NQO1, *GSTM1* AND *GSTT1* POLYMORPHISMS ARE ASSOCIATED WITH CHILDHOOD ACUTE MYELOID LEUKEMIA SUBTYPES AND TYPE I MUTATIONS

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BACKGROUND

Childhood Acute Myeloid Leukemia (c-AML) is a quite heterogeneous disease, regarding its morphology, immunophenotypes, cytogenetics and molecular features. In Brazil, age-adjusted incidence rates range from 13.3 to 24.5 cases per million, which is above the incidence rates in high-income countries. Genetic syndromes are responsible for <10% of c-AML cases and several studies have attempted to unravel environmental causative factors for the disease. Once polymorphic variations in genes that influence the way each individual handles environmental exposures can contribute for a higher attributable risk for cancer, genetic susceptibility has emerged as a low penetrance risk factor for c-AML (Figure 1). For instance, benzene is a environmental pollutant, associated to AML, which is metabolized in humans in cascade, by several enzymes, including CYP2E1, and epoxide hydrolase (EPHX1) which produce more reactive metabolites, and NAD(P)H quinone dehydrogenase 1 (NQO1) and glutathione S-transferases (GSTs), that act as detoxifiers (Figure 2). Therefore, genetic polymorphisms that interfere with those enzymatic functions can contribute to accumulation of reactive metabolites potentially harmful to DNA.

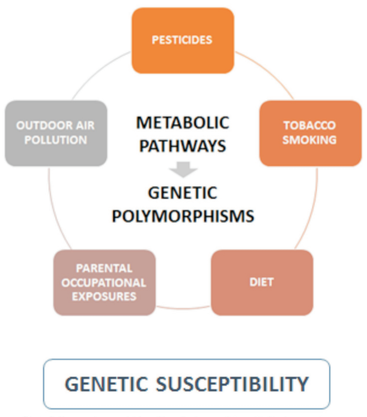

Figure 1. Gene-environment interactions. Environmental xenobiotics, in general, undergo metabolic pathways within human tissues driven by several enzymes, which are highly polymorphic. Those variations confer differences in genetic susceptibility to toxicants among individuals.

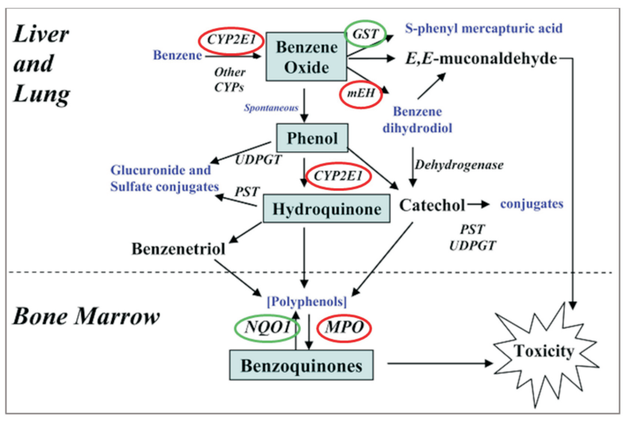

Figure 2. Metabolism of benzene to toxic metabolites. Benzene oxide, the benzoquinones, muconaldehydes, and benzene diol epoxides are electrophiles that readily react with peptides and proteins. Red circles: enzymes that produce more reactive metabolites. Green circles: detoxifying enzymes. CYP2E1, cytochrome P450 2E1. GST, glutathione S-transferases. mEH, epoxide hydrolase 1. MPO, myeloperoxidase. NQO1, NAD(P)H quinone dehydrogenase 1. PST, sulfotransferase 1A1. UDPGT, UDP glucuronosyltransferase 1A1. (SMITH *et al*, 2011 adapted).

