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

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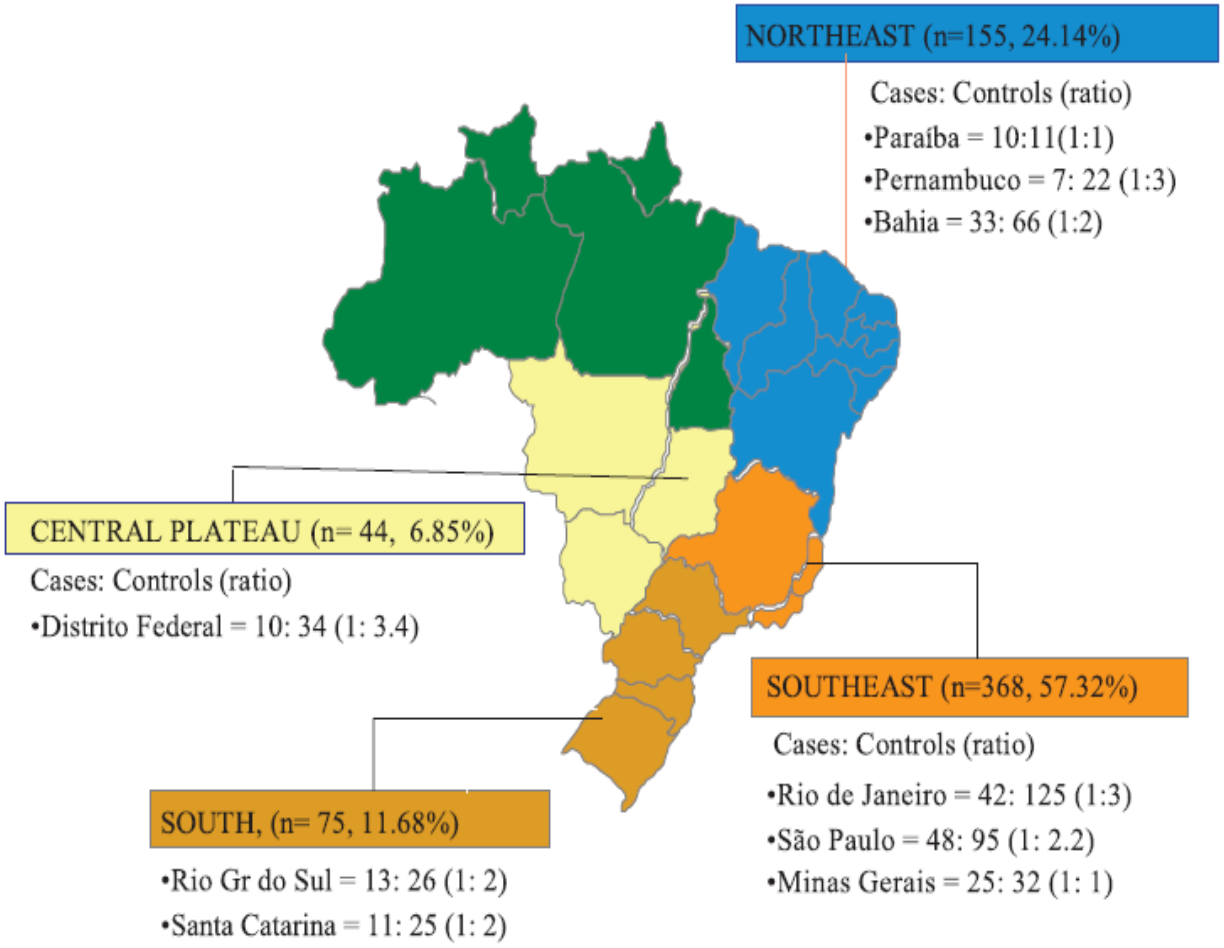

Figure 1. Distribution of cases and controls by geographic regions, Brazil, 1999-2005.

