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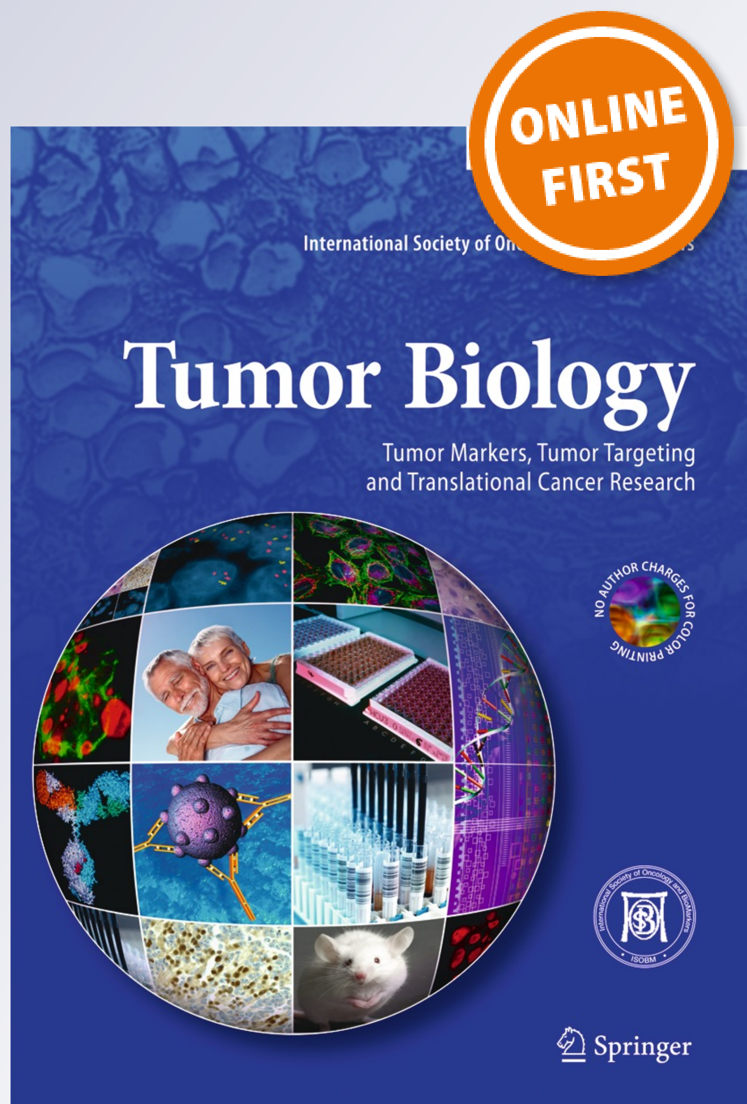

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RESEARCH ARTICLE

N-Acetyltransferase 2 (*NAT2*) polymorphism as a risk modifier of susceptibility to pediatric acute lymphoblastic leukemia

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Abstract *N*-Acetyltransferases (NAT) have been known to modify the risk to a variety of solid tumors. However, the role of *NAT2* polymorphism in risk susceptibility to childhood acute lymphoblastic leukemia (ALL) is still not well known. We performed a case-control study to determine if the common *NAT2* polymorphisms play a role in altering susceptibility to pediatric ALL. DNA of 92 pediatric ALL patients and 312 healthy controls was analyzed for the *NAT2* polymorphisms using the PCR-RFLP method. The wild-type *NAT2*4* was encountered in 8.6 % of patients versus 11.8 % of controls (P=0.23). The rapid acetylators *NAT2*12* 803A>G, AG, GG, and AG/GG were overrepresented in controls (P=0.0001; odds ratio (OR) 0.22, 0.19, and 0.21 respectively). *NAT2*5D* 341T>C and *NAT2*11A* 481C>T were of comparable frequencies. For their combination, *NAT2*5A*, a slow acetylator, both TCTT and CCCT were overrepresented in patients (P<0.001; OR 15.8 and 17.9 respectively). *NAT2*5B* (803A>G, 341T>C, 481C>T) was overrepresented in controls (P<0.001; OR 0.12). Apparently, 803A>G ameliorated the combined effect of 341T>C and 481C>T. A similar effect was obtained with *NAT2*5C* (341T>A, 803A>G) (P<0.0001; OR 0.11). For slow acetylator *NAT2*7A* 857G>A, GA and GA/AA were overrepresented in patients (P=0.009 and 0.01; OR 2.74 and 2.72 respectively). *NAT2*13* 282C>T, *NAT2*6B* 590G>A, and *NAT2*14A* 191G>A were of comparable frequencies. *NAT2* 282C>A in combination with *NAT2* 857G>A (*NAT2*7B*) showed a synergistic effect in patients versus controls (P<0.0001; OR 3.51). In conclusion, *NAT2* gene polymorphism(s) with slow acetylator phenotype is generally associated with the risk of development of ALL in children.