AIMS

In an attempt to identify causative factors underlying the unique epidemiological features of c-AML in Brazil, and considering the lack of information about genetic susceptibility in c-AML, we conducted a case-control study in order to investigate: (1) whether xenobiotic genetic polymorphisms - *CYP2E1* rs3813867, *EPHX1* rs1051740, rs2234922, *NQO1* rs1800566 and *GSTM1/GSTT1* deletions - are associated with c-AML risk; and (2) if any association with recurrent genetic abnormalities would discriminate the differences in frequencies of c-AML subtypes in Brazil.

MATERIAL AND METHODS

Study design is schematized in Figure 3. Hardy-Weinberg equilibrium (HWE) was calculated for controls, comparing observed with expected genotype frequencies. Statistical analysis were performed to estimate odds ratio (OR), age-adjusted OR (adjOR), and 95% confidence interval (95%CI), with chi-square tests, considering P-value < 0.05 as statistically significant. This study was approved by The Ethics and Scientific Committee of Instituto Nacional de Câncer (CEP #186.688), as well as by the Ethics committee of all collaborating Brazilian institutions.

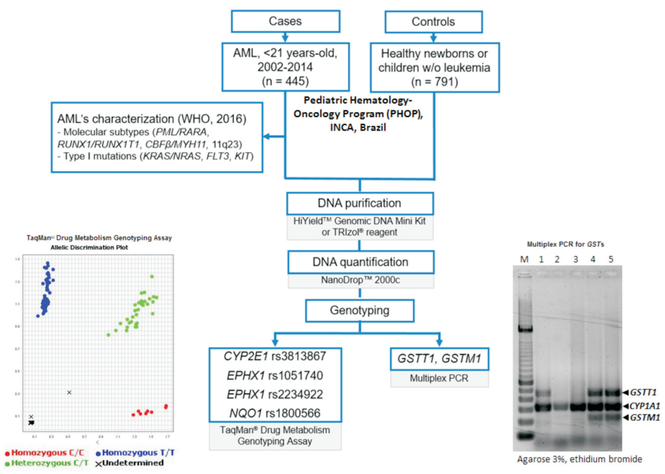

Figure 3. Study design. AML, acute myeloid leukemia.

RESULTS

The majority of cases were 2 to 10 years-old (40.9%), males (56.2%) and non-whites (54.2%). Cases and controls were compared in relation to demographic variables and genotype frequencies. There were no statistical differences between AML and controls in relation to gender (P = 0.680) or ethnicity (P = 0.617), but differed in age groups (P <0.001) **(Table 1)**. Concerning AML subtypes, APL was the most frequent subtype **(Figure 4)**. Somatic mutations in *FLT3* (24.1%), *NRAS* (12.5%), *KRAS* (5.8%) and *KIT* (7.3%) were observed. All the genetic polymorphisms were in accordance with Hardy-Weinberg equilibrium, and we observed that genotype frequencies of *EPHX1* rs1051740 were significantly different between cases and controls **(Table 2)**. The increased risk conferred by rs1051740 was specific to non-whites (OR 1.69, 95%CI 1.17-2.45, P = 0.005), males (OR 1.52, 95%CI 1.05-2.19, P = 0.025) and AML ≥2 - 10 years (OR 1.47, 95%CI 1.03-2.09, P = 0.033), under dominant model. *EPHX1* rs1051740, *NQO1* rs1800566 and GST null genotypes were significantly associated with some specific c-AML subtypes and somatic mutations **(Tables 3 and 4)**. No significant associations were found for other c-AML subtypes, somatic mutations, and *CYP2E1* rs3813867 and *EPHX1* rs2234922 polymorphisms (data not shown).

