The potential effect of gender in combination with common genetic polymorphisms of drug-metabolizing enzymes on the risk of developing acute leukemia

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ABSTRACT

Background and Objectives

We examined common polymorphisms in the genes for glutathione S-transferase (*GST*), cytochrome P450 (*CYP*), quinone oxoreductase (*NQO1*), methylene tetrahydrofolate reductase (*MTHFR*), and thymidylate synthetase (*TYMS*) and the role of gender associated with the susceptibility to *de novo* acute leukemia (AL).

Design and Methods

We conducted a case-control study analyzing the prevalence of the polymorphisms *CYP1A1*2A*, *CYP2E1*5B*, *CYP3A4*1B*, *del{GSTT1}*, *del{GSTM1}*, *NQO1*2*, *MTHFR C6777*, and *TYMS 2R/3R* in 443 patients with AL [302 with acute myeloblastic leukemia (AML) and 141 with acute lymphoblastic leukemia (ALL)] and 454 control volunteers, using polymerase chain reaction (PCR)-based methods.

Results

We found a higher incidence of *del{GSTT1}* in patients with AML than among controls (25.6% vs. 13.7%, OR=2.2, $p<0.001$) and a higher incidence of *NQO1*2* homozygosity (*NQO1*2*$_{hom.}$) in males with the M3 FAB subtype than in control males (8.6% vs. 2.2%, OR=4.9, $p=0.02$). The *del{GSTT1}* and *NQO1*2*$_{hom.}$ polymorphisms increased the risk of ALL (OR=2.2 and 3.0, $p=0.001$ and 0.003, respectively). The higher risk conferred by *NQO1*2*$_{hom.}$ and *del{GSTT1}* mainly affected males (OR=6.1 and 2.4; $p=0.002$ and 0.005, respectively).

Interpretation and Conclusions

Males harboring *NQO1*2*$_{hom.}$ and *del{GSTT1}* polymorphisms showed a higher risk than females of developing AL. Thus, gender might influence the risk of AL associated with these genetic polymorphisms.

Key words: polymorphisms, gender, risk, drug-metabolizing enzymes, acute leukemia.

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Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are the most common acute leukemias (AL) in adults[1] and children.[2,3] On the whole AL is more common in males of all age groups,[4] a fact that remains unexplained. Although the clinical and biological aspects of leukemia are well documented, little is known about the factors that condition an individual's susceptibility to *de novo* leukemia. Normal polymorphic variations in several genes, together with dietary effects, environmental exposure to carcinogens, and individual immune system characteristics are likely to be factors that predispose individuals to develop AL.[5] Polymorphisms could also explain the different incidence of AL observed between the genders.

Genetic polymorphisms in the drug-metabolizing enzymes are extremely common and may contribute to the risk of developing cancers. These polymorphisms could explain differences in the way in which individuals metabolize chemical agents.[6] Studies have included polymorphisms of genes coding for P450 cytochromes (CYP), which are involved in phase I of metabolism (oxidation/activation). Most polymorphisms described for these genes, such as T6235C of *CYP1A1* (*CYP1A1*2A*), C-1019T of *CYP2E1* (*CYP2E1*5B*), and –A290G of *CYP3A4* (*CYP3A4*1B*), are believed to cause an increase in enzymatic activity. They have been implicated in the bioactivation of several chemical carcinogens and the conversion of polyaromatic hydrocarbons from tobacco smoke into intermediate reactive metabolites, some of which can damage DNA.[7] Glutathione S-methyl transferases (GST) are implicated in phase II of metabolism (conjugation/detoxification). Two widespread genetic polymorphisms that involve deletions in the *GSTT1* and *GSTM1* genes, (*del{GSTT1}* and *del{GSTM1}*), have been reported to lead to abrogation of enzyme activity.

