NQO1, *GSTM1* and *GSTT1* variants are associated with pediatric acute myeloid leukemia with genetic abnormalities

Gisele D. Brisson [1], Francianne G. Andrade[1], Filipe V. S. Bueno[1], Bruno de A. Lopes[1]
Bruno A. A. Gonçalves[1], Eugênia Terra-Granado[1], Maria do S. Pombo-de-Oliveira[1]
[1]Pediatric Hematology-Oncology Program, Instituto Nacional de Câncer - INCA, Rio de Janeiro, Brasil

BACKGROUND

Acute myeloid leukemia (AML) is a rare disease in children and little is known about its etiopathology with risk factors. Studies have demonstrated that individual genetic predisposition can modulate the risk of DNA damage due to exogenous exposures. For instance, benzene is a environmental pollutant, associated to AML, which is metabolized in humans in cascade, including CYP2E1, and epoxide hydrolase (EPHX1) which produce more reactive metabolites, and NAD(P)H dehydrogenase quinone 1 (NQO1) and glutathione S-transferases (GSTs), that act as detoxifiers (**Fig.1**). Therefore, genetic polymorphisms that interfere with those enzymatic functions can contribute to accumulation of reactive metabolites potentially harmful to DNA, leading to susceptibility to develop neoplasia.

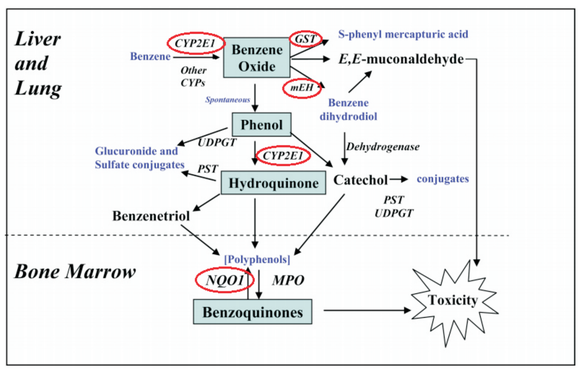

Figure 1. Metabolism of benzene to toxic metabolites. Benzene oxide, the benzoquinones, muconaldehydes, and benzene diol epoxides are electrophiles that readily react with peptides and proteins. The glutathione S-transferases (GST) and NAD(P)H dehydrogenase quinone 1 (NQO1) are responsible for neutralizing those compounds. CYP2E1, cytochrome P450 2E1. mEH, epoxide hydrolase 1. MPO, myeloperoxidase. PST, sulfotransferase 1A1. UDPGT, UDP glucuronosyltransferase 1A1. (SMITH *et al*, 2011)

AIM

The proposal of this study is to investigate the risk associations of *CYP2E1, GSTM1, GSTT1, EPHX1,* and *NQO1* polymorphisms and pediatric AML, characterized by recurrent genetic abnormalities.

METHODS

This is a case-control study, with 450 AML pediatric cases and 782 healthy controls from Brazil (**Fig.2**). The genomic DNA of bone marrow, peripheral or cord blood samples were extracted with QIAamp DNA Blood Mini Kit (QIAGEN) and genotyped for *CYP2E1* rs3813867, *EPHX1* rs1051740, rs2234922, and *NQO1* rs*1800566,* by real time PCR (TaqMan®, Drug Metabolism Genotyping Assays, Applied Biosystems) and for *GSTT1* and *GSTM1* homozygous deletion (null or non-null genotypes) by multiplex PCR. AML subtypes were characterized according to World Health Organization recommendations. Type I mutations – *KRAS/NRAS* (G12, G13), *FLT3* (D835 or ITD) and *KIT* (exons 8 and 17) – were investigated by direct sequencing. Statistical analysis was 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.