Keywords ALL · *NAT2* · Risk susceptibility · Single nucleotide polymorphism

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children, accounting for 25–30 % of all cases of childhood malignancies. Although the clinical, pathological, and immunophenotypic features of the disease are well documented [1], the causes of pediatric acute leukemias are still not well known. The increased incidence of pediatric ALL in Western countries over the past decades was attributed, among other causes, to the introduction of new chemical exposures into the child's environment including parental smoking, pesticides, traffic fumes, paint, and household chemicals [2]. Previous studies have demonstrated that the interaction between genetic background, life style, and these environmental factors plays a critical role in development of ALL in children [3]. The cytochrome P450 family proteins are known as phase I enzymes; in general, they metabolically activate chemical carcinogens [4]. A genotype associated with increased activity might therefore increase the risk of cancer [5]. The *N*-acetyltransferase (NAT) and glutathione *S*-transferase (GST) family proteins are known as phase II enzymes;

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they metabolically inactivate carcinogenic compounds resulting from the action of phase I enzymes.

N-Acetyltransferases metabolize a number of aromatic and heterocyclic amine carcinogens. Both NAT1 and NAT2 catalyze the metabolic activation (via *O*-acetylation) of aromatic and heterocyclic amine carcinogens. Since a single nucleotide change causing substitution of amino acid residues may have the potential to directly affect the biological activity of the gene product [3, 6–8], polymorphism in the *NAT1* and *NAT2* genes may modify the cancer risk.

In the past, seven missense (191G>A, 341T>C, 434A>C, 590G>A, 803A>G, 845A>C, and 857G>A) and four silent (111T>C, 282C>T, 481C>T, and 759C>T) substitutions have been identified in the *NAT2* coding exon. *NAT2*4* is considered the wild-type allele because of the absence of any of these substitutions. However, *NAT2*4* is not the most common allele in many ethnic groups, including Caucasians, Asians, and Africans [9–12]. *NAT2* alleles containing the 191G>A, 341T>C, 434A>C, 590G>A, and/or 857G>A missense substitutions are associated with slow acetylator phenotypes [10, 13].

Three NAT2 phenotypes have been described, fast, intermediate, and slow [7]. Slow acetylators are less efficient in metabolizing aromatic amines and hence may increase the risk of cancer.

NAT2 rapid and slow acetylator alleles have been known to modify the risk to a variety of solid tumors in various ethnic groups [14–28].

However, studies on ALL are few with conflicting results in different ethnic populations [29–37].

In this work, we evaluated, in a case-control study, the impact of *NAT2* polymorphisms on risk susceptibility to pediatric ALL.

Materials and methods

The study included 92 consecutive newly diagnosed pediatric ALL patients who presented to the Pediatric Oncology Department, NCI, Cairo University, in the period between January 2012 and September 2013 and 312 healthy controls. Cases were diagnosed according to standard criteria including clinical, radiological, and laboratory workup. Diagnosis was confirmed by immunophenotyping as previously described [38, 39].

Criteria for inclusion in the study group

(1) Egyptians residing in Egypt area as judged by their names, language, and place of birth
(2) Availability of biological material

The recruited patients comprised 58 males and 34 females with an age range of 1.5 to <18 years with a median of 6 years. Healthy controls (312) were randomly selected from blood donors with an age range of 18–38 years including 250 males and 62 females.