Quinone oxoreductase (NQO1) is a detoxification enzyme that acts in limiting free radical oxidative stress. Two polymorphisms, the C609T (*NQO1*2*) and C465T (*NQO1*3*) substitutions, have been described in the *NQO1* gene; they cause complete loss or reduction of enzyme activity, respectively.[8]

Variations in the activity of genes involved in folic acid metabolism, such as methylene tetrahydrofolate reductase (*MTHFR*) and thymidylate synthetase (*TYMS*), affect dUMP availability and hence DNA repair machinery. In particular, the *MTHFR C677T* polymorphism decreases the activity of the MTHFR enzyme and the double (2R) or triple (3R) 28 base pair repeat polymorphism in the 5´– untranslated region of *TYMS* (*2R/3R TYMS*) modifies the rate of enzyme transcription. Several studies have tried to relate these polymorphisms to the risk of *de novo* leukemia, particularly in patients with ALL. There are only a few reports on AML.[9] For both situations, the results obtained are controversial and require further investigation to confirm or clarify the data obtained. Furthermore, as far as we know, there have been no studies on the possible relationship of these polymorphisms with gender. Such an interaction could explain the unequal risk of AL observed between the sexes.

To clarify these issues we conducted a case-control study to analyze the influence of the genetic polymorphisms *CYP1A1*2A, CYP2E1*5B, CYP3A4*1B, del{GSTT1}, del{GSTM1}, NQO1*2, MTHFR C677T*, and *TYMS 2R/3R* on susceptibility to *de novo* leukemia and to analyze their possible relationships with gender.

Design and Methods

Patients and subjects

We performed a case-control study including 443 patients with AL (302 with AML and 141 with ALL) and a control group composed of 454 individuals without leukemia. The AL samples were from the *La Fe* hospital and from other Spanish hospitals. The samples from *La Fe* hospital were collected sequentially between 1992 and 2005 from patients at the time of diagnosis. Samples from the other Spanish hospitals were not sequential (stored DNA or cellular samples kept frozen). The diagnosis of AML was made in accordance with morphological and cytochemical criteria of the French–American–British (FAB) classification.[10] The diagnosis of ALL was based on immunophenotypic criteria. The main characteristics of the patients are shown in Table 1. Here we studied AML and ALL separately, but grouped together all cases of infant, childhood, and adult AL because we did not find any statistical differences in the incidence of any the polymorphisms between patients aged 16 years or younger (infant and childhood AL) and those older than 16 (adult AL).

The control group consisted of volunteers who had attended the hospital for blood sampling for biochemistry and/or hematologic analyses and who were willing to participate in the study. Subjects with any hematologic or other malignancy were excluded. Informed consent was obtained from all patients and controls in accordance with the recommendations of the Declaration of Human Rights, at the Conference of Helsinki, and also in compliance with institutional regulations (the hospital ethics committee).

Samples and DNA extraction

Venous blood samples were collected from control subjects, or from patients at diagnosis or in complete hematologic remission, into vacuum tubes containing EDTA.K3. The DNA was extracted directly from 500 µL aliquots of whole blood using large volume MagNA Pure LC DNA Isolation kits (Roche, Mannheim, Germany) automatically in the MagNA Pure LC System (Roche).

Cytogenetic analysis

Karyotype analysis was performed using unstimulated short-term cultures and described in accordance with the recommendations of the International System for Human Cytogenetic Nomenclature (ISCN, 1995).[11] Whenever possible, at least 20 metaphases were evaluated. Cytogenetic risk groups were defined by karyotype as follows: *high risk*, –5/del(5q), –7/del(7q), abn 3q, complex aberrations (≥3 independent aberrations), t(9;22), and t(6;9); *low risk*, t(8;21), t(15;17), and inv(16); *intermediate risk*, all other karyotypic aberrations or a normal karyotype.

Genotyping of polymorphisms

We studied the genetic polymorphisms *CYP1A1*2A, CYP2E1*5B, CYP3A4*1B, del{GSTT1}, del{GSTM1}, NQO1*2, MTHFR C677T*, and *TYMS 2R/3R*. Most detection methods used were based on real-time polymerase chain reaction (PCR) performed in a LightCycler (Roche) using primers and fluorescence-labeled hybridization probes, as described below. Genotyping was based on different melting temperatures achieved by the hybridization probes for wild-type and polymorphic alleles. Thus, *CYP1A1*2A* was detected as described by Harth *et al.*,[12] *CYP3A4*1B* as described by Von Ahsen *et al.*,[13] *CYP2E1*5B* as described by Choi *et al.*,[14] and *NQO1*2* as described by Harth *et al.*[15] For the detection of *MTHFR C677T* we followed the method recommended by Roche (Applied Science), using the primers MTHFR_s (cgaagcagggagctttgaggctg) and MTHFR_as (aggacggtgcggtgagagtg) and the hybridization probes MTHFR_LC labeled with Red640 at the 5´ end (LC Red640-cgggagccgatttcatcat-ph) and MTHFR_3FL (tgacctgaagcacttgaaggagaaggtgtc X) labeled with fluorescein. The *del{GSTT1}* and *del{GSTM1}* polymorphisms were detected following the conventional PCR method of Naoe *et al.*,[16] comprising a multiplex PCR that co-amplifies the target genes simultaneously with the β-globin gene used as a reference control gene. The *TYMS 2R/3R* polymorphism was detected using the conventional PCR method described by Villafranca *et al.*[17]

