Polymorphism of CYP1A1 gene and susceptibility to childhood acute lymphoblastic leukemia in Egypt

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Abstract
The origin of acute lymphoblastic leukemia (ALL) may be explained by a combination of genetic susceptibility and environmental exposure. We aimed to study the frequency of CYP1A1 allelic variants in Egyptian patients with ALL, to evaluate their role in the development of ALL and to correlate these allelic variants with clinical and biological characteristics of the patients. Polymorphism of CYP1A1*2A, *2B and *4 alleles was examined in 186 Egyptian children with ALL and 200 normal individuals using polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP). A higher prevalence of the CYP1A1*4 allele was found in patients with ALL than in the normal population (19.4% vs. 10.0%, odds ratio (OR) = 2.160, 95% confidence interval [CI] = 1.200–3.89, \( p = 0.01 \)), especially in the homozygous variant (OR = 6.6, 95% CI = 2.23–19.58, \( p = 0.001 \)) and in male patients (\( p = 0.005 \)), particularly those aged 2–10 years (OR = 5.214, 95% CI = 1.535–17.706, \( p = 0.008 \)). CYP1A1*2A showed a significant difference between age groups (\( p = 0.046 \)), with a higher incidence in the 10–17-year-old group (21.1%). Multivariate analysis showed that only the CYP1A1*4 allele remained as a probable independent risk factor for ALL development (OR = 2.250, 95% CI = 1.244–4.069; \( p = 0.007 \)). Our results suggest that polymorphic variants in the CYP1A1*4 gene may increase the risk of childhood ALL, particularly in male patients aged 2–10 years.

Keywords: CYP1A1, acute lymphoblastic leukemia, polymorphism, mutation, ALL

Background
DNA damage in the hematopoietic precursor cell is the essential prerequisite for the development of acute leukemia [1]. Such damage may result from the interaction of reactive species generated by environmental or endogenous metabolites. Humans vary in their ability to metabolize such reactive intermediates, which may explain differences in leukemia risk as a result of the interplay of genetic susceptibility and exogenous exposure [2]. Xenobiotics are foreign chemical substances; their carcinogenic effect is influenced by a series of genes codifying enzymes involved in oxidation/activation (phase I) and conjugation/detoxification (phase II) of these compounds [3]. Cytochrome P450 (CYP) enzymes are involved in the detoxification of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents and reactive oxygen species [4]. CYP1A1 is a member of the CYP superfamily. The CYP1A1 gene is a polymorphic gene that encodes an enzyme which catalyzes the bioactivation of polycyclic aromatic hydrocarbons (PAHs) [5]. Oxidation of a PAH produces an epoxide, a very reactive electrophilic group which can interact with DNA resulting in the formation of DNA adducts. Usually these epoxides are rapidly hydrolyzed into hydroxyl groups, which are then coupled to glucuronic acid, glutathione or other groups, producing water-soluble compounds that can then be excreted (phase II) [6]. Genetic polymorphisms have been reported for CYP, and may cause lack of functional enzyme [7] or lead to either increased or reduced metabolic activity [8]. These polymorphisms may alter the ability of enzymes to metabolize the chemical carcinogens and mutagens, which may influence the susceptibility of individuals to cancer [9] and can also modify the individual response to cytotoxic treatment [10]. Polymorphisms in the CYP1A1 gene have been described. The T6235C mutation is located 1194 bp downstream of exon 7, and when present alone corresponds to the CYP1A1*2 allele [11]. The A4889G mutation results in replacement of Ile by Val at residue 462 in exon 7, corresponding to the heme-binding region of CYP1A1. This mutation is in linkage disequilibrium with the T6235C mutation (CYP1A1*2B allele) [12]. The C4887A mutation results in the replacement of Thr by Asn in codon 461, near the site of the A4889G mutation (CYP1A1*4 allele) [13]. CYP1A1 and CYP2E1 enzymes show high levels of expression in the myeloblastic and lymphoid cell lines. Also, CYP1A1 and CYP2E1 enzymes are responsible for bioactivation of PAHs in peripheral blood lymphocytes [2]. Molecular epidemiological studies have shown associations between these enzyme variants and altered risk of a variety of cancers, including colorectal, ovarian, bladder and breast [14–16]. Despite much investigation, little is known about
leukemogenesis. In this study, we tried to find new evidence for the hypothesis that acute lymphoblastic leukemia (ALL) is a combined result of environmental exposure and genetic susceptibility. We studied the frequency of CYP1A1 allelic variants in a group of Egyptian patients with ALL and compared frequencies with those in a control group of similar age and sex distribution with a negative history for neoplastic diseases. Allelic variants were then correlated with clinical and biological characteristics of the patients.

