

**Escola Nacional de Saúde Pública  
Fundação Oswaldo Cruz, Ministério da Saúde,  
Rio de Janeiro**



# **Childhood leukemia: genetic-environmental interaction studies in Brazil**

**Sergio Koifman**

# Contents

- Infant acute leukemia: molecular epidemiology
- Brazilian multicentric enrollment
- Hospital based case-control study
- 230 IAL (170 ALL/60 AML) & 464 controls
- Standardized procedures of data collection
- MLL status and other biomarkers ascertainment

# Hypothesis

Benzoquinone  
Etoposids  
Isoflavones  
Anthraquinone  
Podophilin  
Quinolone  
Flavonoid



- Growing fetus is more sensitive to effects of potential of DNA damage insults
- Ligation + Inhibition of *TOPO II* during the early stage of pregnancy
- MLL rearrangements and Hybrid protein



IAL

# AIMS

- ❖ To conduct a molecular case-control study in Brazil, in order to explore the hypothesis that certain environmental exposures could increase the risk of IAL with *MLL* gene rearrangements;
- ❖ To identify molecular-cytogenetic markers that could be associated with maternal exposure to DNA damage substances during pregnancy;

# RECRUITMENT CRITERIA

## Case definition

- diagnosed with ALL or AML according to FAB classifications
- at age 21 months or less
- bone marrow aspirates were available for immunophenotyping and molecular analysis.
- The analyses to characterize *MLL* status were either performed by conventional karyotype, RT-PCR assay and/or by FISH.

## CONTROL SELECTION

All controls were age-frequency matched with IAL cases selected among hospitalized children in the same regional hospitals.

Controls were selected that presented with severe life-threatening conditions:

- trauma (6.3%), cardiopathy (9.1%), infectious diseases (19.7%)
- metabolic disorders (4.1%), neurological diseases (6.5%), sickle cell anemia (12.7%), non-syndrome defects (4.1%), allergy/asthma (14.5%), pneumonia (16.3%), nutritional disturbances, (6.8%)

## Data collection

- Mothers were interviewed in person in the hospital with the aid of a well-structured questionnaire divided in two major sections= i) devoted to childbirth and nursing; ii) to exposures during pregnancy.

- Questions regarding demographics included family income, maternal age and education level;

## Data collection

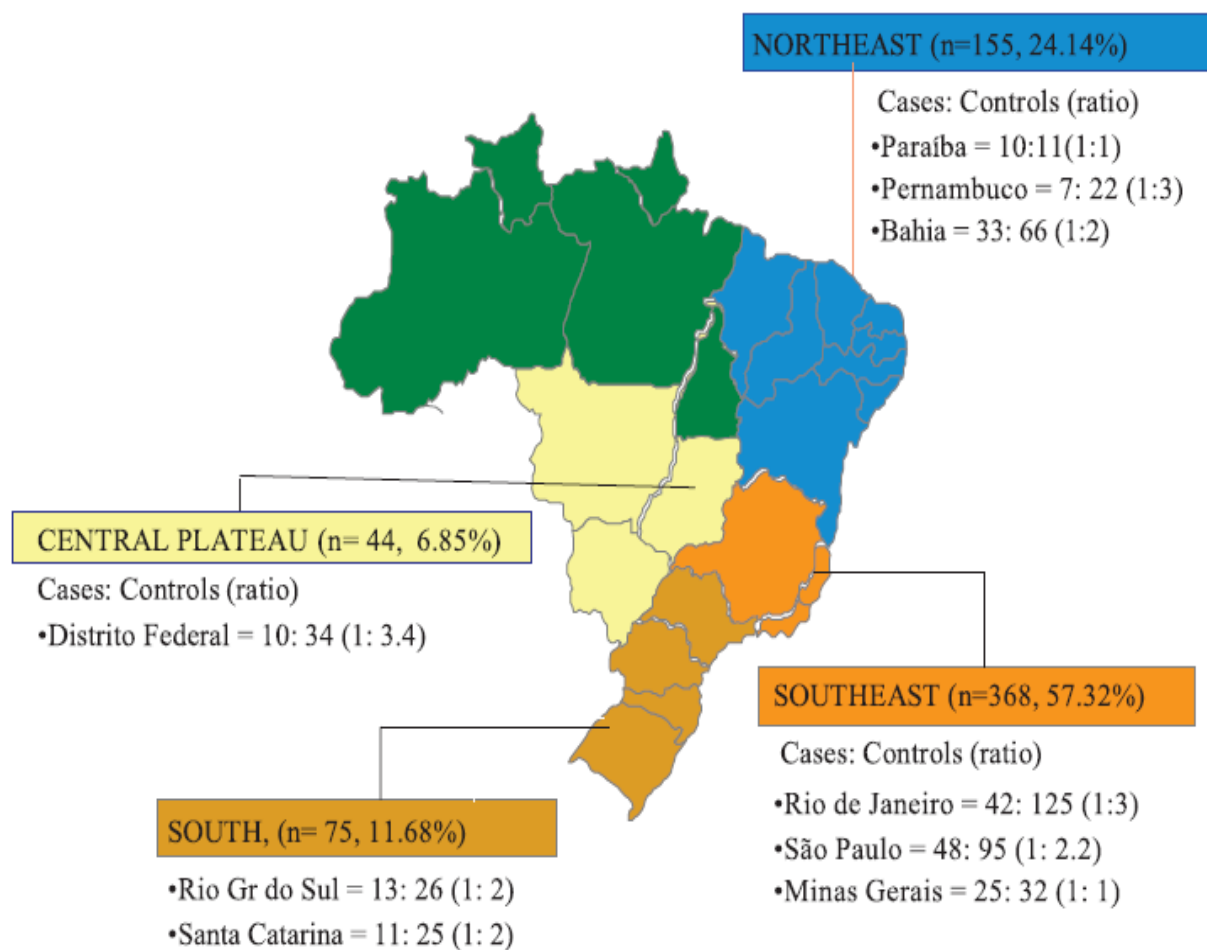
- Maternal history of diseases and reasons for use of medications- different types of drugs taken due to infectious illnesses, previous fetal loss , anemia, backache, others
- Maternal and paternal history of place of work exposures

