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# Genetic polymorphisms of *NQO1*, *CYP1A1* and *TPMT* and susceptibility to acute lymphoblastic leukemia in a Tunisian population

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**Abstract** Acute lymphoblastic leukemia (ALL) is the major pediatric cancer in developed countries. The etiology of ALL remains poorly understood, with few established environmental risk factors. These risks were influenced by co-inheritance of multiple low-risk genetic polymorphisms such as variants within cytochrome P450A1 (*CYP1A1*), NADPH: quinone oxidoreductase (*NQO1*) and Thiopurine methyltransferase (*TPMT*) genes. In this work, we conduct a case-control study to assess the impact of *CYP1A1*\*2A (*CYP1A1* T6235C); *NQO1*\*2 (*NQO1* C609T); *TPMT*\*2 (*TPMT* G238C) and *TPMT* A719G polymorphisms on the risk of developing ALL. The frequencies of *TPMT*\*2, *TPMT* A719G, *NQO1*\*2 and *CYP1A1*\*2 variants were examined in 100 patients with ALL and 106 healthy controls by allele specific PCR and/or PCR-RFLP methods using blood samples. We have found that *NQO1* 609CT genotype was overrepresented in patients and was associated with an aggravating effect compared to the reference group with *NQO1* 609CC genotype ( $p = 0.028$ , OR = 1.41; CI 95 %: 1.04–1.93). However, *TPMT*\*2, *TPMT* 719G and *CYP1A1*\*2 variants did not appear to influence ALL susceptibility ( $p > 0.05$ ). Moreover we have not found a significant correlation between the studied variants and Bcr-Abl transcript. In conclusion we retain that leukemogenesis of ALL is associated with carcinogens metabolism and consequently related to environmental exposures.

**Keywords** Leukemia · Tunisia · *TPMT* · *NQO1* · *CYP1A1*

## Introduction

Acute lymphoblastic leukemia (ALL) is the major pediatric cancer in developed countries [1]. This pathology is characterized by many chromosomal abnormalities that produce aberrant gene fusions or inappropriate expression of oncogenes. Among them rearrangements of the mixed-lineage leukemia (*MLL*) gene localized in 11q23 are common chromosomal abnormalities associated with acute leukemia, especially infant leukemia and secondary leukemia following treatment with DNA topoisomerase II inhibitors. Translocation (9;22) associated with the Bcr-Abl transcript is the most frequent genetic aberration in adult ALL and is found in 20–30 % of patients overall [2]. The etiology of ALL remains poorly understood, with few established environmental risk factors [3–5]. It is, however, likely that the risk of ALL from environmental exposure is influenced by co-inheritance of multiple low-risk variants. For example previous case-control studies have shown that variants within cytochrome p4501A1 (*CYP1A1*), *NQO1* or *TPMT* were associated with increased risk of ALL [6–10].

*CYP1A1* enzyme belongs to the cytochrome P450 family and is a phase I detoxification enzyme involved in the bioactivation of several chemical carcinogens, including cytotoxic drugs. *CYP1A1* transfers electrons onto toxicants to create highly reactive intermediates which are usually coupled to glutathione or other groups, producing water-soluble compounds, but can also interact with DNA, resulting in the formation of DNA adducts [11]. The expression of genes from the CYP1 family increases in lymphoblastic and myeloblastic cell lines and plays a role

Nouha Cherif and Ikbel Bahri contributed equally to this study.

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in the detoxification of environmental factors. Therefore, CYP1A1 enzyme might be responsible for carcinogenesis in haematopoietic cells. This protein is encoded by a polymorphic gene localized in chromosome 15q24.1. There have been many single nucleotide polymorphisms (SNPs) identified in the *CYP1A1* gene. In particular, two common polymorphisms, the T6235C change within the 3' non-coding region of the gene (*CYP1A1*\*2A, rs464690) and the A4889G change in the heme-binding domain of exon 7 (*CYP1A1*\*2C), have been previously described. The *CYP1A1*\*2A variant was associated with an increasing enzymatic activity and/or inducibility and was one of the most studied polymorphisms in association with leukemia [6, 7].

