RESEARCH ARTICLE

Prognostic Value of a CYP2B6 Gene Polymorphism in Patients with Acute Myeloid Leukemia

Nevin M Alazhary[1,3]*, Roxan E Shafik[1], Hanan E Shafik[2], Mahmoud M Kamel[1]

Abstract

  Background: The objectives of this study aimed to detect a CYP2B6 polymorphism in de novo cases of acute myeloid leukemia patients and identify any role in disease progression and outcome. **Materials and Methods:** DNA was isolated from peripheral blood of 82 newly diagnosed acute myeloid leukemia cases and the CYP2B6 G15631T gene polymorphism was assayed by PCR restriction fragment length polymorphism (PCR-RFLP). **Results:** The frequency of the GG genotype (wild type) was 48 (58.5%) and that of the mutant type T allele was 34 (41.9%). GT genotype heterozygous variants were found in 28 (34%), and TT genotype homozygous variants in 6 (7.3%) cases. We found no significant association between the CYP2B6 G15631T polymorphism and complete response (CR) (p-value=0.768), FAB classification (p-value=0.51), cytogenetic analysis (p-value=0.673), and overall survival (p-value=0.325). Also, there were no significant links with early toxic death (p-value=0.92) or progression-free survival (PFS) (p-value=0.245). **Conclusions:** Our results suggest that the CYP2B6 polymorphism has no role in disease progression, therapeutic outcome, patient free survival, early toxic death and overall survival in acute myeloid leukemia patients.

  Keywords: CYP2B6 G15631T - AML - prognosis - PCR/RFLP

Introduction

    Acute myeloid leukemia (AML) is a common complex hematological malignant disorder; genetic abnormalities play indispensable roles in the classification, prognosis, and response to therapy (Long et al., 2014). Although the etiology of AML had developed with the development of molecular biology, cell biology, and risk group medicine into the molecular biology, the exact cause is currently unknown. However, the interaction between environmental factors and genetic factors (such as mutations, epigenetic modifications, and deregulation of gene expression) has been implemented to be a cause for the development of AML (Ruan et al., 2013; Daraki et al., 2014). Several important molecular markers were recently discovered in AML. These markers have helped not only better characterize patients but also improve risk stratification (Daraki et al., 2014).

    Among adults with AML, the survival rates are poor and not improving, and the five-year survival rate after the initial diagnosis is less than 20%, despite advances in understanding the molecular biology of AML and mechanisms of multidrug resistance (MDR). But we are still waiting to discover new therapeutic interventions that consider the main target for successful treatment and improve the survival rate (Anderson et al., 2011).

    The incidence of AML increases with age, with the advancement in aging, prognosis, response to therapy, and overall survival decline (Kayser et al., 2012).

    The response of acute myeloid leukemia (AML) patient to various types of therapy and overall survival (OS) is highly variable, with a median ranging from 6 months to 11.5 years (Simon et al., 2014). Treatment failure in many patients is attributed to relapsed disease and remains the fundamental clinical challenge in these patients. Relapsed AML is encoded by a combination of epigenetic and genetic lesions that are present in a subset of AML cells at diagnosis in response to exposure to cytotoxic drugs. These lesions reinforce disrupting specific biological pathways that directly mediate chemotherapy resistance, and the early detection of these lesions will be predictive of poor outcome.4 Around 20% to 50% of patients with AML are primarily resistant to induction chemotherapy. It has previously been shown that resistance to the first cycle of induction chemotherapy is an independent prognostic factor (Grimwade et al., 2011).

    Drug-metabolizing enzymes or biotransformation enzymes play a crucial role in the detoxification of the mutagenic and neoplastic effects of many chemical carcinogens and metabolizing xenobiotics agents (such as phytochemical and therapeutic drugs). CYP enzymes are the most important groups of biotransformation enzymes; genetic polymorphisms in these enzyme systems resulting in functional allelic variants of the corresponding

[1]Department of Clinical Pathology, [2]Department of Medical Oncology, National Cancer Institute, Cairo University, Cairo, Egypt
[3]Department of Biochemistry and Molecular Medicine, College of Medicine, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia *For correspondence: nevin_elazhary@hotmail.com

enzymes can influence cancer susceptibility when coupled with reactive environmental or endogenous metabolites (Lapinski et al., 2011).

