RESEARCH ARTICLE

Association of CYP3A5*3 and CYP1A1*2C Polymorphism with Development of Acute Myeloid Leukemia in Egyptian Patients

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Abstract

Aim: Cytochrome P450 (CYP) enzyme catalyzes the phase I metabolism reaction which metabolize endogenous and exogenous DNA-reactive chemical compounds and xenobiotics which could induce genotoxicity and increase the risk for leukemia. We aimed to detect frequency of CYP3A5*3 and CYP1A1*2C polymorphisms in Egyptian acute myeloid leukemia (AML) patients and to determine role of allele's variants as a risk factor for developing leukemia. **Patients and Methods:** A case-control study was conducted on seventy acute myeloid leukemia patients and thirty control subjects. Samples were analyzed for prevalence of CYP3A5*3 and CYP1A1*2C polymorphisms using PCR - restriction fragment length polymorphism method. **Results:** CYP3A5*3 polymorphism (3/3) and (1/3) genotype were significantly elevated in AML group compared to control group (p=0.002). However, no statistical significant differences were found between patients and control group as regard CYP1A1*2C polymorphism. **Conclusion:** Our results suggest that Egyptians carrying CYP3A5*3 polymorphism might have an increased risk of AML emphasizing the significance of effective phase I detoxification in carcinogenesis.

Keywords: CYP3A5*3- CYP1A1*2C- AML

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Introduction

Genetic variations are one of the important factors in the development of acute leukemia. Molecular studies have proved that genetic polymorphism of metabolic enzymes influence the risk of a variety of tumors including leukemia (Hatagima et al., 2002).

Functional polymorphisms in the genes encoding xenobiotic-metabolizing enzymes result in inter-individual differences that contribute to leukemia susceptibility. Cytochrome P450 (CYP) enzyme catalyzes the phase I metabolism reaction which metabolize endogenous and exogenous DNA-reactive chemical compounds and xenobiotics which could induce genotoxicity and increase the risk for leukemia (Zanger et al., 2004).

CYP3A5 family is important for the metabolism of more than 100 different drugs, carcinogens and toxic chemicals. The CYP3A5 gene is located on chromosome 7q21.1. Loss of CYP3A5 expression was due to a single nucleotide polymorphism (SNP) at 6,986A>G (CYP3A5*3). This SNP result in a cryptic splice site leading to inclusion of exon 3B followed by splicing which introduces a stop codon. As a result, CYP3A5*3 protein is truncated with reduced enzyme activity in homozygous CYP3A5*3 individuals (Felix et al., 1998).

The CYP1A1 gene (CYP1A1) is another member of the CYP family that is responsible for the metabolism of polycyclic aromatic hydrocarbons such as benzo[a] pyrene (Guengerich et al., 1998). Genetic polymorphisms have been reported for CYP1A1 that alter the function of enzyme and influence the ability of enzymes to metabolize the chemical carcinogens and mutagens (Hung et al., 2003 and Dufour et al., 2005) which might explain susceptibility of individuals to contracting leukemia (Takanashi et al ., 2003).

One of the common genetic mutant site is A2455G (also referred to as CYP1A1*2C, or rs1048943), which has been widely examined with regard to cancer susceptibility (Yamaguti et al., 2010, Swinney et al., 2011 and Zhang et al., 2011). This SNP is characterized by an A to G mutation at nucleotide 2,455 in the region of CYP1A1 (Kawajiri et al., 1990).

The aim of this work was to identify the association of CYP3A5*3 and CYP1A1*2C polymorphisms with the development of acute myeloid leukemia in Egyptian patients.

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Materials and Methods

A case-control study was conducted on 70 consecutive newly diagnosed patients who presented to the Adult Oncology Department, National Cancer Institute (NCI), Cairo University during the period from January 2012 to January 2014.

Thirty age and sex-matched healthy individuals from donors for bone marrow transplantation were included as controls.

The diagnosis of AML was established after clinical, morphological, cytochemical, flow cytometric and cytogenetic analysis. All the cases met the AML diagnosis standards. The study was approved by the Institutional Review board (IRB) ethical committee of the NCI. All subjects were aware by the nature of the study and gave a written informed consent.

