Impact of Cytochromes P450 3A4 and 2B6 gene polymorphisms on predisposition and prognosis of acute myeloid leukemia: an Egyptian case-control study

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Abstract

**Background**: It has been postulated that the interaction between environmental risk factors and genetic susceptibility is a possible cause for the development of acute myeloid leukemia (AML). Cytochrome P450 (CYP) detoxification enzymes are responsible for the elimination of oxidative stress. Genetic polymorphisms in these enzymes may cause AML due to enhanced accumulation of reactive oxygen species. To study the association between CYP3A4 (A290G) and CYP2B6 (G516T) gene polymorphisms and the predisposition and prognosis of AML, 50 upfront AML patients and 50 healthy individuals were genotyped for CYP2B6 (G516T) and CYP3A4 (A290G) single-nucleotide polymorphisms (SNPs) using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) technique. The polymorphisms were evaluated in relation to the response to chemotherapy and survival.

**Results**: CYP2B6 gene mutation carries a threefold risk of developing AML (odds ratio [OR], 3.0; 95% confidence interval [CI], 1.3–6.9), whereas CYP3A4 gene mutation carries approximately fourfold risk (OR, 3.8; 95% CI, 1.4–10.1). The presence of combined gene mutation conferred about 15-fold increased risk of developing AML compared with the presence of a single gene mutation (OR, 14.8; 95% CI, 1.8–124.2). CYP3A4 gene mutation is associated with worse overall survival (P = 0.030).

**Conclusion**: CYP enzyme gene polymorphisms are associated with the development of AML. Elimination of oxidative stress in genetically susceptible individuals may decrease the risk of AML and may improve survival.

**Keywords**: AML, CYP, Gene polymorphism

Background

Acute myeloid leukemia (AML), which is characterized by the infiltration of the bone marrow (BM), blood, and other tissues by proliferative, clonal, abnormally differentiated and sometimes poorly differentiated cells of hematopoietic origin, is the most common type of acute leukemia in adults [1, 2]. The etiology of this disease is almost unknown; however, the interaction between environmental risk factors and genetic susceptibility has been postulated to be a possible cause for the development of AML [3]. Cytochrome P450 (CYP) detoxification enzymes play a crucial role in protecting cells against oxidative damage; CYP metabolizes many exogenous and endogenous genotoxic compounds through the insertion of an atom from molecular oxygen to the substrate, such as hydrogen peroxide [4].

CYP3A4 is the most abundantly expressed CYP in adult human liver; it constitutes approximately 15%–30% of the microsomal P450 pool and is responsible for the metabolism of about 30%–40% of all clinically used
drugs [5, 6]. \textit{CYP2B6} is also a member of the CYP superfamily B that is mainly expressed in the liver [7].

Certain single-nucleotide polymorphisms (SNPs) at the \textit{CYP} genetic loci (\textit{CYP2D6}, \textit{CYP1A1}, \textit{CYP3A5}, and \textit{CYP2E1}) may be considered as risk factors for many types of cancer and hematological malignancies, such as AML, acute lymphoblastic leukemia, and myelodysplastic syndromes, through the inactivation of enzymatic activity [8–14].

\textit{G516T} SNP at the \textit{CYP2B6} gene locus has been identified as a nonsense polymorphism that reduces the activity of functional protein. Thus, individuals carrying the \textit{T} allele (TT) have a lower enzymatic activity than individuals carrying the wild-type \textit{G} allele (GG), whereas individuals carrying the genotype GT show intermediate activity [15].

\textit{CYP3A} enzyme activity varies among ethnicities because of interindividual variability; \textit{CYP3A4} activity may vary by up to 50-fold. Most genetic polymorphisms to \textit{CYP3A4} cause of interindividual variability; SNPs in the \textit{CYP3A4} gene locus has been implicated as a possible cause of this variability; subsequently, it may be considered as a risk factor for cancer, but the effects of this SNP remain not well defined [16–19].

This study aimed to evaluate the association between \textit{CYP2B6} (G515T) and \textit{CYP3A4} (A290G) SNP and AML susceptibility in Egyptian patients and their effect on the response to chemotherapy.