CYP2B6 is a highly variable and polymorphic cytochrome P450 enzyme, which is a phase I enzyme and plays a vital role in the degradation of some endogenous metabolites, xenobiotics, and harmful compounds. The 516G>T single nucleotide polymorphism (SNP) in exon 4 of the CYP2B6 gene may change the CYP2B6 enzyme activity and the gene expression in the liver. Carcinogens' failure to be degraded by CYP2B6 may cause DNA injury and cancer (Yuan et al., 2011).

The CYP2B6 enzyme is composed of 2% to 10% of the total CYP content, and also, it is involved in the metabolism of nearly 25% of drugs and many chemical compounds, in case of any abnormality with this enzyme activity, which may lead to influencing the development of many solid and hematologic malignancies and can also modify individual response to cytotoxic treatment (Berkoz et al., 2009). The G516T CYP2B6 genetic variant due to abnormality in guanine to thymine substitution at nucleotide 516 in exon 4 (rs3745274) and accordingly in glutamine to histidine substitution at 172 amino acid position (Gln172His). This polymorphism affects metabolic activity by aberrant splicing, leading to decreased amounts of the normal mRNA transcript and, therefore, to reduced levels of functional protein (Xu et al., 2012).

Many studies provide evidence for the pathogenic role of the G516T CYP2B6 polymorphism on AML susceptibility, suggesting that the inherited defective function of the CYP2B6 detoxification pathway may be an important genetic determinant of AML risk (Yuan et al., 2011; Turpeinen et al., 2012), but fewer studies were made about the associations between CYP enzyme genotype and disease outcome. No studies were made regarding the relation between CYP2B6 polymorphism and the prognosis of the disease.

Here, we study the G516T CYP2B6 gene-expression signature, which reflects molecular differences between AML with regard to response to therapy. To evaluate their role in predicting the disease course, the therapeutic outcomes and overall survival (OS) rates will be examined. We investigate whether resistance to chemotherapy is represented by gene-expression profiles or not.

Materials and Methods

Before starting the data collection, an informed written consent was obtained from each patient. The study included 82 newly diagnosed AML patients that were presented to the Adult Oncology Department, National Cancer Institute (NCI), Cairo University, between January 2012 and January 2014. Clinical, morphological, cytochemical, and flow cytometric analyses were done for proper diagnosis. The patients were composed of 50 (61.7%) males and 32 (39.02%) females. The CYP2B6 frequency demonstrated a remission status, which was detected after the first cycle, at 28 days, in relation to patient outcome. With respect to all patient privacy, patients were illustrated by code numbers. For each patient, 10 ml of blood was collected

in a sterile heparinized vacutainer.

Genotyping of CYP2B6 polymorphism (G-T transition at position 15631) was determined using a PCR-RFLP method described by (Jamroziak et al., 2004).

The genomic DNA used was extracted from blood using a QIAamp DNA mini isolation kit (QIAGEN). The primers used were as follows: 5'-CTGTGTCCTTGACCTGCTGC-3' as a forward primer and 5'-TCCAGGAGCAGAATAGACATGAAG-3' as a reverse primer. PCR was conducted with 100 ng of genomic DNA, 10 pmol of each primer, and 12.5 ul of Master Mix (Fermentas). PCR was performed with 37 cycles of the following: 95°C for 50 s, 60°C for 50 s, and 72°C for 3 minutes. This resulted in one band at 578 bp. Digestion of the PCR product with 10 U of BstN1 enzyme (New England Biolabs, USA) restriction enzyme in a final volume of 20 μl incubated at 65°C overnight. The wild type genotype (GG) produced a double band at 518 and 60 bp, whereas heterozygotes (GT) produced three bands at 578, 518, and 60 bp. The homozygote polymorphic genotype produced only one band at 578 bp. Polymorphism was detected in 3% agarose gel (see Figures 1 and 2).
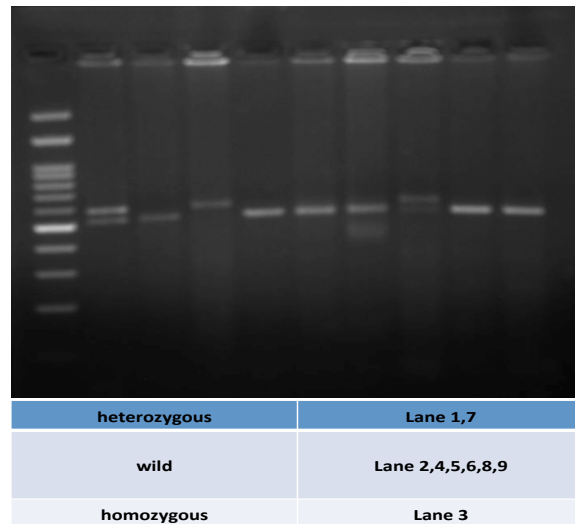