CONCLUSION

Results show that *EPHX1*, *NQO1* and *GSTs* variants are associated with specific AML subtypes, like APL and FLT3 mut. This might explain why APL frequencies are higher in our country than in other populations. *EPHX1* rs1051740 result in 28T>C substitution, which reduces enzymatic activity of epoxide hydrolase in 50%, and could act favouring hydroquinone formation by CYP2E1. In turn, *NQO1* rs1800566, which results in 609C>T substitution and loss of quinone dehydrogenase activity, added to *GSTM1* and *GSTT1* null genotypes, contribute to accumulation of toxic metabolites in the bone marrow that can potentially damage DNA and disrupt myeloid signaling pathways. As perspectives of this work, we intend to conclude genotyping of cases and controls regarding *MPO* polymorphism (rs2333227), and realize gene-environment interaction analysis, using questionnaire data about environmental exposures.

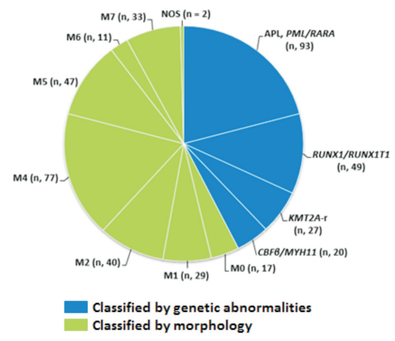

Figure 4. AML subtypes by WHO classification (2016).

Table 1. Main demographic features in controls and AML cases, Brazil (2002-2014).

	Controls, N (%)	Cases, N (%)	*P*-value
Total	791 (100.0)	445 (100.0)	
Age (months)			
< 24	609 (77.0)	102 (22.9)	<0.001
≥ 24-120	139 (17.6)	184 (41.3)	
≥ 120	43 (5.4)	159 (35.7)	
Gender			
Male	431 (54.5)	251 (56.4)	0.680
Female	350 (44.2)	194 (43.6)	
Unknwon	10 (1.3)	0 (0.0)	
Skin color			
White	296 (37.4)	159 (35.7)	0.617
Non-White	421 (53.2)	241 (54.2)	
Unknown	74 (9.4)	45 (10.1)	

Table 2. Genotype frequencies of *CYP2E1, EPHX1, NQO1, GSTM1* and *GSTT1* genetic polymorphisms in controls and AML cases, Brazil (2002-2014).

	Controls, N (%)	Cases, N (%)	OR (95%CI)	*P*-value
Total	791 (100.0)	445 (100.0)		
***CYP2E1* rs3813867 G>C**				
GG	362/414 (87.4)	381/436 (87.4)	1.0*	
GC	48/414 (11.6)	54/436 (12.4)	1.07 (0.71-1.62)	0.753
CC	4/414 (1.0)	1/436 (0.2)	0.24 (0.03-2.14)	0.208
C allele frequency	0.07	0.06		
HW equilibrium	0.101	0.525		
***EPHX1* rs1051740 T>C**				
TT	253/414 (61.1)	232/431 (53.8)	1.0*	
TC	138/414 (33.3)	161/431 (37.4)	1.27 (0.95-1.70)	0.105
CC	23/414 (5.6)	38/431 (8.8)	1.81 (1.05-3.13)	**0.032**
C allele frequency	0.22	0.27		
HW equilibrium	0.467	0.190		
***EPHX1* rs2234922 A>G**				
AA	258/414 (62.3)	280/436 (64.2)	1.0*	
AG	141/414 (34.1)	135/436 (31.0)	0.88 (0.66-1.18)	0.383
GG	15/414 (3.6)	21/436 (4.8)	1.22 (0.61-2.44)	0.566
G allele frequency	0.21	0.20		
HW equilibrium	0.425	0.369		
***NQO1* rs1800566 C>T**				
CC	411/738 (55.7)	237/433 (54.7)	1.0*	
CT	280/738 (37.9)	161/433 (37.2)	0.86 (0.64-1.16)	0.318
TT	47/738 (6.4)	35/433 (8.1)	1.19 (0.68-2.06)	0.550
T allele frequency	0.25	0.27		
HW equilibrium	0.941	0.303		
GSTM1				
Non-null	361/602 (60.0)	245/404 (60.6)	1.0*	
Null	241/602 (40.0)	159/404 (39.4)	1.10 (0.79-1.54)	0.580
GSTT1				
Non-null	455/602 (75.6)	311/404 (77.0)	1.0*	
Null	147/602 (24.4)	93/404 (23.0)	1.00 (0.68-1.48)	0.990

OR, odds ratio (age-adjusted OR for *NQO1* and *GSTM1/GSTT1*). CI, confidence interval. *Reference genotype.