Other metabolic gene variants that have been investigated as risk factors for ALL include polymorphisms in NADPH:quinone oxidoreductase (*NQO1*). The encoded enzyme is a 274-aminoacid flavoprotein that catalyzes the two-electron reduction of quinoid compounds into their reduced form, such as hydroquinones. NQO1 protein protects cells against quinine-induced oxidative stress. Mice lacking the NQO1 protein demonstrated increased sensitivity to benzo(a)pyrene- and 7,12-dimethylbenz(a)-anthracene-induced carcinogenesis of the skin [12, 13]. Moreover, this enzyme activates nitrosoaromatic compounds and heterocyclic amines in tobacco smoke, which may work to induce carcinogenesis [14, 15]. The NQO1 protein is encoded by a polymorphic gene localized in chromosome 16q22.1. There have been more than 93 single nucleotide polymorphisms (SNPs) identified in the *NQO1* gene. The most widely studied SNP of *NQO1* is a C to T change at nucleotide position 609 (rs1800566), also known as *NQO1*\*2 [16]. This results in a proline to serine amino acid change at codon 187 that is associated with a loss of enzyme activity due to instability of the protein product. The *NQO1*\*2 is associated with an increased risk of tobacco-related cancers and ALL [8, 9, 17, 18]. Moreover other polymorphisms were detected in *NQO1* gene and were associated with the modification of the enzyme activity like the *NQO1*\*3. However the frequency of *NQO1*\*3 in different ethnic populations is generally low [19].

The TPMT enzyme catalyses the S-methylation of azathioprine, 6-thioguanine and 6-mercaptopurine (6-MP). These three drugs are used for the treatment of patients with several disorders such as ALL, rheumatoid arthritis, systemic lupus erythematosus or other autoimmune/inflammatory diseases as well as of those undergoing organ transplants [20–22]. This enzyme is encoded by the *TPMT* gene which localized in chromosome 6p22.3. Twenty-four *TPMT* genetic polymorphisms have been identified, and they are associated with decreased levels of TPMT activity and/or thiopurine drug-induced toxicity [23, 24]. Four

mutant alleles, *TPMT*\*2, *TPMT*\*3A, *TPMT*\*3B and *TPMT*\*3C, account for the majority of variant alleles in all human populations studied to date [25]. The distribution of these alleles differs significantly among ethnic populations [26–28].

Considering the role in detoxification played by these enzymes, the existence of common polymorphisms (*NQO1*\*2, *CYP1A1*\*2A, *TPMT*\*2 and *TPMT* A719G) and previous report of their association with other cancers, we aimed at determining whether any association between these polymorphisms and susceptibility to ALL. We have also investigated the correlation between these polymorphisms and the Bcr-Abl transcript.

## Materials and methods

### Subjects

Our analysis included 106 controls and 100 patients with ALL. All patients were recruited at the first diagnosis from the laboratory of molecular and cellular haematology from Pasteur Institute of Tunis, Tunisia. The diagnosis of leukemia subgroups was based on the standard clinico-haematological criteria and molecular analysis. Patients were recruited from different geographic origins, among them 68.00 % were men and the mean age was  $23.35 \pm 7.79$  years. The control group consisted of healthy unrelated volunteers without a medical history of cancer. The control group was approximately matched for gender proportion, geographic origin and age range to those in the case group.

### DNA and RNA extraction

Under informed consent, peripheral blood samples were collected into tubes with EDTA. The red cells were lysed and residual cells were homogenised in 1 ml trizol. For patients, total RNA was extracted from peripheral blood cells by using the acid guanidinium thiocyanate and phenol chloroform method. After having taken the aqueous phase with RNA, we carefully remove the interphase which contains DNA. The DNA extraction was done in accordance with the manufacturer's instructions. For controls subjects, genomic DNA was extracted by the phenol/chloroform protocol. The quality of genomic DNA was controlled by electrophoresis on a 1 % agarose gel stained with ethidium bromide.