heterozygous	Lane 1,7
wild	Lane 2,4,5,6,8,9
homozygous	Lane 3

Figure 1. Run1 for PCR Detection of CYP2B6 G15631T

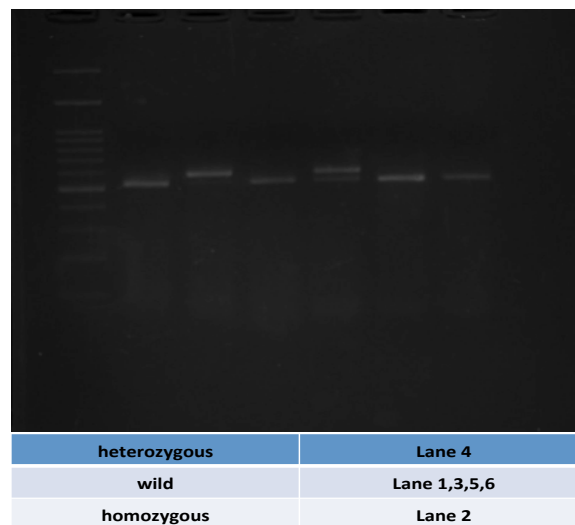| heterozygous | Lane 4 |
| wild | Lane 1,3,5,6 |
| homozygous | Lane 2 |

Figure 2. Run 2 for PCR Detection of CYP2B6 G15631T

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 range. The relationship between CYP and other variables was assessed using a chi-square test. OS was defined as the time from diagnosis to the time of death from any cause. Patients who were alive on the date of the last follow-up were censored on that date. TTP was defined as the time from starting therapy until documented progression. For patients without DP at the time of analysis, the date of the last follow-up was considered right-censored. OS and TTP were estimated using the Kaplan-Meier analysis. Unless otherwise noted, all tests of hypotheses were conducted at an alpha level of 0.05, with a 95% confidence interval (see Figure 1, 2).

Results

In our study, we analyzed the frequency of CYP2B6 G15631T polymorphism through molecular PCR-RFLP technique in 82 newly diagnosed AML cases at diagnosis, then assessed the CYP2B6 polymorphism pattern and its relation to clinical outcome. The group of patients was composed of 50 (61.7%) males and 32 (39.02%) females with a mean age ± SD of 37.7 ± 1.6. Laboratory finding of recruited cases is seen in Table 1. Classification of AML cases according to FAB is as follows: M0 1 (1.2%), M1 20 (24.7%), M2 27 (33.3%), M3 5 (6.2%), M4 25 (30.9%), M5a 1 (1.2%), and M5b 2 (2.5%). One case was missing. The remission status at 28 days is as follows: complete remission 61 (74.4%) (14 cases relapsed), resistant to treatment 13 (15.9%), early death 4 (4.9%), and not assessed 4 (4.9%). The cytogenetic analysis of the cases is seen in Table 2. Frequency of CYP2B6 polymorphism is wild GG type 48 (58.5%), the frequencies of mutant type T allele are 34 (41.9%), which included the GT genotype heterozygous variant found to be 28 (34%), and TT genotype homozygous variant is 6 (7.3%).