# Infant Acute Leukemia and Maternal Exposures during Pregnancy

Maria S. Pombo-de-Oliveira,<sup>1</sup> Sergio Koifman,<sup>2</sup> and Brazilian Collaborative Study Group of Infant Acute Leukemia

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Cancer Epidemiol Biomarkers Prev 2006;15(12). December 2006



**Figure 1.** Distribution of cases and controls by geographic regions, Brazil, 1999-2005.

**Table 2. Maternal exposures during pregnancy, IAL cases and controls, Brazil, 1999-2005**

	IAL, <i>n</i> (%)	Controls, <i>n</i> (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
Tobacco	37 (18.3)	101 (23.0)	0.75 (0.49-1.15)	0.89 (0.631-1.25)
Marijuana	7 (3.5)	17 (3.9)	0.90 (0.37-2.20)	0.87 (0.63-1.20)
Pain relievers				
Dipyrone	124 (61.4)	228 (51.9)	1.48 (1.05-2.08)	1.45 (1.02-2.06)
Others†	50 (24.8)	111 (25.2)		0.97 (0.66-1.43)
Antibiotics				
Amoxicillin	25 (12.4)	62 (14.1)	0.86 (0.52-1.42)	0.88 (0.63-1.25)
Ciprofloxacin (quinolone)	5 (2.5)	11 (2.5)	0.99 (0.34-2.89)	0.94 (0.32-2.77)
Vitamins/iron supplement	73 (36.1)	169 (38.4)	0.77 (0.52-1.14)	0.90 (0.63-1.28)
Folic acid	28 (13.9)	47 (10.7)	1.35 (0.82-2.22)	1.22 (0.73-2.05)
Antiemetic	18 (8.9)	25 (5.7)	1.62 (0.86-3.04)	1.69 (0.87-3.28)
Antifungic (metronidazole)	38 (13.9)	44 (10.1)	1.45 (0.87-2.40)	1.39 (0.82-2.34)
Abortive drugs				
All‡	40 (19.8)	90 (20.5)	0.96 (0.63-1.45)	0.81 (0.53-1.25)
Misoprostol	6 (3.0)	7 (1.8)	1.28 (0.40-4.06)	1.23 (0.38-4.02)
Hormones§	18 (8.9)	4 (0.9)	10.66 (3.56-31.94)	8.76 (2.85-26.93)
Herbal infusions	4 (2.0)	5 (1.1)	1.76 (0.47-6.62)	1.93 (0.49-7.58)
Pesticides	91 (45.3)	119 (27.0)	2.23 (1.58-3.16)	2.18 (1.53-2.13)

\*Adjusted for sex, income, maternal age, and birth weight.

†Paracetamol, aspirin, hyoscine, and codeine.

‡Misoprostol, herbal infusions, and other compounds used as abortive.

§Oral contraceptives, antiabortive progesterone treatment, and thyroid hormones.