Statistical procedures

χ^2 analysis with two-way contingency tables was used to test the association of polymorphisms with other qualitative variables or to check for Hardy–Weinberg equilibrium for each polymorphism in the control group. The influence of each polymorphism on the risk of AL was estimated by applying univariate or multivariate binary logistic regression, including gender and the genotypes of cases (AML or ALL) and controls and estimating the odds ratio (OR) and 95% confidence interval (CI) of the parameters included in the model. Errors generated by multiple testing were corrected using a sharpened step-up Hochberg's procedure to control the experimental error rate,[18] which fixed the limit of significance for two-sided *p*-values at 0.038. To eliminate the effects of empty cells, we computed the OR adding 0.5 to each cell according to Cox *et al.*[19] All analyses were performed using the software package Statistical Program for Social Sciences (SPSS) version 12.0 (Chicago, IL, USA).

Table 1. Characteristics of the study groups.

	Cases		
Variables	*AML (n=302) n (%)*	*ALL (n=141) n (%)*	*Control Group (n=454) n (%)*
Age*			
n;median (range)			
≤ 1 year	3; 3(0.8-1)	2; 2(1-1)	3; 1(1-1)
>1 year and ≤ 16 years	32; 9(1.1-16)	46; 7.5(2-16)	48; 9(2-16)
>16 years and ≤ 60 years	187; 42(17-60)	76; 33(17-59)	336; 37(17-60)
> 60 years	80; 67(61-84)	17; 66(61-82)	67; 68(61-85)
Gender			
Men	166 (54.9)	87 (61.7)	223 (49.1)
Women	136 (45.0)	54 (38.2)	231 (50.8)
FAB type		*Immunophenotype*	
Undiferentiated	3 (0.95)	B-ALL	92 (65.2)
Mixed	1 (0.33)	T-ALL	23 (16.3)
Biphenotypic	1 (0.33)	PreB-ALL	18 (12.7)
M0	11 (3.7)	Ph⁺-ALL	8 (5.6)
M1	48 (15. 9)		
M2	48 (15. 9)		
M3	107 (35.4)		
M4	37 (12.3)		
M5	29 (9.6)		
M6	10 (3.3)		
M7	4 (1.3)		
Not classified	3 (1.0)		
Cytogenetic risk			
high	19 (6.3)	9 (6.3)	
intermediate	119 (39.4)	56 (39.7)	
low	67 (22.2)		
not available	97 (32.1)	76 (53.9)	

*ALL: acute lymphoblastic leukemia; AML: acute myeloblastic leukemia; *at diagnosis of AML or ALL. B-ALL and T-ALL: patients with ALL who showed B antigens or T antigens, respectively. PreB-ALL: patients with ALL who showed B antigens and μ chains. Ph⁺-ALL: patients with ALL who harbored the Philadelphia chromosome.*

Results

Polymorphisms and demographic characteristics

We verified that the genotypic frequencies of polymorphisms in the control group complied with the Hardy–Weinberg equilibrium. We did not find any statistical differences in the incidence of genotypes among defined age groups (<18, 18–40, 41–50, 51–60 and >60 years). We did not find any statistical differences in the genotypic frequencies when stratified by gender for the three study groups (controls, ALL, and AML).