Criteria for inclusion in the control group

(1) Anonymous, healthy, and unrelated individuals
(2) Egyptians residing in Egypt area as judged by their names, language, and place of birth

The study was performed according to the Helsinki declaration. Informed consent was obtained from all participants involved in the study and/or their guardians, and the study was approved by the IRB of the National Cancer Institute, Cairo University.

DNA isolation

DNA was isolated from peripheral blood at diagnosis, using salting out technique [40].

NAT2 genotyping

Analysis of the seven most common *NAT2* alleles was based on the assay previously described [7, 41, 42].

Primer sequences are shown in Table 1

PCR was performed in 20 μL reaction mix containing 20 ng of genomic DNA, 0.5 μmol of each primer, 200 μmol of each dNTP, 10 mmol Tris-HCl (pH 8.3), 50 mmol KCl, 1.5 mmol $MgCl_2$, and 0.5 U of ampliTaq DNA polymerase (Hoffman-LaRoche, Branchburg, NJ). After initial denaturation for 6 min at 95 °C, PCR was performed for 35 cycles of 30 s at 95 °C, 1 min at 58 °C, and 1 min at 72 °C. The last elongation step was extended to 7 min. Subsequently, 5 μL of the PCR product was digested with the proper restriction enzyme at 37 °C overnight.

The restriction enzymes and the resulting restriction fragment patterns for the various polymorphisms are shown in Table 2. The resulting restriction fragments were separated on 1.5–4 % agarose gel electrophoresis. *NAT2 alleles*: **4*, **12A*, **5A*, **5B*, **5C*, **5D*, **6A*, **6B*, **7A*, **7B*, **11A*, **13A*, and **14A* were identified according to the Consensus Human Arylamine N-Acetyltransferase Gene Nomenclature (http://nat.mbg.duth.gr) and Human *NAT2* Alleles (Haplotypes) (http://nat.mbg.duth.gr/Human NAT2 alleles_2013.htm).

Statistical analysis

The level of significance was calculated by Fisher's exact test. Odds ratio (OR) was used to measure the strength of

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Table 1 Primer sequences for detection of various *NAT2* genotypes

Genotype	Primer	Sequence
C481T	*NAT2* P100	5′-GTC ACA CGA GGA AAT CAA ATGC-3′
G857A	*NAT2* P56	5′-GTT TTC TAG CAT GAA TCA CTC TGC-3′
G191A	*NAT2* P100	5′-GTC ACA CGA GGA AAT CAA ATGC-3′
C282T T341C	Mismatch primer	5′-ACC CAG CAT CGA CAA TGT AAT TCC TGC CCT CA-3′
G590A	*NAT2* P87	5′-CCT GGA CCA AAT CAG GAG AG-3′
A803G	*NAT2* P90	5′-ACA CAA GGG TTT ATT TTG TTC C-3′

association between the tested genotypes and ALL risk. Crude OR is given with 95 % confidence interval (CI). All statistical tests were based on two-tailed probability and were performed using SPSS version 12. We corrected results for multiple tests with the Bonferroni method.

Results

The frequencies of *NAT2* polymorphisms are shown in Tables 3 and 4.

The distribution in the control is in accordance with the Hardy-Weinberg principle.

The *NAT2*4* allele (wild) did not show any significant effect on the risk of development of ALL.

The frequency of *NAT2*12A* (803A>**G** genotypes A**G**, **GG**, and A**G**+**GG**) was overrepresented in the control compared to the patient group imposing 2.57-, 2-, and 4.74-fold protection respectively; this was significant for AG and AG+GG.

The frequencies of *NAT2*5D* T341T>**C**, *NAT2*13* 282C>**T**, *NAT2*6B* 590G>**A**, and *NAT2*14A* 191G>**A** polymorphisms among patients and controls were comparable; the differences were statistically insignificant.

Table 4 shows the frequency of *NAT2* allele combinations among patients and controls.

For the slow acetylator *NAT2*5A* (341T>**C**+481C>**T**), only the genotype **CCCT** was significantly more represented in patients compared to controls associated with 2.74-fold increased risk of developing ALL.

For the *NAT2*5B* (341T>**C**, 481C>**T**, 803A>**G**) genotype, the overall frequencies of the mutant alleles were significantly more represented in the controls compared to patients with 2-fold protection. The significance was mainly attributed to T**CC**T**A**G and CC**TTGG** genotypes with 5.26- and 9.8-fold protection respectively.