\section*{Methods}

\subsection*{Clinical demography}
This study included 50 newly diagnosed AML patients and 50 age- and sex-matched healthy individuals. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of our institute (reference number, 1-251014). Informed consent was obtained from each participant.

Diagnosis was established between June 2015 and August 2017, based on the World Health Organization requirements [20] of 20% of BM or peripheral blood blasts, except for cases carrying the recurrent cytogenetic abnormalities t(15;17), t(8;21), inv.(16), or t(16;16). Cases were subjected to full history-taking, thorough clinical examination, routine laboratory investigations including complete blood count and Leishman-stained peripheral blood film examination, BM examination for French-American-British (FAB) classification [21], cytochemistry, immunophenotyping by flow cytometry, cytogenetic studies, and finally genotyping of \textit{CYP2B6} (G516T) and \textit{CYP3A4} (A290G) SNPs using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) technique.

\begin{itemize}
\item Patients were initially treated with 3 + 7 regimen, combining daunorubicin 45 mg/m$^2$ IV at days 1–3 and cytosine arabinoside (ara-C) 100 mg/m$^2$ by continuous infusion from day 1 to day 7 for induction. The response was evaluated by the end of week 4. If the BM was not hypocellular and there was unequivocal residual leukemia, a second course of therapy with HAM regimen (high-dose ara-C [HiDAC] and mitoxantrone) was administered as follows: ara-C 3 g/m$^2$ from day 1 to day 3 for infusion for 3 h and mitoxantrone 12 mg/m$^2$ from day 3 to day 5 by short infusion. After achieving complete remission (CR), consolidation chemotherapy was performed by HiDAC (3 g/m$^2$ for 3 h every 12 h at days 1, 3, and 5). Morphologic CR was defined according to the standard criteria by Cheson [22], namely, an absolute neutrophil count of $\geq$1.0 $\times$ 10$^9$/L, a platelet count of $\geq$100 $\times$ 10$^9$/L, < 5% blasts in BM, and no extramedullary leukemia. The minimum follow-up period was 6 months. Overall survival (OS) was calculated from the date of diagnosis to death from any cause or the date of the final follow-up. Disease-free survival (DFS) was defined as the time from achieving CR to relapse or death or the date of the final follow-up.
\end{itemize}

\subsection*{Molecular analysis}

\textbf{Sampling}
For each patient, 3–5 mL of venous blood was collected on ethylenediaminetetraacetic acid by sterile venipuncture using a sterile Vacutainer tube. Samples were either stored in the same Vacutainer at $-20 \, ^\circ$C or used directly within 24 h for DNA extraction.

\subsection*{DNA extraction}
Genomic DNA was extracted from the whole blood by ready-made isolation kit (TIANamp Genomic DNA Kit catalog no. DP 318-02; TIANGEN Biotech, Beijing, China) according to the manufacturer’s instructions.

\subsection*{Genotyping}
For genotyping of the \textit{CYP2B6} (G516T) SNP, the forward primer sequence was 5’GGTCTGCCCATCTATAAAC3’, and the reverse was 5’CTGATTTCTTCACATGTCTGCCG3’. As for the \textit{CYP3A4} (A290G) SNP, the forward primer sequence was 5’- GGA CAG CCA TAG AGA CAAGGG CC- 3’, and the reverse primer sequence was 5’ - TCACGTACCCCTTT GAG TTC ATA- 3’.

For \textit{CYP2B6} (G516T) SNP, the thermal cycling conditions were initial denaturation for 5 min at 95 $\, ^\circ$C, 30 cycles of 30 s at 95 $\, ^\circ$C, 30 s at 50 $\, ^\circ$C, and 1 min at 72 $\, ^\circ$C with a final step of 10 min at 72 $\, ^\circ$C. For \textit{CYP3A4} (A290G) SNP, the cycling conditions were initial denaturation for 10 min at 95 $\, ^\circ$C, 35 cycles of 45 s at 95 $\, ^\circ$C, 45 s at 59 $\, ^\circ$C, and 1 min at 72 $\, ^\circ$C. The last elongation step...
was extended to 7 min. The resulting PCR products were digested with \textit{BsrI} (New England Biolabs Organic, Beverly, MA, USA) and \textit{MspI} restriction enzyme (New England Biolabs, Ipswich, MA, USA) for the \textit{CYP2B6} (G516T) and the \textit{CYP3A4} (A290G) SNPs, respectively, according to the manufacturer’s instructions. Digested fragments were separated on 2% agarose gel, and RFLP bands were visualized by ethidium bromide staining under ultraviolet light. The \textit{CYP2B6} (G516T) SNP digestion products were three fragments of 241, 268, and 17 bp when the wild-type G allele was present and a single fragment of 509 bp when the T mutant allele was present (Fig. 1). As for the \textit{CYP3A4} (A290G) SNP, two fragments of 142 and 23 bp were produced when the mutant G allele was present and a single band of 165 bp when the wild A allele was present (Fig. 2).

