EPHX1 rs1051740 T>C, *MPO* rs2333227 G>A AND *GSTT1* NULL ARE ASSOCIATED WITH SUSCEPTIBILITY TO CHILDHOOD ACUTE MYELOID LEUKEMIA SUBTYPES

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BACKGROUND

Childhood acute myeloid leukemia (c-AML) is a rare and heterogeneous disease that can manifest in a wide variety of morphological and immunophenotypic subtypes. In Brazil, age-adjusted incidence rates range from 11.3 to 24.5 cases per million children, with a high proportion of acute promyelocytic leukemia. Its etiology is unknown, but some environmental exposures, e.g. tobacco smoke, alcohol consumption, pesticides and topo-II inhibitors exposures, were associated with c-AML risk. Benzene is a ubiquitous environmental pollutant, classified as carcinogenic to humans and associated with myeloid disorders. Its hematotoxic effects are due to the formation of reactive metabolites by human xenobiotic biotransformation pathways. Benzene is metabolized mainly by cytochrome P450 2E1 (CYP2E1), epoxide hydrolase (EPHX1), quinone dehydrogenase 1 (NQO1), myeloperoxidase (MPO) and glutathione S-transferases (GSTs), which are encoded by highly polymorphic genes (**Figure 1**). Considering that, our aim was to investigate the associations of genetic polymorphisms in *CYP2E1*, *EPHX1*, *MPO*, *NQO1*, *GSTM1* and *GSTT1* with c-AML risk.

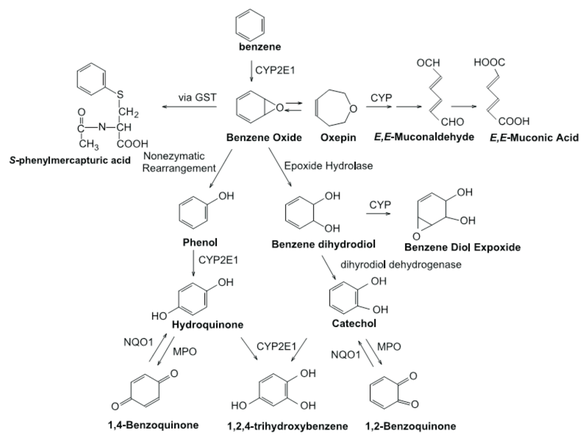

Figure 1. Benzene metabolism pathway (Adapted from KIM *et al*, 2006).

Benzene oxide, benzoquinones, muconaldehydes and benzenediolepoxides are electrophiles that readily react with peptides and nucleic acids (SMITH, 2010).

MATERIAL AND METHODS

This was a case-control study, which included 440 cases (c-AML <21 years) and 416 healthy controls (**Figure 2**). Cases were diagnosed and characterized by morphological, immunophenotypic and molecular-cytogenetic analyses. *CYP2E1* rs3813867, *EPHX1* rs1051740, rs2234922 and *NQO1* rs1800566 were genotyped by real time PCR, *MPO* rs2333227 by Sanger sequencing, and *GSTM1* and *GSTT1* deletions by multiplex PCR. Demographic characteristics and genotypic frequencies were compared by chi-squared or Fisher's test. Odds ratios (OR) with 95% confidence intervals (95%IC) were calculated based on co-dominant, dominant and recessive models. P-value <0,05 was considered statistically significant. INCA's Ethics Committee has approved this study (#186.688).