Materials and methods

Subjects

The study included 186 patients with childhood ALL admitted to the Hematology-Oncology unit of Mansoura Children’s Hospital, between August 2010 and October 2012. The recruited patients comprised 89 males and 97 females between the ages of 1 and 16 years (mean age 5.87 ± 4.29). The distribution of ALL subtypes as determined by immunophenotyping was as follows: 158 cases with B ALL (84.9%) and 28 T-cell ALL (15.1%). A general population healthy control group composed of 200 unrelated subjects with matched age, sex and ethnicity, 107 males and 93 females, were randomly selected. Informed consent was obtained from all participating individuals and/or their parents involved in the study.

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Declaration of Helsinki 1975, as revised in 1983.

CYP1A1 polymorphisms

Peripheral and/or bone marrow samples were obtained according to availability. DNA was extracted from leukocytes by a standard procedure [17]. CYP1A1 mutations T6235C (m1), A4889G (m2) and C4887A (m4) were characterized by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach of Törüner et al. [16]. PCR reactions were performed with a Perkin-Elmer Applied Biosystems 9600 thermocycler (Weiterstadt, Germany). For determination of m1 and m3, an 899 bp DNA fragment was amplified using 1 U Taq polymerase (Perkin-Elmer Applied Biosystems), 10 μmol/L of primers M3F 5′-GGCGAGCAATCTGACCTA and P80 5′-TAGAGTCTGTCTCATGCCT, 0.2 mmol/L deoxynucleotide triphosphate (Boehringer Mannheim, Mannheim, Germany) and 2.4 mmol/L MgCl2 in a total volume of 50 μL. PCR conditions were 35 cycles of 0.5 min at 94°C, 1 min at 63°C and 1 min at 72°C. The PCR product (12.5 μL) was digested with MspI (New England Biolabs; 0.5 U, 65°C overnight) (Figure 2). Mutation m4 could be determined from the same 204 bp fragment but using BsaI (New England Biolabs; 2.5 U, 55°C overnight). Both restriction sites were lost in the case of mutation, and the resulting restricted fragments were evaluated on a 2.0% agarose gel (Figure 3). These mutations were then used to define three distinct alleles, CYP1A1*2A (presence of m1 only), *2B (both m1 and m2) and *4 (m4 only).

Statistical analysis

The statistical significance of differences between groups was calculated using the Pearson χ² and Fisher exact test (two-sided). Unconditional logistic analyses were performed separately for each locus. Odds ratios (ORs) were calculated and are given with 95% confidence intervals (CIs). In multivariate analysis, age groups and gender were included as covariates. All analyses were performed using the SPSS program (SPSS, Inc., Chicago, IL) version 16.

Results

Prevalence of CYP1A1 alleles

The frequency of CYP1A1 alleles, as well as the distribution of its genotypes in patients with childhood ALL and in controls, is given in Table 1. The CYP1A1*2A allele frequency was non-significantly increased in patients with ALL when compared to controls (10.8% vs. 10.0%; p = 0.808), 1.6% homozygous and 9.1% heterozygous compared with 1.0% and 9.0% of controls. The overall carrier frequency of CYP1A1*B genotypes was slightly lower in patients with ALL (7.6%; 1.1% homozygous, 6.5% heterozygous) than in controls (8%; 1% homozygous, 7% heterozygous). The frequency of CYP1A1*C4 genotypes was significantly increased in patients with ALL when compared with controls (19.4% vs. 10%; p = 0.010), 11.8% homozygous and 7.5% heterozygous compared with 2% and 8% of controls. CYP1A1*C4 genotypes and homozygous CYP1A1*C4 genotype were associated with 2.1-fold (95% CI = 1.200–3.89, p = 0.010) and 6.6-fold higher prevalence in patients with ALL than in the normal population (95% CI = 2.225–19.575, p = 0.001), respectively. However, there was no association between the prevalence of CYP1A1*2A and CYP1A1*2B in patients with ALL versus the normal population.