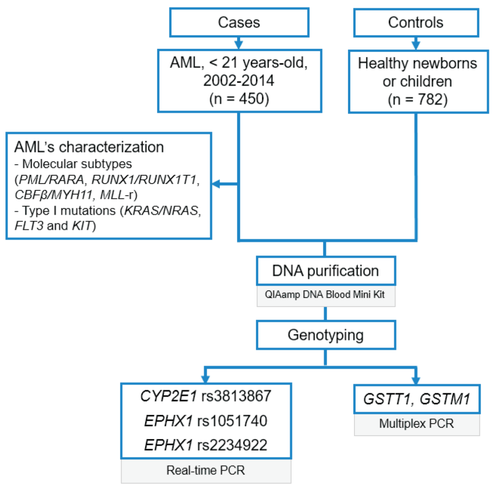

Figure 2. Study design. AML, acute myeloid leukemia

RESULTS

The majority of AML cases were 2-10 years old (40.9%), males (56.2%), and non-whites (54.2%). Cases and controls did not differ in relation to gender (P = 0.597), and skin color (P = 0.758), but did differ in relation to age groups (P <0.001). Frequencies of genetic polymorphisms are detailed in **Table 1**. Preliminary results are shown for *CYP2E1* and *EPHX1* polymorphisms (404 controls and 107 cases). AML's subtypes are shown in **Fig.3**. The frequency of *FLT3*, *NRAS*, *cKIT* and *KRAS* mutations were 24.2%, 12.5%, 7.3% and 5.8% of cases, respectively (**Table 2**). Under recessive model (CC+CT versus TT), *NQO1* 609C>T, as well as *GSTT1* null were associated with increased risk to promyelocytic leukemia (APL) (**Table 3**). *EPHX1* rs1051740 was strongly associated with AML-CBF group. *GSTM1* null genotype was associated with increased risk to APL with *PML/RARα* **(Table 4)** and AML with *FLT3*^{mut} (Table 5). Having *GSTM1* or *GSTT1* null genotype conferred increased risks to AML with *FLT3*^{mut} or any type I mutation. Borderline association between *GSTM1/T1* null genotype and AML with *NRAS*^{mut} were also found **(Table 5),** requiring further tests.

Table 1. Genotype frequencies of *CYP2E1, GSTM1, GSTT1, EPHX1*, and *NQO1* gene polymorphisms in controls and AML cases, Brazil (2002-2014).

	Controls, N (%)	Cases, N (%)	*P*-value
Total	782 (100.0)	450 (100.0)	
CYP2E1* rs3813867			
GG	352 (87.1)	92 (86.0)	0.755
GC	52 (12.9)	15 (14.0)	
CC	0 (0)	0 (0)	
GSTM1			
Non-null	359 (60.2)	245 (60.6)	0.897
Null	237 (39.8)	159 (39.4)	
GSTT1			
Non-null	454 (76.2)	311 (77.0)	0.768
Null	142 (23.8)	93 (23.0)	
EPHX1* rs1051740			
TT	247 (61.1)	62 (58.5)	0.321
TC	135 (33.4)	34 (32.1)	
CC	22 (5.4)	10 (9.4)	
EPHX1* rs2234922			
AA	250 (62.0)	66 (61.7)	0.902
AG	138 (34.2)	36 (33.6)	
GG	15 (3.7)	5 (4.7)	
NQO1* rs1800566			
CC	408 (56.0)	237 (54.7)	0.519
CT	274 (37.6)	161 (37.2)	
TT	46 (6.3)	35 (8.1)	

*All c ontrols were in accordance with Hardy -Weinberg equilibrium (rs3813867, *P*-value 0.167; rs1051740, *P*-value 0.531; rs2234922, *P*-value 0.449; rs1800566, *P*-value 0.999).

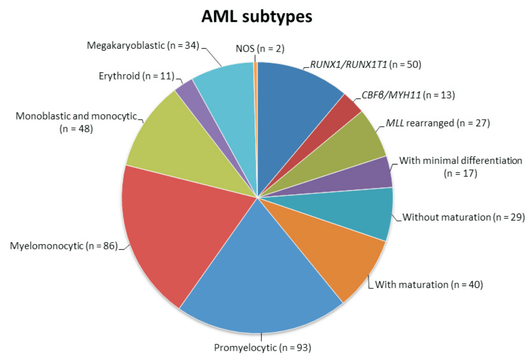

Figure 3. AML's subtypes (number of cases), according to WHO classification (2008).

Table 2. Frequency of Type I mutations in AML cases, Brazil (2002-2014).

Type I mutations	Cases, N (%)
FLT3 ITD or TKD mutations	100/414 (24.2)
NRAS G12D or G13D	43/344 (12.5)
KRAS G12D or G13D	22/382 (5.8)
cKIT exon 8 or 17 mutations	9/124 (7.3)

ITD, in tandem duplication. TKD, tyrosine kinase domain.