\textbf{Statistical methods}

Data were coded and entered using the SPSS statistics version 23 and were summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Unpaired \textit{t}-test was used to compare between quantitative variables and chi-square (\(\chi^2\)) test to compare categorical data. When the expected frequency is < 5, exact test was used instead. Genotype and allele frequencies were compared between the disease and control groups using chi-square tests. Odds ratio (OR) with 95% confidence intervals (CIs) was calculated. \(P < 0.05\) was considered statistically significant.

\textbf{Results}

The study involved 50 newly diagnosed AML patients (AML group) and 50 age- and gender-matched healthy individuals (control group). The median age of patients was 35.9 years (range, 17–64 years); 32 (64%) were male, and 18 (36%) were female.

Upon examination of the AML group, 27 patients (54%) had splenomegaly and 22 (44%) had hepatomegaly. At diagnosis, the mean hemoglobin level among the studied patients was 7.9 ± 1.9 g/dL, the platelet count ranged from 21.4 to 110.5 × 10^3/\mu L and the total leukocyte count ranged from 9.2 to 95.7 × 10^9/L.

According to FAB classification, 19 patients (38%) were classified as FAB M2, 10 (20%) were FAB M1, 6 (12%) were FAB M5, 6 (12%) were FAB M4, 5 (10%) were FAB M3, 3 (6%) were FAB M0, and 1 (2%) was FAB M6.

Cytogenetic analysis of the 50 AML patients revealed abnormal cytogenetics in 32 patients (64%). The frequencies of different cytogenetic abnormalities detected in the AML group are presented in Table 1. The most frequent cytogenetic group was cytogenetically normal (normal cytogenetic [NCG]), which was detected in 18 patients followed by t(15:17). Furthermore, AML patients were stratified into three risk groups according to cytogenetic structure [23]: 14 patients (28%) belonged to favorable-risk group, 29 (58%) belonged to intermediate-risk group, and 7 (14%) had poor prognosis.

The entire AML group received chemotherapy regimens. Notably, 45 patients (90%) received 3 + 7 regimen; 5 patients (10%) received all-trans retinoic acid, whereas 3 patients (6%) received fludarabine+HiDAC+G-CSF therapy. The initial response to therapy was assessed at day 28. CR was attained in 30 patients (60%), whereas 6 patients died before reaching remission.

Regarding the distribution of \textit{CYP2B6} (G516T) gene polymorphism in patients and control group, the wild-
genotype GG was encountered in 23 AML patients (46%) and 36 of the control group (72.0%), the heterozygous mutant genotype GT was encountered in 19 of AML patients (38%) and 12 of the control group (24%), and the homozygous mutant genotype TT was encountered in 8 of AML patients (16%) and 2 of the control group (4%). The mutant genotype frequency distribution was significantly higher in the AML group than control subjects ($P = 0.017$) (Table 2). The presence of CYP2B6 gene mutation (homo- or heterozygous) carries a threefold risk of developing AML (OR, 3.0; 95% CI, 1.3–6.9) (Table 3).

CYP3A4 (A290G) gene polymorphism distribution shows that the wild genotype (AA) was encountered in 31 AML patients (62%) and 43 of the control group (86%), the heterozygous mutant genotype AG was encountered in 16 of AML patients (32%) and 7 of the control group (14%), and the homozygous mutant genotype GG was encountered in 3 of AML patients (6%) and none of the control group. The frequency distribution of the mutant genotypes was significantly higher in the patients than controls ($P = 0.006$) (Table 2). The presence of CYP3A4 gene mutation (homo- or heterozygous) carried an approximately fourfold risk of developing AML (OR, 3.8; 95% CI, 1.4–10.1) (Table 3).