Polymorphisms and characteristics of the AML group

Stratification of the AML group by age at diagnosis, gender, FAB subtype, or cytogenetic risk revealed no differences in genotype frequencies. Females with the M2 FAB subtype showed twice the incidence of the *del{GSTM1}* polymorphism compared with males with the same FAB subtype (59.1% and 21.7%, respectively, χ^2=6.5, p=0.01). We also observed that the *CYP2E1*5B* polymorphism was significantly more frequent in patients with the M3 FAB subtype than in those with non-M3 FAB subtypes (11.2% vs. 2.8%, χ^2=9.0, p=0.01).

Polymorphisms and risk of AML

The del*{GSTT1}* polymorphism was more prevalent in patients with AML than in the control group (25.6% vs. 13.7%, OR=2.2, 95% CI=1.5–3.2, p<0.001; Table 2).

We did not find any statistical difference in the incidence of the *NQO1*2* polymorphism between AML patients and the control group. However, we found a higher incidence of the homozygous ($_{hom.}$) genotype in males with the M3 FAB subtype than in males of the control group [8.6% (5/58) vs. 2.2% (5/219), $OR_{hom.}$=4.9, 95% CI=1.3–18.1, p=0.02; Figure 1]. Conversely, there was no significant statistical difference in the incidence of the *NQO1*2* polymorphism for the M3 FAB subtype among females. This difference in the pattern of incidence of the *NQO1*2* polymorphism between the sexes showed statistically significant interactions ($OR_{het.*male}$=3.1, 95% CI=1.2–8.2, p=0.02 and $OR_{hom.*male}$=8.8, 95% CI=1.2–65.7, p=0.03). When all statistically significant parameters detected in the univariate logistic regression (*del{GSTT1}, NQO1*2*, sex and $NQO1*2*_{sex}$) were included in the multivariate logistic regression model for the entire group of patients with AML (n=274) and the control group (n=438), the *del{GSTT1}* (OR=1.9, 95% CI=1.3–2.9, p=0.001) and the

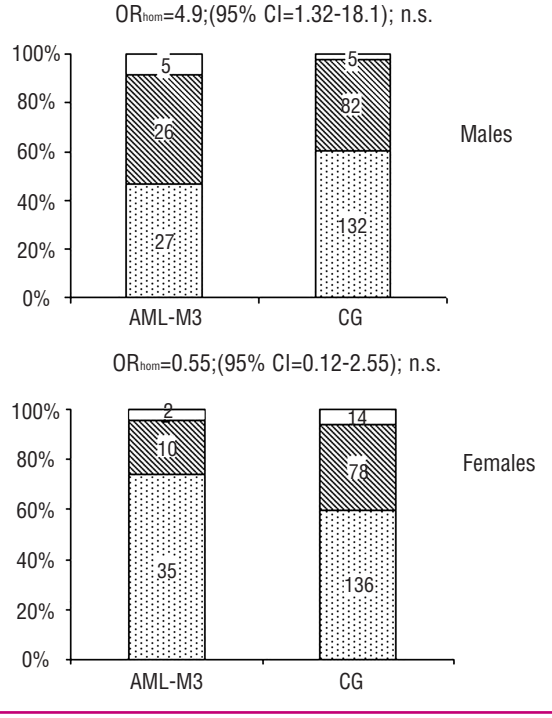

Figure 1. The *NQO1*2* polymorphism in patients with acute myeloid leukemia (AML) subtype M3 of the French–American–British (FAB) classification and the control group (CG). Blank area, homozygous genotypes; dashed area, heterozygous genotypes; dotted area, negative. $OR_{hom.}$ shows the odds ratio for homozygous genotypes; CI: confidence interval; n.s.: not significant.

Table 2. Genotype frequencies of the polymorphisms and odds ratio for acute myeloblastic and lymphoblastic leukemia.