**Table 3. Hormonal intake during preconception and pregnancy and IAL according to MLL status, Brazil, 1999-2005**

Hormones intake	IAL, n (%)	MLL <sup>+ve</sup> , n (%)	MLL <sup>-ve</sup> , n (%)	Controls, n (%)	IAL vs controls, OR (95% CI)*	MLL <sup>+ve</sup> vs controls, OR (95% CI)*	MLL <sup>-ve</sup> vs controls, OR (95% CI)*
Preconception <sup>†</sup>							
Present	24 (12.2)	14 (21.5)	6 (7.7)	23 (5.3)	2.26 (1.21-4.21)	3.34 (1.51-7.36)	1.13 (0.40-3.14)
Absent	173 (87.8)	51 (78.5)	72 (92.3)	409 (94.7)			
1st trimester							
Present	17 (8.4)	5 (7.4)	4 (5.0)	3 (0.7)	11.35 (3.20-40.20)	10.57 (2.33-47.91)	7.55 (1.50-37.94)
Absent	185 (91.6)	63 (92.6)	76 (95.0)	438 (99.3)			
2nd trimester							
Present	7 (3.5)	1 (1.5)	2 (2.5)	3 (0.7)	4.49 (1.07-18.87)	2.62 (0.15-17.56)	3.52 (0.51-24.02)
Absent	195 (96.5)	67 (98.5)	78 (97.5)	438 (99.3)			
3rd trimester							
Present	6 (3.0)	1 (1.5)	3 (3.8)	3 (0.7)	2.32 (0.60-8.98)	1.02 (0.10-9.93)	3.94 (0.80-19.28)
Absent	196 (97.0)	67 (98.5)	77 (96.2)	438 (99.3)			

\*Reported hormonal intake 1 year before pregnancy.

<sup>†</sup>MLL status and hormonal exposure OR (case-case approach) adjusted for sex, income, maternal age, and birth weight.

**Table 4. Association of selected environmental exposures during pregnancy and *MLL* status, IAL cases, in a case-case analysis, Brazil, 1999-2005**

Exposure	Exposed and <i>MLL</i> <sup>+ve</sup> (n)	Unexposed and <i>MLL</i> <sup>+ve</sup> (n)	Exposed and <i>MLL</i> <sup>-ve</sup> (n)	Unexposed and <i>MLL</i> <sup>-ve</sup> (n)	Crude OR (95% CI)*	Adjusted OR <sup>†</sup> (95% CI)
Dipyrene	47	23	44	34	1.58 (0.80-3.08)	1.45 (0.75-2.86)
Metronidazole	12	54	7	72	2.29 (0.84-6.19)	1.72 (0.64-4.58)
Quinolones	2	65	1	79	2.43 (0.21-27.41)	2.25 (0.70-25.70)
Hormones	6	57	4	76	2.00 (0.54-7.42)	1.88 (0.50-7.01)
Misoprostol	3	17	2	10	0.88 (0.12-6.21)	0.44 (0.50-7.01)

\*Interaction OR between *MLL* gene status and selected exposures (case-only approach).

†ORs for *MLL* gene status and selected exposures adjusted for sex, income, maternal age, and birth weight.

## Full Paper

# High birth weight as an important risk factor for infant leukemia

**S Koifman<sup>1</sup>, MS Pombo-de-Oliveira<sup>\*2</sup> and The Brazilian Collaborative Study Group of Infant Acute Leukemia<sup>3</sup>**

<sup>1</sup>National School of Public Health/FIOCRUZ, Rua Leopoldo Bulhões, 1480, Rio de Janeiro, RJ 21041-210, Brazil; <sup>2</sup>Divisão de Medicina Experimental, Centro de Pesquisa – Brazilian National Cancer Institute, Rua André Cavalcanti, 37, CEP, Rio de Janeiro, RJ 20231-050, Brazil

In this paper, we compared the birth weight distribution among 201 infant leukaemia (IL) cases with that of 440 noncancer controls enrolled in Brazil in 1999–2005. Compared with the general population and the stratum 2500–2999 g as reference, IL cases weighing 3000–3999 g presented an odds ratio (OR) of 1.68 (95% CI: 1.03–2.76), and those of 4000 g or more, an OR of 2.28 (95% CI: 1.08–4.75),  $P_{\text{trend}} < 0.01$ . Using hospital-based controls, the OR for 4000 g or more, compared to 2500–2999 g, was 1.30 (95% CI: 1.02–1.43) after adjusting for confounders (gender, income, maternal age, pesticide and hormonal exposure during pregnancy). The results suggest that high birth weight is associated with increased risk of IL.