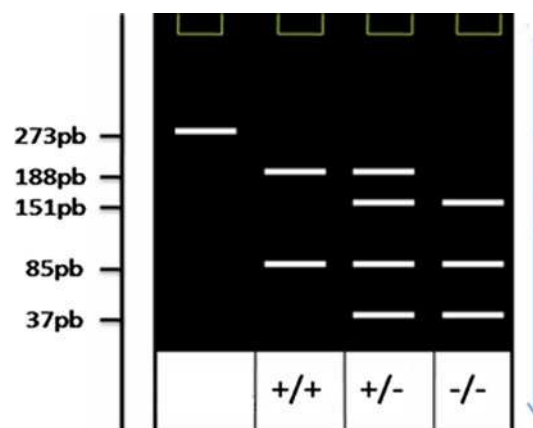
### Bcr-abl and abl transcripts analysis for ALL patients

*bcr-abl* and *abl* transcripts was identified as previously reported by Menif et al. [29]. The two transcripts were quantified using a commercially available fusion Quant kit

(IPSOGEN) developed according to the EAC network protocol. cDNA was amplified by 50 cycles of Q-PCR using the ABI 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA) and taqMAN® Universal PCR Master Mix in accordance with the manufacturer's instructions in a final reaction volume of 25 µl. The *bcr-abl* and *abl* probes were dual labeled with FAM and TAMRA; the probe and primer concentrations for *abl* mRNA quantification were 200 and 300 nM, respectively.

Genotyping of *NQO1* C609T, *CYP1A1* T6235C, *TPMT* G238C and *TPMT* A719G

The amplified fragment size and the primers used for all amplifications were shown in Table 1. For *NQO1* and *CYP1A1* genes, we screened the *NQO1* C609T and *CYP1A1* T6235C mutations by PCR/RFLP approach. The PCR reactions were performed in a final volume of 25 µl containing 100 ng of genomic DNA, 2.5 µl of 10× PCR buffer, 1.25 µl of MgCl<sub>2</sub> at 50 mM, 5 pmol of each primer and 1 U of Taq DNA polymerase (Invitrogen). PCR was carried out as follows: an initial denaturing step (94 °C for 10 min), was followed by 30 cycles consisting of denaturing (94 °C for 60 s), primer annealing (at 58 °C for *NQO1* and at 62 °C for *CYP1A1* for 30 s) and extension (72 °C for 60 s). The mixture was then heated at 72 °C for 10 min. To detect the *NQO1* C609T transition, the PCR products were digested at 37 °C overnight with 10 U of *HinfI*. The size of obtained fragments after *HinfI* digestion and the expected genotypes for *NQO1* gene were summarized in Fig. 1. To detect the *CYP1A1* T6235C transition, the PCR product was digested at 37 °C overnight with 5 U of *MSPI*. After *MSPI* digestion, the wild allele (*CYP1A1* 6235\*T) was characterized by the presence of only one band of 343 pb, however the mutated allele (*CYP1A1* 6235\*C) was marked by the presence of two band of 209 and 134 pb. After PCR/RFLP, the presence of mutated alleles (*NQO1* 609\*T and *CYP1A1* 6235\*C) was confirmed by direct sequencing.



**Fig. 1** Sizes of obtained fragments after *HinfI* digestion and the expected genotypes for *NQO1* gene. ++ homozygous wild genotype, ± heterozygous genotype, -- homozygous mutated genotype

For *TPMT* gene the *TPMT*\*2 (G238C) variant was detected by allele specific PCR and the *TPMT* A719G mutation was investigated by direct sequencing of purified PCR products with the Big Dye Terminator kit V 1.1 (Applied Biosystems).

#### Statistical analysis

The Hardy–Weinberg equilibrium test was calculated by the software package Arlequin (version 3.01). Statistical analysis was performed by SPSS 16.0 software.