The distribution of the combined CYP2B6 and CYP3A4 gene mutations among patients and controls revealed only 1 case of combined gene mutation in the control group compared with 14 cases in the AML group ($P = 0.003$). The presence of combined gene mutation conferred about a 15-fold increased risk of developing AML compared with the presence of a single gene mutation (OR, 14.8; 95% CI, 1.8–124.2) (Table 3).

The gene polymorphism of both CYP2B6 and CYP3A4 did not show any association with demographic, clinical,

Table 1 Frequency of cytogenetic characteristics in AML group

| Cytogenetic type (n = 50) | Frequency (n = 50) | Percent |
|--------------------------|--------------------|---------|
| t(15:17)*                | 5                  | 10.0    |
| t(8;21)*                 | 6                  | 12.0    |
| Inv16*                   | 3                  | 6.0     |
| Others                   | 36                 | 72.0    |

*Favorable prognosis cytogenetic abnormalities

Table 2 CYP2B6 gene polymorphism distribution in the two studied groups

| Genotype   | AML group n = 50 | Control group n = 50 | p value |
|------------|------------------|----------------------|---------|
| CYP2B6 gene polymorphism |                     |                      |         |
| GG         | 23 (46%)         | 36 (72%)             | 0.017   |
| GT         | 19 (38%)         | 12 (24%)             |         |
| TT         | 8 (16%)          | 2 (4%)               |         |
| GT + TT    | 27 (54%)         | 14 (28%)             | 0.008   |
| CYP3A4 gene polymorphism |                     |                      |         |
| AA         | 31 (62.0%)       | 43 (86%)             | 0.006   |
| AG         | 16 (32.0%)       | 7 (14%)              |         |
| GG         | 3 (6.0%)         | 0 (0%)               |         |
| AG + GG    | 19 (38%)         | 7 (14%)              | 0.006   |
or laboratory characteristics of the AML patients (Table 4).

Cytogenetic risk stratification showed a significant association between \textit{CYP3A4} gene mutation and unfavorable cytogenetic risk \((P = 0.031)\). The presence of \textit{CYP3A4} mutation was found to be a risk factor for unfavorable cytogenetic profile in AML patients \((OR, 2.8; 95\% CI, 0.8–10.2)\). However, there was no significant association between \textit{CYP2B6} gene mutation and cytogenetic risk stratification (Table 5).

**Overall survival**

The cumulative OS proportion of AML patients was 48.5\% at 9 months. The median survival time was 9 months \((95\% CI, 7.8–10.2\,\text{months})\). \textit{CYP3A4} mutation was found to be a risk factor for worse OS \((P = 0.030)\) (Fig. 3). In contrast, OS was not affected by the presence of \textit{CYP2B6} gene mutations \((P = 0.827)\) (Table 6).

Those with combined \textit{CYP2B6} and \textit{CYP3A4} gene mutations had worse OS than AML patients with a single gene mutation: the median survival time was 12.0 months \((95\% CI, 8.7–15.3)\) in cases with wild \textit{CYP3A4} genotype, whereas 9.0 months \((95\% CI, 7.8–10.2)\) in the mutant genotype \((P = 0.030)\) (Fig. 4).

**Disease-free survival**

At 9 months, the cumulative DFS proportion of the 30 AML patients who achieved CR was 44.6\%. The median DFS was 9 months \((95\% CI, 5.8–12.2\,\text{months})\). DFS was not affected by the presence of \textit{CYP2B6} gene mutations \((P = 0.352)\) or \textit{CYP3A4} mutation \((P = 0.195)\). In addition, no significant difference was found in DFS among patients with a single gene mutation of one of the \textit{CYP2B6} and \textit{CYP3A4} genes compared with those with combined mutations \((P = 0.265)\) (Table 7).