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

	IAL, *n* (%)	Controls, *n* (%)	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)
Ciprofloxacilin (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^{+ve}, *n* (%)	MLL^{-ve}, *n* (%)	Controls, *n* (%)	IAL vs controls, OR (95% CI)*	MLL^{+ve} vs controls, OR (95% CI)*	MLL^{-ve} vs controls, OR (95% CI)*
Preconception †							
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.
†*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 MLL^{+ve} (*n*)	Unexposed and MLL^{+ve} (*n*)	Exposed and MLL^{-ve} (*n*)	Unexposed and MLL^{-ve} (*n*)	Crude OR (95% CI)*	Adjusted OR† (95% CI)
Dipyrone	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.

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Full Paper

High birth weight as an important risk factor for infant leukemia

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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), $P_{trend}<0.01$. 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*$^{+ve}$		*MLL*$^{-ve}$	
		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	100	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)[a]	13	2.28 (1.08–4.75)[b]	0	—	8	2.68 (0.99–7.15)[c]	5	1.31 (0.41–3.89)[d]

ALL = acute lymphoblastic leukaemia; AML = acute myeloblastic leukaemia; CI = confidence interval; IL = infant leukaemia; OR = odds ratio. [a]χ^2 for trend = 4.60, $P = 0.03$. [b]χ^2 for trend = 6.66, $P < 0.01$. [c]χ^2 for trend = 3.56, $P = 0.059$. [d]χ^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[a] OR (95% CI)	*n*	Crude OR (95% CI)	Adjusted[a] OR (95% CI)	*n*	Crude OR (95% CI)	Adjusted[a] 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)[b]
>3999	23	13	2.28 (0.96–5.37)	1.20 (1.02–1.43)[c]	13	3.26 (1.32–8.01)	1.31 (1.09–1.57)[d]	—		

