RESEARCH COMMUNICATION

Association of the GSTP1 gene (Ile105Val) Polymorphism with Chronic Myeloid Leukemia

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Abstract

The GSTP1 enzyme plays a key role in biotransformation and bioactivation of certain environmental pollutants such as benzo[a]pyrene-7, 8-diol-9,10-epoxide (BPDE) and other diol epoxides of polycyclic aromatic hydrocarbons. It catalyses the detoxification of base propanols that arise from DNA oxidation thus offering cellular protection against oxidative stress. A single nucleotide polymorphism at codon 105 results in the substitution of isoleucine (Ile) to valine (Val) causing a metabolically less active variant of the enzyme. We here assessed the impact of the GSTP1 codon 105 polymorphism in chronic myeloid leukemia (CML) development and therapy response. The Ile105Val polymorphism was analyzed using a PCR-RFLP technique. Two hundred and sixty patients with CML and 248 healthy, age and sex matched controls were included in the study of associations with patient characteristics and treatment outcome. The GSTP1 Ile105Val polymorphism was significantly associated with CML development ($\chi 2 = 9.57$; df = 2; p = 0.0084). With respect to clinical phase, CML patients in advanced phase (accelerated and blast crisis) had higher frequency of heterozygous (Ile/Val) genotype (47.62%) compared to chronic phase (36.5%). Further 54.5% of patients in blast crisis carried valine allele as compared to those in chronic phase (36.5%). The frequency of combined genotypes (Ile/Val, Val/Val) was elevated in cytogenetic poor (41.6%) and minor (53.57%) responders as compared to major (38.51%) responders. Hence the present study suggests that GSTP1 Ile105Val polymorphism with reduced GSTP1 enzyme activity might influence CML development, progression and response rates.

Keywords: GSTP1 gene - chronic myeloid leukemia - single nucleotide polymorphism - PCR-RFLP

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Introduction

The glutathione S-transferases (GSTs) are a family of enzymes belongs to phase II enzymes involved in detoxification of xenobiotics (carcinogens, pesticides, antitumor agents, & environmental pollutants), in which the glutathione is conjugated to the electrophilic center of the compound via sulfhydryl group on a wide variety of substrates to facilitate their excretion (Boyer, 1989). Hence GSTs play significant role in the cellular defense. GSTs fall into two distinct superfamilies: membrane bound microsomal GSTs and the soluble or cytosolic GSTs. The cytosolic glutathione S-transferase were classified into eight classes on the basis of sequence diversity and designated as Alpha (α), Mu (μ), Pi (л), Kappa (K), Theta (θ), Omega (O), Sigma (ε) and Zeta (Z) (Mannervik et al., 1985). These cytosolic enzymes play major role in detoxification of activated carcinogens (Rebbeck et al., 1997).

Glutathione S-transferase P1 (GSTP1) belongs to the pi class gene family, located on chromosome 11q13 (Autrup, 2000). It spans 2.48kb of DNA and comprises of 7 exons (Morrow et al., 1989 and Bora et al., 1997) that encode for cytosolic GST enzyme. GSTP1 is considered as major antioxidant present in both the epidermis and the dermis, overexpressed in a variety of preneoplastic and neoplastic tissues (Moscow et al., 1989; Landi, 2000). GSTP1 was found to be commonly overexpressed in tumors and elevated levels had been found in tumors of stomach, colon, bladder, oral, breast, skin, and lung (Moscow et al., 1989; Howie et al., 1990; Singh et al., 1990; Peters et al., 1990; Peters et al., 1992; Singh et al., 1994; Mulder et al., 1995; Shimizu et al., 1995). In some cancer models, GSTP1 expression was considered as preneoplastic tumor marker. Increased levels of GSTP1 in tumors might account for part of the inherent drug resistance, which was observed in many tumors suggesting its role in cancer etiology and therapy (Tsuchida and Sato, 1992). GSTP1 gene possesses two variations in coding region, an A→G transition at 105 codon and a C→T transition at 114 codon (Ali-Osman et al., 1997; Watson et al., 1998). GST polymorphisms may alter the ability of enzymes to metabolize the chemical carcinogens and mutagens. It had been suggested that these differences in the ability

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to metabolize carcinogens and mutagens might influence the susceptibility to cancer (Taspinar et al., 2008).

