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Brief communication

The role of methylenetetrahydrofolate reductase in acute lymphoblastic leukemia in a Brazilian mixed population

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Abstract

The polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene are associated with leukemogenesis. In order to investigate the influence of two polymorphisms in the *MTHFR* gene, 677C > T and 1298A > C, on the risk of acute lymphoblastic leukemia (ALL) we performed a case–control study in children from different Brazilians' regions. Genotyping of 176 ALL and 199 unselected healthy subjects was performed using PCR-RFLP assay. There was no association between the 677C > T or 1298A > C and risk of ALL in total case–control sample. However, 677T allele was linked to a decrease risk of ALL [odds ratio (OR), 0.43; 95% confidence interval (CI), 0.22–0.86], whereas the 1298A > C polymorphism presents an elevated risk factor [OR, 2.01; 95% CI, 1.01–3.99] in non-White children. Our investigation provides interesting data concerning the opposite effect of A1298C polymorphisms, particularly in the light of relatively scarce data regarding the MTHFR role in leukemia susceptibility in different populations.

Keywords: *MTHFR*; Polymorphism; Childhood acute leukemia

1. Introduction

Recently, inheritance of mutant alleles of methylenetetrahydrofolate reductase (*MTHFR*) has been linked to the risk of molecularly defined acute lymphoblastic leukemia (ALL) subtypes according to epidemiological studies [1]. The rational is that the folate metabolism pathways lead to the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, toward methionine and DNA synthesis. Folate deficiency has been associated with uracil miss incorporation into DNA, leading to double strand DNA breaks during uracil excision repair and increasing the risk of chromosomal aberrations that is presumably the onset of the leukemogenic process [2]. Furthermore, the value of folate supplementation during pregnancy was shown to prevent acute leukemia in children. The results strongly suggest that genetic polymorphisms and environment interaction might play a role in the susceptibility to childhood ALL [3]. In order to understand the multi-factors that influence the leukemogenesis in a heterogeneous population, we investigated the influence of two polymorphisms of the *MTHFR* gene, 677C > T and 1298A > C, on the risk of ALL in three different regions in Brazil.

Abbreviations: ALL, acute lymphoblastic leukemia; BpALL, B-cell precursor acute lymphoblastic leukemia; CI, confidence interval; GBTLI, Grupo Brasileiro para Tratamento de Leucemias Infantil; *MTHFR*, methylenetetrahydrofolate reductase; na, not applied; OR, odds ratio; T-ALL, T-cell acute lymphoblastic leukemia

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Table 1
Main characteristics of ALL and controls

Characteristic	Case, $n = 176$ (%)	Control, $n = 199$ (%)
Sex		
Male	92 (52.3)	83 (41.7)
Female	84 (47.7)	116 (58.3)
Race		
White	94 (53.4)	120 (60.3)
Non-White	82 (46.6)	79 (39.7)
Acute leukemia		
BpALL	167 (94.9)	na
T-ALL	9 (5.1)	na
Regions of Brazil		
South	15 (8.5)	57 (28.7)
Southeast	79 (44.9)	59 (29.6)
Northeast	82 (46.6)	83 (41.7)
Total	176 (100)	199 (100)

n: total number; BpALL: B-cell precursor acute lymphoblastic leukemia; T-ALL: T-cell acute lymphoblastic leukemia; na: not applied.

2. Methods

2.1. Subjects

A series of 176 children with ALL was subjected to immune-molecular studies including genetic polymorphism analysis before any chemotherapeutic treatment. Cases evaluated in this study came from three different Brazilian regions: (i) Rio de Janeiro, São Paulo and Minas Gerais (Southeast region); (ii) Pernambuco, Paraíba and Bahia (Northeast region); (iii) Santa Catarina e Rio Grande do Sul (South region). The main clinical and demographical characteristics of cases and controls are shown in Table 1. The majority of the cases (95%) were B-cell precursor ALL (BpALL) with the mean age of 6.2 years old, whereas in control group, the mean age was 25 years old. The proportion of ALL enrolled in the *Grupo Brasileiro para Tratamento de Leucemia Infantil* [GBTLI-93,99] clinical trials was estimated in 85% of cases.

Controls consisted of blood samples obtained from unselected healthy subjects from the same regions of cases ($n = 199$). Informed consent was obtained from all parents or responsible for the subject, after easy-to-understand explanations of the issues related to this study. The race variables were considered according to the definition of skin colour complexion provided by the mother of each child and by the interviewers of the cases and controls. Because of the heterogeneous phylogeography of the Brazilians, the distribution in this study was categorized into two major groups: Whites (mainly Brazilians from European descent) and non-Whites (admixture of Amerindians, Europeans and Africans).

2.2. Genotyping of MTHFR variants

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism technique on DNA from peripheral blood samples as previously described by Wiemels et al. [1].

2.3. Statistical analysis

Maximum likelihood method was used to estimate the allelic frequencies and the goodness of fit of phenotype distribution to Hardy–Weinberg equilibrium was tested by chi-square. Comparison among race and region groups was performed through contingency tables analyzed by chi-square or Fisher exact test. The analysis was carried out using the SPSS Statistical Package (Version 11.5) to estimate odds ratios (OR) and confidence intervals at 95% (95% CI) significance level.