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

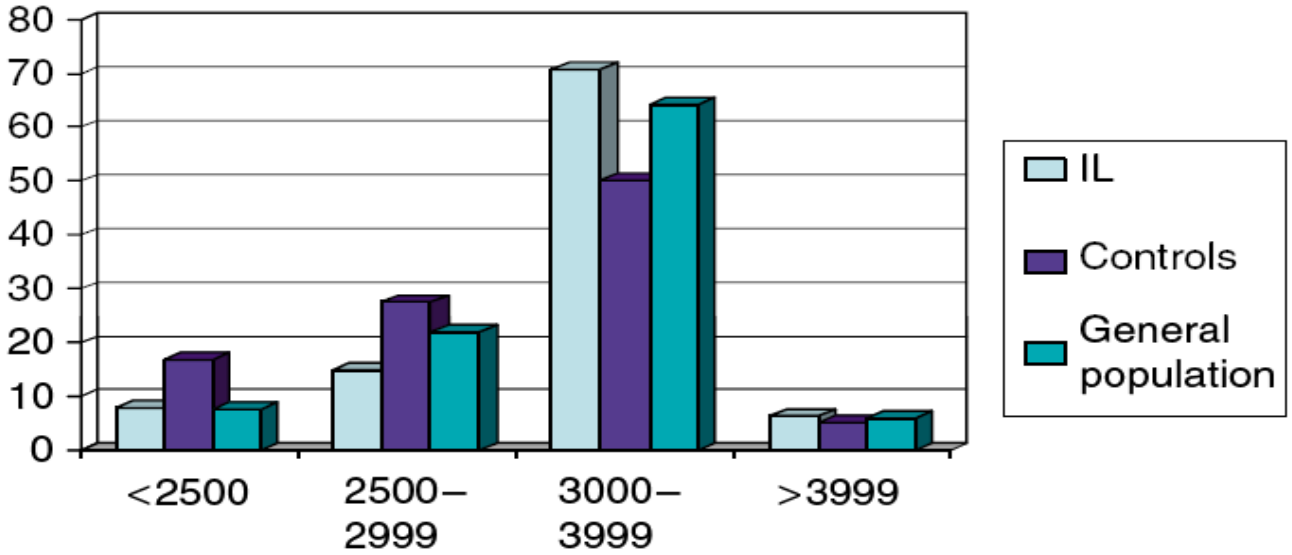