**Discussion**

AML is a form of cancer characterized by the infiltration of malignant cells to the blood, BM, and other tissues. Fifty years ago, AML was incurable; however, a few years ago, the prognosis improved and now AML is curable in 35\%–40\% of adult patients aged \(\leq 60\) years [23].

The exact causes of leukemia are still unknown despite widespread studies that investigated the mechanisms of the disease. It is believed that the exposure of DNA to reactive oxygen species in the hematopoietic stem cells is an important factor in the development of leukemia [24]. CYP enzymes play an important role in the elimination of oxidative stress.

Genetic polymorphisms in these enzyme systems are associated with the reduction of CYP enzymes; thus, it can influence cancer susceptibility [8, 9].

The current work studied the possible association between \textit{CYP2B6} (G516T) and \textit{CYP3A4} (A290G) gene polymorphisms and susceptibility to AML in the Egyptian population and the association between \textit{CYP} genotypes and the clinical presentation, hematological and cytogenetic findings, and treatment response in AML patients.

### Table 3 Risk estimate for AML in the two studied genes

| Gene Polymorphism | AML | Control | OR  | 95% CI   | p-value |
|-------------------|-----|---------|-----|---------|---------|
| CYP2B6 genotype   |     |         |     |         |         |
| GG                | 23 (46%) | 36 (72%) | 3.0 | 1.3-6.9 | 0.008   |
| GT + TT           | 27 (54%) | 14 (28%) |     |         |         |
| CYP3A4 genotype   |     |         |     |         |         |
| AA                | 31 (62%) | 43 (86%) | 3.8 | 1.4-10.1| 0.006   |
| AG + GG           | 19 (38%) | 7 (14%)  |     |         |         |
| CYP2B6 + CYP3A4   |     |         |     |         |         |
| Combined          | 14 (43.7%) | 5 (15%)  | 14.8| 1.8-124.2| 0.003   |
| Single            | 18 (56.3%) | 19 (95.0%) |     |         |         |

**Table 4** Relation of CYP2B6 gene polymorphism and CYP3A4 gene polymorphism with demographic, clinical and laboratory data in AML patients

|                  | CYP2B6 gene polymorphism | CYP3A4 gene polymorphism |
|------------------|--------------------------|--------------------------|
|                  | Wild         | Hetero + Homozygous | p value | Wild        | Hetero + Homozygous | p value |
| Median Age (years) | 32.4 | 37.6 | 0.379 | 35.4 | 40.1 | 0.418 |
| Sex, No (%)       |     |     |     |     |     |     |
| Male              | 14 (48.2%) | 15 (51.8%) | 0.704 | 19 (65.5%) | 10 (34.5%) | 0.547 |
| Female            | 9 (42.8%) | 12 (57.2%) |     | 12 (51.7%) | 9 (42.9%) |     |
| Splenomegaly, No. (%) | 15 (55.5%) | 12 (44.5%) | 0.141 | 17 (63.0%) | 10 (37.0%) | 0.879 |
| Hepatomegaly, No. (%) | 12 (54.5%) | 10 (45.5%) | 0.282 | 14 (63.6%) | 8 (36.4%) | 0.833 |
| Hemoglobin (g/dL) | 7.5 ± 1.4 | 7.9 ± 2.0 | 0.476 | 7.8 ± 1.9 | 8.1 ± 1.9 | 0.535 |
| Median TLC (×10^3/mm^3) | 42.8 | 56.4 | 0.722 | 60.3 | 53.4 | 0.542 |
| Median Platelet count (×10^3/mm^3) | 68.4 | 50.8 | 0.784 | 78.6 | 66.4 | 0.810 |
cases to justify whether such polymorphisms have any effect on the disease prognosis.

In this study, the mutant CYP2B6 genotypes were significantly higher in patients than controls ($P = 0.017$). Calculated risk estimation revealed that the mutant genotypes conferred a threefold increased risk of developing AML (OR, 3.3; 95% CI, 1.3–6.9).