Sample collection and DNA extraction

Bone marrow samples (1ml) from patients and controls were collected on EDTA, bone marrow was treated with erythrocyte lysis solution; leukocytes were collected and stored in buffer RLT (1x107leukocytes) at -80 °C till use for complete DNA extraction.

Total genomic DNA was extracted from bone marrow cells of patients with AML and Control using QIAamp DNA Mini isolation kit (QIAGEN) (Cat No.: 51304) following standard procedures according to the manufacturer's instructions.

PCR - restriction fragment length polymorphism Analysis

PCR followed by enzymatic digestion of the PCR products (CYP3A5*3and CYP1A1*2C) was used for the genotyping of the CYP3A5*3and CYP1A1*2C polymorphisms. Approximately 50 ng of genomic DNA was used as template in each of the PCR amplifications. The 50-ul reaction also consisted of each dNTP at 150 uM, 10pM of each primer, 1.5–2.0 mM MgCl2, and 2 units of Amplitaq Gold (PE Applied Biosystems, Warrington, United Kingdom) in the manufacturer's buffer. After an initial heat activation step at 95°C for 10 min, amplification was performed in a PTC-100TM Programmable Thermal Controller using the following conditions; CYP3A5*3, denaturation at 94°C for 30 sec, annealing at 58°C for 1 min, extension at 72°C for 1 min for a total of 35 cycles ending with a final extension at 72°C for 10 min. The conditions for the CYP1A1*2C PCR were as described except an annealing temperature of 65°C was used.

CYP3A5*3. CYP3A5*3was amplified in a 293-bp product using the following primers: CYP3A5*3-F (5'-CATGACTTAGTAGACAGATGA-3') and CYP3A5*3-R (5'-GGTCCAAACAGGGAAGAAATA-3'). Ten ul of PCR products were digested at 37°C overnight with 10 units of SspI (SibEnzyme) (New England Biolabs, USA) in 1X buffer supplied with the enzyme and supplemented with 100 ng/ul Bovine Serum Albumin (BSA). The presence of the SspI restriction site yields 148, 125, and 20 bp bands corresponded to the AA (homozygous wild type genotype), 168, 148, 125 and 20 bp bands correspond to the AG (heterozygous genotype) and 168, 125 bp band corresponded to the GG (homozygous variant genotype) as shown in Figure 1.

CYP1A1 *2C. CYP1A1 *2C polymorphism was amplified in a 204-bp fragment using the following primers: CYP1A1 *2C F (5'-CTGTCTCCCTCTGGTTAAGGAAGC-3') and CYP1A1 *2C R (5'-TTCCACCCGTTGCAGGATAGCC-3'). Ten ul of PCR products were digested with 10 units of BsrDI (SibEnzyme) (New England Biolabs, USA) in the manufacturer's buffer. The polymorphic allele eliminates the BsrDI restriction site, and, therefore, the digestion of polymorphic samples resulted in a single band at 204 bp, whereas the wild-type allele resulted in two bands at 149 bp and 55 bp. While the heterozygous genotype resulted in 204, 149, and 55 bp bands as shown in Figure 2.

The digested products were resolved on 3% agarose gels for CYP1A1 *2C and 4% agarose gel for CYP3A5*3 stained with ethidium bromide and analyzed under UV light.

Statistical analysis

Data management and analysis was performed using SPSS, version 20. Categorical data were summarized as percentages; numerical data were summarized using means and standard deviation or medians and ranges. Relation between Cyp3A5*3 and Cyp1A1*2C polymorphisms and other variables was assessed using Chi-square test. Overall survival (OS) was defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of last follow-up were censored on that date. Disease free survival (DFS) was defined as the time from complete remission until documented relapse or death. For patients without disease relapse at the time of analysis, the date of last follow-up was considered right-censored. OS and DFS were estimated using the Kaplan-Meier analysis. Log rank test was used to compare survival curves. All tests of hypotheses were conducted at the alpha of 0.05 Level, with a 95% confidence interval (Dawson et al., 1994).