For the *NAT2*5C* (341T>**C**, 803A>**G**) genotype, the overall frequencies of the mutant alleles were significantly more represented in the controls compared to patients with 2.42-fold protection. The significance was mainly attributed to T**C**A**G** and **CCGG** genotypes with 5.26- and 3.33-fold protection respectively.

For the *NAT2*6A* (282C>**T**, 590G>**A**) genotype, the overall frequencies among patients and controls were comparable. However, the *NAT2*6A* genotype C**TAA** was associated with 10-fold increased risk; it was encountered in 3 (3.3 %) of ALL patients compared to 1 (0.3 %) of normal controls (P value=0.04; OR=10.247; 95 % CI=1.154–99.73), yet with Bonferroni correction, it was not statistically significant.

For the *NAT2*7B* (282C>**T**, 857G>**A**) genotypes, the overall frequencies of the mutant alleles were significantly overrepresented among patients compared to the control. The significance was mainly attributed to the C**T**G**A** genotype with 2.49-fold increased risk. The other genotypes were uncommon.

Table 2 Restriction fragment pattern of various *NAT2* polymorphisms

Polymorphism	Enzyme	Units	BP fragments	
			Wild	Homozygous
481C>T	*Kpn 1*	6 U	665, 546	1211
857G>A	*BamH 1*	7 U	930, 281	1211
191G>A	*Msp 1*	2 U	181, 168, 93	274, 168
282C>T	*Fok 1*	3 U	342, 100	442
341T>C	*Dde 1*	5 U	216, 163, 63	184, 163, 63, 32
590C>A	*Taq 1*	4 U	170, 139, 112	309, 112
803A>G	*Dde 1*	5 U	301, 120	301, 97, 23

Digestion of 5 μL of the PCR product at 37 °C overnight

Discussion

ALL in children offers a unique opportunity to examine the effect of carcinogen-metabolizing genes in the risk of pediatric cancers. The young age of patients and, thus, a short latency period between the appearance of the initiating mutation and the detection of tumor cells should facilitate the identification of risk factors, as compared with adult cancer patients in whom many factors come into play because of long latency periods [31].

In utero and postnatal exposures to various carcinogens may play a role in the etiology of ALL [37, 43]. *N*-Acetyltransferases, encoded by *NAT2* genes, are involved in

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Table 3 *NAT2* allele frequencies among ALL cases and controls

NAT2 allele	Genotype[a]	Control		Patients		*P* value[b]	OR	95 % CI
		N	%	*N*	%			
*NAT2*4* Rapid	Wild	23	11.2	8	8.6	0.502	0.749	0.322–1.745
*NAT2**12A	AA	83	26.6	54	58.6			
803A>G	**AG**	*157*	*50.2*	*26*	*28.2*	*0.000*	*0.389*	*0.235–0.645*
rs1208	**GG**	72	23	12	13	0.037	0.500	0.258–0.967
Rapid	AG+**GG**	*229*	*73.3*	*38*	*41.3*	*0.000*	*0.211*	*0.129–0.345*
*NAT2*5D*	TT	89	30.2	27	29.6			
341T>C	**TC**	124	42.1	47	51.6	0.462	1.249	0.724–2.157
rs1801280	**CC**	61	20.7	17	18.7	0.862	0.919	0.461–1.829
Slow	TC+**CC**	185	67.5	64	70.3			
*NAT2*11A*	CC	103	40.6	28	30.4			
C481C>T	**CT**	104	40.9	51	55.5	0.034	1.243	1.056–3.082
rs1799929	**TT**	47	18.5	13	14.1	0.735	1.017	0.484–2.139
Rapid	CT+**TT**	154	59.9	64	69.6	0.103	1.559	0.936–2.596
*NAT2*13A*	CC	129	42.1	42	45.7			
282C>T	**CT**	147	48.1	42	45.7	0.620	0.878	0.538–1.431
rs1041983	**TT**	30	9.8	8	8.6	0.834	0.819	0.349–1.924
Rapid	CT+**TT**	177	57.8	50	54.3	0.812		
*NAT2*6B*	GG	152	48.9	41	44.6			
590G>A	**GA**	135	43.4	42	45.6	0.536	1.181	0.726–1.921
rs1799930	**AA**	24	7.7	9	9.8	0.496	1.390	0.600–3.221
Slow	GA+**AA**	159	51.1	51	55.4	0.478	1.212	0.761–1.932
*NAT2*7A*	GG	179	91.8	74	80.4			
857G>A	**GA**	*15*	*7.7*	*17*	*18.4*	*0.009*	*2.741*	*1.301–5.776*
rs1799931	**AA**	1	0.5	1	1.1	0.503	2.419	0.149-39-187
Slow	GA+**AA**	*16*	*8.2*	*18*	*19.5*	*0.010*	*2.721*	*1.317–5.624*
*NAT2*14A*	GG	304	97.7	88	96.8			
191G>A	**GA**	8	2.3	3	3.2	0.436	1.481	0.926–1.921
rs1801279	**AA**	0	0	0	0			
Slow	GA+**AA**	8	2.6	3	3.2	0.436	1.481	0.926–1.921