#### Results

All samples were found to be in Hardy–Weinberg equilibrium for *CYP1A1*, *NQO1* and *TPMT* genotypes (Table 2). The alleles frequencies for *CYP1A1* 6235\*C, *NQO1* 609\*T, *TPMT* 238\*C and *TPMT* 719\*G (mutated alleles) in control population were respectively estimated at 0.05, 0.22, 0.11 and 0.004. Genotypes and alleles distributions for *CYP1A1*,

**Table 1** Primer sequences, amplified fragment sizes and annealing temperatures

Studied polymorphisms	Sequences	Fragment sizes (pb)	Annealing T (°C)
<i>NQO1</i> *2 (C609T)	F: 5'-AGTGGCATTCTGCATTTCTGTG-3' R: 5'-GATGGACTTGCCCAAGTGATG-3'	273	58
<i>CYP1A1</i> *2A (T6235C)	F: 5'-CAGTGAAGAGGTGTAGCCGCT-3' R: 5'-TAGGAGTCTTGTCTCATGCCT-3'	343	62
<i>TPMT</i> *2 (G238C)	PC: 5'-TAAATAGGAACCATCGGACAC-3' PW: 5'-GTATGATTTTATGCAGGTTTG-3' PM: 5'-GTATGATTTTATGCAGGTTTC-3'	256	57
<i>TPMT</i> A719G	F: 5'-GAGACAGAGTTTCACCATCTTGG-3' R: 5'-CAGGCTTTAGCATAATTTCAATTCCTC-3'	373	62

F forward primer, R reverse primer, PC common primer, PW wild primer, PM mutated primer

**Table 2** Hardy–Weinberg equilibrium test for *NQO1*\*2, *CYP1A1*\*2A, *TPMT*\*2 and *TPMT* A719G polymorphisms

Variant	Group					
	Controls			Cases		
	Obs. Het	Exp. Het	<i>p</i> value	Obs. Het	Exp. Het	<i>p</i> value
<i>NQO1</i> *2	0.301	0.351	0.16	0.450	0.433	0.81
<i>CYP1A1</i> *2A	0.103	0.098	1.00	0.180	0.196	0.32
<i>TPMT</i> *2	0.198	0.209	0.632	0.240	0.242	1.00
<i>TPMT</i> *A719G	0.009	0.009	1.000	0.01	0.01	1.00

Obs. Het observed heterozygous genotype, Exp. Het expected heterozygous genotype

*NQO1* and *TPMT* genes in cases and controls were summarized in Table 3.

For the *NQO1* C609T genotype, significant differences in genotype frequencies between cases and controls were detected with considering both co-dominant and dominant models (Table 3). For this locus, *NQO1* 609CT genotype was found to be overrepresented in patients and associated with an aggravating effect compared to reference group harbouring *NQO1* 609CC genotype ( $p = 0.028$ , OR = 1.41; CI 95 %: 1.04–1.93). We have also shown that the *NQO1* 609\*T allele increases significantly the risk of ALL in the Tunisian population ( $p = 0.049$ ). For the *CYP1A1* T6235C polymorphism, we have shown that the *CYP1A1* 6235\*C allele was overrepresented in ALL patients (Table 3). However we have not found a significant statistical difference (corrected  $p$  value is 0.06).

With respect to *TPMT* variation we have found that the frequencies of *TPMT*\*2 and *TPMT* 719\*G alleles did not differ significantly between cases and controls. These results were obtained for the dominant, co-dominant and recessive models (Table 3). These results suggest that *TPMT*\*2 and *TPMT* 719\*G variants were not implicated in genetic susceptibility to ALL for the Tunisian population.