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**Keywords:** infant acute leukaemia; intrauterine factors; birth weight; *MLL* rearrangements

**Table 1** Birth weight distribution, ALL, AML, MLL status (1999–2005) and all births in Brazil (general population), 2000

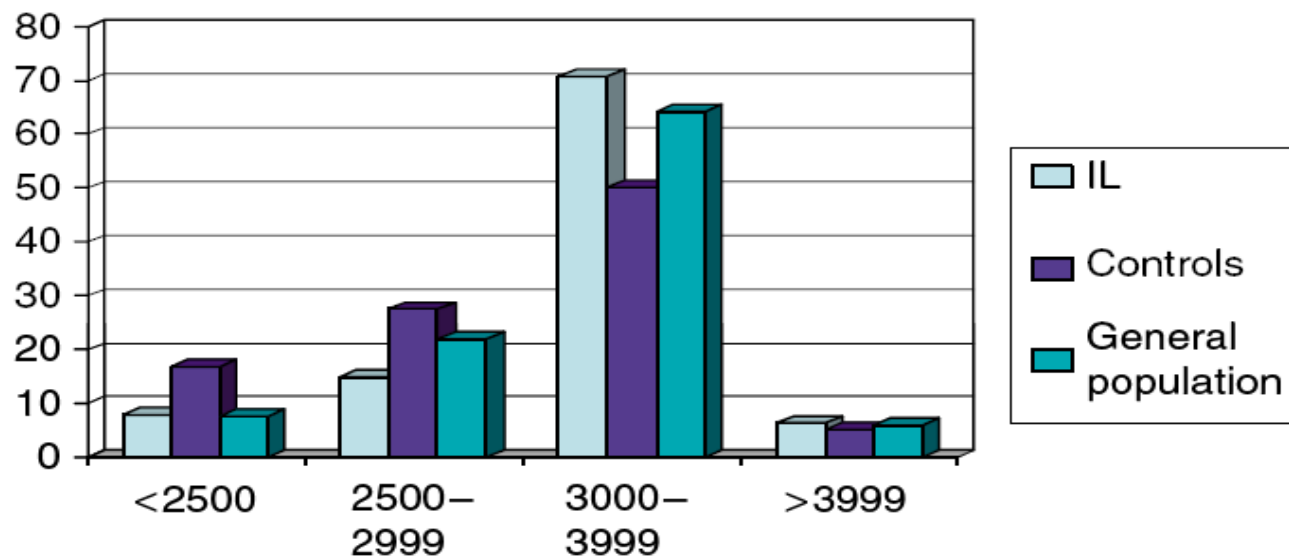
Birth weight (in g)	General population (all births)	IL		ALL		AML		MLL <sup>+ve</sup>		MLL <sup>-ve</sup>	
		n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
<2500	243835	16	1.52 (0.80–2.89)	11	1.50 (0.72–3.11)	5	1.59 (0.46–5.16)	6	1.56 (0.51–4.54)	3	0.61 (0.14–2.26)
2500–2999	696925	30	1.00	21	1.00	9	1.00	11	1.00	14	1.00
3000–3999	2036925	143	1.63 (1.08–2.47)	103	1.68 (1.03–2.76)	39	1.48 (0.64–3.28)	43	1.34 (0.67–2.75)	58	1.42 (0.77–2.66)
>3999	189476	13	1.59 (0.79–3.17) <sup>a</sup>	13	2.28 (1.08–4.75) <sup>b</sup>	0	—	8	2.68 (0.99–7.15) <sup>c</sup>	5	1.31 (0.41–3.89) <sup>d</sup>

ALL = acute lymphoblastic leukaemia; AML = acute myeloblastic leukaemia; CI = confidence interval; IL = infant leukaemia; OR = odds ratio. <sup>a</sup> $\chi^2$  for trend = 4.60,  $P = 0.03$ . <sup>b</sup> $\chi^2$  for trend = 6.66,  $P < 0.01$ . <sup>c</sup> $\chi^2$  for trend = 3.56,  $P = 0.059$ . <sup>d</sup> $\chi^2$  for trend = 0.91,  $P = 0.34$ .