Wild type: 191GG, 282CC, 341TT, 481CC, 590GG, 803AA, 857GG

[a] Bold font denotes mutant allele

[b] Italics denotes statistical significance at $P \leq 0.0169524$

the biotransformation of aromatic amines present in tobacco smoke, the environment, and the diet.

Here, we determined the frequencies of *NAT2* allelic variants in 92 ALL cases and 312 Egyptian unrelated healthy controls. Our controls, recruited from blood donors, were not matched for age. However, the genetic makeup of an individual does not change by age and being adults guarantees that they did not and will not develop pediatric ALL. Table 5 shows the comparison of *NAT2* variant allele frequencies among our study control population and other ethnic groups. The *NAT2*4* rapid acetylator genotype showed a significantly lower frequency in our cohort ($P=0.032$) while the second rapid acetylator genotype (*NAT2*12A*) showed a much higher frequency ($P<0.001$). On the other hand, the slow acetylator variant alleles (*NAT2*5A* and *NAT2*5C*) are more frequent among our population ($P<0.001$ to $P<0.0001$), compared to those of other populations. The frequency of *NAT2*5B* among our population is significantly higher than that of other populations. The frequencies of the other slow acetylator genotypes *NAT2*6A* among our population are significantly higher than those of US Caucasians and French-Canadians ($P<0.001$ and $P<0.001$) [44, 45] while *NAT2*7B* is significantly higher than that of US Caucasians and French-Canadians ($P=0.0014$ and $P=0.004$) [41, 45] and similar to that of Caucasians [30] and German Caucasians [30]. The variation among different ethnic groups and even within the same country was recently emphasized in a study involving different areas of India [44]. This study also emphasized the impact of environment on the *NAT2* allele distribution where slow acetylators were more associated with vegetarian dietary habits and rapid

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Table 4 *NAT2* allele's combinations among ALL cases and controls