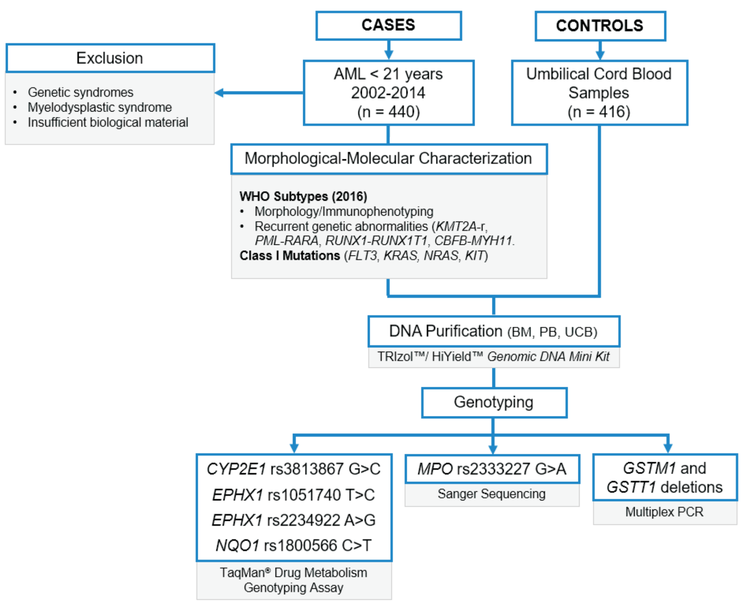

Figure 2. Study design.

AML, acute myeloid leukemia. BM, bone marrow. PB, peripheral blood. PCR, polymerase chain reaction. UCB, umbilical cord blood. WHO, World Health Organization.

RESULTS

1- Characteristics of the Study Population

Table 1. Demographic characteristics and genotypic frequencies of controls and c-AML cases, Brazil (2002-2014).

	Controls, n (%)	Cases, n (%)	*P*-value
Total	416 (100.0)	440 (100.0)	
Age (years)			
< 2	416 (100.0)	102 (23.2)	NA
= 2 – 10	-	179 (40.7)	
= 10	-	159 (36.1)	
Sex			
Male	237 (57.0)	250 (56.8)	0.964
Female	179 (43.0)	190 (43.2)	
Skin Color			
White	162 (38.9)	158 (35.9)	0.663
Non-White	232 (55.8)	241 (54.8)	
Unknown	22 (5.3)	41 (9.3)	
***CYP2E1* rs3813867 G>C**			
GG	362 (87.4)	376 (87.2)	0.354
GC	48 (11.6)	54 (12.5)	
CC	4 (1.0)	1 (0.2)	
***EPHX1* rs1051740 T>C**			
TT	253 (61.1)	231 (54.2)	0.057
TC	138 (33.3)	157 (36.9)	
CC	23 (5.6)	38 (8.9)	
***EPHX1* rs2234922 A>G**			
AA	258 (62.3)	277 (64.3)	0.457
AG	141 (34.1)	133 (30.9)	
GG	15 (3.6)	21 (4.9)	
***MPO* rs2333227 G>A**			
GG	206 (50.9)	218 (56.8)	0.153
GA	175 (43.2)	140 (36.5)	
AA	24 (5.9)	26 (6.8)	
***NQO1* rs1800566 C>T**			
CC	227 (54.8)	234 (54.7)	0.994
CT	154 (37.2)	159 (37.1)	
TT	33 (8.0)	35 (8.2)	
GSTM1			
Non-null	237 (59.7)	243 (60.6)	0.795
Null	160 (40.3)	158 (39.4)	
GSTT1			
Non null	307 (77.3)	309 (77.1)	0.927
Null	90 (22.7)	92 (22.9)	

c-AML, childhood acute myeloid leukemia. NA, not applicable.
All genotype frequencies are in accordance with Hardy-Weinberg equilibrium (*P*-value > 0.05).

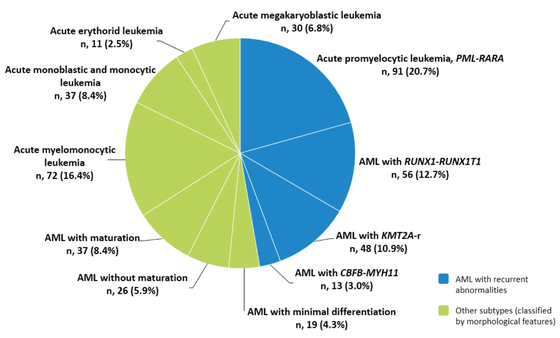

Figure 3. Frequency of childhood AML subtypes, according to World Health Organization Classification of Myeloid Neoplasms (2016). Total n = 440.

