RESEARCH COMMUNICATION

Analysis of CYP3A5*3 and CYP3A5*6 Gene Polymorphisms in Indian Chronic Myeloid Leukemia Patients

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Abstract

CYP3A5 is a member of the CYP3A gene family which metabolizes 50% of therapeutic drugs and steroid hormones. CYP3A5*3 and CYP3A5*6 polymorphisms exhibit inter-individual differences in CYP3A5 expression. The CYP3A5*3 allele (A6986G transition in intron 3) results in loss of CYP3A5 expression and the CYP3A5*6 allele (G14690A transition in exon 2, leading to the skipping of exon 7) is associated with lower CYP3A5 catalytic activity. The aim of the present study was to investigate their influence on susceptibility to chronic myeloid leukemia (CML). 265 CML cases and 241 age and sex matched healthy controls were analyzed by the PCR-RFLP technique. The frequencies of homozygous 3/3 genotype and CYP3A5*3 allele were elevated significantly in the CML group compared to controls ($\chi 2=93.15$, df=2, p=0.0001). With respect to clinical parameters, CYP3A5*3 allele frequency was increased in patients with advanced phase of the disease (0.71) as compared to those in chronic phase (0.65). Patients without hematological response (minor/poor) had higher frequency of 3/3 genotype (54.54%) as compared to those with major hematological response (41.2%). CYP3A5*6 allele was not observed in cases as well as in controls. Our study suggests that the CYP3A5*3 gene polymorphism is significantly associated with the risk of CML development and disease progression.

Keywords: CYP3A5 gene - chronic myeloid leukemia - single nucleotide polymorphism - PCR-RFLP - response

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Introduction

CYP3A is the major drug-metabolizing enzyme in the gastrointestinal tract and liver (Wilkinson et al., 1997). It has broad substrate specificity and can metabolize approximately 50% of therapeutic drugs (nifedipine and cyclosporine), steroid hormones (testosterone, progesterone and androstenedione) and several xenobiotics (Paulussen et al., 2000). It is highly inducible and can also be inhibited by numerous drugs. The CYP3A5 gene is a part of a cluster of cytochrome P450 genes: CYP3A4, 3A5, 3A7 and 3A43, localized on chromosome 7q21.1 position (Gellner et al., 2001). CYP3A5 gene exhibit interindividual variations in expression levels. The most frequent polymorphisms identified in all ethnic populations involving splicing defects are CYP3A5*3 and CYP3A5*6 (Kuehl et al., 2001).

CYP3A5*3 polymorphism in intron 3 of the CYP3A5 gene can reduce the expression of CYP3A5 to less than 1/1000 of that found in carriers of the wild type allele (CYP3A5*1). CYP3A5*3 allele (A to G substitution at position 6986) produces a cryptic splice site and encodes for an abnormally spliced mRNA with a premature stop codon, while CYP3A5*1 allele (A at position 6989) produces a normal mRNA, resulting in a high expression of this enzyme. Another polymorphism CYP3A5*6 (G14690A substitution in exon 2, leading to the skipping of exon 7) cause alternate splicing and protein truncation leading to complete absence of CYP3A5 from tissues of some people (Kuehl et al., 2001). Since CYP3A5 is the primary extrahepatic CYP3A enzyme, the polymorphic variation in CYP3A5 might be influencing the disposition of endogenous steroids or xenobiotics in these tissues (kidney, prostrate, lung, breast, and leukocytes) which might increase the risk to develop disease condition.

Imatinib (tyrosine kinase inhibitor) is the target drug used in chronic myeloid leukemia (CML) therapy, whose metabolism is mainly mediated by CYP3A4 and CYP3A5, to some extent by other isoenzymes such as CYP2D6, CYP2C9, CYP1A2 and CYP2C19 (Peng et al., 2004). The present study on the association of CYP3A5*3 and *6 gene polymorphisms with CML was carried out in order to understand the involvement of CYP3A5 polymorphisms in the origin of CML and also their impact on imatinib response.

Materials and Methods

The present study includes 265 CML and 241 control samples. Blood samples were collected from CML patients

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being treated at NIMS (Nizam's Institute of Medical Sciences), Hyderabad. The age and sex matched control samples without history of cancer were randomly selected from different localities in Andhra Pradesh. Patient's clinical data like phase of the disease and response to therapy was noted from the tumor registry file with the help of oncologist. Response status (hematological and cytogenetic) was classified on the basis of the total leukocyte count, percentage of ph+ve cells and duration of response to imatinib therapy (Druker et al., 2005). Genomic DNA was isolated by using salting-out method (Lahiri and Nurnberger, 1991) and used for polymorphic analysis using PCR-RFLP technique.

*Genotyping of CYP3A5*3 and CYP3A5*6 polymorphisms (Kuehl et al., 2001)*

CYP3A5*3 (G6986A) polymorphism was analysed using a set of gene specific primers Forward 5'- gtt gta cgc cac aca gca cc - 3' and Reverse 5'- ctc ttt aaa gag ctc ttt tgt ctc tca- 3' to get 155bp PCR product. 5μl of PCR product was subjected to restriction digestion with one unit of Dde1 enzyme and genotyping was done on 3% agarose gel. CYP3A5*1 (wild allele) was identified by the presence of two fragments of size 121, 34 bps whereas CYP3A5*3 (mutant allele) by fragments of sizes 97, 34, and 24 bps.

A 268 bp fragment was amplified for analysis of

Table 1. Characteristics of the CML Patients (n=265)

Item	No	%
Gender		
Males	183	69.05
Females	82	30.94
Age		
< 20 Yrs	25	9.43
20-30 Yrs	68	25.66
30-40 Yrs	60	22.64
> 40 Yrs	112	42.26
Disease Phase		
Chronic	216	81.50
Accelerated	19	7.16
Blast Crisis	23	8.67

Table 2. Distribution of CYP3A5*3 Polymorphism in Chronic Myeloid Leukemia with Respect to Epidemiological and Clinical Parameters

parameters	1/1	1/3	3/3	Allele frequency	
	No %	No %	No %	1	3
Cases (265)	33 (12.5)	115 (43.4)	117 (44.2)	0.341	0.658
Controls (241)	124 (51.5)	71 (29.5)	46 (19.1)	0.661	0.338
		$\chi2 = 93.15$; df = 2, p = <0.0001			
Age of the proband					
< 20 Yrs (25)	3 (12.0)	11 (44.0)	11 (44.0)	0.340	0.66
20-30 Yrs (68)	12 (17.6)	30 (44.1)	26 (38.2)	0.397	0.602
30-40 Yrs (60)	8 (13.3)	22 (36.7)	30 (50.0)	0.316	0.683
> 40 Yrs (112)	10 (8.9)	52 (46.4)	50 (44.6)	0.321	0.678
		$\chi2 = 4.52$; df = 6, p = 0.6067			
Sex of the Proband					
Males (183)	20 (10.9)	83 (45.4)	80 (43.7)	0.336	0.663
Females (82)	13 (15.9)	32 (39.0)	37 (45.1)	0.353	0.646
		$\chi2 = 1.65$; df = 2, p = 0.438			
Phase of CML					
Chronic (216)	28 (13.0)	95 (44.0)	93 (43.1)	0.349	0.650
Accelerated (19)	1 (5.3)	11 (57.9)	7 (36.8)	0.342	0.657
Blast Crisis (23)	3 (13.0)	7 (30.4)	13 (56.5)	0.282	0.717
		$\chi2 = 3.73$; df = 4, p = 0.4438			
Hematological response					
Major (187)	26 (13.9)	84 (44.9)	77 (41.2)	0.363	0.636
Minor (11)	1 (9.1)	4 (36.4)	6 (54.5)	0.272	0.727
Poor (22)	1 (4.5)	9 (40.9)	12 (54.5)	0.250	0.75
		$\chi2 = 2.86$; df = 4, p = 0.5815			
Cytogenetic Response					
Major (139)	19 (13.7)	58 (41.7)	62 (44.6)	0.345	0.654
Minor (30)	2 (6.7)	16 (53.3)	12 (40.0)	0.333	0.666
Poor (41)	8 (19.5)	20 (48.8)	13 (31.7)	0.439	0.560
		$\chi2 = 4.29$; df = 4, p = 0.3682			

CYP3A5*6 (A14690G) polymorphism using primers Forward 5'- gag aga aat aat gga tct aag aaa cc -3' and Reverse 5'- gat agt tct gaa agt ctg tgg c - 3'. The amplified product (268 bp fragment) was digested with one unit of DdeI enzyme (New England Biolabs) at 37°C for overnight and electrophoresed on 14% PAGE. CYP3A5*1 wild allele produces fragments of different sizes 120, 103, 25 &20 and CYP3A5*6 mutant allele produces 128, 120 & 20 bp fragments.

Statistical Analysis

All the statistical analyses were performed with Statistical Package for the Social Science (SPSS) 15.0. Chi square test was calculated to test the significance of genotype association with the occurrence of CML and its prognosis. All the P values were two sided and the level of significance was taken as $p<0.05$.