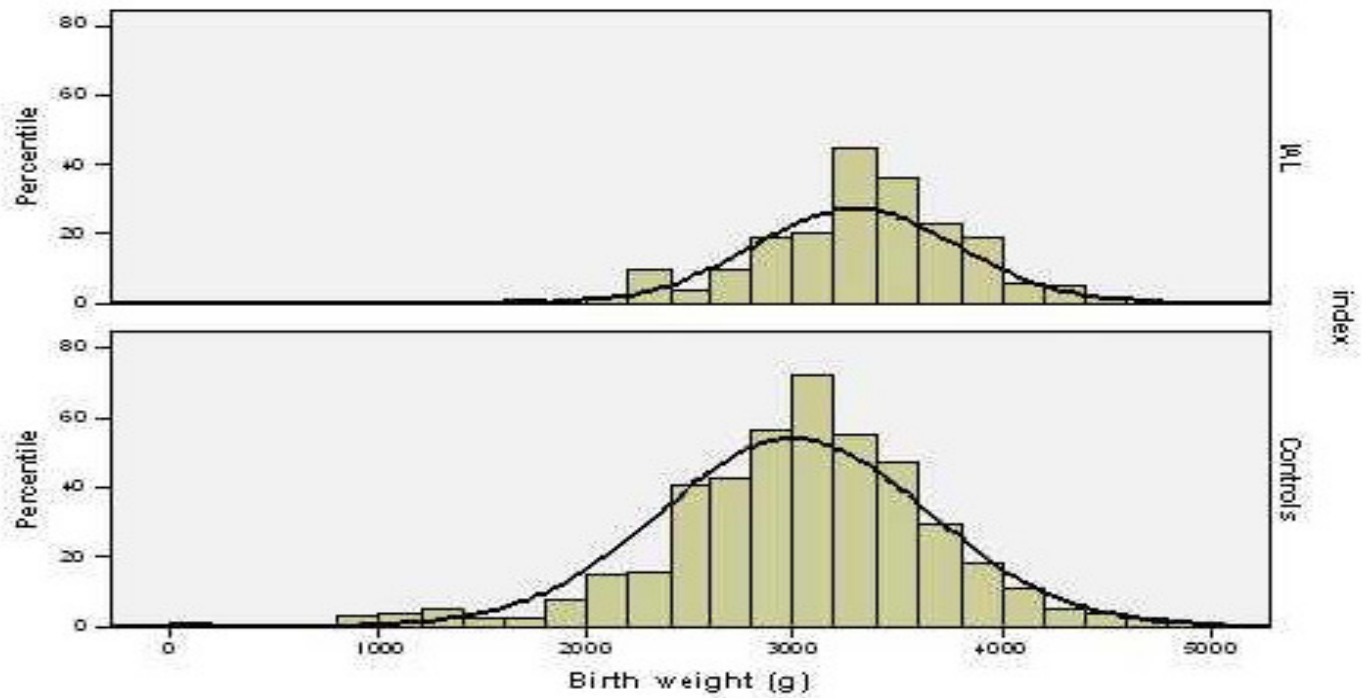
**Table 2** Birth weight distribution by weight strata, IL and hospital-based controls, Brazil, 1999–2005

Birth weight (in g)	Hospital-based controls	All IL			ALL			AML		
		n	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)	n	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)	n	Crude OR (95% CI)	Adjusted <sup>a</sup> OR (95% CI)
<2500	74	16	0.87 (0.42–1.79)	0.88 (0.41–1.86)	11	0.86 (0.36–1.99)	0.99 (0.41–2.42)	5	0.91 (0.25–3.12)	0.74 (0.21–2.55)
2500–2999	121	30	1.00	1.00	21	1.00	1.00	9	1.00	1.00
3000–3499	150	81	2.18 (1.31–3.63)	1.23 (1.04–1.46)	57	2.19 (1.18–2.93)	1.24 (1.02–1.50)	24	2.15 (0.91–5.20)	1.20 (0.91–1.58)
3500–3999	72	62	3.47 (1.00–6.08)	1.29 (1.12–1.48)	46	3.68 (1.96–6.96)	1.30 (1.11–1.52)	15	2.80 (1.08–7.36)	1.23 (0.98–1.55) <sup>b</sup>
>3999	23	13	2.28 (0.96–5.37)	1.20 (1.02–1.43) <sup>c</sup>	13	3.26 (1.32–8.01)	1.31 (1.09–1.57) <sup>d</sup>	—	—	—

ALL = acute lymphoblastic leukaemia; AML = acute myeloblastic leukaemia; CI = confidence interval; IL = infant leukaemia; OR = odds ratio. <sup>a</sup>Adjusted for sex, income, maternal age, pesticide exposure and hormonal intake during pregnancy. <sup>b</sup> $\chi^2$  for trend = 5.51,  $P = 0.018$ . <sup>c</sup> $\chi^2$  for trend = 16.70,  $P = 0.00004$ . <sup>d</sup> $\chi^2$  for trend = 18.14,  $P = 0.00002$ .