The GSTP1 polymorphism at codon 105 (exon 5) is an A-to-G transition resulting in an amino acid substitution of isoleucine by valine (Zimniak et al., 1994). Isoleucine 105 form exhibited lower catalytic activity towards several carcinogenic diol epoxides as compared to valine 105 form (Hu et al., 1997). The substitution of the less bulkier and more hydrophobic valine results in substrate-dependent alterations of GSTP1 catalytic activity (Ali-Osman et al., 1997; Sundberg et al., 1998). The polymorphic enzyme (Val 105) has a 7-fold higher diol epoxide activity, and a 3-fold lower 1-chloro-2, 4-dinitrobenzene activity, when compared to the wild-type protein (Ile 105) (Harries et al., 1997). Individuals with the GST P1 valine allele showed a significant higher levels of DNA adducts (Ryberg et al., 1997).

Hence an attempt was made to find out whether GSTP1 gene (Ile105Val) polymorphism might be a predictive factor in development and therapy response in chronic myeloid leukemia (CML) patients.

Materials and Methods

In the present study 260 CML samples were chosen from Nizam's Institute of Medical Sciences, Hyderabad, reported during 2004-2006. 248 healthy age and sex matched individuals living in different areas of Andhra Pradesh without family history of CML or any other cancers were selected to serve as control group. Informed consent was taken from all the individuals recruited for the study, after obtaining ethical committee clearance. All ph+ve CML patients were treated with Abl specific tyrosine kinase targeted drug, imatinib. Response status of the CML patients (hematological and cytogenetic responses) were classified into major, partial/minor or poor response (Druker et al., 2005). 5ml of blood was collected in an EDTA vaccutainer from patients and controls. DNA was isolated and used for analysis of GSTP1Ile105Val polymorphism (Lahiri and Nurnberger, 1991)

Genotyping of GSTP1 polymorphism (Ile105Val)

Genotyping of GSTP1 polymorphism Ile105Val was analyzed using PCR-RFLP technique. A set of primers forward 5'- GTA GTT TGC CCA AGG TCA AG – 3'and reverse 5'- AGC CAC CTG AGG GGT AAG –3' were used to amplify 436bp product. PCR reaction mixture (25μl) consisted of genomic DNA -150ng, primers - 15 pmol/l, dNTPs- 200μmol/l, tris buffer -20 mmol/l, MgCl2 - 2.5 mmol/l, taq DNA polymerase - 0.5 U, and deionized water. PCR cycling conditions consisted of initial denaturation at 94°C for 3 minutes followed by 5 cycles of denaturation at 94°C for 15s, annealing at 60°C for 30s, extension at 72°C for 60s and followed by 5 cycles of denaturation at 94°C for 15s, annealing at 60°C for 30s, extension at 72°C for 60s and final extension at 72°C for 5 minutes. After PCR, the products were checked on 1% agarose gel for presence of amplification. All samples were subjected for restriction digestion with Bsm A1 enzyme (Fermentas, India). Isoleucine variant at 105 position produces 2 fragments 329, 107 bps and valine variant at 105 position produces 3 fragments 216, 113, 107 bps.

Statistical analysis

The allele and genotype frequencies were calculated by direct counting. Differences in genotype frequency distribution between patients and controls were studied using 2*2 contingency x2 test and x2 test for heterogeneity. All the P values were two sided and the level of significance was taken as $p<0.05$.

Results and Discussion

GST is also expressed in erythrocytes though the function is not known, but the red cell membrane contains transport systems that actively transport GSH-xenobiotic conjugates from the erythrocytes. Thus GST may serve to rid the red cell and perhaps to scavenge foreign molecules from the blood stream. Red cell GST also binds heme, an extremely hydrophobic molecule, in a manner analogous to the binding of bilirubin by liver GST enzymes. It had been suggested that its function may involve intracellular transport of heme within the developing erythroid cell (Beutler et al., 1988).

The frequency distribution of GSTP1 gene (Ile105Val)

Table 1. Distribution of GSTP1 Gene (Ile105Val) Polymorphism in Chronic Myeloid Leukemia with respect to Epidemiological and Clinical Parameters