Table 3. Genotype frequencies and age-adjusted risk associations of *CYP2E1, GSTM1, GSTT1, EPHX1*, and *NQO1* gene polymorphisms in controls and APL or other AML subtypes, Brazil (2002-2014).

	Controls, N (%)	APL			Other AML subtypes		
		Cases, N (%)	aOR (95%CI)	*P*-value	Cases, N (%)	aOR (95%CI)	*P*-value
***CYP2E1* rs3813867**							
GG	352 (87.1)	24 (85.7)	1.0*		68 (86.1)	1.0*	
GC	52 (12.9)	4 (14.3)	1.13 (0.38-3.38)	0.773	11 (13.9)	1.10 (0.54-2.21)	0.799
GSTM1							
Non-null	359 (60.2)	48 (55.8)	1.0*		197 (61.9)	1.0*	
Null	237 (39.8)	38 (44.2)	1.81 (0.92-3.56)	0.084	121 (38.1)	1.10 (0.78-1.56)	0.590
GSTT1							
Non-null	454 (76.2)	60 (69.8)	1.0*		251 (78.9)	1.0*	
Null	142 (23.8)	26 (30.2)	**2.33 (1.16-4.69)**	**0.018**	67 (21.1)	0.89 (0.59-1.34)	0.570
***EPHX1* rs1051740**							
TT	247 (61.1)	15 (53.6)	1.0*		47 (60.3)	1.0*	
TC+CC	157 (38.9)	13 (46.4)	1.36 (0.63-2.94)	0.428	31 (39.7)	1.04 (0.63-1.70)	0.884
TT+TC	382 (94.6)	26 (92.9)	1.0*		70 (89.7)	1.0*	
CC	22 (5.4)	2 (7.1)	1.34 (0.30-5.99)	0.663	8 (10.3)	1.98 (0.85-4.64)	0.123
***EPHX1* rs2234922**							
AA	250 (62.0)	16 (57.1)	1.0*		50 (63.3)	1.0*	
AG+GG	153 (38.0)	12 (42.9)	1.23 (0.57-2.66)	0.607	29 (36.7)	0.95 (0.58-1.56)	0.833
AA+AG	388 (96.3)	28 (100.0)	1.0*		74 (93.7)	1.0*	
GG	15 (3.7)	0 (0)	-	0.613	5 (6.3)	1.75 (0.62-4.96)	0.349
***NQO1* rs1800566**							
CC	408 (56.0)	52 (57.8)	1.0*		185 (53.9)	1.0*	
CT+TT	320 (44.0)	38 (42.2)	0.94 (0.55-1.59)	0.803	158 (46.1)	0.93 (0.70-1.25)	0.646
CC+CT	682 (93.7)	80 (88.9)	1.0*		318 (92.7)	1.0*	
TT	46 (6.3)	10 (11.1)	**2.76 (1.12-6.78)**	**0.027**	25 (7.3)	1.19 (0.66-2.12)	0.566

APL, acute promyelocytic leukemia. AML, acute myeloid leukemia. aOR, age-adjusted odds ratio. CI, confidence interval. *Reference genotype.

Table 4. Genotype frequencies and age-adjusted risk associations of *CYP2E1, GSTM1, GSTT1, EPHX1*, and *NQO1* gene polymorphisms in controls and AML cases, according to recurrent genetic abnormalities, Brazil (2002-2014).