Finally we have used the multinomial logistic regression to investigate relation between genetic polymorphisms and bcr-abl status. Information about Bcr-Abl transcript status was available for only 77 patients. Of them Bcr-Abl was detected on only 17 patients. These patients were distributed according to transcript type as 11.7 % (9/77) with b3a2, 6.5 % (5/77) with e1e2 and 3.9 % (3/77) with b2a2 variant. The comparison of *CYP1A1*, *NQO1* and *TPMT* genotypes distribution between patients stratified according to the Bcr-Abl transcript status does not show any significant statistical difference (Table 4).

## Discussion

Acute lymphoblastic leukemia is the major pediatric cancer in developed countries. The risk of developing ALL may

be influenced by polymorphisms of xenobiotic metabolizing enzymes. Thus, in this population-based, case–control study, we investigated the association between xenobiotic-metabolizing gene polymorphisms and susceptibility to ALL in a Tunisian population.

The frequency of *NQO1*\*2 variant in our control population was estimated at 0.22. This value is in the range of reported frequencies for African populations. Indeed Kelsey et al. [30] estimated the frequency of *NQO1*\*2 in a African-American group at 0.22. Moreover a recent study focusing the analysis of *NQO1* polymorphisms and susceptibility to atopy and airway hyperresponsiveness has reported that the frequency of *NQO1*\*2 variant in African children was estimated at 0.15 [31]. However these frequencies are slightly higher than those usually found in Caucasian populations [32] and lower to which founded for the Chinese population [33]. When we compare the frequencies of *NQO1*\*2 variant between patients and controls we have found that *NQO1* 609CT genotype was associated with a 1.41-fold increased risk of developing ALL. This result confirms the studies of Smith et al. [9] and Krajcinovic et al. [18]. Moreover a recent a meta-analysis of Vijayakrishnan and Houlston [34] has revealed a significant association between *NQO1*\*2 variant and ALL (OR = 1.24, 95 % CI: 1.02–1.50). We explain the positive association between *NQO1*\*2 variant and ALL by reducing detoxifying power for toxic quinone and free radicals and/or by decreasing stability of p53. Indeed, it is well known that the wild *NQO1* enzyme protects the cell against the oxidative stress and toxic quinone [35, 36] and stabilizes the p53 protein [37, 38]. In opposition to our results other authors have found a positive association between the *NQO1* CC (homozygous wild genotype) and the increased risk of ALL [39]. Authors explain this association by the role of wild *NQO1* enzyme in activating carcinogens aside from detoxification. This hypothesis is supported by the review of Ross [36] who has postulated that some endogenous metabolites can be transformed by *NQO1* to yield more active products that can produce reactive oxygen or alkylating species, thus attacking nucleophilic sites within essential biomolecules such as DNA. These apparent discrepancies might reflect differences in the chemical carcinogens involved in leukaemogenesis in different countries. In the other hand the correlation between *NQO1*\*2 variant and Bcr-Abl transcript does not show any significant statistical difference which confirms the study of Kracht et al. [40]. In this context others studies have examined the *NQO1*\*2 distribution according to MLL rearrangement status and have produced conflicting result. While Lanciotti et al. [8] indicate that only the infantile ALL patients without MLL rearrangements had a significantly higher frequency of *NQO1* genotypes associated with low/null activity enzyme. Wiemels et al. [41] have