NAT2 allele	Mutation [a]	Control		Patients		*P* value[b]	OR	95 % CI
		N	%	*N*	%			
*NAT2*5A*	341,481							
Slow[#]	**TCCT**	82	36.7	33	35.8	0.880	0.962	0.580–1.595
	TCTT	1	0.4	4	4.3	0.027	10.091	1.112–91.543
	CCCT	*2*	*0.89*	*7*	*7.6*	*0.001*	*9.100*	*1.853–44.680*
	CCTT	41	18.3	18	19.5	0.782	1.171	0.876–2.456
	Total	126	56.0	62	67.3	0.325	1.321	0.435–2.936
*NAT2*5B*	341,481,803							
Slow[##]	**TCCTAG**	*81*	*36.3*	*9*	*9.8*	*<0.001*	*0.190*	*0.091–0.398*
	TCCTGG	2	0.9	2	2.2	0.583		
	TCTTAG	0		1				
	TCTTGG	0		1				
	CCCTAG	1		0				
	CCCTGG	11	4.9	4	4.3	0.827		
	CCTTAG	11	4.9	4	4.3	0.827		
	CCTTGG	*40*	*17.9*	*2*	*2.2*	*<0.001*	*0.102*	*0.024–0.430*
	Total	*146*	*60.5*	*23*	*25*	*0.002*	*0.542*	*0.325–0.896*
*NAT2*5C*	341.803							
Slow[#]	**TCAG**	*121*	*44.2*	*12*	*13*	*<0.001*	*0.19*	*0.099–0.364*
	CCAG	11	4.0	5	5.4	0.754		
	TCGG	2	0.7	3	3.3	0.104		
	CCGG	**59**	**21.5**	**7**	**7.6**	**0.003**	**0.30**	**0.132–0.683**
	Total	*193*	*70.5*	*27*	*29.3*	*0.011*	*0.41*	*0.311–0.763*
*NAT2*6A*	282.590							
Slow[#]	CTGA	*120*	*39.3*	*29*	*31.5*	0.174		
	TTGA	*6*	*2.0*	*2*	*2.2*	0.902		
	CTAA	1	0.3	3	3.3	0.04	10.25	1.154–99.73
	TTAA	*23*	*7.5*	*6*	*6.7*	0.854		
	Total	*150*	*49.1*	*40*	*43.4*	0.478	0.821	0.699–1.28
*NAT2*7B*	282,857							
Slow[#]	CTGA	*10*	*5.2*	*12*	*12*	*0.004*	*2.485*	*1.21–6.085*
	CTAA	**0**		**0**				
	TTGA	2	1.0	1	1.1	0.995		
	TTAA	1	0.5	1	1.1	0.542		
	Total	13	6.7	14	15.2	0.02	3.244	1.378–7.634

[a] Bold font denotes mutant allele

[b] Italics denote statistical significance

[#] *P*=≤0.0125; [##] *P*=≤0.0063912

acetylators with non-vegetarian habits. The variation in allele frequency between our population and other populations might question the appropriateness of using adults as controls; the difference in age could possibly be associated with a difference in potential admixtures with other populations. However, this assumption is invalidated by many factors. An admixture from Europe is not possible because of the religious barriers not only for Moslems but also for Egyptian Christians who belong to a different church. It is true that some Egyptians get married to spouses belonging to other nationalities, mostly Arab and occasionally Western, but those actually leave Egypt and settle in the spouses' country. Besides, the blood donors are all from donation campaigns in universities and factories all in the age of 20s, maximum 30s. Accordingly, they are not really from a different generation (the generation is 33 years).

Our results showed that children carrying *NAT2* slow-acetylation genotypes *NAT2*7A* G857G>**A** and **7B*

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Table 5 Frequencies of NAT2 polymorphisms among Egyptians compared to other ethnic groups

Population (reference)	No.	*NAT2 genotype: %*							*P* value
		**4*	**12A*	**5A*	**5B*	**5C*	**6A*	**7B*	
Egyptians									
No.		23/206	72/312	126/223	146/223	193/274	150/305	13/193	
%		11.2	23.1	56.5	65.5	70.5	49.1	6.7	
French-Canadian population [45]	274	25.9*	0.3**	2.4**	44.2**	1.0**	24.9**	1.2***	*0.032, **<0.001, ***0.0014
US Caucasians [42]	211	23.2*	2.3**	6.8**	37.2***	NA	25***	1.2****	*0.039, **<0.001, ***<0.001, ****0.004
Caucasians [30]	74	23.4*	NA	2.5**	38.5**	2.9**	31.1***	1.4	*0.039, **<0.001, ***0.0053
German Caucasians [30]	128	21.1*	NA	3.1*	37.9**	2.3*	32.8***	2.7	*<0.001, **<0.001, ***0.0062

Rapid alleles, *NAT2*4*, *12A; slow alleles, *NAT2*5A*, **5B*, **5C*, **6A*, **7B*. *P* value: compared to Egyptians (current study)

NA not available

(282C>**T**, 857G>**A**) are at 2.7- and 3.2-fold increased risk of developing ALL respectively. A comparable 2.9-fold increased risk was reported for *NAT2*7B* in a French-Canadian population [45].

In this work, the *NAT2* rapid acetylator *NAT2*12A* (*not NAT2*4*) imposed 2.4-fold protection. A 1.67-fold protection was reported with the other rapid acetylator allele *NAT2*4* in a French-Canadian population [31].

