Evaluation of *CYP2B6 G15631T* polymorphism as a risk factor for development of chronic myeloid leukemia

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Objectives
The objective of this study was to investigate the possible relationship between *CYP2B6 G15631T* gene polymorphism and chronic myeloid leukemia (CML) risk.

Background
The cytochrome P450 (CYP) enzymes constitute one of the biggest gene families and play a vital role in the metabolism of endogenous biomolecules, drugs, and xenobiotics. One of the members of this family, CYP2B6, plays a very important role in metabolizing carcinogens and medications. *CYP2B6 G15631T* gene polymorphism reduces CYP2B6 enzyme activity.

Patients and methods
Fifty CML patients and 32 matched healthy controls were enrolled in this study. *CYP2B6 G15631T* polymorphic variant was detected by PCR-restriction fragment length polymorphism.

Results
The frequencies of GG genotype (wild type) were 60% and 43% in CML and control groups, respectively. The frequencies of polymorphic GT genotype (heterozygous variant) were found to be 32% in CML patients and 37.5% in controls (P = 0.608). The TT genotype (homozygous variant) was 8% in CML cases and 18.8% in the control group (P = 0.147). The frequency of the T allele was 24% in CML patients and 37.5% in healthy individuals (P = 0.064). We did not find any association between *CYP2B6 G15631T* polymorphic variant and CML risk.

Conclusion
CYP2B6 G15631T is not a risk factor for CML. Further studies on this polymorphism using large number of cases may provide valuable information.

Keywords:
chronic, cytochrome P450 CYP2B6, genetic, leukemia, polymorphism

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Introduction

Chronic myeloid leukemia (CML) is an acquired hematopoietic stem-cell disease, characterized by an increased production of immature granulocytes, which accumulate in the bone marrow and interfere with the normal blood cell production, accounting for 30% of adult leukemias. The symptoms of CML include bone marrow hypercellularity, anemia, splenomegaly, and leukocytosis [1,2]. CML progresses slowly in three phases, chronic phase, accelerated phase, and blast phase, which are differentiated by the number of blast cells in the blood and the bone marrow, and the severity of the symptoms. In 95% of CML cases, chromosomal translocation resulting in the formation of the Philadelphia chromosome is observed, which in turn leads to the formation of the BCR-ABL fusion gene. This reciprocal translocation, creating an elongated chromosome 9 der (9) and a truncated chromosome 22 (Philadelphia chromosome), is the hallmark of CML [3,4].

Cytochrome P450 (CYP) superfamily comprises phase I detoxification enzymes that metabolize many exogenous and endogenous genotoxic compounds, such as dibenzanthracene, 6-aminochryse, styrene, nicotine, and vinyl chloride [5–9], by insertion of an atom from molecular oxygen into the substrate, acting as monooxygenases, oxidases, and peroxidases [10]. CYP detoxification enzymes play a key role in protecting cells against oxidative damage. It has been demonstrated that single nucleotide polymorphisms at the CYP genetic loci inactivate enzymatic activity and may be associated with many types of cancers including hematological malignancies, such as acute lymphoblastic leukemia, myelodysplastic syndromes, and acute myeloid leukemia [11–14].

The CYP2B6 enzyme plays a key role in the biotransformation of many xenobiotics [5]. Although, a few years ago, CYP2B6 was thought to play only a small role in drug and xenobiotic metabolism,

nowadays, it is understood that CYP2B6 enzyme is composed of 2–10% of total CYP content, and it is also involved in the metabolism of nearly 25% of drugs and many chemical compounds [4,5]. In the course of biotransformation, these chemicals convert to reactive metabolites by CYP2B6 and other phase I enzymes, but, if there is a problem with CYP2B6 enzyme activity due to any reason, these reactive metabolites can make interactions with DNA, RNA, protein, and other biological materials and can achieve DNA adducts. If these DNA adducts contact hematopoietic stem cells, some hematological malignancies can occur [2].

In conclusion, we aimed to detect the possible relationship between CYP2B6 G15631T gene polymorphism and risk for development of CML.