3. Results

The frequencies of mutant alleles (±standard error) in ALL and controls are 0.25 ± 0.02 and 0.31 ± 0.02 for MTHFR 677T and 0.28 ± 0.02 and 0.25 ± 0.02 for MTHFR 1298C, respectively. Both groups, ALL cases and controls, showed a good fit to Hardy–Weinberg's genetic equilibrium model in relation to observed and expected genotypic frequencies for MTHFR 677C > T and 1298A > C polymorphisms (data not shown). Table 2 shows the frequencies of MTHFR 677 genotypes, as well as, MTHFR 1298 genotypes and, MTHFR 677C > T and 1298A > C polymorphisms distribution in cases and controls, in different Brazilian regions. There was no association between the 677C > T or 1298A > C and risk of ALL in total case–control sample, but the OR for ALL linked to MTHFR 677CT heterozygotes was 0.49 (95% CI, 0.23–1.00) in Northeast region. The distribution of MTHFR genotypes according to race is shown in Table 3. In non-White children a protective effect for 677C > T was found for both heterozygotes [OR, 0.46; 95% CI, 0.22–0.97] and overall (CT + TT) [OR, 0.43; 95% CI, 0.22–0.86]. On the other hand, an increased risk of ALL was observed in 1298A > C for both heterozygotes [OR, 2.10; 95% CI, 1.03–4.29] and overall (AC + CC) [OR, 2.01; 95% CI, 1.01–3.99]. No associations were observed in White children group in all analysis performed. In order to identify which factor (geographical or race) was responsible for the associations found, an independent analysis of the Northeast region, stratified by race, was performed in Table 4. This resulted in a drawback related to the small number of subjects. Significant associations were observed only in non-White children [OR, 0.33; 95% CI, 0.13–0.82] for 677C > T and [OR, 2.93; 95% CI, 1.22–7.08] for 1298A > C.

4. Discussion

The causes of the majority of ALL are unknown and commonly involve gene–environment interactions that may result in chromosome translocations. This study showed a

Table 2
Distribution of *MTHFR* genotypes in ALL and controls according to region of Brazil

Polymorphism	*MTHFR*	ALL, *n* (%)	Controls, *n* (%)	ALL vs. controls, OR (95% CI)
South				
677C > T	CC	8 (57.2)	29 (51.8)	1.0[a]
	CT	5 (35.7)	20 (35.7)	0.91 (0.22–3.7)
	TT	1 (7.1)	7 (12.5)	0.52 (0.02–5.54)
	CT + TT	6 (42.8)	27 (48.2)	0.81 (0.21–3.02)
1298A > C	AA	4 (36.4)	30 (52.6)	1.0[a]
	AC	5 (45.4)	23 (40.3)	1.63 (0.33–8.35)
	CC	2 (18.2)	4 (7.1)	3.75 (0.34–40.39)
	AC + CC	7 (63.6)	27 (47.4)	1.94 (0.44–9.04)
Southeast				
677C > T	CC	39 (53.4)	27 (45.7)	1.0[a]
	CT	29 (39.8)	25 (42.4)	0.80 (0.36–1.77)
	TT	5 (6.8)	7 (11.9)	0.49 (0.12–1.99)
	CT + TT	34 (46.6)	32 (54.3)	0.74 (0.35–1.55)
1298A > C	AA	40 (52.0)	31 (52.5)	1.0[a]
	AC	31 (40.2)	23 (39.0)	1.04 (0.48–2.27)
	CC	6 (7.9)	5 (8.5)	0.93 (0.22–3.94)
	AC + CC	37 (48.0)	28 (47.5)	1.02 (0.49–2.14)
Northeast				
677C > T	CC	49 (62.9)	40 (48.2)	1.0[a]
	CT	22 (28.2)	37 (44.6)	0.49 (0.23–1.00)
	TT	7 (8.9)	6 (7.2)	0.95 (0.26–3.53)
	CT + TT	29 (37.1)	43 (51.8)	0.55 (0.28–1.08)
1298A > C	AA	39 (48.7)	50 (60.2)	1.0[a]
	AC	38 (47.5)	30 (36.2)	1.62 (0.82–3.23)
	CC	3 (3.8)	3 (3.6)	1.28 (0.19–8.55)
	AC + CC	41 (51.2)	33 (39.8)	1.59 (0.82–3.11)
Total				
677C > T	CC	96 (58.2)	96 (48.5)	1.0[a]
	CT	56 (33.9)	82 (41.4)	0.68 (0.43–1.09)
	TT	13 (7.9)	20 (10.1)	0.65 (0.29–1.46)
	CT + TT	69 (41.8)	102 (51.5)	0.68 (0.44–1.05)
1298A > C	AA	83 (49.4)	111 (55.8)	1.0[a]
	AC	74 (44.1)	76 (38.2)	1.30 (0.83–2.04)
	CC	11 (6.5)	12 (6.0)	1.23 (0.48–3.15)
	AC + CC	85 (50.6)	88 (44.2)	1.29 (0.84–1.99)

