

# Association of CYP3A5\*3 polymorphism with development of acute leukemia

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**BACKGROUND:** CYP3A5 was observed to be an important genetic contributor to inter individual differences in CYP3A-dependent drug metabolism in acute leukemic patients. Loss of CYP3A5 expression was mainly conferred by a single nucleotide polymorphism at 6986A>G (CYP3A5\*3). We investigated the association between CYP3A5\*3 polymorphism and acute leukemia.

**MATERIALS AND METHODS:** Two hundred and eighty nine acute leukemia cases comprising of 145 acute lymphocytic leukemia (ALL), 144 acute myeloid leukemia and 241 control samples were analyzed for CYP3A5\*3 polymorphism using PCR-RFLP method. Statistical analysis was performed with SPSS version (15.0) to detect the association between CYP3A5\*3 polymorphism and acute leukemia.

**RESULTS:** The CYP3A5\*3 polymorphism 3/3 genotype was significantly associated with acute leukemia development ( $\chi^2$ - 133.53; df-2, *P* 0.000). When the data was analyzed with respect to clinical variables, mean WBC, blast % and LDH levels were increased in both ALL and AML cases with 3/3 genotype. The epidemiological variables did not contribute to the genotype risk to develop either AML or ALL.

**CONCLUSION:** The results suggest that the CYP3A5\*3 polymorphism might confer the risk to develop ALL or AML emphasizing the significance of effective phase I detoxification in carcinogenesis. Association of the polymorphism with clinical variables indicate that the 3/3 genotype might also contribute to poorer survival of the patients.

**Key words:** Acute leukemia, CYP3A5, drug metabolism, polymorphism

## Introduction

Cytochrome P450 enzymes are involved in initial oxidation, reduction or alkylation of xenobiotics that convert inactive carcinogens into active electrophiles which have the capacity to interact with biological molecules. CYP genes convert several anti-neoplastic agents into intermediate reactive metabolites, some of which can damage DNA.<sup>[1]</sup> Polymorphisms in the CYP genes have been associated with increased or decreased risk of certain cancers, because of extensive or poor metabolism of carcinogens or drugs.<sup>[2]</sup> The genetic variations which result in altering the activity of these enzymes had been found to be associated with the risk of developing secondary cancers and t-AML/t-MDS.<sup>[3]</sup> CYP3A5 family plays a significant role in the metabolism of more than 100 different drugs, carcinogens and toxic chemicals.<sup>[4]</sup>

The CYP3A5 gene is located on chromosome 7q21.1. CYP3A5 was observed to be an important genetic contributor to inter individual differences in CYP3A-dependent drug metabolism. Loss of CYP3A5 expression was mainly conferred by a single nucleotide polymorphism at 6986A>G (CYP3A5\*3). This SNP produces a cryptic splice site resulting in the inclusion of exon 3B followed by splicing which consequently introduces a stop codon. As a result, CYP3A5\*3 protein is truncated with greatly reduced enzyme activity in homozygous CYP3A5\*3 individuals.

In the present study, attempt has been made to identify the association of CYP3A5\*3 polymorphism with the development of acute leukemia.

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**Materials and Methods**

*Study subjects*

The study includes 289 acute leukemia cases comprising of 145 acute lymphocytic leukemia (ALL), 144 acute myeloid leukemia (AML) and 241 control samples. Blood samples were collected from patients being treated at NIMS (Nizams Institute of Medical Sciences), Hyderabad. The age and sex matched control samples were randomly selected from different areas from the local population. Patient’s clinical data like WBC count, blast%, platelet count, Hb, LDH, complete remission rate (CR) and disease free survival (DFS) was noted from the tumor registry file with the help of a medical oncologist. Genomic DNA was isolated by using salting-out method.<sup>[5]</sup>

*Restriction fragment length polymorphism*

CYP3A5\*3 polymorphism was analyzed through PCR-RFLP (restriction fragment length polymorphism) method. 121 bp fragment was amplified using specific primer sequences: Forward: 5'- GTT GTA CGC CAC ACA GCA CC -3'; Reverse: 5'- CTC TTT AAA GAG CTC TTT TGT CTC TCA - 3'. The 25 µl PCR reaction mixture consists of approximately 100-150 ng of genomic DNA, 15 pmol/l of each primer, 200 µmol/l of dNTPs, 20 mmol/l of tris HCl, 50 mM of KCl, 2.5 mmol/l of MgCl<sub>2</sub>, 0.5 U of Taq DNA polymerase and deionized water (varied).

The PCR cycling conditions include initial denaturation at 95°C for 12 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 20 s, extension at 72°C for 40 s and final extension at 72°C for 6 min. After amplification, PCR products were subjected for restriction digestion using Dde 1 enzyme (New England Biolabs, USA). The samples were genotyped on 3% agarose gel. CYP3A5\*3 wild type allele has no restriction site and

produces fragment of size 121bp whereas mutant allele with restriction site produces two fragments of size 97 and 24 base pairs.