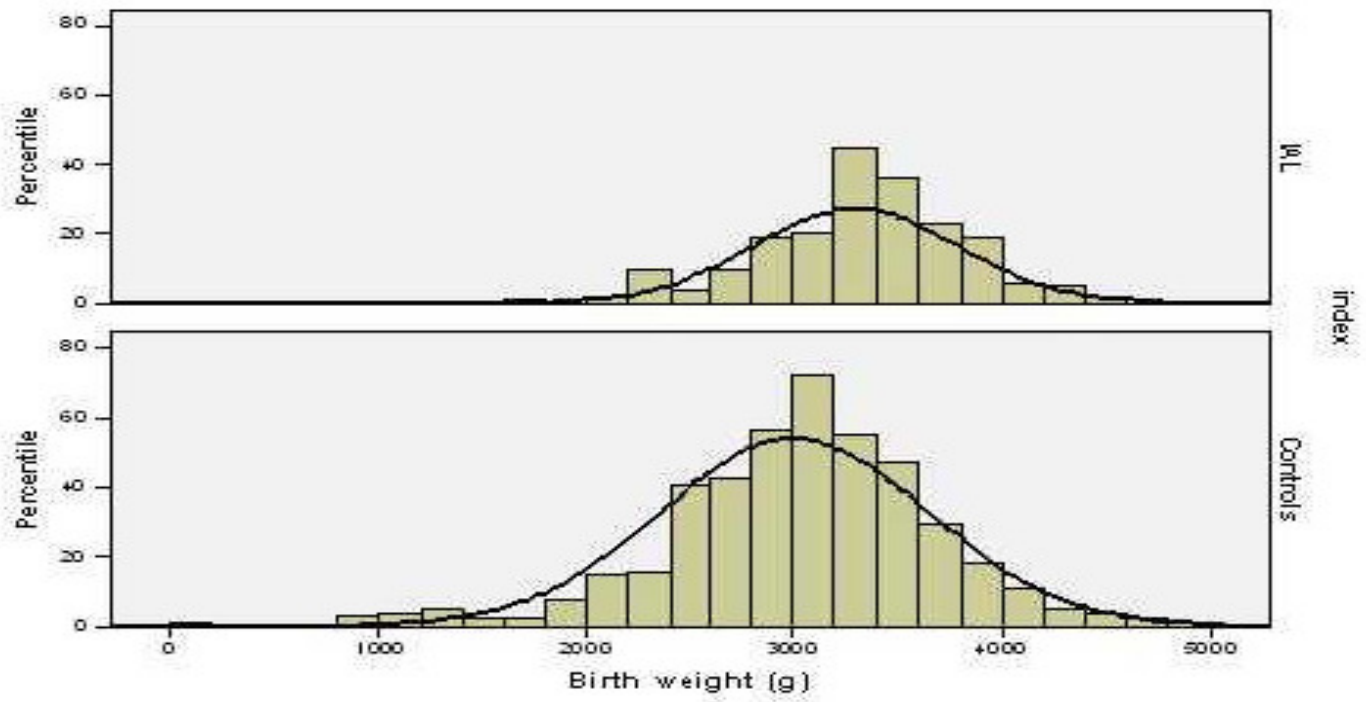
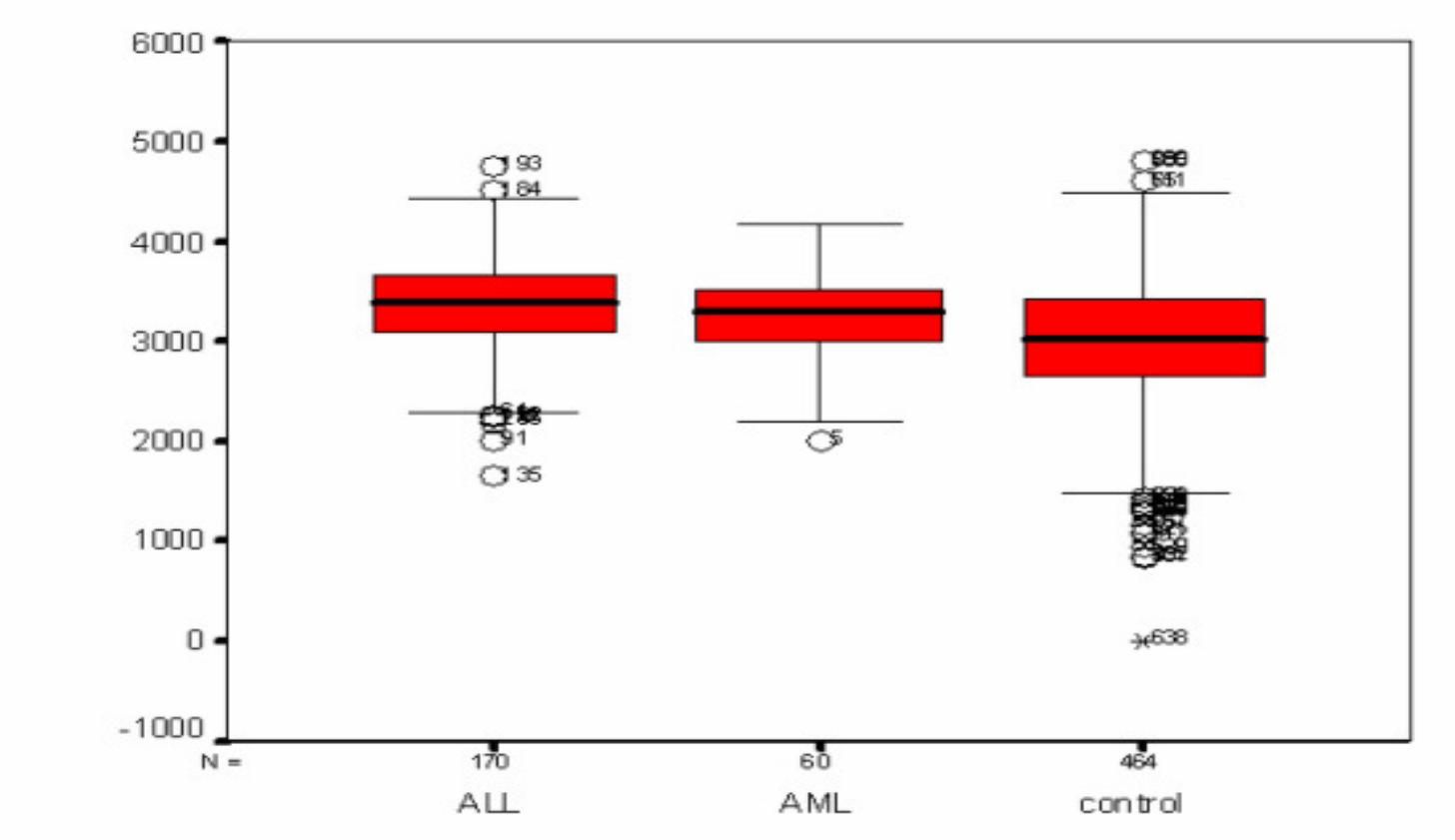