**Table 3** Genotypes distribution for *NQO1*, *CYP1A1* and *TPMT* in cases and controls from Tunisia

Polymorphism	Genotypes	Controls ( <i>n</i> = 106)	Cases ( <i>n</i> = 100)	<i>p</i> value (Yates correction)	OR (95 % CI)
<i>NQO1</i> *2 ( <i>C609T</i> )	Co-dominant model				
	CC	66	46	–	1*
	CT	32	45	0.028	1.41 (1.04–1.93)
	TT	8	9	0.510	–
	Dominant model				
	CC	66	46	–	1*
	CT + TT	40	54	0.028	1.38 (1.05–1.83)
	Recessive model				
	TT	8	9	–	1*
	CT + CC	98	91	0.900	–
	Alleles				
<i>CYP1A1</i> *2A ( <i>T6235C</i> )	C (wild)	164	137	–	1*
	T (mutated)	48	63	0.049	1.26 (1.00–1.60)
	Co-dominant model				
	TT	95	80	–	1*
	TC	11	18	0.152	–
	CC	0	2	0.413	–
	Dominant model				
	TT	95	80	–	1*
	TC + CC	11	20	0.08	–
	Recessive model				
	CC	0	2	–	1*
<i>TPMT</i> *2 ( <i>G238C</i> )	TC + TT	106	98	0.452	–
	Alleles				
	T (wild)	191	178	–	1*
	C (mutated)	11	22	0.065	–
	Co-dominant model				
	GG	83	74	–	1*
	GC	21	24	0.572	–
	CC	2	2	1.00	–
	Dominant model				
	GG	83	74	–	1*
	GC + CC	23	26	0.575	–
<i>TPMT</i> A719G	Recessive model				
	CC	2	2	–	1*
	GC + GG	104	98	1.00	–
	Alleles				
	G (wild)	187	172	–	1*
	C (mutated)	25	28	0.602	–
	Co-dominant model				
	AA	105	99	–	1*
	AG	1	1	1.00	–
	GG	0	0	–	–
	Dominant model				
	AA	105	99	–	1*
<i>TPMT</i> A719G	AG + GG	1	1	1.00	–
	Recessive model				
	GG	0	0	–	1*
	AG + AA	106	100	–	–
	Alleles				
	A (wild)	211	199	–	1*
	G (mutated)	1	1	1.00	–

1\* reference group, OR odds ratio, CI confidence interval



**Table 4** Logistic regression effect of *NQO1*, *CYP1A1* and *TPMT2* polymorphisms on Bcr-Abl status

Transcrit <sup>a</sup>	df	Sig.	Exp (B)	95 % confidence interval for Exp (B)	
				Lower bound	Upper bound
[ <i>NQO1</i> *2=C/C]	1	0.615	0.654	0.125	3.423
[ <i>NQO1</i> *2=C/T]	1	0.367	0.445	0.077	2.583
[ <i>NQO1</i> *2=T/T]	0	–	–	–	–
[ <i>CYP1A1</i> *2A=C/C]	1	0.993	3.109	0.000	–
[ <i>CYP1A1</i> *2A=T/C]	1	0.421	1.700	0.467	6.185
[ <i>CYP1A1</i> *2A=T/T]	0	–	–	–	–
[ <i>TPMT</i> *2=C/C]	1	0.995	3.316	0.000	– <sup>b</sup>
[ <i>TPMT</i> *2=G/C]	1	0.409	–	0.135	2.257
[ <i>TPMT</i> *2=G/G]	0	–	–	–	–
[ <i>TPMT</i> A719G=A/A]	1	–	3,597	3.597	35.970
[ <i>TPMT</i> A719G=A/G]	0	–	–	–	–

This reduced model was obtained after adjustment to sex and age

Logistic regression: number of observation= 77,  $\chi^2 = 88.409$ ,  $p = 0.000$

Df degree of freedom, Sig.  $p$  value, Exp (B) expected odds ratio

<sup>a</sup> The reference category is patients without Bcr-Abl

<sup>b</sup> This parameter is set to zero because it is redundant

**Table 5** Summary of some studies reporting the association between *NQO1* and ALL

First author [reference]	Population/country	Cases (n)	Controls (n)	Variant		Tumours types	Odds ratio (95 % CI)
				% <i>NQO1</i> *2 <sup>a</sup>	% <i>NQO1</i> *2 <sup>b</sup>		
Present study	Tunisian	106	100	31.50	22.64	ALL	1.26 (1.0–1.60)
Rimando et al. [39]	Filipino	60	60	0.20	0.39	ALL	4.82 (2.1–10.6) <sup>d</sup>
Lanciotti et al. [8]	Italian	18	147	72 <sup>c</sup>	38 <sup>c</sup>	ALL <sup>e</sup>	4.22 (1.43–12.49)
Kracht et al. [40]	Austria, Germany, Czechoslovakia (Caucasian)	138	190	0.36	0.35	ALL <sup>f</sup>	NS
Krajinovic et al. [18]	French-Canadian	174	323	22.4	17.8	ALL	1.3 (1.0–1.8)
Smith et al. [9]	United Kingdom	67	108	43.48 <sup>c</sup>	32.40 <sup>c</sup>	ALL	1.93 (0.96–3.87)
Wiemels et al. [41]	United Kingdom	36	100	0.32	0.17	ALL/AML <sup>g</sup>	2.54 (1.08–5.96)