We found no significant association between CYP2B6 G15631T polymorphism with FAB and cytogenetic

Table 1. Laboratory Finding of AML Cases

	Mean ± SD
TLC	65.3 ± 8.3
HB	7.65 ± 0.2
PLAT	79.4 ± 19.4
Blasts in blood	56.8 ± 2.6
Blasts in marrow	67.6 ± 2.3
Bone marrow cellularity	N (%)
Normocellular	9 (11.3)
Hypercellular	71 (88.8)
FLT status	N=77 (%)
Wild	49 (63.6)
Mutant	28 (36.4)

Table 2. Cytogenetic Analysis of the AML Cases

Genotype	N=82 (%)
Normal karyotype	62 (79.5%)
t (18:21)	8 (10.3%)
inv 16	2 (2.6%)
t (15:17)	2 (2.6%)
PML-RAR	3 (3.8%)
t (9:22)	1 (1.3%)

Table 3. Relation between CYP with FAB Classification and Cytogenetics

	GG genotype	T allele	P-value
FAB	N=47 (%)	N=34 (%)	0.51
M0	0 (0)	1 (2.9%)	
M1	9 (19.1%)	11 (32.4%)	
M2	17 (36.2%)	10 (29.4%)	
M3	3 (6.4%)	2 (5.9%)	
M4	15 (31.9%)	10 (29.4%)	
M5a	1 (2.1%)	0 (0%)	
M5b	2 (4.3%)	0 (0%)	
Cytogenetics	N=45 (%)	N=33 (%)	0.673
Normal karyotype	36 (80%)	26 (78.8%)	
t (18:21)	4 (8.9%)	4 (12.1%)	
inv 16	2 (4.4%)	0 (0)	
t (15:17)	1 (2.2%)	1 (3%)	
PML-RAR	2 (4.4%)	1 (3%)	
t (9:22)	0	1 (3%)	

Table 4. Relation between CR and CYP

	GG genotype N=48	T allele N=34	P-value
Treatment outcome	N(%)	N(%)	0.768
Not assessed	3 (6.2%)	1 (2.9%)	
Complete remission	34 (70.8%)	27 (79.4%)	
Resistant	8 (16.7%)	5 (14.7%)	
Early death	3 (6.2%)	1 (2.9%)	
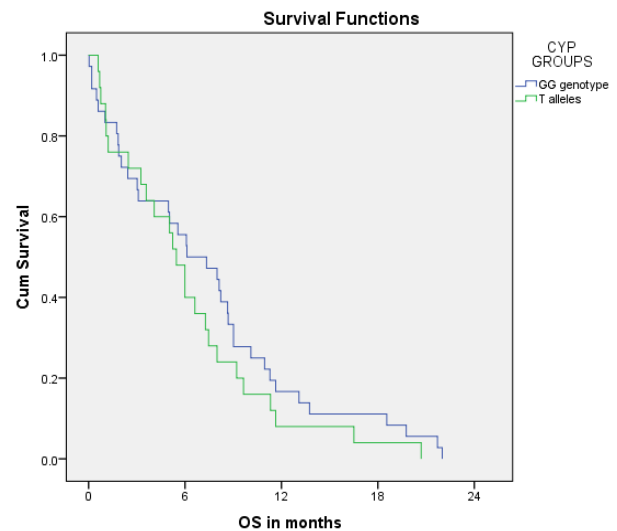
Figure 3. Overall Survival, p=0.325

Table 5. Toxic death* CYP groups Cross Tabulation

	GG genotype N=48	T allele N=34
Toxic death	6 (12.5%)	4 (11.8%)
Others	42 (87.5%)	30 (88.2%)

*Ten (12.2%) patients were exposed to early death within six weeks probably due to toxic drugs therapy (p=0.92)
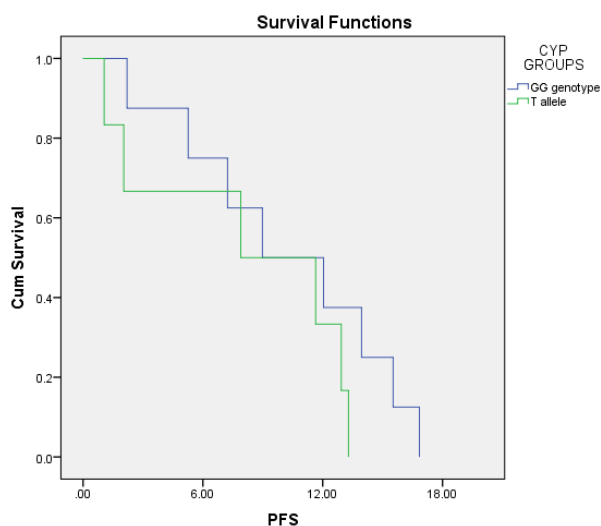
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Figure 4. Patient Free Survival, p=0.245