In 2009, Bekroz et al. investigated the association between CYP2B6 gene polymorphism and the susceptibility to acute leukemia in the Turkish population. Their study included 80 acute leukemia patients (44 of the

| CYP2B6          | Favorable (cytogenetic) (n = 14) | Intermediate + poor (cytogenetic) (n = 36) | $P$ value | OR (95%CI) |
|-----------------|----------------------------------|--------------------------------------------|-----------|------------|
| Wild (GG)       | 9 (64.3%)                        | 14 (18.5%)                                 | 0.106     | 2.8 (0.8–10.2) |
| Mutant (GT + TT)| 5 (35.7%)                        | 22 (81.5%)                                 |           |            |

| CYP3A4          | Favorable (cytogenetic) (n = 14) | Intermediate + poor (cytogenetic) (n = 36) | $P$ value | OR (95%CI) |
|-----------------|----------------------------------|--------------------------------------------|-----------|------------|
| Wild (AA)       | 12 (85.7%)                       | 19 (52.8%)                                 | 0.031     | 5.4 (1.0–27.5) |
| Mutant (AG + GG)| 2 (14.3%)                        | 17 (47.2%)                                 |           |            |

**Fig. 3** Overall survival curves of AML patients with and without mutations of CYP3A4 gene
patients had acute lymphocytic leukemia [ALL] and 36 had AML) and 100 control subjects [25]. Logistic regression analyses revealed a significant correlation between the CYP2B6 (G516T) polymorphism and patients with acute leukemia (OR, 2.481; 95% CI, 1.353–4.551; \( P = 0.003 \)). In addition, in agreement with our results, Yuan et al. (2011) reported that the GT and GT + TT genotype frequencies of c.516G > T SNP were higher in ALL (37.5% and 42.7%, respectively; \( P < 0.01 \)) and AML (37.2% and 40.9%, respectively; \( P < 0.01 \)) than control subjects [13].

Regarding the CYP3A4 (A290G) SNP, the mutant genotypes were overrepresented in patients compared with the control subject (\( P = 0.006 \)). The presence of the mutation, whether homozygous or heterozygous, carried an approximately fourfold increased risk of developing AML (OR, 3.8; 95% CI, 1.4–10.1).

In agreement with our data, Ali et al., in a case-control study involving 77 newly diagnosed AML cases and 72 age- and sex-matched healthy controls, reported a high frequency of the homozygous genotype (GG) in AML cases, although not statistically significant (\( P = 0.999 \)).

### Table 6 Overall survival of AML patients in relation to gene polymorphism of CYP2B6 and CYP3A4

|                      | N   | 9 months Cumulative Survival (%) | Median Survival time (95%CI) (months) | \( p \) value |
|----------------------|-----|----------------------------------|--------------------------------------|--------------|
| Whole group          | 50  | 48.5                             | 9.0 (7.8-10.2)                       |              |
| CYP2B6               |     |                                  |                                      |              |
| Wild                 | 23  | 64.3                             | 10.0 (7.8-12.2)                      |              |
| Mutant               | 27  | 34.9                             | 9.0 (8.3-9.7)                        | 0.827        |
| CYP3A4               |     |                                  |                                      |              |
| Wild                 | 31  | 63.5                             | 12.0 (8.7-15.3)                      | 0.030        |
| Mutant               | 19  | 28.7                             | 9.0 (7.8-10.2)                       |              |
| Single vs. Combined  |     |                                  |                                      |              |
| Single Mutation      | 18  | 62.2                             | 11.0 (8.1-13.9)                      | 0.030        |
| Combined Mutations   | 14  | 16.1                             | 8.0 (6.3-9.7)                        |              |

![Fig. 4 Overall survival curves of AML patients with single and combined mutations of CYP2B6 and CYP3A4 genes](image-url)
The G allele was significantly frequent in AML cases ($P = 0.001$; OR, 17.9; 95% CI, 4.041–78.903) [26].

Similar to our results, Voso et al., in a case-control study involving 160 cases of AML and 162 matched controls, reported a significantly higher prevalence of the polymorphic variant CYP3A4 (A290G) genes in AML cases than controls (9.4% vs 3.1%; $P = 0.04$), increasing the risk of developing AML to 3.2-fold (95% CI, 1.1–11.5) [14].