Polymorphisms Genotypes	*Control group n/total (%)*	*AML n/total (%)*	*OR (95% CI)*	*p value*	*ALL n/total (%)*	*OR (95% CI)*	*p value*
*CYP1A1*2A*							
Heterozygous	84/403 (20.8)	31/199 (15.6)	0.69 (0.43-1.09)	n.s.	13/92 (14.1)	0.62 (0.32-1.17)	n.s.
Homozygous	2/403 (0.5)	0/199 (0.0)	0.40* (0.02-8.42)	n.s.	0/92 (0.0)	0.87* (0.04-18.23)	n.s.
*CYP2E1*5B*							
Heterozygous	23/390 (5.9)	13/225 (5.8)	0.96 (0.48-1.98)	n.s.	8/118 (6.8)	0.86 (0.37-1.98)	n.s.
Homozygous	0/390 (0.0)	1/225 (0.4)	5.21* (0.21-128.5)	n.s.	0/118 (0.0)	Undefined	
*CYP3A4*1B*							
Heterozygous	29/357 (8.1)	14/222 (6.3)	0.77 (0.39-1.48)	n.s.	6/128 (4.7)	0.56 (0.23-1.38)	n.s.
Homozygous	0/357 (0.0)	1/222 (0.5)	4.84* (0.20-119.3)	n.s.	1/128 (0.8)	8.41* (0.34-207.68)	n.s.
del{GSTM1}							
Homozygous	232/451 (51.4)	143/295 (48.5)	0.88 (0.66-1.191)	n.s.	64/140 (45.7)	0.79 (0.54-1.16)	n.s.
del{GSTT1}							
Homozygous	61/455 (13.7)	74/289 (25.6)	2.17 (1.48-3.16)	<0.001	36/141 (25.5)	2.15 (1.35-3.43)	0.001
*NQO1*2*							
Heterozygous	160/447 (35.8)	94/273 (34.4)	0.96 (0.71-1.33)	n.s.	41/120 (34.2)	1.05 (0.68-1.63)	n.s.
Homozygous	19/447 (4.3)	16/273 (5.9)	1.38 (0.69-2.77)	n.s.	14/120 (11.7)	3.03 (1.44-6.37)	0.003
MTHFR C677T							
Heterozygous	160/331 (48.3)	80/163 (49.1)	0.98 (0.64-1.50)	n.s.	53/117 (45.3)	0.72 (0.45-1.13)	n.s.
Homozygous	65/331 (19.6)	29/163 (17.8)	0.87 (0.51-1.51)	n.s.	15/117 (12.8)	0.49 (0.26-0.96)	0.038 (n.s.)
TYMS							
2R3R	162/347 (46.7)	94/169 (55.6)	1.19 (0.73-1.94)	n.s.	42/108 (38.9)	0.77 (0.43-1.37)	n.s.
3R3R	117/347 (33.7)	42/169 (24.9)	0.74 (0.43-1.27)	n.s.	43/108 (39.8)	1.08 (0.60-1.95)	n.s.

*ALL: acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; n.s.: not significant. (n.s.) limit of significance. *The adjusted odds ratio (OR) was computed by adding 0.5 to each cell to eliminate empty cells.*

interaction *NQO1*2*$_{het*male}$ (OR=3.6, 95% CI=1.2–10.9, p=0.02) were the only independent parameters that conferred an increased risk of leukemia.

When we introduced polymorphisms stratified by gender into the multivariate logistic regression, we found that *NQO1*2*$_{hom.}$ (OR=3.4, 95% CI=1.1–10.2, p=0.03) was the only independent risk factor among males (control group=214 and AML=151), whereas *del{GSTT1}* (OR=2.3, 95% CI=1.3–3.9, p=0.005) was the only independent risk factor for females (control group=224 and AML=123).

Polymorphisms and susceptibility to ALL

Stratification of patients with ALL by age at diagnosis, gender, B- or T-cell lineage, or cytogenetic risk revealed no statistically significant differences in genotype frequencies. We found a higher incidence of the *del{GSTT1}* in patients with ALL than in the control group (25.5% vs. 13.7%, OR=2.2, p=0.001; Table 2). This difference was mainly because of males with ALL, who showed a 24.5% (24/87) incidence of the polymorphism compared with a 13.7% (30/218) incidence among the males in the control group (OR=2.4, p=0.005; Figure 2). Likewise, we observed a higher incidence of the *NQO1*2*$_{hom.}$ in patients with ALL than in the control group (11.7% vs. 4.3%, OR$_{hom.}$=3.0, p=0.003; Table 2). This statistical difference was because 12.5% (9/72) of the males with ALL were homozygous for this polymorphism compared to only 2.2% (5/219) of the control group (OR$_{hom.}$=6.1, p=0.002; Figure 3). This difference was not, however, observed in females.