Table 3. Genotype frequencies of *CYP2E1, EPHX1, NQO1, GSTM1* and *GSTT1* gene polymorphisms in controls and AML cases, according to WHO classification, Brazil (2002-2014).

	Controls, N (%)	Acute promyelocytic leukemia, *PML-RARα*			AML with abnormal marrow eosinophils, *CBFB/MYH11*			AML with minimal differentiation or without maturation			Acute myelomonocytic leukemia or Acute monoblastic and monocytic leukemia		
		Cases, N (%)	OR (95%CI)	*P*-value	Cases, N (%)	OR (95%CI)	*P*-value	Cases, N (%)	OR (95%CI)	*P*-value	Cases, N (%)	OR (95%CI)	*P*-value
***CYP2E1* rs3813867 G>C**													
GG	362 (87.4)	80 (87.9)	1.0*	0.902	18 (94.7)	1.0*	0.491	43 (93.5)	1.0*	0.231	108 (87.1)	1.0*	0.920
GC+CC	52 (12.6)	11 (12.1)	0.96 (0.48-1.92)		1 (5.3)	0.39 (0.05-2.96)		3 (6.5)	0.49 (0.15-1.62)		16 (12.9)	1.03 (0.57-1.88)	
GG+GC	410 (99.0)	91 (100.0)	1.0*	1.000	19 (100.0)	1.0*	1.000	46 (100.0)	1.0*	1.000	123 (99.2)	1.0*	1.000
CC	4 (1.0)	0 (0.0)	-		0 (0.0)	-		0 (0.0)	-		1 (0.8)	0.83 (0.09-7.53)	
***EPHX1* rs1051740 T>C**													
TT	253 (61.1)	45 (50.6)	1.0*	0.066	7 (38.9)	1.0*	0.059	19 (41.3)	1.0*	**0.010**	72 (58.5)	1.0*	0.608
TC+CC	161 (38.9)	44 (49.4)	1.54 (0.97-2.43)		11 (61.1)	2.47 (0.94-6.50)		27 (58.7)	**2.23 (1.20-4.15)**		51 (41.5)	1.11 (0.74-1.68)	
TT+TC	391 (94.4)	84 (94.4)	1.0*	1.000	13 (72.2)	1.0*	**0.004**	42 (91.3)	1.0*	0.332	114 (92.7)	1.0*	0.469
CC	23 (5.6)	5 (5.6)	1.01 (0.37-2.74)		5 (27.8)	**6.54 (2.15-19.92)**		4 (8.7)	1.62 (0.53-4.91)		9 (7.3)	1.34 (0.60-2.98)	
***EPHX1* rs2234922 A>G**													
AA	257 (62.2)	57 (63.3)	1.0*	0.844	12 (66.7)	1.0*	0.703	31 (67.4)	1.0*	0.492	89 (71.8)	1.0*	0.051
