elevated (100%) among the IM responders compared to the non-responders (99.02%). Although the allelic frequency of *CYP2B6*6* polymorphism in IM responders was higher (36.3%) than in the IM resistant group (32.9%), this difference was not significant. In contrast, the frequency of the *CYP3A4*18* allele was significantly elevated in the poor responders (100%) compared to the good responders (97.1%; *P*=0.036; Table II).

Interestingly, for the *CYP3A5*3* polymorphism, most of the participants (99.93%) in both groups were found to possess homozygous mutant (GG) and only one subject was identified with wild type (AA). The frequency of *3/*3* (GG) genotype in good responders (100%) was not significantly different from the non-responders (99.02%). Our analysis did not reveal homozygous mutant genotype (CC) for *CYP3A4*18*. The frequencies of the homozygous wild type genotype (TT) were considerably lower (*P*=0.013) among responsive patients (94.12%) compared to resistant patients (100%). However, the frequency of heterozygous genotype (TC) was notably higher in the IM-responders (5.88 *vs*. 0%).

For *CYP2B6*, the genotypic frequencies of all three types of inheritance (GG, GT and TT) were elevated in the poor responders compared to the good responders; nevertheless, the difference was not statistically significant (Table II).

We performed a binary logistic regression to determine the risk association of polymorphisms with imatinib outcome. Heterozygous (GT) genotype carriers of *CYP2B6*6* demonstrated a higher risk of acquiring resistance (OR 1.04; 95% CI: 0.492-2.218), whereas homozygous genotype (TT) demonstrated a lower risk of unresponsiveness towards the drug (OR 0.72; 95% CI: 0.283-1.836), but the associations were not significant (*P*=0.909 and *P*=0.491, respectively).

In addition, the relationship of SNPs with the epidemiological and clinical parameters was assessed of patients with CML. No significant differences were observed between *CYP3A5*3* and *CYP3A4*18* polymorphisms and age, gender or CML phases in the present study. However, criteria such as risk groups demonstrated an association with the given polymorphisms (*P*=0.035 and *P*=0.032, respectively) (Table III). As for *CYP2B6*6*, no significant differences were detected between the polymorphisms and mentioned parameters (Table III).

Sequencing results confirmed the successful performance of RFLP analysis of *CYP3A5*3*, *CYP3A4*18* and *CYP2B6*6* (Figure).

Discussion

IM possesses numerous advantageous characteristics, including a balanced dose-response relationship and very high bioavailability. However, despite being the recommended type of therapy, quite often, cancer cells develop a survival strategy

SNP	Frequency (%)		P (χ^2)
	IM good response (n=51), n (%)	IM resistant (n=102), n (%)	
CYP3A5*3 (rs776746)			
Genotype			
Homozygous wild type (AA)	0	1 (0.98)	0.478[δ]
Heterozygous (AG)	0	0	
Homozygous mutant (GG)	51 (100)	101 (99.02)	
Allele			
A	0	2 (0.98)	0.316[δ]
G	102 (100)	202 (99.02)	
CYP3A4*18 (rs28371759)			
Genotype			
Homozygous wild type (TT)	48 (94.12)	102 (100)	0.013[δ]
Heterozygous (TC)	3 (5.88)	0	
Homozygous mutant (CC)	0	0	
Allele			
T	99 (97.1)	204 (100)	0.036[δ]
C	3 (2.9)	0	
CYP2B6*6 (rs3745274)			
Genotype			
Homozygous wild type (GG)	24 (47.06)	50 (49.02)	0.737 (0.61)
Heterozygous (GT)	17 (33.33)	37 (36.27)	
Homozygous mutant (TT)	10 (19.61)	15 (14.71)	
Allele			
G	65 (63.7)	137 (67.1)	0.609 (0.357)
T	37 (36.3)	67 (32.9)	

Table II. Genotype and allele frequencies of *CYP3A5*3, CYP3A4*18* and *CYP2B6*6*

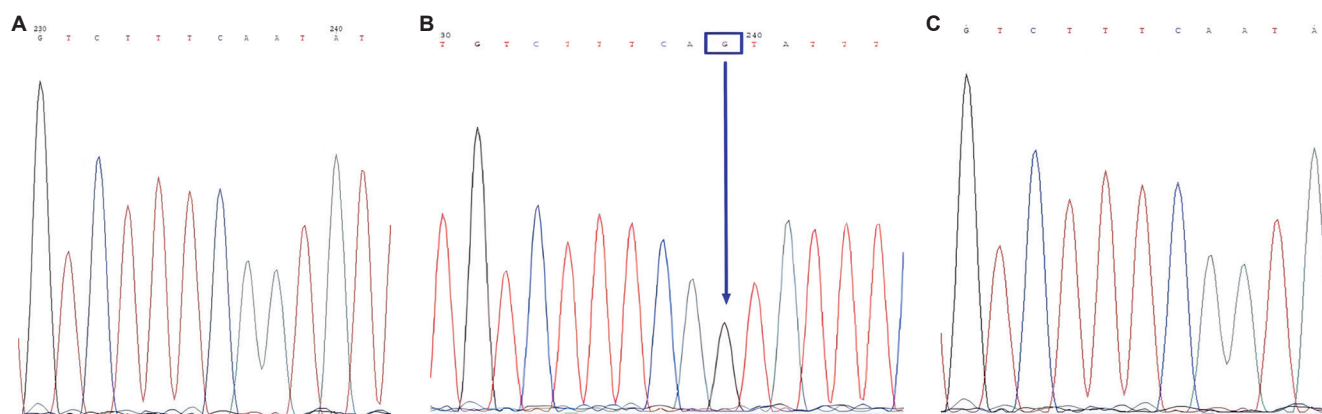[δ]Test statistic not reported for Fisher's exact test. IM, imatinib mesylate

Figure. Confirmation of the *CYP3A5*3* allele by the Sanger sequencing method. Each peak in electropherogram represents a single nucleotide in the DNA sequence. (**A**) sequencing of the sample with wildtype genotype (*CYP3A5*1/*1*), (**B**) sequencing of the mutant genotype (*CYP3A5*3/*3*) revealed a single base substitution of adenine with guanine (A>G) in intron 3, and (**C**) matched reference sequence. PCR, polymerase chain reaction.

Table III. Distribution of CYP3A5*3, CYP3A4*18 and CYP2B6*6 polymorphisms in chronic myeloid leukaemia patients with respect to epidemiological and clinical parameters

Genotype	Age (yr)		Gender		Sokal risk score			CML stages		
	<50 (n-103), n (%)	≥50 (n=51), n (%)	Male (n=74), n (%)	Female (n=79), n (%)	High (n=19), n (%)	Medium (n=87), n (%)	Low (n=47)	Chronic (n=129), n (%)	Accelerated (n=13), n (%)	Blast crisis (n=11), n (%)
*CYP3A5*3*										
Homozygous wild type (AA)	1 (0.98)	0	1 (1.4)	1 (1.2)	1 (5.2)#	0	0	1 (0.8)	0	0
Heterozygous (AG)	0	0	0	0	0	0	0	0	0	0
Homozygous mutant (GG)	101 (99.02)	51 (100)	73 (98.6)	78 (98.8)	18 (94.8)#	87 (100)#	47 (100)#	128 (99.2)	13 (100)	11 (100)
*CYP3A4*18*										
Homozygous wild type (TT)	100 (98.04)	50 (98.04)	72 (97.2)	78 (98.8)	19 (100)δ	87 (100)δ	44 (93.6)δ	126 (97.7)	13 (100)	11 (100)
Heterozygous (TC)	2 (1.96)	1 (1.96)	2 (2.8)	1 (1.2)	0	0	3 (6.4)δ	3 (2.3)	0	0
Homozygous mutant (CC)	0	0	0	0	0	0	0	0	0	0
*CYP2B6*6*										
Homozygous wild type (GG)	49 (48)	25 (49)	41 (55.4)	33 (41.7)	7 (36.8)	43 (49.4)	24 (51.1)	63 (49)	5 (35.7)	6 (54.6)
Heterozygous (GT)	37 (36.4)	17 (33.3)	22 (29.7)	32 (40.5)	9 (47.4)	29 (33.3)	16 (34)	45 (340)	7 (50)	2 (18.2)
Homozygous mutant (TT)	16 (15.6)	9 (17.7)	11 (14.9)	14 (17.8)	3 (15.8)	15 (17.3)	7 (14.9)	21 (17)	1 (14)	3 (27.3)

#$P<0.05$ for CYP3A5*3; δ$P<0.05$ for CYP3A4*18 polymorphism, respectively. CML, chronic myeloid leukemia

causing resistance after a few months or years of therapy[19]. Among the identified mechanisms to develop resistance, alterations or amplification of the target protein play a significant role. Thus, mutations in major imatinib metabolizers CYP3A5 and CYP3A4 may correlate with IM treatment response.