	Controls, N (%)	AML with CBF alterations**			AML with *PML/RARα*			AML with *MLL* rearrangements		
		Cases, N (%)	aOR (95%CI)	*P*-value	Cases, N (%)	aOR (95%CI)	*P*-value	Cases, N (%)	aOR (95%CI)	*P*-value
***CYP2E1* rs3813867**										
GG	352 (87.1)	9 (75.0)	1.0*		12 (85.7)	1.0*		6 (100.0)	1.0*	
GC	52 (12.9)	3 (25.0)	2.26 (0.59-8.61)	0.203	2 (14.3)	1.13 (0.25-5.18)	0.700	0 (0)	-	1.000
GSTM1										
Non-null	359 (60.2)	31 (59.6)	1.0*		18 (48.6)	1.0*		14 (66.7)	1.0*	
Null	237 (39.8)	21 (40.4)	1.57 (0.75-3.28)	0.228	19 (51.4)	**3.06 (1.17-7.98)**	**0.022**	7 (33.3)	0.77 (0.31-1.96)	0.587
GSTT1										
Non-null	454 (76.2)	44 (84.6)	1.0*		28 (75.7)	1.0*		17 (81.0)	1.0*	
Null	142 (23.8)	8 (15.4)	0.65 (0.26-1.66)	0.371	9 (24.3)	1.31 (0.47-3.67)	0.612	4 (19.0)	0.75 (0.25-2.28)	0.615
***EPHX1* rs1051740**										
TT	247 (61.1)	3 (25.0)	1.0*		6 (42.9)	1.0*		4 (66.7)	1.0*	
TC+CC	157 (38.9)	9 (75.0)	**4.72 (1.26-17.70)**	**0.016**	8 (57.1)	2.10 (0.71-6.16)	0.169	2 (33.3)	0.79 (0.14-4.35)	1.000
TT+TC	382 (94.6)	8 (66.7)	1.0*		13 (92.9)	1.0*		6 (100.0)	1.0*	
CC	22 (5.4)	4 (33.3)	**8.68 (2.43-31.07)**	**0.004**	1 (7.1)	1,34 (0.17-10.68)	0.553	0 (0)	-	1.000
***EPHX1* rs2234922**										
AA	250 (62.0)	10 (83.3)	1.0*		9 (64.3)	1.0*		4 (66.7)	1.0*	
AG+GG	153 (38.0)	2 (16.7)	0.33 (0.07-1.51)	0.224	5 (35.7)	0.91 (0.30-2.76)	0.864	2 (33.3)	0.82 (0.15-4.51)	1.000
AA+AG	388 (96.3)	11 (91.7)	1.0*		14 (100.0)	1.0*		6 (100.0)	1.0*	1.000
GG	15 (3.7)	1 (8.3)	2.35 (0.28-19.42)	0.380	0 (0)	-	1.000	0 (0)	-	
***NQO1* rs1800566**										
CC	408 (56.0)	34 (55.7)	1.0*		19 (51.4)	1.0*	0.413	18 (66.7)	1.0*	0.190
CT+TT	320 (44.0)	27 (44.3)	0.95 (0.53-1.70)	0.860	18 (48.6)	1.37 (0.65-2.88)		9 (33.3)	0.57 (0.24-1.32)	
CC+CT	682 (93.7)	58 (95.1)	1.0*		34 (91.9)	1.0*	0.121	27 (100.0)	1.0*	0.998
TT	46 (6.3)	3 (4.9)	1.21 (0.33-4.47)	0.781	3 (8.1)	2.98 (0.75-11.86)		0 (0)	-	

AML, acute myeloid leukemia. aOR, age-adjusted odds ratio. CI, confidence interval. Reference genotype. AML with *CBFB-MYH11* or *RUNX1-RUNX1T1*.

Table 5. Genotype frequencies and age-adjusted risk associations of *CYP2E1, GSTM1, GSTT1, EPHX1*, and *NQO1* gene polymorphisms in controls and AML cases with Type I mutations, Brazil (2002-2014).