Results

In the present study, the association of epidemiological, clinical and molecular variables with the origin of CML was evaluated. The mean age at onset of CML was found to be 36.6 years with age at onset ranging from 9-70 years. Stronger male preponderance was observed in the present study with a sex ratio of 2.2 :1 indicating higher risk for male sex to develop CML. Higher frequency of patients was observed in the age group>40 years (42.26%) as compared to other age groups. 81.50% of the patients were found to be in chronic phase as compared to accelerated (7.16%) and blast phases (8.67%) (Table 1).

The frequency distribution of CYP3A5*3 polymorphism in CML patients and controls was shown in Table 2. The frequency of 3/3 genotype was significantly elevated in the CML patients (44.2%) as compared to controls (19.1%) with corresponding increase in CYP3A5*3 allele frequency indicating that presence of CYP3A5*3 allele might confer increased risk of developing CML ($\chi 2$- 93.15, df- 2, $p<0.0001$). No significant association was observed between CYP3A5*3 polymorphism and age at onset of CML and sex of the proband which indicates that CYP3A5*3 polymorphism might be independent risk factor and not influenced by age and sex of the patient.

When clinical phase of CML was considered, CYP3A5*3 allele frequency exhibited increasing trend with disease progression from chronic to blast crisis. Patients in the advanced phases had higher frequency of CYP3A5*3 allele (0.71) compared to those in chronic phase (0.65). The significance of CYP3A5*3 gene polymorphism with respect to Imatinib therapy was evaluated in terms of hematological and cytogenetic responses. Patients without hematological response (minor/poor) had higher frequency of 3/3 genotype (54.54%) as compared to those with major hematological response (41.17%). Whereas no association of CYP3A5*3 with cytogenetic response was observed (Table 2).

Discussion

In the present study, mean age at onset of CML was found to be 36.6 years with modal class being>40 years. Stronger male preponderance was observed with a sex ratio of 2.1 :1 indicating higher risk for male sex to develop CML. Chronic myeloid leukemia may occur at any age, but the incidence of CML increases exponentially with age, with presentation being most common among adults above the age of 40 years (Sawyers, 1999; Faderl et al., 1999). Other studies revealed variability in incidence of CML in different populations (Au et al., 2009). In the present study, most of the CML patients were diagnosed in chronic phase as compared to accelerated and blast phases as the duration for chronic phase varies from several months to years. Previous study (Kumar et al., 2003) (2003), also reported the elevated frequency of patients in chronic phase (88.5%) compared to those in accelerated and blast crisis (11.5%). Patients in the early phase had increased response rates compared to accelerated and blast crisis phases. Hence phase of the disease was considered as important factor in determining the progression of the disease CML.

CYP3A5*3 polymorphism with reduced mRNA expression leads to drug toxicity and subsequent DNA damage which might be responsible for disease progression. In the present study, significant elevation of 3/3 homozygous genotype and CYP3A5*3 allele frequency in CML group was observed which indicated that the loss of CYP3A5 expression associated with mutant allele might be responsible for the accumulation of endogenous steroids or xenobiotics in different tissues which might induce genotoxicity that confer the risk for disease susceptibility. Further 3/3 genotype was also associated with progression of disease. On contrary, previous studies reported similar frequencies of CYP3A5*3 allele in both the leukemia group and controls (Liu et al., 2002; Blanco et al., 2002; Aplenc et al., 2003; Bajpai et al., 2009). Shen et al., (2008) failed to observe significant association between CYP3A5 polymorphism and morbidity of acute leukemia patients, but the expression of CYP3A5 in acute leukemia patients was closely associated with the chemotherapeutic effect and prognosis (Shen et al., 2008).

However previous studies on solid tumors reported a significant association of CYP3A5*3 polymorphism with esophageal (Dandara et al., 2005) non-small cell lung (Nogal et al., 2007) and prostate cancers (Varala et al., 2008). Petrova et al, (2007) reported that CYP3A5 variants were not associated with the occurrence of colorectal cancer in Bulgarian population (Petrova et al., 2007).

All Ph positive CML patients recruited in the present study were treated with imatinib. Genetic variation in CYP3A activity might influence the rate of metabolism and elimination of CYP3A substrates such as Imatinib. The association of 3/3 genotype with poor hematological response but not with cytogenetic response might indicate the involvement of much more complex interaction in imatinib metabolism.

In the present study, CYP3A5*6 allele was not observed in both of the CML patients and controls (results

not included). Our results are in correspondence with other studies from Caucasian (Kuehl et al., 2001) and Japanese population (Fukuen et al., 2002).

Our study suggests that CYP3A5*3 gene polymorphism in CML patients might be associated in conferring increased risk for CML development and disease progression but not significant with imatinib response. Hence the analysis of CYP3A5 gene polymorphisms might be helpful in maintaing the CML patients for progression.

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