AG+GG	156 (37.8)	33 (36.7)	0.95 (0.59-1.53)		6 (33.3)	0.82 (0.30-2.24)		15 (32.6)	0.80 (0.42-1.52)		35 (28.2)	0.65 (0.42-1.01)	
AA+AG	398 (96.4)	87 (96.7)	1.0*	1.000	18 (100.0)	1.0*	1.000	46 (100.0)	1.0*	0.382	119 (96.0)	1.0*	0.790
GG	15 (3.6)	3 (3.3)	0.92 (0.26-3.23)		0 (0.0)	-		0 (0.0)	-		5 (4.0)	1.12 (0.40-3.13)	
***NQO1* rs1800566 C>T**													
CC	411 (55.7)	52 (57.8)	1.0*	0.792	11 (55.0)	1.0*	0.935	21 (47.7)	1.0*	0.375	59 (48.0)	1.0*	0.295
CT+TT	327 (44.3)	38 (42.2)	0.93 (0.55-1.59)		9 (45.0)	1.04 (0.41-2.67)		23 (52.3)	1.35 (0.70-2.59)		64 (52.0)	1.25 (0.82-1.90)	
CC+CT	691 (93.6)	80 (88.9)	1.0*	**0.028**	18 (90.0)	1.0*	0.263	42 (95.5)	1.0*	0.965	110 (89.4)	1.0*	**0.017**
TT	47 (6.4)	10 (11.1)	**2.75 (1.12-6.76)**		2 (10.0)	2.53 (0.50-12.87)		2 (4.5)	0.97 (0.21-4.50)		13 (10.6)	**2.38 (1.17-4.83)**	
GSTM1													
Non-null	361 (60.0)	48 (55.8)	1.0*	0.086	10 (55.6)	1.0*	0.088	27 (67.5)	1.0*	0.991	71 (59.2)	1.0*	0.147
Null	241 (40.0)	38 (44.2)	1.81 (0.92-3.55)		8 (44.4)	2.80 (0.86-9.09)		13 (32.5)	1.01 (0.45-2.26)		49 (40.8)	1.44 (0.88-2.34)	
GSTT1													
Non-null	455 (75.6)	60 (69.8)	1.0*	**0.019**	17 (94.4)	1.0*	0.145	29 (72.5)	1.0*	0.309	97 (80.8)	1.0*	0.484
Null	147 (24.4)	26 (30.2)	**2.31 (1.15-4.65)**		1 (5.6)	0.21 (0.03-1.73)		11 (27.5)	1.56 (0.66-3.66)		23 (19.2)	0.81 (0.45-1.46)	
GSTM1/M1													
Non-null[1]	536 (89.0)	73 (84.9)	1.0*	0.099	18 (100.0)	1.0*	0.997	35 (87.5)	1.0*	0.442	105 (87.5)	1.0*	0.472
Null[2]	66 (11.0)	13 (15.1)	2.15 (0.87-5.32)		0 (0.0)	-		5 (12.5)	1.56 (0.50-4.85)		15 (12.5)	1.31 (0.63-2.76)	