We then analyzed the frequency of the CYP1A1 alleles in patients and controls grouped according to sex and age (0–2, >2–10, >10–17). We divided our study samples into several subgroups to investigate whether results were consistent

![Figure 1](image_url)
across the strata, as this would describe how the effect on risk varies across subgroups of subjects with different characteristics. No significant differences were found between cases and control subjects regarding age and sex distribution in *2A and *2B alleles (data not shown). However, we found a higher frequency of the CYP1A1*4 allele in male patients compared with male controls among all age groups (24.7% vs. 9.3%, p = 0.005). Further, subgrouping males according to age modified this risk: the CYP1A1*4 allele was significantly higher in male patients aged >2–10 years (OR = 5.214, 95% CI = 1.535–17.706, p = 0.008) (Table II).

**Patients’ characteristics and CYP1A1 polymorphism**

We examined the CYP1A1 polymorphism distribution according to patients’ characteristics. The carrier frequency of CYP1A1*2A, *2B and *4 did not differ significantly between male and female patients (p = 0.498, 0.200, 0.076, respectively). A statistically significant difference was observed in the distribution of CYP1A1*2A genotypes among all age groups of patients with ALL (p = 0.046), with a higher frequency of this allele in age group >10–17 years (21.1%) than in age groups 0–2 years (11.3%) and >2–10 years (6.3%).

The frequency of the CYP1A1*4 allele was higher in B-cell ALL at 20.9% than in T-cell ALL with a frequency of only 10.7% (p = 0.300). In addition, the CYP1A1*4 allele was higher in patients with a white blood cell (WBC) count >10 × 10^9/L (p = 0.400) and with blast cell percentage ≤ 50% (p = 0.840), but without statistical significance.

Multivariate analysis using unconditional logistic regression was performed to determine which of the covariates (age, sex and CYP1A1*4 allele) continued to show a significant difference between cases and controls in the presence of the others. Only the CYP1A1*4 allele (OR = 2.250, 95% CI = 1.244–4.069, p = 0.007) remained as a probable significant indicator of ALL risk (Table III).

**Discussion**

There are many reports published to date to clarify the pathogenic significance of some polymorphisms in the genes of carcinogen-metabolizing enzymes in the development of various forms of cancer [18–21]. However, no consistent conclusion has yet been established, especially on the role of these polymorphisms in the development of leukemia [22].

Epidemiologic studies have led to the suggestion that in utero and postnatal exposures to various biological, physical and chemical factors may be important determinants of childhood ALL [23,24]. The apparent association of parental exposures to various chemicals [25,26] suggests that variability at loci encoding xenobiotic-metabolizing enzymes could influence susceptibility to ALL. Infants and children may be at greater risk than adults from a variety of environmental toxicants, due to differential exposure and/or physiologic immaturity [24]. Xenobiotics enter the placenta through the maternal circulation [27]. Therefore, alterations in placental metabolism could potentially result in exposure of the developing fetus to harmful events [28].

This study characterized the CYP1A1 genotypes in Egyptian children with ALL as well as control subjects. The frequency of CYP1A1 alleles in the control subjects is in accordance with data from other studies [29–31].