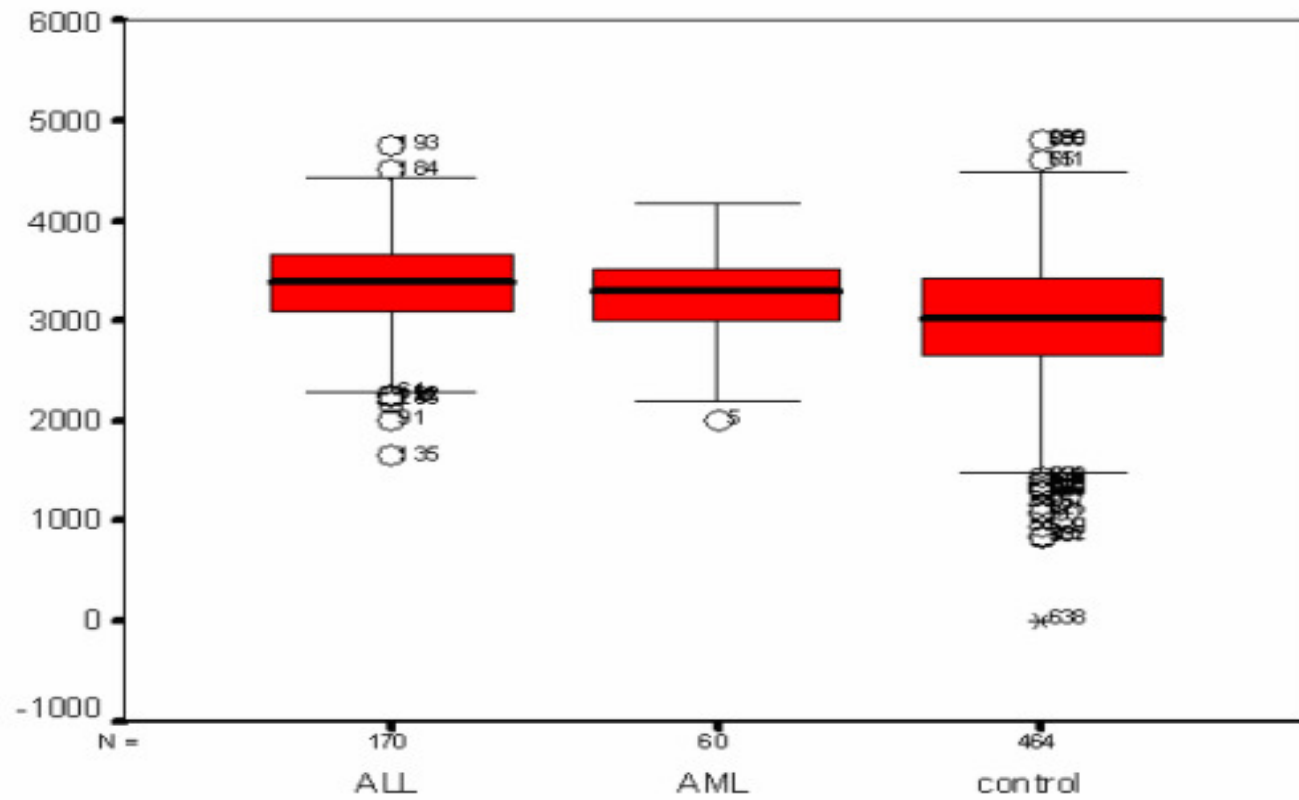


**Figure 1** Birth weights (in g) strata distribution, IL cases, controls and the general population, Brazil.

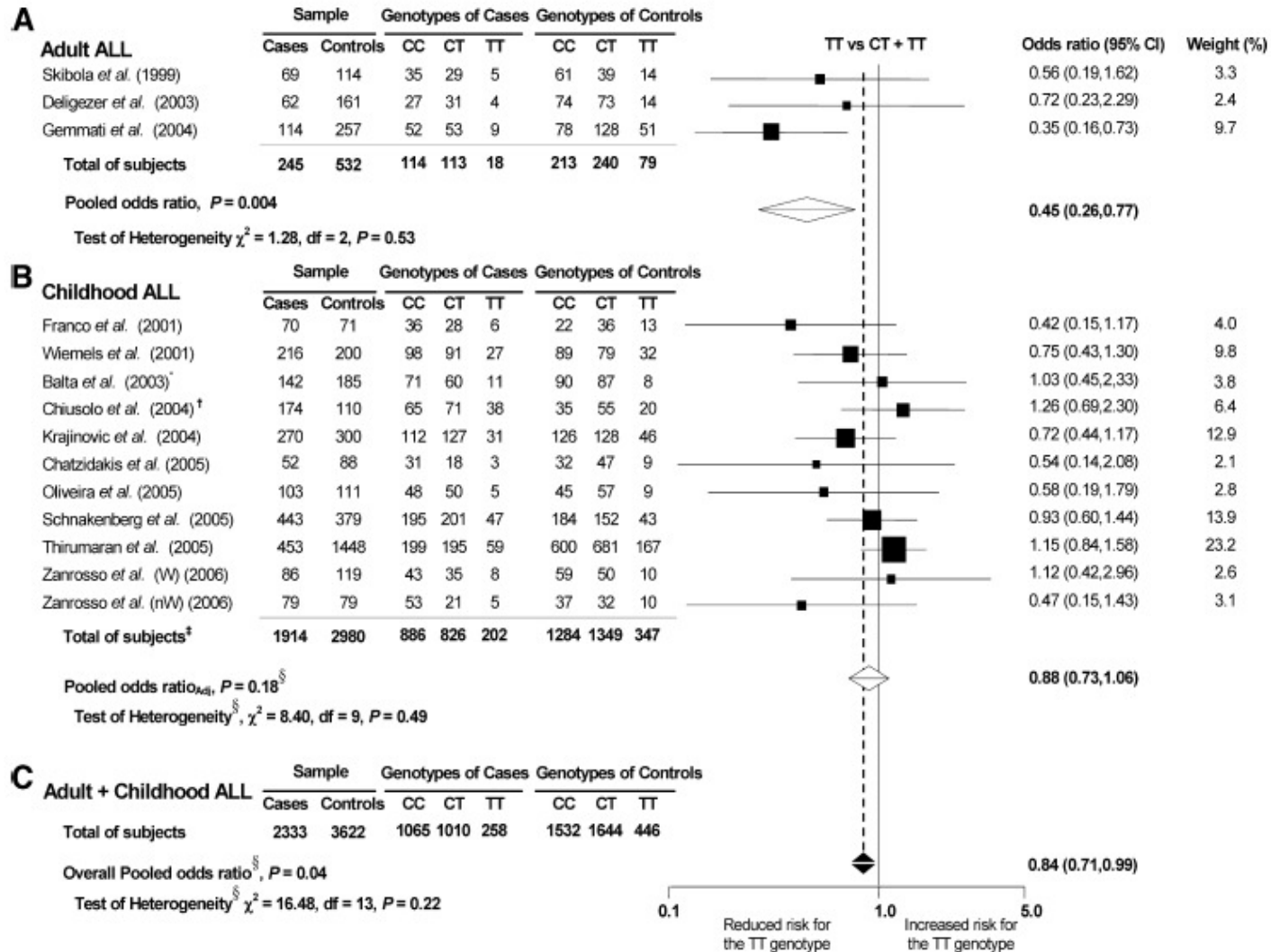


Percentile	IAL (g)	Controls (g)
10	2650	2250
20	2960	2562
30	3109	2750
40	3250	2917
50	3310	3026
60	3458	3180
70	3527	3323
80	3708	3500
90	3897	3750

Figure 2 - Birth weight distribution, IAL subtypes and controls, Brazil, 1999-2006



**MTHFR** Polymorphisms and ALL Risk: A Meta-analysis



**POTENTIAL CONTRIBUTION OF CURRENT BRAZILIAN STUDIES ON THE  
EPIDEMIOLOGY OF CHILDHOOD LEUKEMIA**

Maria S. Pombo de Oliveira <sup>1</sup>, Sergio Koifman<sup>2</sup>, Gisele M. Vasconcelos <sup>1</sup>, Mariana Emerenciano <sup>1</sup>, Cristiane de Oliveira Novaes<sup>1,2</sup>, and the Brazilian Collaborative Study Group of Infant Acute Leukemia <sup>3</sup>

1. Hematology-Oncology Pediatric Program, Research Center, Instituto Nacional de Câncer, Rio de Janeiro, Brazil;

2. National School of Public Health, Oswaldo Cruz Foundation (FIOCRUZ), Ministry of Health of Brazil, Rio de Janeiro, Brazil;

# Brazilian scenario

- Ethnic admixture & genetic mixing
- Migration and environmental exposures following industrialization
- Epidemiological transition: overlapping sets
- Lifestyle changes

Pombo-de-Oliveira MS, Koifman S, 2008  
*Blood Cells Molecules and Diseases.*

To resume, such massive diversity from a genetic and environmental conditions observed in the Brazilian population, including a recent massive population displacement within the country, family changing patterns according to size and new lifestyle profiles, new occupational and environmental exposures, among others, creates a special framework allowing to explore the natural history of cancer in general, and of childhood leukemia in particular.