analysis (available cases: 47 wild and 34 mutant) (p-value=0.51 and 0.673, respectively) (Table 3). The relation between CR and CYP2B6 G15631T is identified in Table 4; it has no significant value (p-value=0.768). Ten patients (six were wild and four were mutant, which represented 12.2%) were exposed to early death within six weeks after beginning of therapy probably due to toxic death. Then we correlated them with CYP2B6 G15631T polymorphism. We found no significant value (p-value=0.92), as seen in Table 5. Figure 3 shows the overall survival (OS) time, with no significant value (p-value=0.325). Figure 4 shows patient free survival (PFS), with no significant value (p-value=0.245). Ten (12.2%) patients were exposed to early death within six weeks probably due to toxic drugs therapy (p=0.92).

Discussion

Acute myeloid leukemia (AML) considers one of the most divergent malignancies among other hematological malignancies. Last decade has shown immense improvement in identification of new molecular structure which are of high clinical relevance (Dunna et al., 2012; Firoz et al., 2014).

CYP2B6 is a member of the biggest and most crucial family of cytochrome P450 (CYP) enzymes, which have an indispensable role in metabolism of many biomolecules, medication, carcinogens, and xenobiotics. Polymorphisms of these genes have been shown to effectuate the jeopardy of several solid tumors and hematologic malignancies and can also change malignant patient response to his therapeutic treatment (Jamroziak et al., 2004; Kayser et al., 2012; Daraki et al., 2014).

Many research have been conducted about CYP2B6 enzyme expression, different population, inhibitors, inductors, structure, and their involvement as a risk factor in the capability of an individual to develop acute leukemia (Lamba et al., 2003; Shimada et al., 2006; Zanger et al., 2007; Eyada et al., 2007), but here in our study, we assessed the frequency of CYP2B6 polymorphism in 82 patients with de novo AML and analyzed their

association with the remission status, FAB classification, genetic status of the patients, early toxic death, patient free survival, and overall survival (OS).

Our results indicate that the frequency of GG genotype (wild type) is 48 (58.5%), polymorphic GT genotype (heterozygous variant) is 28 (34%), and the TT genotype (homozygous variant) is 6 (7.3%). We found no significant association among CYP2B6 G15631T polymorphism, cytogenetic analysis (p-value=0.673), and FAB classification (p-value=0.51); and also, no value was found between remission status and CYP2B6 G15631T value (p-value=0.768). Regarding PFS and early toxic death, no significance was detected in the p-value (respectively 0.245 and 0.92). Finally, no significant value was found with overall survival (p-value=0.325).

These results are in agreement with those of (Vanessa da et al., 2009) who reported in a study (which included 95 pediatric patients) that there is no association between cytochrome P450 enzyme with the prognosis and response to therapy. Another study done by Palodetto et al.17 also found no correlation between CYP2B6 G15631T and NQO1 C609T polymorphisms and MDS progression. (Gamal et al., 2014) predicted that no statistically significant finding was found among the CYP3A4-A-290G polymorphism with clinical, laboratory indices as well as response to treatment, overall survival, and disease-free survival.

However, these results are in disagreement with those of (MT Voso et al., 2005) who analyzed the polymorphisms in drug and carcinogen metabolizing enzymes in acute myeloid leukemia patients and their prognostic value, they reported that polymorphic variant of the cytochrome P450 CYP1A1*2A were present in 11.3% of patients, and their presence premeditated an independent bad prognostic marker for complete remission and overall survival. Also, (Daraki et al., 2014) identified that a high frequency of TT genotype was observed in patients classified to be a poor risk group, also suggesting a possible role of the CYP2B6 genetic background in the development of specific chromosomal aberrations. (Nageswararao et al., 2010) studied the PM genotype of CYP2D6*4 that were associated with clinical variables, such as elevated WBC, blast%, LDH, and failure to CR, thus contributing to poorer survival. This was can be explained by studies that investigate the relation among other members of cytochrome P450 (CYP) enzymes and not on CYP2B6 per se on prognosis.

In conclusion, we hypothesize that CYP2B6 has no role as a prognostic factor on AML patients. However, further studies on this polymorphism in association with the patients' response in large scale are crucial to gain insight and explore their role in disease progression and outcome.

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