	Controls, N (%)	AML with *FLT3* mutation			AML with *NRAS* mutation			AML with any Type I mutation**		
		Cases, N (%)	aOR (95%CI)	*P*-value	Cases, N (%)	aOR (95%CI)	*P*-value	Cases, N (%)	aOR (95%CI)	*P*-value
***CYP2E1* rs3813867**										
GG	352 (87.1)	22 (88.0)	1.0*		8 (88.9)	1.0*		34 (87.2)	1.0*	
GC	52 (12.9)	3 (12.0)	0.92 (0.27-3.19)	1.000	1 (11.1)	0.85 (0.10-6.90)	1.000	5 (12.8)	0.99 (0.37-2.66)	0.993
GSTM1										
Non-null	359 (60.2)	47 (52.8)	1.0*		23 (54.8)	1.0*		83 (55.7)	1.0*	
Null	237 (39.8)	42 (47.2)	**2.42 (1.17-5.00)**	**0.017**	19 (45.2)	1.75 (0.79-3.86)	0.167	66 (44.3)	1.66 (0.97-2.83)	0.065
GSTM1/T1										
Non-null[a]	533 (89.4)	71 (79.8)	1.0*		33 (78.6)	1.0*		124 (83.2)	1.0*	
Null[b]	63 (10.6)	18 (20.2)	**2.66 (1.05-6.76)**	**0.040**	9 (21.4)	2.76 (0.99-7.75)	0.053	25 (16.8)	**2.14 (1.02-4.52)**	**0.045**
***EPHX1* rs1051740**										
TT	247 (61.1)	15 (60.0)	1.0*		5 (55.6)	1.0*		22 (56.4)	1.0*	
TC+CC	157 (38.9)	10 (40.0)	1.05 (0.46-2.39)	0.910	4 (44.4)	1.26 (0.33-4.76)	0.741	17 (43.6)	1.22 (0.63-2.36)	0.564
TT+TC	382 (94.6)	23 (92.0)	1.0*		8 (88.9)	1.0*		36 (92.3)	1.0*	
CC	22 (5.4)	2 (8.0)	1.51 (0.33-6.82)	0.642	1 (11.1)	2.17 (0.26-18.13)	0.406	3 (7.7)	1.45 (0.41-5.07)	0.474
***EPHX1* rs2234922**										
AA	250 (62.0)	14 (56.0)	1.0*		5 (55.6)	1.0*		20 (51.3)	1.0*	
AG+GG	153 (38.0)	11 (44.0)	1.28 (0.57-2.90)	0.547	4 (44.4)	1.31 (0.35-4.94)	0.736	19 (48.7)	1.55 (0.80-3.00)	0.188
AA+AG	388 (96.3)	24 (96.0)	1.0*		8 (88.9)	1.0*		36 (92.3)	1.0*	
GG	15 (3.7)	1 (4.0)	1.08 (0.14-8.51)	1.000	1 (11.1)	3.23 (0.38-27.53)	0.302	3 (7.7)	2.16 (0.60-7.80)	0.206
***NQO1* rs1800566**										
CC	408 (56.0)	52 (53.6)	1.0*		23 (53.5)	1.0*		81 (51.6)	1.0*	
CT+TT	320 (44.0)	45 (46.4)	1.04 (0.60-1.78)	0.897	20 (46.5)	1.06 (0.54-2.07)	0.868	76 (48.4)	1.10 (0.72-1.69)	0.669
CC+CT	682 (93.7)	94 (96.9)	1.0*		42 (97.7)	1.0*		151 (96.2)	1.0*	
TT	46 (6.3)	3 (3.1)	0.94 (0.23-3.83)	0.926	1 (2.3)	0.60 (0.08-4.80)	0.630	6 (3.8)	0.86 (0.30-2.45)	0.780

AML, acute myeloid leukemia. aOR, age-adjusted odds ratio. CI, confidence interval. *Reference genotype. **_FLT3, NRAS, KRAS_ or _cKIT_ mutations.
[a]Non-null genotype for *GSTT1* and/or *GSTM1*. [b]Null genotype for both *GSTT1* and *GSTM1*.

CONCLUSION

Our results show that *NQO1* and *GSTs* variants are associated with specific AML subtypes (APL and FLT3^{mut}). EPHX1 rs1051740 variant has also been associated with specific AML (AML with CBF alterations). Since *GSTs* and *NQO1* encode for detoxifying enzymes, being essential for protecting bone marrow from reactive metabolites, (e.g. benzene derivatives), and *EPHX1* encodes for an hydrolase which acts in biotransformation of epoxides, genetic variants in this pathway can modulate the risk of DNA damage, leading to specific AML subtypes.

REFERENCES

1. WIEMELS, J., Perspectives on the causes of childhood leukemia. Chem Biol Interact, 2012. 196(3): 59-67.
2. SMITH, M. T. et al. Benzene, the Exposome and Future Investigations of Leukemia Etiology. Chem Biol Interact, 2011. 192(1-2): 155–159.
3. SWERDLOW, S. H. et al. WHO Classification of Tumors of Haemtopoetic and Lymphoid Tissues. Lyon, France: International Agency for Research on Cancer; 2008.

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