Results

We examined the frequencies of Cyp3A5*3 and Cyp1A1*2C polymorphisms in 70 AML patients and 30 control subjects matched for age and sex.

The patients' characteristics are shown in Table 1. The genotype distributions in AML and control together with the adjusted ORs are shown in Table 2.

A higher prevalence of the variant allele at CYP3A5*3 was found in cases, when compared with controls (81.5% vs 14%, P = 0.002). This was translated into a 4.72-fold risk for AML in carriers of variant heterogenous and 5.23 fold risk for AML in carriers of variant homogenous CYP3A5*3 alleles. No differences between patients and controls were found when looking at Cyp1A1*2C polymorphisms.

Stratification of the AML group by age at diagnosis, gender, FAB subtype, or cytogenetic risk revealed no differences in genotype frequencies. Significance correlation was seen between Cyp1A1*2C with hemoglobin

Table 1. Clinical and Laboratory Characteristics of 70 AML Patients

Patients characteristics	N=70	(%) ±SD
Age	34.09	±12.77
Sex		
Male	41	(58.6)
Female	29	(41.4)
Splenomegaly	26	(37.1)
Hepatomegaly	29	(41.4)
Lymphadenopathy	7	(10)
Laboratory finding	Mean	+/- SD
TLC	66.5	+/-78.03
HB	7.49	+/-1.84
Platelet	66.34	+/-73.48
Peripheral blood blasts%	56	+/-24.10
Bone marrow blasts%	66.63	+/-19.69
Bone marrow cellularity	N	(%)
Normo-cellular	60	(85.7)
Hyper-cellular	10	(14.3)
FLT status	N=67	(%)
Wild	40	(59.7)
Mutant	27	(40.3)
FAB		
M1	19	(27.1)
M2	24	(34.3)
M4	24	(34.3)
M5	3	(4.3)
Cytogenetics	67	(%)
Normal karyotype	58	(86.6)
t (8:21)	7	(10.4)
Inv(16)	2	(3)

level and near significance with hepatosplenomegaly and lymphadenopathy (Table 3).

Thirty seven patients (52.85%) achieved complete remission (CR) after induction chemotherapy, 17 (24.29%) were resistant to treatment and 16 (22.86%) died before day 28. No statistically significant effect of CYP3A5*3 or CYP1A1*2C genotypes on response to induction chemotherapy was noticed (p=0.37 and 0.93 respectively) (Table 4).

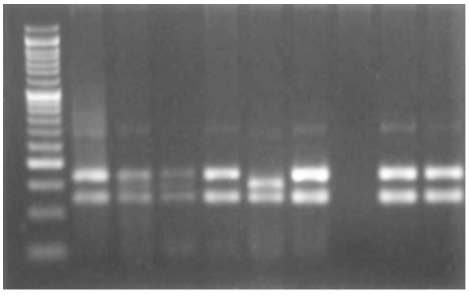


Figure 1. Gel Electrophoresis of PCR Products of CYP3A5*3 after Digestion by SspI enzyme.
Lane 1, molecular weight marker 50 bp with the reference bands size 200 bp and 500bp; Lane 2, 5, 7, 9 and 10, The homozygous variant genotype (168 and 125 bp bands); Lane 3, 4, The heterozygous variant genotype (168,148 and 125 bp bands); Lane 6, The wild genotype (148,125 bp bands)

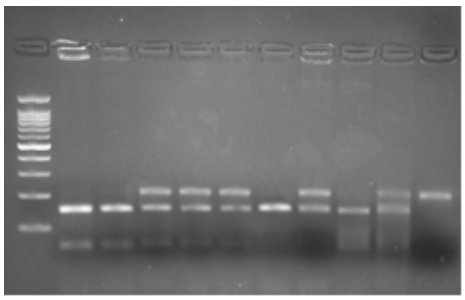


Figure 2. Gel Electrophoresis of PCR Products of CYP1A1*2C after Digestion by BsrDI Enzyme.
Lane 1, molecular weight marker 100bp with reference band 500bp; Lane 2, 3, 7, 9, The wild type of CYP1A1*2C polymorphism (149 and 55 bp bands); Lane 4, 5, 6, 8 and 10, The variant heterozygous type (204,149 and 55 bp bands); Lane 11, The variant homozygous type (204 bp band)

Median DFS for all patients was 2.6 months (95% CI 0-5.54). 17.8 % of the patients who reached CR were disease free at 2 years. Median OS for all patients was 4.17 months (95% CI 0.75-7.58) with 13.2% of patients surviving at 2 years (Figure 3).