Other studies, however, reported no impact of *NAT2* acetylator genotypes on the risk of developing acute leukemia either in children [30, 34, 36] or in adults [32]. Also, none of the published GWAS have documented *NAT2* as a risk factor in ALL susceptibility [46], yet the studied populations were all of Western origin. All other ethnic groups were deliberately excluded.

In the current study, we found that the slow acetylator *NAT2*5B* (314T>**C**, 481C>**T**, and 803A>**G**) and **5C* (314T>**C**, 803A>**G**) were associated with a lower risk of ALL development (1.8- and 2.4-fold protection respectively). This is in contrast to a study that reported that the slow acetylation activity of *NAT2* is associated with 1.5-fold increased risk [31].

As regards the other slow acetylator alleles *NAT2*5A* and *NAT2*6A*, our results did not show any significant impact on susceptibility to ALL. In agreement with our results, two more studies reported similar findings [31, 32].

However, the T**CTT** and **CCCT** alleles of *NAT2*5A* (341T>**C** and 481C>**T**) were found to be associated with 10- and 9-fold increased risk respectively though only **CCCT** achieved statistical significance.

Other studies reported the association of *NAT2*5D* with increased susceptibility to both ALL and AML and the association of *NAT2*14*, **5A*, and **5C* with increased risk susceptibility to ALL in a Brazilian population [47]; this study reported the risk to be 7.9-fold in children <1 year and 1.5-fold in children 1–10 years. However, another Brazilian study reported no significant differences between the groups analyzed regarding the *NAT2* variants [37]. A 1.8-fold increased risk was reported in association with *NAT2* 481CC, 341TT, and 590GG in a Russian population [34] and in association with *NAT2**5 in a French population [38].

The discrepancy between our study and others with regard to the effect of *NAT2* alleles may be related to several factors:

(1) *N*-Acetyl transferase metabolizes a number of aromatic and heterocyclic amine carcinogens that produce tumors, aromatic and hydrazine (*N*-acetylation=deactivation) and *N*-hydroxy-aromatic and heterocyclic amines (*O*-acetylation=activation). So the genetic polymorphism of *NAT2* may modify the cancer risk related to these carcinogens either by increasing or decreasing the risk.
(2) There are tissue-specific expression of NAT2 acetylation allozymes that also affect the cancer risk, e.g., the rapid acetylator phenotype has been reported to be associated with colorectal and lung cancers; however, head and neck and bladder cancers seem to be associated with slow acetylator phenotype [8].
(3) The expression of the acetylator phenotype depends on the substrate specificity, e.g., the NAT2*7B allozyme has higher affinity than other NAT2 allozymes for sulfamethazine and dapsone but not for 2-aminofluorene [48].
(4) Some studies reported a phenotypic difference within the slow acetylator genotypes [10, 49, 50].
(5) The effect of NAT2 phenotype is influenced by a number of factors including diet, disease, and drug therapy [7].

It is worth mentioning that our population showed a higher incidence of *NAT2* 803A>**G** allele which in turn affected the slow acetylator alleles *NAT2*5B* and *NAT2*5C* making the association with decreased risk of ALL.

In conclusion, taking in consideration the limitation of the relatively small sample size, our findings showed that the rapid acetylator genotype *NAT2*12A* 803A>G, which has a high detoxifying activity, showed 3.3-fold protection while

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the slow acetylator *NAT2*5A* (TCTT and CCCT genotypes only) was found to be associated with 10- and 9-fold increased risk respectively, while the overall frequency of *NAT2*5A* did not show any significant impact on ALL risk. Also, the slow acetylator polymorphisms *NAT2*7A* and **7B* were found to be associated with 2.7- and 3.2-fold increased risk of ALL respectively.

Finally, it is clear that ALL is a multifactorial disease where a number of risk factors play a significant role increasing or decreasing its incidence. Risk modification is affected by gene-gene and gene-environmental interaction as well as by ethnic variations. Studies addressing this issue have to be performed locally in each population to verify the significant factors in each according to their ethnic constitution and local environmental exposures.

Conflicts of interest None

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