Figure 1 Birth weights (in g) strata distribution, IL cases, controls and the general population, Brazil.



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



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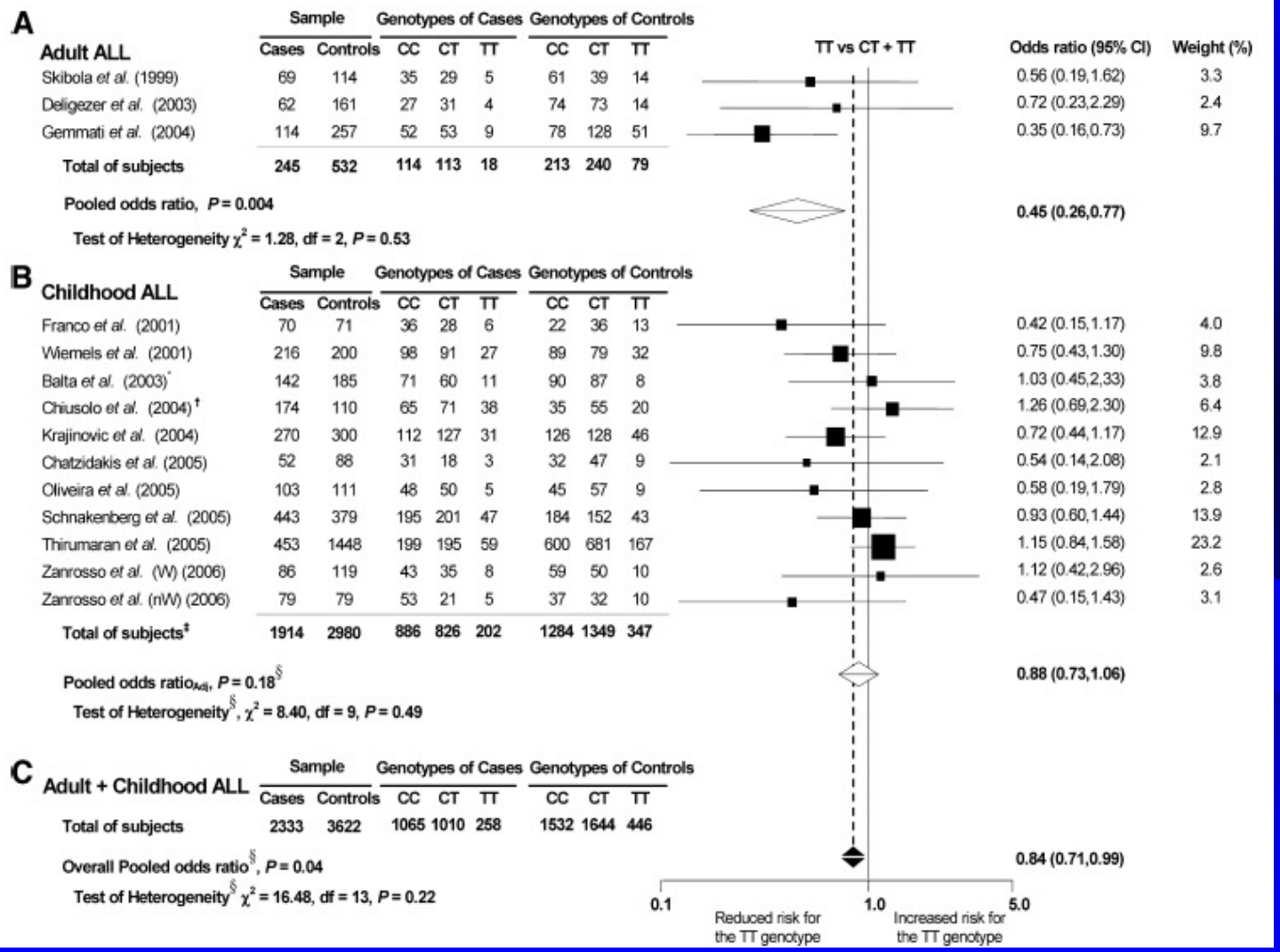
MTHFR Polymorphisms and ALL Risk: A Meta-analysis



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

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

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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.

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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
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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 leukemais
- ADH, ALDH and alcohol intake
- XME, DNA repair and parental occupational exposures
- transportes 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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