There was no statistically significant difference in median DFS and OS between patients carrying one or both mutated genes and those with both genes wild (p=0.159, 0.12 respectively) (Figure 4).

Table 2. Genotype Distribution of Cyp3A5*3 and Cyp1A1*2C Polymorphisms in 70 AML Patients and 30 Controls

	Cases (n=70)		Control (n=30)		OR	95.0% C.I		P value
	No.	%	No.	%		LL	UL	
Cyp3A5*3								
Wild	13	18.5	16	53.3	Ref			0.002*
heterogenous Variant	23	32.9	6	20	4.72*	1.48	15.03	
homo Variant	34	48.6	8	26.7	5.23*	1.81	15.14	
Cyp1A1*2C								
Wild	49	70	23	76.7	Ref			0.801
heterogenous Variant	16	22.9	6	20	1.25	0.43	3.63	
homo Variant	5	7.1	1	3.3	2.35	0.26	21.26	

*Significant P value ≤ 0.05

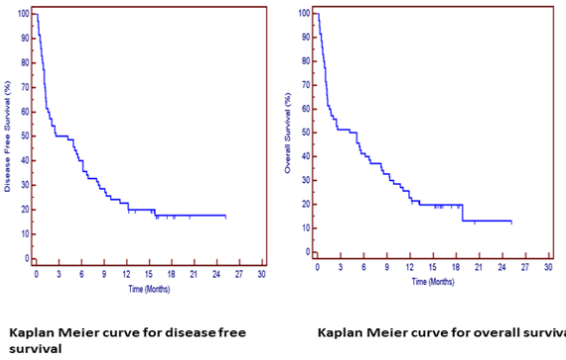
Table 3. Characteristics of 70 AML Patients according to Genotype Distribution of Cyp3A5*3 and Cyp1A1*2C Polymorphisms