# Funding Support

- Brazilian National Research Council (CNPq)
- Ministry of Health
- Swiss Bridge Foundation
- Bank of Brazil ( Programa Criança e Vida)

# **The Brazilian Collaborative Study Group of Infant Acute Leukemia: Participating Centers**

- INCA, Fiocruz, UFRJ, HSE - Rio de Janeiro
- Centro Boldrini, Campinas, Sao Paulo
- HMG and Oncology Society, Bahia
- UFSC, Santa Catarina
- Hospital N. Laureano, Paraiba
- UFSMa, Rio Grande do Sul
- UFMG, Minas Gerais
- Hosp. Oswaldo Cruz, Pernambuco
- USP Ribeirao Preto, Sao Paulo
- Hospital A.C. Camargo, Sao Paulo
- Hospital de Apoio, Brasilia, DF

*Brazilian Collaborative Study Group of Infant Acute Leukemia*

Dora Márcia Alencar<sup>3</sup>, Alexandre Apa<sup>2</sup>, Jozina Aquino<sup>1</sup>, Alejandro Mauricio Arancibia<sup>12</sup>,  
Flávia Nogueira Araújo<sup>1</sup>, Paulo Ivo C Araújo<sup>2</sup>, Rosania Maria Basegio<sup>16</sup>, Silvia R.  
Brandalise<sup>3</sup>, Lilian Burlemaqui<sup>1</sup>, Eni Guimarães Carvalho<sup>4</sup>, Synara No Seara Cordeiro<sup>2</sup>,  
José Carlos Córdoba<sup>5</sup>, Virginia M. Coser<sup>6</sup>, Imaruí Costa<sup>6</sup>, Maria Lydia D'Andrea<sup>14</sup>, Tereza  
Cristina Cardoso Fonseca<sup>17</sup>, Fernando Augusto de Freitas<sup>3</sup>, Maria Célia Moraes Guerra<sup>7</sup>,  
Mônica Lankszner<sup>15</sup>, Maria Lucia de Marino Lee<sup>8</sup>, Luis Fernando Lopes<sup>10</sup>, Isis Q.  
Magalhães<sup>5</sup>, Carmen M. Mendonça<sup>10</sup>, Núbia Mendonça<sup>1</sup>, Flávia Nogueira<sup>1</sup>, Waldir Pereira<sup>7</sup>,  
Vitória P. Pinheiro<sup>5</sup>, Gilberto Ramos<sup>11</sup>, Elisângela Ribeiro<sup>13</sup>, Terezinha J M Salles<sup>9</sup>, Elaine  
Sobral<sup>7</sup>, Jose Andréa Yunes<sup>5</sup>.

# Perspectives

Suggested *environmental exposures* to be explored:

- parental tobacco smoke
- maternal alcohol intake
- maternal and child's diet
- parental occupational exposures
- household chemicals (pesticides)
- persistent organic pollutants
- ionizing radiation (mother and child)
- traffic density
- hazardous air pollutants

# Perspectives

Suggested list for *birth characteristics* to explore:

- birthweight (% of optimal BW)
- previous fetal loss
- birth length
- defects present at birth
- maternal weight gain during pregnancy
- parental age at birth

# Perspectives

Suggested list for *Infection & Immunity* to be explored:

- birth order and parity
- social groups (daycare, playgroups)
- infection history (child, mother)
- vaccination history
- breastfeeding
- Maternal illnesses during pregnancy
- Contact with pets and farm animals
- Rural/urban (with residential histories)
- Serological measures (AB to infections, etc).
- Allergies

# Perspectives

Suggested list for *Genetic Susceptibility* to explore:

- folate pathway
- immune response pathway
- xenobiotic metabolism
- DNA repair,
- Oxidative stress
- Membrane transportation
- Ancestry in admixed populations

# Perspectives

Suggested list for *Family History* to be explored:

- hematological cancers
- other cancers
- other heritable diseases
- congenital abnormalities
- autoimmune diseases
- allergies

# Perspectives

Suggested list for *Gene-environment* to explore:

- 1-carbon metabolism genes, folate intake, alcohol
- xenobiotic metabolism genes and pesticides
- xenobiotic metabolism genes and parental smoking
- immune function genes and infection
- DNA repair genes and translocation-positive leukemias
- ADH, ALDH and alcohol intake
- XME, DNA repair and parental occupational exposures
- transporters and pesticides and PAHs
- growth factors and birthweight

# Perspectives

Suggested list for Family History to be explore

- hematological cancers
- other cancers
- other heritable diseases
- congenital abnormalities
- autoimmune diseases
- allergies

# Perspectives

Suggested environmental exposures to be explored:

- parental tobacco smoke
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