*Statistical analysis*

All the statistical analyses were performed with SPSS version 15. 0. (Statistical Package for the Social Science). Chi square values and Odds ratios were calculated to test the significance of genotype association with the occurrence of acute leukemia. t test was done to test the significance of association of clinical variables with the risk of acute leukemia. All the P values were two sided and the level of significance was taken as P <0.05.

**Results**

CYP3A5\*3 homozygous genotype (3/3) and 3 allele frequency were significantly elevated in both ALL and AML groups as compared to control group. The genotype distribution of CYP3A5\*3 polymorphism in AML group did not deviate from Hardy–Weinberg equilibrium, whereas the control group showed significant deviation from Hardy–Weinberg equilibrium ( $\chi^2$ -28.1) [Table 1]. Odds ratios were significant for 1/1 vs 1/3 and 1/3 vs 3/3 groups among AML cases and for 1/1 vs 1/3 and 1/1 vs 3/3 groups among ALL cases when compared with controls [Table 2].

When the genotype data was analyzed with respect to epidemiological variables like sex, age at onset, none of the variables showed significant association with CYP3A5\*3 [Tables 3-6]. However, 3/3 genotype frequency was found to be elevated among ALL patients with age at onset >20 years (28.2%) as compared to those with age at onset <10 years (14.3%) [Table 5]. In AML, 3/3 genotype frequency was elevated considerably in early age at onset group (41.7%) as compared to later age at onset group (29.7%) [Table 6].

**Table 1: Genotype distribution of CYP3A5\*3 polymorphism in acute leukemia and controls**

CYP3A5*3	Genotype frequency			Total	Allele frequency	
	1/1 No %	1/3 No %	3/3 No %		1	3
ALL	9 (6.2)	110(75.9)	26 (17.9)	145	0.151	0.848
AML	12 (8.3)	84 (58.3)	48 (33.3)	144	0.38	0.63
Total	21 (7.3)	194 (67.1)	74 (25.6)	289	0.41	0.59
Controls	124 (51.5)	71 (29.5)	46 (19.1)	241	0.609	0.390

ALL vs controls  $\chi^2$ - 59.295; df - 2, P-0.000\*, AML vs controls  $\chi^2$ - 73.60; df-2, P-0.000\*, Total cases vs controls  $\chi^2$ - 133.53; df-2, P-0.000\*, Hardy–Weinberg equilibrium for i) ALL  $\chi^2$ -42.03\* ii) AML  $\chi^2$ - 8.6 \* iii) Total cases  $\chi^2$ -43.8\* iv) Controls  $\chi^2$ -28.16\*, \* $\chi^2$ -value significant at P<0.05

**Table 2: Odds ratios (OR) in disease versus controls**

	ALL		AML	
	OR	CI	OR	CI
1/1 vs. 1/3	0.101*	0.064 to 0.16	0.129*	0.079 to 0.21
1/3 vs. 3/3	2.586*	1.551 to 4.6405	1.133	0.678 to 1.891
1/1 vs. 3/3	1.388	0.511 to 3.772	0.112*	0.062 to 0.205

\*OR significant ( $P < 0.05$ )

**Table 4: CYP3A5\*3 polymorphism and sex in AML group**

AML	Genotype frequency						Total	Allele frequency	
	1/1		1/3		3/3			1	3
	n	%	n	%	n	%			
Males	7	8.5	52	59.1	29	33.0	88	0.375	0.625
Females	5	9.3	30	55.6	19	35.2	54	0.370	0.630

$\chi^2$ -0.189; df-2, ( $P > 0.05$ )

**Table 6: CYP3A5\*3 polymorphism and age at onset in AML group**

AML	Genotype frequency						Total	Allele frequency	
	1/1		1/3		3/3			1	3
	n	%	n	%	n	%			
<20	1	4.2	13	54.2	10	41.7	24	0.313	0.687
20-30	6	11.1	29	53.7	19	35.2	54	0.380	0.620
>30	5	7.8	40	62.5	19	29.7	64	0.391	0.609

$\chi^2$ -2.251; df-4, ( $P > 0.05$ ).

When the data was analyzed with respect to clinical variables, there was a considerable elevation in mean WBC count, blast% and LDH level and reduction in mean DFS of ALL patients with 3/3 genotype [Table 7]. Similar increasing trend in the values of WBC, blast% and LDH were observed in AML patients with 3/3 genotype without any difference in DFS values [Table 8]. 35% of AML patients with 3/3 genotype failed to achieve complete remission as compared to 5% of patients with 1/1 genotype [Table 9].

There was no variation with respect to platelet count, Hb in both ALL and AML patients.

## Discussion

Polymorphic variants in the CYP3A5 gene have functional effect on the activation of environmental pollutants. Accordingly, the significant association of CYP3A5\*3 allele with ALL and AML observed in the present study indicates the importance of proper detoxification mechanism in preventing carcinogenesis. Elevation in the frequency of homozygous genotype

**Table 3: CYP3A5\*3 polymorphism and sex in all groups**

ALL	Genotype frequency						Total	Allele frequency	
	1/1		1/3		3/3			1	3
	n	%	n	%	n	%			
Males	4	4.1	75	76.5	19	19.4	98	0.423	0.577
Females	5	10.6	35	74.5	7	14.9	47	0.479	0.521

$\chi^2$ -2.576; df-2, ( $P > 0.05$ )

**Table 5: CYP3A5\*3 polymorphism and age at onset in all groups**

ALL	Genotype frequency						Total	Allele frequency	
	1/1		1/3		3/3			1	3
	n	%	n	%	n	%			
<10	3	7.1	33	78.6	6	14.3	42	0.464	0.536
10-20	4	6.3	51	79.7	9	14.1	64	0.461	0.539
>20	2	5.1	26	66.7	11	28.2	39	0.385	0.615

$\chi^2$ -3.873; df-4, ( $P > 0.05$ ).

3/3 and 3 allele indicated that CYP3A5\*3 genotype with lower CYP expression might result in accumulation of xenobiotics leading to leukemogenesis. Further, it might not be metabolizing the antileukemic drugs effectively which would result in drug toxicity and poor survival.

Previous studies reported the lack of association of CYP3A5\*3 with development of myeloid leukemia (CML, AML).<sup>[8]</sup> No significant association was reported between CYP3A5\*3 polymorphism and morbidity of acute leukemia patients, but it was closely associated with the chemotherapeutic effect and prognosis.<sup>[9]</sup>

The results also indicated that selection might be operating in the population against \*3 allele, since loss of CYP3A5 enzyme activity is associated with defective detoxification of xenobiotics. These selective forces might be responsible for deviation from Hardy–Weinberg equilibrium in the control group. Hence, increased frequency of \*3 allele in disease group and also the lack of deviation of the same from Hardy–Weinberg equilibrium indicated the importance

of selection in elimination of ineffective allele. However, sample size and method of sampling also contribute to deviation from equilibrium.

In conclusion, our results suggested that the CYP3A5\*3 polymorphism might confer the risk to develop ALL or AML. The 3/3 genotype can be considered as a risk genotype to develop acute leukemia and also

**Table 7: Mean values of clinical variables with respect to CYP3A5\*3 polymorphism in all groups**

Clinical variables	1/1		1/3		3/3		Total
	Mean ±SE	n	Mean ±SE	n	Mean ±SE	n	
Mean age	16.78±5.06	9	15.55±0.94	110	16.54±1.72	26	145
Mean WBC (thousand)	17.93±4.88	9	55.18±7.28	110	60.26±11.61*	26	145
Mean blast%	42.00±12.38	9	49.05±3.13	110	57.38±6.45	26	145
Mean platelet count (lakhs)	0.99±0.26	9	0.83±0.067	110	0.88±0.15	26	145
Mean HB	10.42±1.10	9	8.87±0.25	110	8.15±0.44**	26	145
Mean LDH	903.56±493.77	9	736.04±54.32	110	1033.46±131.71***	26	145
Mean DFS	37.44±5.57	9	28.56±2.05	100	21.13±2.08****	23	132

\*t=2.10, P< 0.05 (as compared to 1/1 group), \*\*t=2.31, P< 0.05 (as compared to 1/1 group), \*\*\*t=2.31, P< 0.05 (as compared to 1/3 group), \*\*\*\*t=3.42, P< 0.05 (as compared to 1/1 group)

**Table 8: Mean values of clinical variables with respect to CYP3A5\*3 polymorphism in AML group**

Clinical variables	1/1		1/3		3/3		Total
	Mean ±SE	n	Mean ±SE	n	Mean ±SE	n	
Mean age	32.17±4.34	12	34.00±1.84	82	29.63±1.87	48	142
Mean WBC (thousand)	13.70±2.27	12	48.64±7.79	82	65.46±12.96*	48	142
Mean blast%	51.75±7.66	12	62.76±2.87	82	61.69±3.93	48	142
Mean platelet count (lakhs)	1.06±0.48	12	1.02±0.14	82	1.02±0.17	48	142
Mean HB	7.19±0.82	12	8.47±0.28	82	8.30±0.33	48	142
Mean LDH	253.08±26.55 <sup>#</sup>	12	454.77±31.43 <sup>#</sup>	82	588.85±64.83 <sup>#</sup>	48	142
Mean DFS	13.00±4.56	8	11.40±1.31	50	10.80±1.51	25	83

\*t=1.98, P<0.05 (as compared to 1/1 group), <sup>#</sup>F=5.33, P<0.05

**Table 9: CYP3A5\*3 polymorphism and complete remission rates in all and AML groups**

ALL	CYP3A5*3						Total
	1/1		1/3		3/3		
	n	%	n	%	n	%	
CR + VE	9	6.9	99	75.6	23	17.6	131
CR-VE	0	0	5	100	0	0	5
χ <sup>2</sup> - 1.597; df-2, (P>0.05)							
AML	1/1		1/3		3/3		Total
	n	%	n	%	n	%	
CR+VE	8	12.7	37	58.7	18	28.6	63
CR-VE	2	5	24	60	14	35	40

χ<sup>2</sup>-1.826; df-2, (P>0.05)

influences clinical variables such as WBC count, blast%, LDH levels and contribute to poorer survival.

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