	Cyp3A5*3			p value	Cyp1A1*2C			P value
	wild	hetero	Homo		wild	hetero	homo	
Number	13	23	34		49	16	5	
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Sex				0.91				0.47
Male	8 (61.5)	14 (60.9)	19 (55.9)		28 (57.1)	11 (68.8)	2 (40.0)	
Female	5 (38.5)	9 (39.1)	15 (44.1)		21 (42.9)	5 (31.3)	3 (60.0)	
Age				0.778				0.799
Mean±SD	34.85±12.7	35.30±15.0	32.97±11.3		34.24±13.3	32.69±12.8	37.0±5.96	
TLC				0.57				0.48
<30	6 (46.2)	12 (52.2)	13 (38.2)		24 (49.0)	5 (31.3)	3 (40.0)	
≥30	7 (53.8)	11 (47.8)	21 (61.8)		25 (51.0)	11 (68.8)	2 (60.0)	
Hb level				0.58				0.01*
<8	9 (69.2)	14 (60.9)	18 (52.9)		34 (69.4)	5 (31.3)	2 (40.0)	
≥8	4 (30.8)	9 (39.1)	16 (47.1)		15 (30.6)	11 (68.8)	3 (60.0)	
Platelets				0.19				0.65
<100,000	12 (92.3)	18 (78.3)	23 (67.6)		37 (75.5)	13 (81.3)	3 (60.0)	
≥100,000	1 (7.7)	5 (21.7)	11 (32.4)		12 (24.5)	3 (18.8)	2 (40.0)	
Peripheral blasts				0.29				0.84
<50	2 (15.4)	8 (36.4)	13 (39.4)		17 (35.4)	4 (26.7)	2 (40.0)	
≥50	11 (84.6)	14 (63.6)	21 (60.6)		31 (64.6)	11 (73.2)	3 (60.0)	
Bone marrow cellularity				0.9				0.23
Normocelluler	1 (7.7)	4 (17.4)	5 (14.7)		6 (12.2)	2 (12.5)	2 (40.0)	
Hypercelluler	12 (92.3)	19 (82.6)	29 (85.3)		43 (87.8)	14 (87.5)	3 (60.0)	
Bone marrow blasts				0.44				1
>50	3 (23.1)	2 (8.7)	7 (20.6)		8 (16.3)	3 (18.8)	1 (20.0)	
≥50	10 (76.9)	21 (91.3)	27 (79.4)		41 (83.7)	13 (81.3)	4 (80.0)	
FAB classification				0.85				0.84
M1	3 (23.1)	7 (30.4)	9 (26.5)		11 (22.4)	6 (37.5)	2 (40)	
M2	4 (30.8)	10 (43.5)	10 (29.4)		19 (38.8)	4 (25)	1 (20)	
M4	5 (38.5)	6 (26.1)	13 (38.2)		16 (32.7)	6 (37.5)	2 (40)	
M5	1 (7.7)	0 (0.0)	2 (5.8)		3 (6.1)	0 (0.0)	0 (0.0)	
Extramedullary infiltration								
Hepatomegaly	6 (46.2)	10 (43.5)	13 (38.2)	0.86	24 (49.0)	5 (31.3)	0 (0.0)	0.06
Splenomegaly	4 30.8	9 39.1	13 38.2	0.87	22 44.5	4 25.0	0 0.0	0.08
Lymph nodes	1 15.4	1 8.7	3 8.8	0.75	3 6.1	4 25.0	0 0.0	0.08
Cytogenetic				0.068				0.47
T(8:21)	2 (16.7)	4 (17.4)	1 (3.1)		7 (14.9)	0 (0.0)	0 (0.0)	
Inv 16	0 (0.0)	2 (8.7)	0 (0.0)		2 (4.3)	0 (0.0)	0 (0.0)	
Normal karyotype	10 (83.3)	17 (73.9)	31 (96.6)		38 (80.9)	15 (100.0)	5 (100.0)	
FLT				0.98				0.91
Wild	7 (58.3)	13 (59.1)	19 (61.3)		26 (57.8)	10 (66.7)	3 (60.0)	
Mutant	5 (41.7)	9 (40.9)	12 (38.7)		19 (42.2)	5 (33.3)	2 (40.0)	

Discussion

Although the clinical and pathological aspects of leukemia are well understood, the effect of genes that influence susceptibility to this disease is not clear. Drug-metabolizing enzymes play an important role in regulating the toxic, mutagenic and neoplastic effects of chemical carcinogens as well as metabolizing other xenobiotics and endogenous compounds (Nishimura et al., 2006).

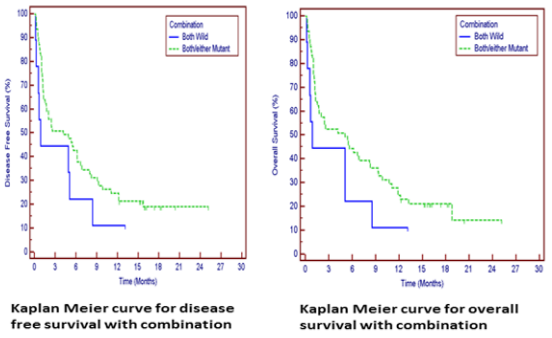
In this regard, CYP enzymes are the main group of biotransformation enzymes, so we conducted this



Kaplan Meier curve for disease free survival

Kaplan Meier curve for overall survival

Figure 3. Kaplan Meier Curves for Disease Free Survival and Overall Survival for 70 AML Patients



Kaplan Meier curve for disease free survival with combination

Kaplan Meier curve for overall survival with combination

Figure 4. Kaplan Meier Curves for Disease Free Survival and Overall Survival Of 70 AML Patients in Combination with the Polymorphisms.

