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Short Communication

Methylenetetrahydrofolate reductase polymorphisms in myeloid leukemia patients from Northeastern Brazil

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Abstract

Methylenetetrahydrofolate reductase (MTHFR: EC 1.5.1.20) polymorphisms are associated to acute lymphoid leukemia in different populations. We used the polymerase chain reaction and the restriction fragment length polymorphism method (PCR-RFLP) to investigate *MTHFR* C677T and A1298C polymorphism frequencies in 67 patients with chronic myeloid leukemia (CML), 27 with acute myeloid leukemia FAB subtype M3 (AML-M3) and 100 apparently healthy controls. The *MTHFR* mutant allele frequencies were as follows: CML = 17.2% for C677T, 21.6% for A1298C; AML-M3 = 22.2% for C677T, 24.1% for A1298C; and controls = 20.5% for C677T, 21% for A1298C. Taken together, our results provide evidence that MTHFR polymorphisms have no influence on the development of CML or AML-M3.

Key words: AML, CML, *MTHFR* polymorphisms.

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Leukemias are clonal diseases which commonly arise as a result of genetic damage deregulating blood cell development or hematopoiesis. The risk of leukemia may be increased by both quantitative and qualitative changes in folate metabolism (Hur *et al.*, 2006). Methylenetetrahydrofolate reductase (MTHFR: EC 1.5.1.20) is a key enzyme in folate and homocysteine (Hcy) metabolism which catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the predominant circulatory form of folate and carbon donor for the remethylation of homocysteine to methionine (Skibola *et al*., 1999). Various studies have described an association between the presence of the C677T and A1298C MTHFR polymorphisms and the risk of leukemias (Skibola *et al.*, 1999; Franco *et al.*, 2001; Wiemels *et al.*, 2001; Moon *et al.*, 2007; Zanrosso *et al.*, 2006), suggesting that alteration of folate metabolism in the presence of enzyme variant forms can protect against acute lymphoid leukemia (ALL) but increase the risk of chronic myeloid leukemia (CML). However, this association was not related to acute myeloid leukemia (AML) in British (Skibola *et al.*, 1999) and Spanish populations (Bolufer *et al.*, 2007).

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Folate deficiency has been associated with uracil misincorporation into DNA, increasing the risk of chromosomal aberrations and presumably the onset of the leukemogenic process (Wiemels *et al.*, 2001).

We investigated the *MTHFR* C677T and A1298C polymorphism frequency among AML-M3 and CML patients from Salvador in the Northeastern Brazilian state of Bahia. The frequency of these polymorphisms vary according to the population, being less common among Africans at 1.6% for 677TT and 4.4% for 1298CC (Gueant-Rodriguez *et al.*, 2006) than in other ethnic groups such as Caucasians in which the frequency is 12.3% for 677TT and 11% for 1298CC (Skibola *et al.*, 1999).

We carried out a cross-sectional study of 94 patients with myeloid leukemia, of which 67 (30 (45.5%) female and 36 male (54.5%); median age 44 y, range 09 y to 93 y) had chronic myeloid leukemia (CML) and 27 (15 female (53.6%) and 13 male (46.4%); median age 27 y, (range 06 y to 70 y) with acute myeloid leukemia FAB subtype M3 (AML-M3) selected between 1999 and 2003 from several institutions treating patients with malignant diseases, the institutions being: Oncology Clinic; San Raphael Hospital;

Edgard Santos Federal University Hospital and Cehon Clinic. All these institutions are in the city of Salvador, Bahia, Brazil. The control group consisted of 100 (47 female (47%) and 53 male(53%); median age 29 y, range 18 y to 40 y) apparently healthy individuals randomly chosen from the general population. All individuals included in this study were from Bahia, Brazil, a state which has a highly mixed population, mainly of African origin. The study was approved by the Oswaldo Cruz Research Foundation's human research ethics Committee. Bone marrow and peripheral blood samples were taken only after signed informed consent forms were obtained from patients or an official responsible.

We isolated DNA and RNA from bone marrow cells or peripheral blood leukocytes, using Trizol^{R} (Gibco-BRL, USA) for RNA extraction and a Genomic Blood DNA Purification Kit (Amersham Pharmacia Biotech, USA) according to the guidelines of the manufacturer. The translocations t(9;22)(q34;q11) for CML patients and t(15;17)(q22;q12-21) for AML-M3 were investigated using reverse transcriptase PCR (RT-PCR) as previously described (Borrow *et al.*, 1992; Artigas *et al.*, 2002).

The MTHFR C677T and A1298C polymorphisms were investigated using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique and the *Hinf I* and *Mbo II* restriction enzymes (New England Biolabs, USA) (Wiemels *et al.*, 2001).

Descriptive analyses included gene and allelic frequencies and we also calculated the odds ratio (OR) as an estimate of relative risk and the 95% confidence interval (CI). Moreover, the Hardy-Weinberg equilibrium was tested. Significance was considered for $p < 0.05$. All analyses were carried out using the EPI INFO software, version 6.04, that is a public domain software produced by the Centers for Disease Control (CDC) and the World Health Organization (WHO).

All 67 CML patients had t(9;22)(q34;q11) translocations and all 27 AML-M3 patients had t(15;17)(q22;q11-21) translocations. The total *MTHFR* mutant allele frequencies were as follows: CML = 17.2% for C677T, 21.6% for A1298C; AML-M3 = 22.2% for C677T, 24.1% for A1298C; and controls = 20.5% for C677T, 21% for A1298C. The C677T and A1298C polymorphism frequency distributions were in agreement with the Hardy-Weinberg equilibrium.