[a] Reference group (OR = 1.0).

protective role of MTHFR 677C > T polymorphism in non-White children, linked to a significant 2.18-fold decreased risk of developing ALL, whereas the 1298A > C polymorphism demonstrated a significant 2.01-fold increased risk for ALL. These risks were noteworthy when only the Northeast region was analyzed: 3.00-fold decreased risk for MTHFR 677C > T and 2.93-fold increased risk for 1298A > C of developing ALL, in non-White children. The frequencies of the different subtypes of ALL have been related to age, ethnicity and social conditions in different countries and folate deficiency has been associated with uracil misincorporation into DNA and the increased risk of chromosomal aberrations [2]. For instance, a case–control study with Australian children demonstrated that folate supplementation during pregnancy reduced the risk of ALL, and that the protective effect of the MTHFR polymorphisms depended on adequate folate intake [4]. Franco et al. also demonstrated a reduced risk of ALL [OR, 0.4 (0.2–0.8)] associated with 677T allele, and no significant association with 1298A > C genotypes, however their data suggested a trend to risk factor to 1298A > C [5]. This Brazilian study was small and characterized in its majority by White children, while in our cohort the non-White children are well represented.

The opposite effect of 1298A > C polymorphisms in our study was surprising when compared to other reports about leukemia susceptibility. In addition, malnutrition in lower socio-economic status was associated with ethnicity of African descent in Brazil [6]. Therefore, further studies are necessary to explain how these combined risk factors, 1298A > C and lack of folate supplementation influence the high risk in the leukemogenesis process.

Table 3
Distribution of *MTHFR* genotypes in ALL and controls according to race

Polymorphism	*MTHFR*	ALL, *n* (%)	Controls, *n* (%)	ALL vs. controls, OR (95% CI)	*P*, Yates corrected
White					
677C > T	CC	43 (50.0)	59 (49.6)	1.0[a]	
	CT	35 (40.7)	50 (42.0)	0.96 (0.50–1.80)	
	TT	8 (9.3)	10 (8.4)	1.10 (0.36–3.34)	
	CT + TT	43 (50.0)	60 (50.4)	0.98 (0.54–1.78)	
1298A > C	AA	48 (53.3)	62 (51.7)	1.0[a]	
	AC	35 (38.9)	50 (41.6)	0.90 (0.49–1.67)	
	CC	7 (7.8)	8 (6.7)	1.13 (0.34–3.75)	
	AC + CC	42 (46.7)	58 (48.3)	0.94 (0.52–1.68)	
Non-White					
677C > T	CC	53 (67.0)	37 (46.8)	1.0[a]	
	CT	21(26.6)	32 (40.5)	0.46 (0.22–0.97)	0.040
	TT	5 (6.4)	10 (12.7)	0.35 (0.09–1.24)	
	CT + TT	26 (32.9)	42 (53.2)	0.43 (0.22–0.86)	0.015
1298A > C	AA	35 (44.9)	49 (62.0)	1.0[a]	
	AC	39 (50.0)	26 (32.9)	2.10 (1.03–4.29)	0.040
	CC	4 (5.1)	4 (5.1)	1.40 (0.27–7.28)	
	AC + CC	43 (55.1)	30 (38.0)	2.01 (1.01–3.99)	0.046

[a] Reference group (OR = 1.0).

Table 4
Distribution of *MTHFR* genotypes in childhood leukemia and controls according to race in the Northeast region

Polymorphism	*MTHFR*	ALL, *n* (%)	Controls, *n* (%)	ALL vs. controls, OR (95% CI)	*P*, Yates corrected
Northeast, White					
677C > T	CC	13(46.4)	16 (51.6)	1.0[a]	
	CT	11(39.3)	14(45.1)	0.97 (0.29–3.26)	
	TT	4(14.3)	1(3.2)	4.92 (0.41–131.28)	
	CT + TT	15 (53.6)	15 (48.4)	1.23 (0.39–3.89)	
1298A > C	AA	20 (68.9)	17 (54.8)	1.0[a]	
	AC	9 (31.1)	13 (41.9)	0.59 (0.18–1.94)	
	CC	0 (0)	1 (3.3)	0.0 (0.0–16.21)	
	AC + CC	9 (31.1)	14 (45.2)	0.55 (0.17–1.78)	
Northeast, non-White					
677C > T	CC	36 (72.0)	24 (46.2)	1.0[a]	
	CT	11 (22.0)	23 (44.2)	0.32 (0.12–0.84)	0.018
	TT	3 (6.0)	5 (9.6)	0.40 (0.07–2.19)	
	CT + TT	14 (28.0)	28 (53.8)	0.33 (0.13–0.82)	0.014
1298A > C	AA	19 (37.3)	33 (63.5)	1.0[a]	
	AC	29(56.8)	17 (32.7)	2.96 (1.20–7.36)	0.016
	CC	3 (5.9)	2 (3.8)	2.61 (0.31–24.96)	
	AC + CC	32 (62.7)	19 (36.5)	2.93 (1.22–7.08)	0.014

[a] Reference group (OR = 1.0).

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