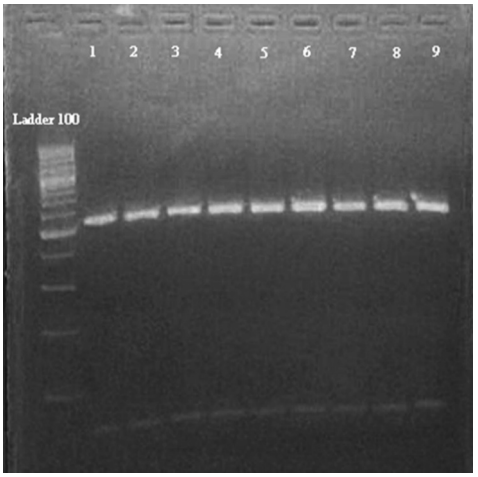
Patients and methods

Fifty CML patients admitted to the Clinical Oncology Department, Menoufia University Hospitals, Egypt, were enrolled in a random manner for the study. Controls included 32 age-matched healthy participants. This study was approved by the ethical committee of the Faculty of Medicine, Menoufia University. All patients provided signed a written informed consent to provide a blood sample and to review their medical record for research purposes.

DNA was extracted from peripheral leukocytes using a GeneJET Genomic DNA Purification Kit. Identification of the *CYP2B6 G15631T* polymorphic variant was performed by PCR-restriction fragment length polymorphism. PCR was conducted using the primer pair: 5-CTGTGTCCTTGACCTGCTGC-3 as a forward primer and 5-TCCAGGAGC AGAATAGACATGAAG-3 as a reverse primer [15]. PCR conditions were 95°C for 3 min, followed by 40 cycles of 95°C for 50 s, 60°C for 50 s, 72°C for 3 min, and a final extension step at 72°C for 5 min. The obtained PCR products showed a single fragment at 578 bp (Fig. 1). PCR products were then digested with 10 U of BsrI restriction enzyme Fermentas (Waltham, Massachusetts, USA) at 65°C for 4 h. Digestion products were visualized on a 3% agarose gel containing ethidium bromide. Wild -type genotype (GG) produced a double band at 518 and 60 bp, and heterozygotes (GT) produced three bands at 578, 518, and 60 bp. Homozygote polymorphic genotype (TT) produced only one band at 578 bp (Fig. 2).

The analysis was performed with SPSS, version 20, statistical software (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ± SD. Qualitative data were expressed as number and percentage and analyzed by applying χ^2 test. *P* value less than 0.05 was considered statistically significant. The odds ratio and 95% confidence interval were also calculated.

Figure 1



CYP2B6 G15631T amplified gene product at 578 bp.

Results

The mean age of CML patients was 44.84±14.67 years (23 male patients and 27 female patients) and that of controls was 40.62 ± 12.25 years (16 male individuals and 16 female individuals). The frequencies of GG genotype in CML cases and in the control group were 60 and 43.8%, respectively. The frequency of the GT was 32% in CML cases and 37.5% in the control group. Furthermore, the TT homozygote genotype showed a frequency of 8% in CML cases and 18.8% in the control group (Table 1). No significant differences were found between CML cases and the control group with regard to all genotype variants ($P > 0.05$).

To evaluate the effect of the minor allele as a risk factor in CML incidence, we assessed the minor allele's distribution in patients and healthy individuals. The frequency of the T allele was not significantly different between CML patients and healthy individuals (24 and 37.5%, respectively; $P = 0.064$; odds ratio 0.526; 95% confidence interval = 0.27–1.04) (Table 1).