Parameters	Ile/Ile	Ile/Val	Val/Val	Allele frequency	
	No%	No%	No%	I	V
Cases(260)	141(54.2)	102(39.2)	17(6.5)	0.74	0.26
Controls(248)	140(56.5)	105(42.3)	3(1.2)	0.78	0.22
	$\chi2 = 9.57$; df = 2; p = 0.0084				
Age of the proband					
< 20 Yrs(24)	8(33.3)	14(58.3)	2(8.3)	0.625	0.375
20-30 Yrs(66)	40(60.6)	23(38.4)	3(4.5)	0.780	0.219
30-40 Yrs(60)	33(55.0)	24(40.0)	3(5.0)	0.75	0.25
> 40 Yrs(110)	60(54.5)	41(37.3)	9(8.2)	0.731	0.268
	$\chi2 = 6.63$; df = 6; p = 0.390				
Sex of the Proband					
Males(178)	100(56.2)	68(38.2)	10(5.6)	0.752	0.247
Females(82)	41(50.0)	34(41.5)	7(8.5)	0.707	0.292
	$\chi2 = 1.28$; df = 2; p = 0.527				
Phase of CML					
Chronic(211)	118(55.9)	77(36.5)	16(7.6)	0.741	0.258
Accelerated (20)	11(55.0)	8(40.0)	1(5.0)	0.75	0.25
Blast Crisis(22)	10(45.5)	12(54.5)	---	0.727	0.272
	$\chi2 = 3.91$; df = 4; p = 0.418				
Hematological response					
Major(183)	103(56.3)	65(35.5)	15(8.2)	0.74	0.26
Minor (10)	5 (50.0)	5 (50.5)	---	0.72	0.25
Poor(21)	14(66.7)	6(28.6)	1(4.8)	0.81	0.19
	$\chi2 = 2.4$; df = 4; p = 0.662				
Cytogenetic Response					
Major(135)	83(61.5)	41(30.4)	11(8.1)	0.77	0.23
Minor(28)	13(46.4)	11(39.3)	4(14.3)	0.66	0.33
Poor(41)	24(58.5)	16(39.0)	1(2.4)	0.78	0.22
	$\chi2 = 4.97$; df = 4; p = 0.290				

polymorphism was represented in table 1. In our study, GSTP1 Val/Val genotype frequency was found to be significantly elevated in the CML (6.5%) compared to controls (1.2%) ($\chi 2$ - 9.57, df – 2, p – 0.0084) indicating that this genotype might confer risk to develop CML. Valine genotype has decreased enzyme activity which might be due to altered catalytic activity and thermal stability of the enzyme. This leads to less detoxifying efficiency for the ultimate carcinogens like polycyclic aromatic hydrocarbons (PAH) which would induce DNA adducts and ultimately lead to carcinogenesis (Hayes and Pultford, 1995). Other studies had also reported significant association of valine allele with susceptibility to develop tumors of bladder, breast, lung, and multiple myeloma (Ryberg et al., 1997; Helzlsouer et al., 1998; Maggini et al., 2008). Previous studies showed that GSTP1 105 val genotype had been associated with favourable prognosis following chemotherapy with drugs known to be GSTP1 substrates in a variety of malignancies such as pediatric acute lymphoblastic leukemia, breast and colon cancers (Stanulla et al., 2000; Sweeney et al., 2000; Dasgupta et al., 2003).

With respect to age of the proband, heterozygote frequency (Ile105Val) for valine was found to be elevated in the group of patients below 20 years with corresponding increase in valine allele frequency (0.375) compared to patients in higher age groups. Very interesting observation was that 66.6% of CML patients with age at onset <20years were found to carry valine allele. Our results suggested that presence of valine allele confers increased risk to develop CML at early age, which might be due to reduced rate of detoxification of metabolites derived due to UVR-derived oxidative stress and other environmental carcinogens. When sex of the proband was considered, the valine allele frequencies were found to be more or less equal in both sexes.

When clinical phase was considered, CML patients in advanced phase (accelerated and blast crisis) had higher frequency of heterozygous Ile105Val genotype (47.62%) compared to chronic phase (36.5%). Further 54.5% of patients with blast crisis carried valine allele as compared to those in chronic phase (36.5%) suggesting that valine allele predispose the individuals to develop advanced disease.

When response to imatinib was considered, haematological response did not show association with GSTP1 polymorphism, whereas the frequency of combined genotypes (Ile/Val, Val/Val) was elevated in cytogenetic poor (41.6%) and minor (53.57%) responders as compared to major (38.51%) responders. These results had suggested that GSTP1 Ile105Val polymorphism with reduced GSTP1 enzyme activity might result in accumulation of intermediate metabolites in the body leading to additional mutations which might influence disease progression and response rates.

Hence the study of GSTP1Ile105Val polymorphism would be helpful in assessing the risk for disease progression and drug responses in CML.

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