Our result of a significantly higher CYP1A1*4 allele level in patients with ALL than in controls is in accordance with that reported by others for Caucasians [32]. Yet others have reported a higher prevalence of the CYP1A1*4 allele in patients with acute myeloid leukemia (AML) than in controls [33]. No statistical difference was found in the genotype frequencies of CYP1A1*2A and CYP1A1*2B between patients with ALL and controls. CYP1A1*2A allele frequency was found to be significantly increased in children with ALL as compared to normal controls in a French-Canadian population [1]. However, there was no difference in the frequency of this allele between Turkish children with ALL and healthy volunteers [34]. A Thai population had a high prevalence of CYP1A1*2A and *2B variants when compared to Caucasians or African Americans [35]. However, others were unable to identify differences in the genotype frequencies of CYP1A1 between cases and controls [36]. This could reflect
differences in the allele frequencies of CYP1A1 or environmental exposures in each ethnic group. Additionally, the substrates metabolized by CYP1A1 may not be the major carcinogens involved in the pathogenesis of childhood ALL. Our results showed that CYP1A1*2A and *2B are not associated with susceptibility to develop ALL, in accordance with others from Turkey [37], Thailand [36], India [38], Spain [39], France [40] and Korea [41], whereas others reported significant associations between the risk of developing ALL and CYP1A1*2A [42–44] as well as CYP1A1*2B mutation [42–45]. Swinney et al. found an increased risk of developing ALL in a Hispanic group for CYP1A1*2B but not in other ethnic groups [46]. On the other hand, further studies reported that the CYP1A1*2B allele appeared to confer a protective advantage against a parental smoking effect on childhood ALL [47]. The discrepancy between studies may be due to the relatively small numbers of patients in previously published studies. Furthermore, the impact of this polymorphism on the risk of ALL may also vary from population to population because of genetic, environmental, socioeconomic and nutritional factors. We found that patients with ALL carrying the CYP1A1*4 allele may be at greater risk to develop ALL. On the other hand, others proved that CYP1A1*4 is not a significant indicator of ALL risk [48]. However, some studies reported that CYP1A1*4 had a significantly higher risk to develop AML [33,49], breast cancer [44] and endometrial carcinoma [50]. The CYP1A1*4 variant might influence the activation of PAHs and estradiol metabolism, which has been considered a critical event leading to oncogenic mutations [50]. Moreover, when analyzing the frequency of the CYP1A1*4 allele in patients and controls, grouped according to sex and age, we found a probable higher risk to develop ALL in male patients among all age groups, especially in the age group 2–10 years (p = 0.008). These findings could partly explain the higher prevalence of ALL in males [51,52]. Others reported a protective effect of this allele against ALL in female patients [1,48]. However, other studies did not find any statistical difference in genotypic frequencies when stratified by gender [39]. Such imprinting of sex-specific cytochrome P450 forms is controlled by the levels and modes of excretion of androgens, estrogens and growth hormone [53]. Because the human fetus and embryo are exposed to numerous chemicals throughout gestation, it is theoretically possible that the imprinting of cytochrome P450 enzymes also occurs. Several results suggest a possible role of sex steroids in control of the proliferation of leukemic cells [54]. Furthermore, the distinct effects of the polymorphism in sex might also explain the poorer treatment response observed in boys with ALL than girls.

Table I. Distribution of CYP1A1 genotypes in patients with ALL and control group.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genotype</th>
<th>Total no. (n = 186)</th>
<th>Control (n = 200)</th>
<th>p-Value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>*2A (m1)</td>
<td>–/–</td>
<td>166 (89.2%)</td>
<td>180 (90%)</td>
<td>0.947</td>
<td>1.024</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td>–/+</td>
<td>17 (9.1%)</td>
<td>18 (9.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>3 (1.6%)</td>
<td>2 (1.0%)</td>
<td>0.597</td>
<td>1.627</td>
<td>0.268</td>
</tr>
<tr>
<td>*2B (m1 and m2)</td>
<td>–/–</td>
<td>172 (92.5%)</td>
<td>184 (92.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>–/+</td>
<td>12 (6.5%)</td>
<td>14 (7.0%)</td>
<td>0.831</td>
<td>0.917</td>
<td>0.413</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>2 (1.1%)</td>
<td>2 (1.0%)</td>
<td>0.947</td>
<td>1.070</td>
<td>0.149</td>
</tr>
<tr>
<td>*4 (m4)</td>
<td>–/–</td>
<td>150 (80.6%)</td>
<td>180 (90.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>–/+</td>
<td>14 (7.5%)</td>
<td>16 (8.0%)</td>
<td>0.898</td>
<td>1.050</td>
<td>0.496</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>22 (11.8%)</td>
<td>20 (10.0%)</td>
<td>0.001</td>
<td>6.600</td>
<td>2.255</td>
</tr>
<tr>
<td></td>
<td>–/+ +/+</td>
<td>36 (19.4%)</td>
<td>20 (10.0%)</td>
<td>0.010</td>
<td>2.160</td>
<td>1.200</td>
</tr>
</tbody>
</table>

**Note:** ALL, acute lymphoblastic leukemia; –/–, null; –/+, heterozygous; +/+, homozygous; –/+, +/+, combined heterozygous and homozygous; OR, odds ratio; CI, confidence interval.