For *MTHFR C677T*, we observed a lower incidence of homozygosity among the ALL patients than among the control group (12.8% vs. 19.6%, OR=0.5, p=0.038), although this difference only just reached statistical significance.

When polymorphisms found to have statistical significance by univariate logistic regression, (*del{GSTT1}* and *NQO1*2*), were introduced into a multivariate logistic model, *NQO1*2* was the only independent risk factor for ALL (*OR*$_{NQO1*2hom.}$=3.2, 95% CI=1.5–6.7, p=0.003). When these polymorphisms were stratified by gender, the *NQO1*2*$_{hom.}$ genotype was the only independent risk factor for ALL in males (72 ALL and 214 control group; OR=5.9, 95% CI=1.8–18.8, p=0.002). However, we could not find any polymorphism with statistical significance for risk among females (48 ALL and 224 control group).

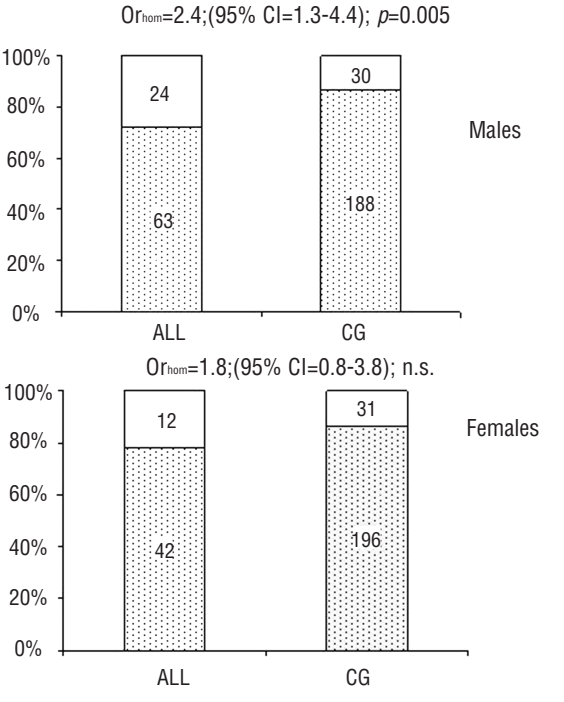

Figure 2. The *del{GSTT1}* polymorphism in patients with acute lymphoblastic leukemia (ALL) and the control group (CG). Blank area, homozygous genotypes; dotted area, negative genotypes. OR$_{hom.}$, odds ratio for homozygous genotypes; CI: confidence interval; n.s.: not significant.

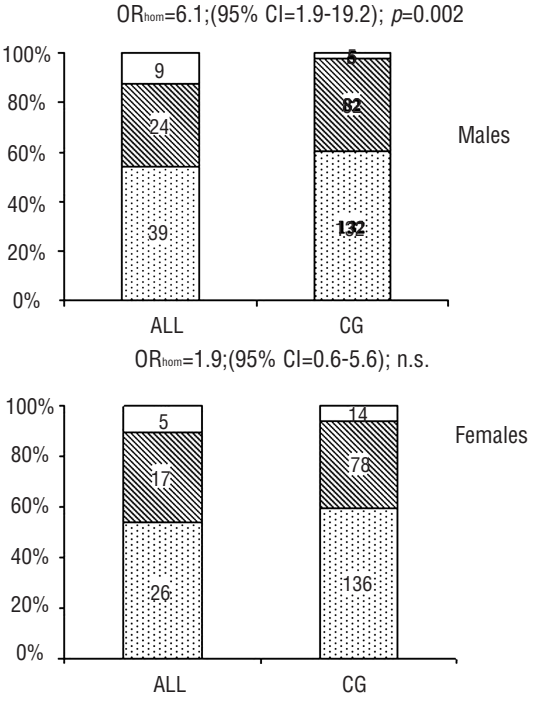

Figure 3. The *NQO1*2* polymorphism in patients with acute lymphoblastic leukemia (ALL) and the control group (CG). Blank area, homozygous genotypes; dashed area heterozygous genotypes; dotted area, negative. OR$_{hom.}$, odds ratio for homozygous genotypes; CI: confidence interval; n.s.: not significant.