CYP3A5*3 is the most frequent and well studied variant allele of CYP3A5. CYP3A5*1 is known to be associated with higher expression of CYP3A5 protein. Interestingly, a recent study of CYP3A5*3 polymorphism in healthy Azerbaijani participants revealed homozygous mutant genotype (*3/*3) frequency as 95 per cent, which is significantly lower than in this investigation (99%), indicating that elevated homozygous mutant genotype (*3/*3) may contribute to the condition. No significant difference was found between CYP3A5*3 and molecular response to IM in patients with CML. Our findings correlate with the results of different studies on various ethnic populations investigating this problem. For instance, recent investigations of CYP3A5*3 and dose-adjusted imatinib trough concentrations[20,21] in different Asian populations did not reveal a significant correlation between the genotypes and the imatinib plasma trough concentrations and clinical response frequencies. Similar studies of the association between the polymorphism and the imatinib trough plasma levels also demonstrated an absence of the correlation[22,23].

Nevertheless, our results are not in agreement with the results of recent investigations in the Egyptian and Indian populations, which concluded affirmatively about an association of CYP3A*1/*1 genotype with IM efficacy and *3/*3 genotype with inferior outcome[9,24]. Other related experiments with opposite outcomes were performed in the Canadian population and Indian population, where AA genotype in rs776746 was strongly associated with major or complete cytogenetic response to Imatinib[8,10]. Although we identified no significant relationship between CYP3A5*3 and risks of acquiring resistance to IM, studies suggest that the drug's low clearance and high oral bioavailability may be caused by the homozygous mutant genotype. Thus, carriers of *3/*3 may have a decreased enzyme activity, leading to a better response to imatinib[11].

Being one of the most abundant SNPs of CYP3A4, CYP3A4*18 may affect the overall protein structure and cause rearrangement of substrate recognition sites, which are essential for substrate recognition and substrate access to the active site. Alterations of that type may lead to disrupted metabolism of many drugs[12]. Since this SNP contributes to the slower metabolism of xenobiotics, it raises the suggestion of its involvement in imatinib resistance. For instance, the CYP3A4*18 variant in this study considerably influences IM therapy (P=0.013). In contrast, a study of the association between the genotypes of CYP3A4*18 and the imatinib plasma trough concentrations in the Korean population did not reveal statistically significant differences[21].

Similarly, the investigation of the CYP3A4*18 and IM levels in Indian population also did not show any association. Interestingly, although high interindividual variability in the trough concentration of IM was observed, none of the patients were found to be carriers of the CYP3A4*18 allele[25]. Moreover, Maddin et al[11] identified that patients with CML carrying the heterozygous genotype CYP3A4*18 showed a lower risk of acquiring IM resistance, but the association was not significant. An inconsistency with previously reported results in various populations highlights the demand for further studies in larger cohorts. However, similar to the studies from different ethnic populations[11,26,27] and in healthy Azerbaijani volunteers[28], no homozygous mutant variant was detected (CC) among our patients.

Increasing interest in G15631T has been stimulated by the revelations of a specific CYP2B6 genotype, which greatly affects the metabolism of various drugs, with the purpose of increasing drug efficacy and avoiding adverse drug reactions[29]. Furthermore, as this SNP causes the transition of glutamine (Glu) amino acid to histidine (His), it may block carcinogen substrates' transformation into harmless metabolites[14]. This study found no statistically significant association between CYP2B6*6 polymorphism and IM therapy response in patients with CML. In accordance with our results, a study of enzyme functionality of human P450 stated that CYP2B6 had not been implicated in IM metabolism[30]. Differently from us, according to the investigation of the impact of CYP2B6 15631G>T on IM response and CML susceptibility, SNP has been revealed as a good therapeutic predictor due to its strong association with clinical and cytogenetical outcomes[31].

To best of our knowledge, the present investigation is the first to report allelic and genotypic frequencies of CYP SNPs and their impact on IM response in the population of Azerbaijan. These findings are an attempt

to provide preliminary evidence that pre-treatment genotyping of *CYP3A4*18* may help predict the therapeutic response of IM in patients with CML. Nevertheless, these alterations may be population specific and should be profiled in a larger cohort to evaluate clinical relevance.

In conclusion, despite the absence of correlation with IM response, polymorphisms of *CYP3A5*3* are common among the Azerbaijani population. Contrastingly, SNPs of *CYP3A4*18* are significantly associated with IM response suggesting a risk of being resistant to IM therapy.

Financial support & sponsorship: None.

Conflicts of Interest: None.

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