In contrast, Pakakasama et al. analyzed 107 children with ALL and 320 healthy controls for CYP3A4 (A290G) polymorphism and reported an insignificant difference in the distribution of the polymorphism between patients and controls [25]. This difference may be explained by the variability of the risk factor for acute leukemia between children and adults.

Naoe et al. studied the CYP3A4 (A290G) polymorphism in 58 patients with t-AML/t-MDS and 150 Japanese healthy individuals and found that all subjects had the wild genotype, except 1 case that had a heterogeneous genotype, indicating a very low frequency or a lack of CYP3A4 (A290G) polymorphism in the Japanese population [27]. This may be explained by ethnic variability.

The distribution of combined CYP2B6 and CYP3A4 gene mutations was also significantly increased in patients compared with controls ($P = 0.003$). The presence of combined gene mutation carried an approximately 15-fold increased risk of developing AML compared with the presence of a single gene mutation. To the best of our knowledge, no published study investigated the risk of this combined gene polymorphism and AML.

Similar to these results are those reported by Alazhary NM et al. (2015), who found no significant association between CYP2B6 polymorphism and CR, cytogenetic analysis, and OS [28]. A cytogenetic analysis of 50 AML patients revealed abnormal cytogenetics in 32 patients (64%) and NCG in 18 patients (36%). Risk stratification according to the cytogenetic makeup of AML patients revealed 14 favorable (28%) and 36 nonfavorable patients (72%). In this study, no significant association was found between the presence of mutation in the CYP2B6 gene and cytogenetic risk stratification of AML patients. In contrast, a significant association was found between CYP3A4 gene mutation and unfavorable cytogenetic risk.

In another study, Sophia et al. (2014) reported a higher frequency of the mutant CYP2B6 (G516T) allele in patients with poor prognosis based on cytogenetic findings, indicating that the presence of the variant allele is probably related to specific chromosomal abnormalities conferring a poor prognosis [29].

Furthermore, we found that CYP3A4 (A290G) mutation was significantly associated with worse OS ($P = 0.030$). In contrast, OS was not affected by the presence of CYP2B6 gene mutations ($P = 0.827$). Patients carrying mutation of both genes had worse OS ($P = 0.030$) than patients with a single gene mutation.

DFS was not affected by the presence of CYP2B6 gene mutations ($P = 0.352$) or CYP3A4 gene mutation ($P = 0.195$). In addition, there was no significant difference in DFS between patients with a single gene mutation of one of the CYP2B6 and CYP3A4 genes compared with those with combined mutations ($P = 0.265$).

Similarly, Ali et al. (2013) reported that there was no significant association between CYP3A4 (A290G) SNP and different clinical or laboratory parameters and the early response to treatment, OS, and the DFS [26].

**Conclusion**

The results of this study provide an evidence for the possible pathogenetic role of the CYP2B6 and CYP3A4 polymorphisms on the risk of developing AML. Moreover, having combined mutation in both genes increases such risk. CYP3A4 polymorphism was associated with an unfavorable cytogenetic risk and worse OS, suggesting that CYP3A4 may have a role in disease progression.
therapeutic outcome, DFS, and OS in AML patients, whereas CYP2B6 mutations were not associated with such findings. Oxidative stress is an important risk factor for the development of AML. The elimination of oxidative stress in genetically susceptible individuals may decrease the risk of AML and may improve survival.

Further studies are needed to be conducted on a larger sample size and on other populations to verify the prognostic implications of the CYP2B6 and CYP3A4 polymorphisms on treatment outcome and disease progression.

Abbreviations
Cyp: Cytochrome p 450; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism; PCR: Polymerase chain reaction; FAB: French-American-British; DFS: Disease free survival

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Competing interests
The authors have no conflicts of interest to disclose.

Authors’ contributions
NG: Study design. SB: Revision of the manuscript. GS: Interpretation of data. HL: Data analysis. MS: Study design, drafting the work. All authors have read and approved the manuscript. All authors agreed both to be personally accountable for the author’s own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Availability of data and materials
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Declarations
Ethics approval and consent to participate
The study was approved by the Research Ethics Committee (REC) of Cairo University Hospitals. (reference number, I-251014) A written informed consent was obtained from each participant.

Consent for publication
Not applicable.

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The authors have no conflicts of interest to disclose.

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