Table II. Distribution of CYP1A1*4 allele according to sex and age.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Age</th>
<th>Hetero- or homozygous</th>
<th>Male</th>
<th>Female</th>
<th>p-Value</th>
<th>OR (95% CI)</th>
<th>Male</th>
<th>Female</th>
<th>p-Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*4</td>
<td>All ages</td>
<td>ALL</td>
<td>89</td>
<td>22</td>
<td>24.7</td>
<td>97</td>
<td>14</td>
<td>14.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>107</td>
<td>10</td>
<td>9.3</td>
<td>93</td>
<td>10</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Value OR (95% CI)</td>
<td>0.005</td>
<td>0.447</td>
<td>1.414</td>
<td>7.327</td>
<td>1.400</td>
<td>0.588</td>
<td>3.300</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALL</td>
<td>3.219</td>
<td>29</td>
<td>6</td>
<td>20.7</td>
<td>24</td>
<td>2</td>
<td>8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>3</td>
<td>15.0</td>
<td>19</td>
<td>1</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Value OR (95% CI)</td>
<td>0.566</td>
<td>0.952</td>
<td>1.414</td>
<td>7.327</td>
<td>1.400</td>
<td>0.588</td>
<td>3.300</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–2</td>
<td>ALL</td>
<td>1.582</td>
<td>46</td>
<td>12</td>
<td>26.1</td>
<td>49</td>
<td>9</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>61</td>
<td>4</td>
<td>6.6</td>
<td>58</td>
<td>6</td>
<td>10.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Value OR (95% CI)</td>
<td>0.008</td>
<td>0.397</td>
<td>1.535</td>
<td>17.706</td>
<td>1.638</td>
<td>0.523</td>
<td>5.137</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10–17</td>
<td>ALL</td>
<td>5.214</td>
<td>14</td>
<td>4</td>
<td>28.6</td>
<td>24</td>
<td>3</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>26</td>
<td>3</td>
<td>11.5</td>
<td>16</td>
<td>3</td>
<td>18.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-Value OR (95% CI)</td>
<td>0.145</td>
<td>0.594</td>
<td>1.535</td>
<td>17.706</td>
<td>1.638</td>
<td>0.523</td>
<td>5.137</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–2</td>
<td>ALL</td>
<td>3.767</td>
<td>3.632</td>
<td>22.459</td>
<td>0.610</td>
<td>0.099</td>
<td>3.747</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** ALL, acute lymphoblastic leukemia; OR, odds ratio; CI, confidence interval.
in girls with ALL [55]. Surprisingly, this allele was found to be of higher prevalence in female patients than in female controls in AML [33].

Patients’ characteristics showed no significant associations between CYP1A1 polymorphisms and sex, WBC count, immunophenotyping and marrow blast percentage at diagnosis, in agreement with others regarding age, sex and immunophenotype [34]. In contrast, a statistically significant difference was observed in the distribution of CYP1A1*2A genotype among age groups of patients with ALL, with a higher prevalence of this allele in the age group >10–17 years. Some studies did not find any statistical differences in the incidence of genotypes among age groups [39]. The discrepancies between published results regarding the association of leukemia risk and gene polymorphisms could result from differences between the populations of patients studied, as polymorphic variants might be associated with certain factors of the patients’ characteristics.

The limitations of this study, as in other single studies, include the low statistical power, particularly in detecting an association with the homozygous rare variant genotype. A further limitation might be population mixing, i.e. inclusion of individuals from populations with different genetic backgrounds, although we restricted our analyses to the Egyptian population. Nevertheless, the bias resulting from population mixing is likely to be of small magnitude. Because we did not have information about environmental factors that mothers were exposed to during pregnancy, such as smoking habit, or environmental factors that children were exposed to before developing ALL, we could not assess the interactions between those environmental factors and CYP1A1 polymorphisms.

In conclusion, these results suggest that there are no significant associations between CYP1A1 polymorphisms and ALL except for CYP1A1*4 genotype, which may be a probable risk factor for ALL in Egyptian children, especially male children aged 2–10 years, although the causation of ALL is merely inference. Further studies are needed in a larger number of patients with childhood ALL to replicate our results and to evaluate independent associations with CYP1A1 polymorphisms.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

References
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Table III. Relationship between CYP1A1*4 allele, age, sex and risk of childhood ALL*

<table>
<thead>
<tr>
<th></th>
<th>p-Value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.126</td>
<td>0.788</td>
<td>0.581</td>
</tr>
<tr>
<td>Sex</td>
<td>0.195</td>
<td>1.308</td>
<td>0.871</td>
</tr>
<tr>
<td>CYP1A1*4</td>
<td>0.007</td>
<td>2.250</td>
<td>1.244</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; OR, odds ratio; CI, confidence interval.

*Age groups: 0–2, >2–10, >10–17; sex groups: male and female; *4 groups: null, heterozygous or homozygous. Heterozygous and homozygous mutant genotypes are considered together in estimates of p-value and OR.

Baseline group for variables. OR is adjusted OR.

CYP1A1 polymorphism and susceptibility to ALL
enzyme (CYP1A1) and aryl hydrocarbon receptor (AhR) in the placenta.