Discussion

We found a higher incidence of the *del{GSTT1}* polymorphism among patients with AML than among controls, which is consistent with previous reports.[20,21] In contrast to Smith *et al.*,[22] in the present study we found

no association of the *NQO1*2* polymorphism with an increased risk of AML. However, males with the M3 FAB subtype who were homozygous for the *NQO1*2* polymorphism showed an increased risk of AML, an effect not manifest in females. Although as far as we know this association has not been reported previously, there are supporting data. Reynolds *et al.*[23] reported that the myeloperoxidase (*MPO*) polymorphism *MPO*2*, consisting of a G–642A variation in the promoter region of *MPO*, was overrepresented in patients with the AML-M3 and M4 FAB subtypes. Furthermore, the *MPO*2* genotype enhances MPO expression and cooperates with *NQO1*2* homozygosity, increasing susceptibility to benzene poisoning.[24]

The statistical results obtained here for patients with AML mainly represent the two thirds of patients who were under 30 or over 50 years of age. Cartwright *et al.*[25] reported a clear predominance of AML among males in these age ranges, whereas the disease was more prevalent among women aged between 30 and 50 years. Thus, the greater susceptibility to AML conferred by *NQO1*2* in males and the interaction between genders for the incidence of *NQO1*2* in M3 FAB that we found here may help explain the higher male-linked risk of leukemia.

In contrast with other reports,[26–30] we found here that the *del{GSTT1}* genotype was associated with an increased risk of ALL. This discrepancy could be explained in part by differences in the age of the study populations: the subjects in the present study were mostly adults whereas in the other studies they were mostly children. Moreover, the higher incidence of the *del{GSTT1}* polymorphism we found in our study was mainly among males with ALL. Our results for the *NQO1*2* polymorphism in patients with ALL were concordant with the data for patients with the M3 FAB subtype. Thus, the presence of the *NQO1*2* polymorphism increased the risk of ALL only in males, but did not modify it in females. The increased risk of ALL conferred by *NQO1*2* homozygosity found in this study is in agreement with some studies carried out in children with ALL;[31–33] however, other reports did not support these results.[34,35]

The statistical results obtained here for patients with ALL are based mainly on subjects older than 10 years (100/141; 71%). For this age group, Cartwright *et al.*[25] reported a clear predominance of ALL among males, whereas the incidence of ALL in children was similar in the two series. In this regard, our findings of the increased risk of ALL in males conferred by the *del{GSTT1}* polymorphism and, in particular, by the *NQO1*2* polymorphism, provides a genetic basis for the higher incidence of ALL reported in males. Because there is no current environmental hypothesis to explain the higher incidence of AL in males, it could be hypothesized that females are genetically better protected than males against environmental and toxic agents that cause AL. In this regard, a study carried out among Malaysians[36] suggested the presence of a gene located near the ABO locus on chromosome 9, which could protect women with a group O blood type against AL. Additionally, several results suggest a possible role of sex steroids in the control of the proliferation of leukemic cells. For example, it has been reported that the antiproliferative effect of 17-β estradiol on the human monoblastic cell line U937 is more powerful than that of testosterone.[37] Furthermore, the distinct effects of the polymorphism in the sexes might also explain the poorer treatment response observed in boys with ALL than in girls with ALL.[38]

Our data also support the importance of *NQO1*2* homozygosity and the *del{GSTT1}* genotype in increased susceptibility to AL, which is particularly evident in males. This association suggests that gender might influence the risk of AL when associated with genetic polymorphisms in drug-metabolizing enzymes. Furthermore, the interaction of gender with such polymorphisms could contribute to a better understanding of the higher incidence of AL in males. However, the relevance of gender as a modifier of the risk of developing leukemia clearly requires further experimental study.

Authors' Contributions

PB was the principal investigator and takes primary responsibility for the paper. MC performed the laboratory assays. MAS and MJC contributed to the conception and design of the study, and to the statistical analysis. EB performed the statistical analysis and interpreted the results. PB and MAS wrote the manuscript. DC, JC and JRG contributed to the revision and scientific review of the manuscript. The order of the authorship was a joint decision by the authors. All authors approved the final version of this article. JC and MJC prepared Table 1, and MC prepared Table 2; the figures were prepared by EB.

Conflict of Interest

The authors reported no potential conflicts of interest.

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