The *MTHFR* C677T and A1298C polymorphisms showed a similar distribution for the heterozygous and homozygous genotypes among the leukemia (CML and AML-M3) and control groups (Table 1). The double heterozygous frequencies for both *MTHFR* polymorphisms was 6.4% in the leukemia group and 5.0% in control groups. There was no double homozygous for both polymorphisms (Table 2).

The MTHFR enzyme and its involvement in folate metabolism have been considered a cancer susceptibility factor and the presence of the *MTHFR* polymorphism has been associated to DNA hypomethylation and chromosomal breaks and damage (Krajinovic *et al.*, 2004). Skibola *et al* (1999) showed that variant *MTHFR* genotypes, including 677TT, 1298AC or 1298CC have a lower risk of developing acute lymphoid leukemia (ALL) in adulthood although it did not influence AML patients of the same age. Their observation was supported by subsequent studies performed in childhood AML (Wiemels *et al.*, 2001). However, a similar study carried out among a highly mixed population of Brazilian leukemia patients reported the opposite effect for the A1298C polymorphism, which was associated with an elevated risk factor for non-white AML children which could also be associated with the high

Table 1 - Methylenetetrahydrofolate reductase gene (*MTHFR*) C677T and A1298C polymorphisms genotype distributions among 67 patients with chronic myeloid leukemia (CML), 27 with acute myeloid leukemia FAB subtype M3 (AML-M3) and 100 apparently healthy controls. The odds ratio (OR) column shows the 95% confidence interval in parentheses.

MTHFR polymorphisms	Number of individuals (%)			Odds ratio (95% CI)	
	AML-M3 (%)	CML (%)	Controls (%)	AML-M3 *vs.* controls	CML *vs.* controls
C677T					
CC	17 (63.0)	46 (68.7)	65 (65.0)	1.0[a]	1.0[a]
CT	8 (29.6)	19 (28.3)	29 (29.0)	1.03 (0.37 to 2.85)	0.97 (0.46 to 2.03)
TT	2 (7.4)	2 (3.0)	6 (6.0)	1.25 (0.16 to 7.55)	0.48 (0.07 to 2.76)
CT + TT	10 (37.0)	21 (31.3)	35 (35.0)	1.09 (0.41 to 2.87)	0.85 (0.42 to 1.73)
A1298C					
AA	15 (55.6)	41 (61.1)	63 (63.0)	1.0[a]	1.0[a]
AC	11 (40.7)	23 (34.3)	32 (32.0)	1.46 (0.56 to 3.81)	1.11 (0.55 to 2.25)
CC	1 (3.7)	3 (4.6)	5 (5.0)	0.73 (0.03 to 6.97)	0.89 (0.16 to 4.48)
AC + CC	12 (44.4)	26 (38.8)	37 (37.0)	1.36 (0.53 to 3.50)	1.08 (0.54 to 2.14)

[a]Reference group (OR = 1.0).

Table 2 - Methylenetetrahydrofolate reductase gene (*MTHFR*) C677T and A1298C polymorphisms genotypic distributions in 94 patients (67 with chronic myeloid leukemia and 27 with acute myeloid leukemia FAB subtype M3) and 100 apparently healthy controls.

MTHFR polymorphisms		Number of individuals (%)	
MTHFR C677T	*MTHFR* A1298C	Leukemia patients	Controls
CC	AA	34 (36.0)	33 (33.0)
CC	AC	29 (30.8)	27 (27.0)
CC	CC	2 (2.1)	5 (5.0)
CT	AA	17 (18.1)	24 (24.0)
CT	AC	6 (6.4)	5 (5.0)
CT	CC	2 (2.1)	0
TT	AA	4 (4.3)	6 (6.0)
TT	AC	-	-
TT	CC	-	-
Total		94 (100)	100 (100)

frequency of malnutrition and lower socioeconomic status of African descendants in Brazil (da Costa Ramos *et al.*, 2006). A sample population (n = 6,864) from Northeastern Brazilian showed an estimated 97% interethnic panmixia, possibly due to the intensive African slave trade when more than five million people from several African countries were brought to Brazil between 1550 and 1850, principally between the eighteenth and nineteenth centuries (Krieger *et a*l., 1965). However, Chiusolo *et al* (2004) found no evidence of an association between the *MTHFR* C677T and A1298C genotypes and susceptibility to ALL among Italian patients.

Our present findings demonstrate a similar *MTHFR* polymorphism frequency distribution among CML and AML-M3 patients and controls, while Hur *et al.* (2006) demonstrated a significant decrease in CML risk among Koreans with the A1298C polymorphism. These differences indicate the possible influence of racial, ethnic and nutritional factors in several population groups and the *MTHFR* polymorphisms in the relationship between *MTHFR* polymorphisms and leukemia (Zanrosso *et al.*, 2006). Moreover, lymphoid cells may require more folate and be more susceptible to folate deficiency, resulting in higher DNA damage in this lineage than in myeloid cells (Skibola *et al*., 1999).

The *MTHFR* polymorphism frequencies described here, together with the absence of double homozygotes for the C677T/A1298C mutations, suggest the rarity of these mutations in *cis* which are probably incompatible with fetus development (Skibola *et al.*, 1999; Chiusolo *et al.,* 2004; Zanrosso *et al.*, 2006).

In conclusion, we found no association between the *MTHFR* C677T and A1298C polymorphisms among AML-M3 and CML patients from the Northeastern Brazilian state of Bahia. Therefore, further studies are necessary to consider the interaction of folate nutritional status and genes associated to folate metabolism, in order to establish its role in leukemogenesis.

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