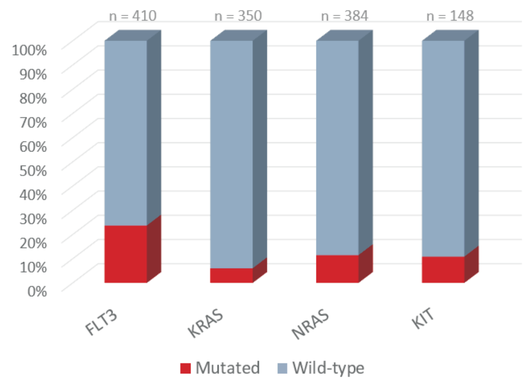

Figure 4. Frequency of Class I mutations in childhood AML cases. *FLT3* (internal tandem duplication or D835 mutation), *NRAS* and *KRAS* (mutations in codons G12 or G13), *KIT* (mutations in exons 8 or 17).

2- Associations of Genetic Polymorphisms with c-AML risk

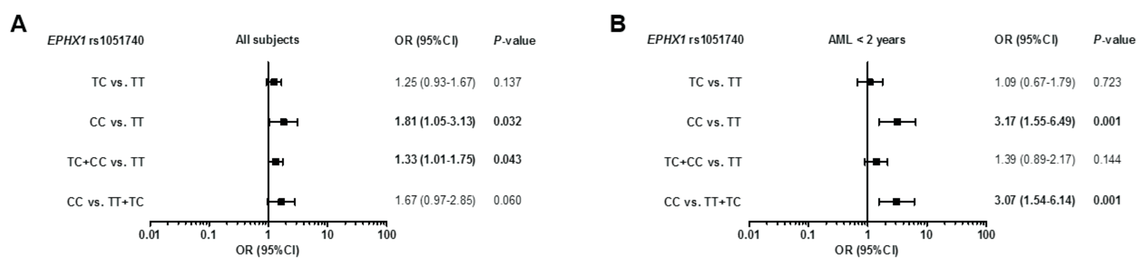

Figure 5. Risk associations of *EPHX1* rs1051740 with childhood AML.
A, All AML cases versus controls. B, AML aged up to 2 years versus controls. C, AML with *CBFB-MYH11* versus controls. D, AML with *KMT2A* rearrangement versus controls. E, AML without maturation versus controls. F, AML with *FLT3* mutated versus controls. AML, acute myeloid leukemia. OR, odds ratio. 95%CI, 95% confidence interval.