Discussion

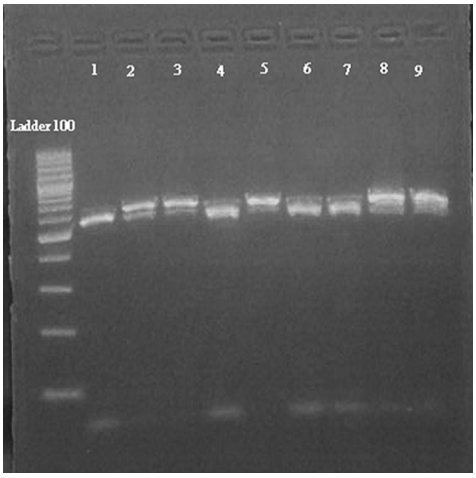
Biotransformation enzymes play a major role in regulating the toxic, mutagenic, and neoplastic effects of chemical carcinogens as well as metabolizing other xenobiotics (phytochemical and therapeutic drugs) and endogenous compounds (steroid hormones) [3,16–19].

CYP enzymes are the main group of biotransformation enzymes. Genetic polymorphisms in these enzyme

Table 1 Distribution of CYP2B6 G15631T genotype

	Cases (*n*=50) [*n* (%)]	Control (*n*=32) [*n* (%)]	*P*	OR	95% CI
Genotype					
GG	30 (60)	14 (43.8)		Reference	-
GT	16 (32)	12 (37.5)	0.608	0.622	0.23-1.66
TT	4 (8)	6 (18.8)	0.147	0.311	0.076-1.281
GG + GT	46 (92)	26 (81.3)		Reference	-
TT	4 (8)	6 (18.8)	0.147	0.377	0.09-1.46
GG	30 (60)	14 (43.8)		Reference	-
GT + TT	20 (40)	18 (56.3)	0.150	0.519	0.21-1.27
Allele					
G	76 (76)	40 (62.5)		Reference	-
T	24 (24)	24 (37.5)	0.064	0.526	0.27-1.04

Figure 2



CYP2B6 G15631T gene product after enzyme digestion.

systems can influence cancer susceptibility when coupled with the relevant carcinogen exposures [20].

Certain gene polymorphisms, altered forms of genes that differ by a single nucleotide base pair, have been shown to change the risk for development of leukemia, and these variations can interact with diet, other environmental exposures, and individual immune function to be major determinants of susceptibility [21–23].

CYP2B6 is one of the members of the CYP gene family [2,3]. CYP2B6 G15631T polymorphism changes glutamine (Glu) amino acid to histidine (His) amino acid [24–26], reduces the CYP2B6 enzyme activity, and can block the transformation of carcinogen substrates to harmless metabolites [24,27,28].

Some research has been conducted about CYP2B6 enzyme activity, expression, and polymorphisms, but there is no study that shows the susceptibility to the risk of CML [2,4,5,29–31]. In this study, we aimed to investigate the possible relationship between CYP2B6 polymorphism and CML.

According to the current results, the frequency of polymorphic GT genotype in controls is 37.5%, but it is 32% in CML patients. Furthermore, the TT genotype showed a frequency of 8% in CML cases and 18.8% in the controls. These data show that CYP2B6 G15631T polymorphism does not increase the CML risk. Moreover, the frequency of the T allele was not significantly different between CML patients and healthy individuals (24 and 37.5%, respectively).

Although there are no any studies about CYP2B6 polymorphism and CML susceptibility, while there are some respected studies about the relationship between this gene polymorphism and other hematologic malignancy risks. Berköz and Yalin [15] reported that GT genotype may be an important genetic determinant for acute leukemias. Furthermore, Daraki *et al.* [29] identified that a high frequency of TT genotype was observed in patients classified to be a poor-risk group of AML, also suggesting a possible role of the CYP2B6 genetic background in the development of specific chromosomal aberrations. However, in the Alazhary *et al.* [30] study, the CYP2B6 polymorphism has no role in disease progression, therapeutic outcome, patient survival, early toxic death, and overall survival in acute myeloid leukemia patients. Palodetto *et al.* [31] also reported no correlation between CYP2B6 G15631T and NQO1 C609T polymorphisms and MDS progression.

Conclusion

In conclusion, we hypothesize that CYP2B6 has no role as a risk factor in CML patients. However, further studies on this polymorphism on a large scale are crucial to gain insight and explore their role in disease risk, progression, and outcome.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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