AML, acute myeloid leukemia. OR, odds ratio (age-adjusted OR for *NQO1* and *GSTM1/GSTT1*). CI, confidence interval. *Reference genotype. [1]Non-null genotype for *GSTT1* and/or *GSTM1*. [2]Null genotype for both *GSTT1* and *GSTM1*.

Table 4. Genotype frequencies of *CYP2E1, EPHX1, NQO1, GSTM1* and *GSTT1* gene polymorphisms in controls and AML cases, according to Type I mutations, Brazil (2002-2014).

	Controls, N (%)	AML with *FLT3* mutation			AML with *NRAS* mutation			AML with any Type I mutation**		
		Cases, N (%)	OR (95%CI)	*P*-value	Cases, N (%)	OR (95%CI)	*P*-value	Cases, N (%)	OR (95%CI)	*P*-value
***CYP2E1* rs3813867 G>C**										
GG	362 (87.4)	88 (90.7)	1.0*	0.370	35 (81.4)	1.0*	0.264	139 (88.5)	1.0*	0.722
GC+CC	52 (12.6)	9 (9.3)	0.71 (0.34-1.50)		8 (18.6)	1.59 (0.70-3.62)		18 (11.5)	0.90 (0.51-1.60)	
GG+GC	410 (99.0)	97 (100.0)	1.0*	1.000	43 (100.0)	1.0*	1.000	157 (100.0)	1.0*	0.579
CC	4 (1.0)	0 (0.0)	-		0 (0.0)	0.91 (0.88-0.93)		0 (0.0)	-	
***EPHX1* rs1051740 T>C**										
TT	253 (61.1)	47 (49.0)	1.0*	**0.029**	25 (58.1)	1.0*	0.704	82 (52.9)	1.0*	0.076
TC+CC	161 (38.9)	49 (51.0)	**1.64 (1.05-2.56)**		18 (41.9)	1.13 (0.60-2.14)		73 (47.1)	1.40 (0.96-2.03)	
TT+TC	391 (94.4)	89 (92.7)	1.0*	0.515	40 (93.0)	1.0*	0.726	144 (92.9)	1.0*	0.490
CC	23 (5.6)	7 (7.3)	1.34 (0.56-3.21)		3 (7.0)	1.28 (0.37-4.43)		11 (7.1)	1.30 (0.62-2.73)	
***EPHX1* rs2234922 A>G**										
AA	257 (62.2)	63 (65.6)	1.0*	0.535	27 (62.8)	1.0*	0.942	103 (66.0)	1.0*	0.402
AG+GG	156 (37.8)	33 (34.4)	0.86 (0.54-1.38)		16 (37.2)	0.98 (0.51-1.87)		53 (34.0)	0.85 (0.58-1.25)	
AA+AG	398 (96.4)	94 (97.9)	1.0*	0.752	41 (95.3)	1.0*	0.669	151 (96.8)	1.0*	0.805
GG	15 (3.6)	2 (2.1)	0.57 (0.13-2.51)		2 (4.7)	1.29 (0.29-5.86)		5 (3.2)	0.88 (0.31-2.46)	
***NQO1* rs1800566 C>T**										
CC	411 (55.7)	52 (53.6)	1.0*	0.906	23 (53.5)	1.0*	0.879	81 (51.6)	1.0*	0.687
CT+TT	327 (44.3)	45 (46.4)	1.03 (0.60-1.78)		20 (46.5)	1.05 (0.54-2.06)		76 (48.4)	1.09 (0.71-1.68)	
CC+CT	691 (93.6)	94 (96.9)	1.0*	0.924	42 (97.7)	1.0*	0.628	151 (96.2)	1.0*	0.774
TT	47 (6.4)	3 (3.1)	0.93 (0.23-3.81)		1 (2.3)	0.60 (0.08-4.78)		6 (3.8)	0.86 (0.30-2.44)	
GSTM1										
Non-null	361 (60.0)	47 (52.8)	1.0*	**0.018**	23 (54.8)	1.0*	0.171	83 (55.7)	1.0*	0.068
Null	241 (40.0)	42 (47.2)	**2.41 (1.17-4.99)**		19 (45.2)	1.74 (0.79-3.84)		66 (44.3)	1.65 (0.96-2.82)	
GSTT1										
Non-null	455 (75.6)	58 (65.2)	1.0*	0.067	32 (76.2)	1.0*	0.728	109 (73.2)	1.0*	0.473
Null	147 (24.4)	31 (34.8)	2.01 (0.95-4.22)		10 (23.8)	1.17 (0.48-2.87)		40 (26.8)	1.24 (0.69-2.23)	
GSTM1/M1										
Non-null[1]	536 (89.0)	71 (79.8)	1.0*	**0.042**	33 (78.6)	1.0*	0.058	124 (83.2)	1.0*	0.051
Null[2]	66 (11.0)	18 (20.2)	**2.62 (1.03-6.65)**		9 (21.4)	2.70 (0.97-7.56)		25 (16.8)	2.10 (0.99-4.40)	

AML, acute myeloid leukemia. OR, odds ratio (age-adjusted OR for *NQO1* and *GSTM1/GSTT1*). CI, confidence interval. *Reference genotype. [1]Non-null genotype for *GSTT1* and/or *GSTM1*. [2]Null genotype for both *GSTT1* and *GSTM1*.

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