Table 4. Response to Induction Therapy according to Genotype Distribution of Cyp3A5*3 and Cyp1A1*2C Polymorphisms in 70 AML Patients

	CYP3A5*3			P Value	CYP1A1*2C			P Value
	Wild	Hetero	Homo		Wild	Hetero	Homo	
	13	23	34		49	16	5	
	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
CR	5 (38.5)	16 (69.6)	16 (47.1)	0.37	26 (53.1)	9 (56.3)	2 (40.0)	0.93
Resistant	4 (30.8)	4 (17.4)	9 (26.5)		11 (22.4)	4 (25.0)	2 (40.0)	
Early death Before d28	4 (30.8)	3 (13.0)	9 (26.5)		12 (24.5)	3 (18.8)	1 (20.0)	

*Significant P value ≤ 0.05

case-control study to detect the effect of polymorphism in CYP3A5*3 and CYP1A1*2C in developing AML. Up to the best of our knowledge, we are the first to study these polymorphisms in Egypt.

CYP3A5 was observed to be an important genetic contributor to inter-individual difference 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) (Rao et al., 2011)

In this study, patients with AML and healthy controls were analyzed for CYP3A5*3 polymorphism in attempt to identify the association of CYP3A5*3 polymorphism with the development of AML.

Three genotypes of CYP3A5 polymorphism were detected. CYP3A5*1/*1 wild type, CYP3A5*1/*3 heterozygous and CYP3A5*3/*3 homozygous genotype.

The homozygous genotype (3/3) and the heterozygous genotype (1/3) were significantly elevated in AML group compared to control group (p=0.002) a result which suggests that the CYP3A5*3 polymorphism might confer the risk to develop AML emphasizing the significance of effective phase I detoxification in carcinogenesis.

Similar data was detected by (Rao et al., 2011) who found an elevation in CYP3A5*3 homozygous genotype (3/3) in AML group as compared to control group. Also (Elgari, et al., 2015) observed a significant increase in CYP3A5*3 homozygous genotype (3/3) in patients with acute leukemia.

On the contrary, (Liu et al., 2002) reported similar frequencies of CYP3A5*3 polymorphism in leukemic patients and normal controls. This controversy is most probably due to different ethnic population and exposure to different carcinogens in different environments.

In our study, no significant association was detected between CYP3A5*3 polymorphism and demographic, clinical, laboratory data, response to treatment or survival. On the contrary, (Rao et al. ,2011) found that 3/3 genotype influences clinical variables such as WBC count, blast percentage, lactate dehydrogenase (LDH) level and contribute to poorer survival. Also, (Shen et al., 2008) reported that CYP3A5 genotype is closely associated with the chemotherapeutic effect and prognosis.

CYP1A1 is one of the less known members of the CYP gene family. We also investigate the relationship between CYP1A1*2C polymorphism and the possibility of developing AML.

Three genotypes were observed in both patients and control group, CYP1A1*2C*1/*1 wild type, CYP1A1*2C *1/*3 heterozygous and CYP1A1*2C *3/*3 homozygous variant genotype. No statistical significant differences in frequency were found between AML patients and control group. Our results are in concordance with results observed by the meta-analysis done by (Lu et al., 2015) who found no association between A2455G and risk of AML among Asians and Caucasians. In contrary to our findings, (Pelloso et al., 2013) found that CYP1A1*2C polymorphisms were more frequent in control group than AML group.

In our study, no significant associations were detected between CYP1A1*2C polymorphism and demographic, clinical, laboratory data, response to treatment or survival.

In conclusion, this study suggests that the Egyptians who carry the CYP3A5*1/*3 heterozygous and CYP3A5*3/*3 homozygous genotype might have an increased risk of AML and CYP3A5*3 mutations can

be used as one of the markers to predict a person's risk for AML. This could be beneficial for screening of high risk groups like myelodysplastic syndrome patients, patients with chronic myeloproliferative disorders such as polycythemia vera, essential thrombocythemia, and idiopathic myelofibrosis and patients with cancer who are treated with certain chemotherapy or high-dose radiation exposure. However, further large studies are recommended in order to confirm the findings of this study.

Statement conflict of Interest

The authors have declared no conflict of interest.

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