ALL acute lymphoblastic leukemia

<sup>a</sup> Variant frequencies in cases

<sup>b</sup> Variant frequencies in controls

<sup>c</sup> Percentage of low/null activity CT + TT genotype

<sup>d</sup> Positive association with *NQO1* homozygous wild genotype, NS no statistical significant association

<sup>e</sup> MLL negative

<sup>f</sup> ALL carrying distinct fusion genes

<sup>g</sup> MLL positive

found that low *NQO1* activity genotypes (heterozygous CT or homozygous TT) were associated with ALL containing the *MLL* gene rearrangements. Table 5 summarizes our data and some studies on *NQO1*\*2 and the risk of ALL.

The frequency of *CYP1A1*\*2A variant in our control population was estimated at 0.05. This frequency is lower to which reported by other studies [6, 7]. The comparison

of patients and controls according to *CYP1A1*\*2 variant does not show a statistical significant difference (corrected  $p$  value = 0.06). Our observation is compatible with the findings of Liu et al. [42]. In contradiction Krajinovic et al. [43] and Clavel et al. [44] suggest that individuals harbouring the *CYP1A1*\*2 variant may be at an increased risk for ALL. Indeed the *CYP1A1*\*2 variant is associated with

elevated enzymatic activity supporting the hypothesis of linking the risk of ALL with the inducibility of the xenobiotic metabolizing enzyme CYP1A1. Consequently, carriers of variant alleles are expected to present greater risk when exposed to carcinogens such as polycyclic aromatic hydrocarbons. With considering the *TPMT* variations we have found that the frequencies of *TPMT*\*2 variant and “low” *TPMT* genotypes in the healthy Tunisian population were higher to which reported for the Caucasian populations [45–47]. Moreover high frequency of *TPMT* “low” alleles was observed for ALL patients. For these patients low *TPMT* activity can cause toxic concentration of thio-guanine nucleotides in hematopoietic cells after treatment with thiopurine drugs. The greatest risk of hematopoietic toxicity is associated with homozygous *TPMT* variants. However, patients known to be *TPMT*-deficient can be successfully treated with reduced dosages of thiopurines [48]. Although the high frequency of *TPMT* “low” alleles in Tunisian population in both cases and controls we have found that these variants were not implicated in genetic susceptibility to ALL. This result was in contradiction with a previously reported study of Samochatova et al. [10]. In this study we explain the absence of positive association between *CYP1A1*\*2 variant, *TPMT* low allele and ALL by differences in the chemical carcinogens involved in leukemogenesis in different countries.

Finally, although some of the results presented here are novel and presented for the first time for the Tunisian population, this study has some limitations. The primary limitation of our study is the small sample size and, as a consequence, low statistical power to detect associations. The second limitation of this study is the absence of epidemiologic information such as tobacco status and other environmental risk factors. Besides that, in the future, enlargement of sample sizes in the Tunisian population (which is already ongoing) will be essential to assess the role that non genetic baseline factors together with the genetic factors play as predictors of differential susceptibility to the malignancy.

## Conclusion

In this study we find statistical evidence that *NQO1*\*2 variant was associated with an increased risk of ALL in a Tunisian population. This finding suggests that leukemogenesis of ALL is associated with carcinogen metabolism and consequently related to environmental exposures.

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**Conflict of interest** All authors would like to declare that they have no conflict of interest.

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