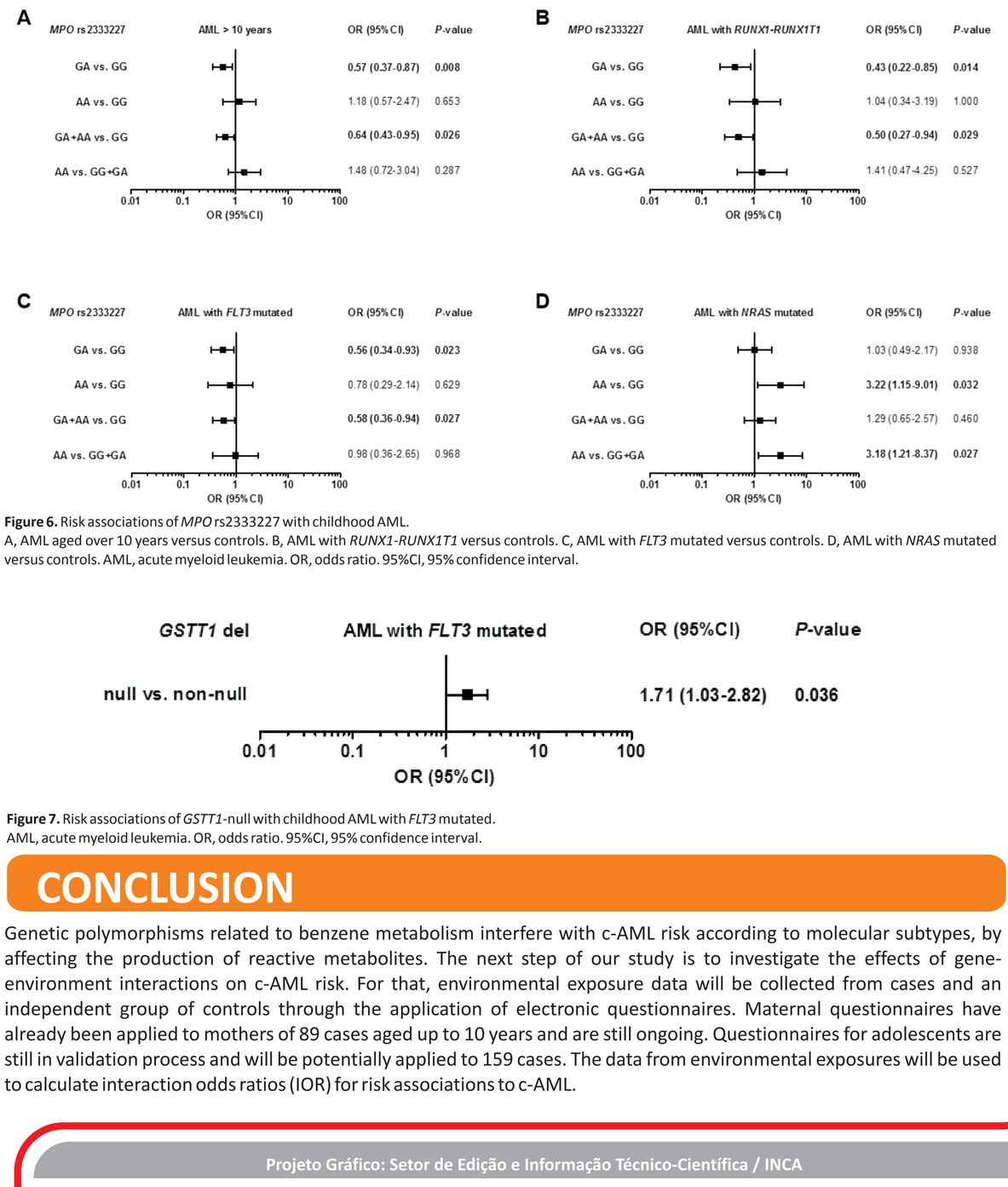

Figure 6. Risk associations of *MPO* rs2333227 with childhood AML.
A, AML aged over 10 years versus controls. B, AML with *RUNX1-RUNX1T1* versus controls. C, AML with *FLT3* mutated versus controls. D, AML with *NRAS* mutated versus controls. AML, acute myeloid leukemia. OR, odds ratio. 95%CI, 95% confidence interval.

Figure 7. Risk associations of *GSTT1*-null with childhood AML with *FLT3* mutated.
AML, acute myeloid leukemia. OR, odds ratio. 95%CI, 95% confidence interval.

CONCLUSION

Genetic polymorphisms related to benzene metabolism interfere with c-AML risk according to molecular subtypes, by affecting the production of reactive metabolites. The next step of our study is to investigate the effects of gene-environment interactions on c-AML risk. For that, environmental exposure data will be collected from cases and an independent group of controls through the application of electronic questionnaires. Maternal questionnaires have already been applied to mothers of 89 cases aged up to 10 years and are still ongoing. Questionnaires for adolescents are still in validation process and will be potentially applied to 159 cases. The data from environmental exposures will be used to calculate interaction odds ratios (IOR) for risk associations to c-AML.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA