RESEARCH COMMUNICATION

Association of an MDR1 Gene (C3435T) Polymorphism with Acute Leukemia in India

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Abstract

The multidrug resistance (MDR1) gene product P-glycoprotein is a membrane bound protein that functions as an ATP-dependent efflux pump, transporting exogenous and endogenous substrates from the cells. Since it plays an important role in chemotherapy, there is an increasing interest in the possible significance of genetic variation in MDR1. Our main objective was to study the MDR1gene polymorphism at C3435T with reference to development and progression of acute leukemia. The present study included 290 acute leukemia cases, comprising of 147 acute lymphocytic leukemia (ALL), 143 acute myeloid leukemia and 249 age-sex matched control samples for the analysis of *MDR1 C3435T* polymorphism, by the PCR-RFLP method. The MDR1 genotype distribution revealed an elevated frequency of the TT genotype in ALL cases (51.7%) as compared to controls (28.9%), whereas AML group did not show any association. The mean white blood cell count, blast% and LDH levels were increased in ALL patients with the CC genotype. No deviation was observed with respect to haemoglobin, platelet count and disease free survival in ALL patients. The association of CC genotype with clinical variables in ALL indicated that the CC genotype with high expression might be eliminating antileukemic drugs (anthracyclines, Daunorubicin, Vincristeine, Mitoxanthrone) which are P-gp substrates, leading to lower intra cellular drug concentrations and a poor prognosis. Such an association with the CC genotype was not observed in AML. In conclusion, these results suggested that the *MDR1* TT genotype might influence risk of development of acute lympoblastic leukemia and the CC genotype might be linked to a poor prognosis of ALL.

Keywords: MDR1 gene - ALL - AML - C3435T polymorphism - PCR-RFLP

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Introduction

The human multi drug resistance gene (MDR1), located on chromosome 7q21, comprises 28 exons and encodes a 170 KD integral membrane protein product P-glycoprotein (Pgp), which belongs to the ATP binding cassette (ABC) super family of transporters resides in the plasma membrane and functions as an efflux transporter of a wide variety of natural compounds and lipophilic xenobiotics (Lin et al., 2003).

P-gp seemed to have a role in transporting phospholipids across the cell membrane and might function as an antiapoptotic molecule. P-gp could inhibit the activation of down stream caspases 8 and 3 conferring resistance to apoptosis. Moreover P-gp conferred resistance to cell lysis induced by activated compliment factor (Johonstone et al., 2000). Over expression of Pgp in cancer cells causes a decrease in drug accumulation, thereby mediating cellular resistance to a variety of anti-cancer agents, whereas reduced expression leads to accumulation of carcinogens.

P-gp drug substrates include anthracyclines and epidophyllotoxins that had been shown to be efficacious in the treatment of AML (Bradshaw et al., 1998). A reduced intra cellular concentration of cytotoxic drugs attributable to the action of P-gp in AML blasts may therefore be related to resistant disease and failure of AML therapy. Indeed 30% of younger patients (<60 years) and 50% of older patients (>60 years) suffering from AML have been shown to express readily detectable levels of P-gp on their blast cells (Leith et al., 1994; Chauncey et al., 2000).

Since P-gp plays pivotal role in protecting cells from harmful chemicals and active metabolites, it might be plausible that this expression might contribute significantly in cellular response to stress stimuli. In the promoter region of *MDR1* gene, the putative binding sites for various transcription factors were identified. Hence, these sites may be targets of environmental signaling. These evidences clearly point out the possibilities of *MDR1* gene regulation by a myriad of environmental signals. Further evidence had indicated that protein kinase C activation which increases P-gp activity also exhibit positive impact on *MDR1* gene expression. The three most frequent SNPs in the Caucasian population observed were in exons 12, 21 and 26 at nucleotide positions 1236, 2677,

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and 3435. Moreover, it was shown in healthy volunteers that these changes were in linkage disequilibrium and might therefore be associated with transcriptional regulation of *MDR1* mRNA (Kim et al., 2001). The *MDR1* gene polymorphism in exon 26 at 3435 C>T position is a synonymous polymorphism located near nucleotide binding site of transmembrane P-gp. This synonymous polymorphism (C3435) could affect the function of the P-gp protein by various mechanisms like alterations in *MDR1* mRNA folding and stability.

Materials and Methods

The present study includes 290 acute leukemia cases comprising of 147 acute lymphocytic leukemia (ALL), 143 acute myeloid leukemia (AML) and 249 control samples. Blood samples were collected from freshly diagnosed patients with acute leukemia who were reporting at NIMS (Nizams Institute of Medical Sciences), Hyderabad. The age and sex matched control samples were randomly selected from different areas of Andhra pradesh. Patient's clinical data like WBC count, blast%, platelet count, Hb, LDH, complete remission (CR) response to therapy and period of disease free survival (DFS) was noted from the tumor registry files with the help of medical oncologist during followup. Genomic DNA was isolated by using salting-out method (Lahiri and Nurnberger, 1991) and used for genotyping of MDR C3435T polymorphism through PCR-RFLP analysis. The present study has been planned to identify the association of MDR1 C3435T polymorphism with the development of ALL, AML and their progression.

Genotyping of the MDR1 C3435T polymorphism

MDR1 C3435T polymorphism was analyzed through PCR-RFLP (restriction fragment polymorphism length polymorphism) method. 244bp fragment was amplified using specific primer sequences: Forward: 5'-GAT CTG TGA ACT CTT GTT TTC A-3''Reverse: 5'- GAA GAG AGA CTT ACA TTA GGC-3' The 25μl PCR reaction mixture consists of approximately 100-150ng of genomic DNA, 15pmol/l of each primer, 200μmol/l of dNTPs, 20 mmol/l of Tris HCl, 50 mM of KCl, 2.5mmol/l of $MgCl_2$, 1 U of Taq DNA polymerase and deionized water (varied). The PCR Cycling Conditions include initial denaturation at 94°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72˚C for 1 minute and final

Table 1. Estimation of Relative Risk for ALL and AML Associated with MDR1 C3435T Genotype

Genotype	Control (n=249)	ALL Cases Value (n=147)	χ^2	P	AML Cases Value (n=143)	χ^2	P
Frequency Genotype (n, %)							
CC	49 (19.7)	18 (12.2)			32 (22.4)		
CT	128 (51.4)	53 (36.1)	20.624#	0.00	73 (51.0)	0.550	0.779
TT	72 (28.9)	76 (51.7)			38 (26.6)		
Allele							
C	0.45	0.3			0.48		
T	0.55	0.7			0.52		
Odds Ratios		ORs	(95%CI)		ORs	(95%CI)	
CC vs CT		0.88	0.47-1.65		1.14	0.67-1.95	
CT vs TT		0.39*	0.25-0.62		1.08	0.67-0.18	
CC vs TT		0.37*	0.21-0.66		1.24	0.68-2.24	

χ^2 value significant; *Odds ratios significant

Table 2. MDR1 C3435T Polymorphism with Respect to Demography of Sex of the Proband with Acute Leukemia

Genotype distribution with respect to Sex of the proband in ALL

ALL	Genotype Frequency							Allele Frequency	
	CC		CT		TT		Total		
	n	%	n	%	n	%		C	T
Males	12	12.0	32	32.0	56	56.0	100	0.28	0.72
Females	6	12	21	44.7	20	42.6	47	0.35	0.65

χ^2=2.56; df =2, p=0.27

OR (CI 95%) : CC vs CT : 1.3125 (0.4265 to 4.0388)
OR (CI 95%) : CT Vs TT : 0.5442 (0.2569 to 1.153)
OR (CI 95%) : CC Vs TT : 0.7143 (0.2366 to 2.1567)

Genotype distribution with respect to Sex of the proband in AML

AML	Genotype Frequency							Allele Frequency	
	CC		CT		TT		Total		
	n	%	n	%	n	%		C	T
Males	18	20.5	43	48.9	27	30.7	88	0.45	0.55
Females	14	26.4	29	54.7	10	18.9	53	0.54	0.46

χ^2=2.49; df=2, p=0.28

OR (CI 95%) : CC vs CT :0.8671 (0.3734 to 2.0133)
OR (CI 95%) : CT Vs TT : 0.5492 (0.2312 to 1.3044)
OR (CI 95%) : CC Vs TT : 0.4762 (0.1739 to 1.3037)

Table 3. MDR1 C3435T Polymorphism with Respect to Demography of Age at Onset with Acute Leukemia

Genotype distribution with respect to Age at onset in ALL

ALL	Genotype Frequency							Allele Frequency	
	CC		CT		TT		Total		
	n	%	n	%	n	%		C	T
<10	6	14.3	17	40.5	19	45.2	42	0.35	0.65
10-20	6	9.5	22	34.9	35	55.6	63	0.27	0.73
>20	6	14.3	14	33.3	22	52.4	42	0.31	0.69
χ^2=1.52; df=4, p=0.82									

Genotype distribution with respect to Age at onset in AML

AML	Genotype Frequency							Allele Frequency	
	CC		CT		TT		Total		
	n	%	n	%	n	%		C	T
<20	4	16.7	11	45.8	9	37.5	24	0.39	0.61
20-30	13	23.6	30	54.5	12	21.8	55	0.51	0.49
>30	15	24.2	31	50.0	16	25.8	62	0.49	0.51
χ^2=2.31; df=4, p=0.67									

Table 4. Mean Values of Clinical Variables with Respect to MDR1 C3435T Polymorphism in ALL and AML Cases

Clinical Variable	ALL		AML	
Mean Age	n	Mean±S.E	n	Mean ±S.E
CC	18	18.89±3.37	32	34.63±3.24
CT	53	15.57±1.32	72	32.33±1.73
TT	76	15.12±1.01	37	29.78±2.22
	147		141	
Mean WBC(thousands)				
CC	18	74.23±17.88	32	37.04±9.71
CT	53	53.82±12.80	72	61.02±9.82
TT	76	49.09±6.11	37	45.53±12.50
	147		141	
Mean Blast%				
CC	18	63.89±6.74#	32	59.25±5.05
CT	53	47.32±4.56	72	59.53±3.20
TT	76	47.11±3.83	37	66.73±3.69
	147		141	
Mean Platelet(lakhs)				
CC	18	0.87±0.21	32	0.98±0.23
CT	53	1.06±0.10	72	0.85±0.13
TT	76	0.68±0.06	37	1.39±0.25
	147		141	
Mean HB				
CC	18	8.60±0.71	32	8.42±0.37
CT	53	8.72±0.40	72	8.20±0.28
TT	76	8.98±0.25	37	8.59±0.47
	147		141	
Mean LDH				
CC	18	1007.6±170.27*	32	431.84±58.20
CT	53	677.53±51.15	72	504.0±45.81
TT	76	819.75±80.97	37	475.57±47.5
	147		141	
Mean DFS				
CC	17	27.65±4.83	22	11.59±1.60
CT	47	26.13±2.34	36	11.94±1.78
TT	71	28.07±2.42	26	10.92±1.56
	135		84	

t test values: # as compared with CT or TT, p<0.05; * as compared with CT, p<0.05

extension at 72°C for 5 minutes. After amplification, PCR products were subjected for restriction digestion using DpnII enzyme (New England Biolabs USA) for 1 hour at 55°C. For 3435-C allele, two fragments of sizes 172 bp and 72 bp were obtained whereas a fragment of size 244 was observed for 3435Tallel. After digestion, the products were electrophoresed on a 3% agarose gel for genotyping.

Statistical Analysis

All the statistical analyses were performed with SPSS 15.0 (Statistical Package for the Social Science). Chi square test was done to test the significance of genotype association with the occurrence of acute leukemia and its prognosis. All the P values were two sided and the level

of significance was taken as P <0.05.

Results and Discussion

In the present study 3435 TT genotype of MDR1 was significantly associated with ALL as compared to controls. The frequency of 3435 TT genotype was significantly elevated in ALL (51.7%) as compared to controls (28.9%). Similar significant association was not observed with AML (Table 1). This indicated that MDR1 C3435 TT polymorphism might confer an increased risk for developing acute lymphoblastic leukemia. 3435 TT genotype with low P-gp expression levels had less efficiency to efflux the toxins, resulting in higher intracellular concentrations of mutagens or toxins leading to DNA damage and accumulation of mutations resulting in disease progression. Previous studies had also reported an increased risk of acute lymphoblastic leukemia (ALL) associated with TT genotype, possibly due to a quantitative change in the MDR1 gene expression resulting from genetic polymorphisms (Jamroziak et al., 2004; Hattori et al., 2007).When the distribution of genotype frequencies were analyzed with respect to sex of the proband, MDR1 TT genotype was associated with male sex in ALL and AML patients (56.0%, 30.7%) (Table 2). MDR1 TT genotype frequency was found to be increased with age at onset more than 10 yrs in ALL and with age at onset <20yrs in AML (Table 3). With respect to clinical variables Mean WBC, mean blast% and mean LDH levels were increased in ALL patients with CC genotype. The association of CC genotype with clinical variables in ALL indicated that CC genotype with high expression might be eliminating the antileukemic drugs (anthracyclines, Daunorubicin, Vincristeine, Mitoxanthrone) which are P-gp substrates more effectively leading to low intra cellular drug concentration and poor prognosis. No such association was observed in AML, indicating MDR1 C3435 polymorphism did not appear to have significant clinical implications in AML which was in accordance with earlier report (Hur et al., 2008).

In conclusion these results suggested that MDR1 TT genotype might confer risk to development of acute lympoblastic leukemia and CC genotype with poor prognosis.

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