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PRELIMINARY REPORT

Association Between the 5,10-MTHFR 677C>T and RFC1 80G>A Polymorphisms and Acute Lymphoblastic Leukemia

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Background. Polymorphisms in folate-related genes are closely related to the development of cancer. The 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are associated with an increased risk of susceptibility to pediatric ALL.

Objective. The aim of this study was to illustrate the association between 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms and ALL in a Mexican population.

Materials and Methods. This study was conducted in 60 pediatric ALL patients and 60 healthy individuals. The 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms were detected by the PCR-RFLP method.

Results. Our investigation revealed that the 5,10-MTHFR 677 C/T and 5,10-MTHFR 677T/T genotypes are associated with susceptibility to pediatric ALL (OR = 1.9, 95% IC = 1.36–12.09, $p = 0.012$ and OR = 2.8, 95% CI = 1.49–22.82, $p = 0.011$, respectively). Likewise, the G/A genotype from the RFC1 80G>A polymorphism showed an increased ALL risk compared to RFC1 G80G genotype (OR = 3. 3, 95% CI = 1.75–8.87, $p = 0.002$).

Conclusion. Therefore, our results suggest that the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are factors involved in the susceptibility to ALL in Mexican population.

Key Words: Acute lymphocytic leukemia, MTHFR, RFC1, Polymorphism.

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease and is the type of pediatric cancer most common in children from developed countries, accounting for approximately 26.8% of all pediatric cancers (1). In Mexico, the frequency of ALL is the highest in the world (2). The etiology of ALL continues to be explored, and multiple factors have been associated, being the genetic alteration the most relevant features that clearly play a critical role in the development of leukemia (3,4). Rosales-Rodríguez B, et al. (2016) showed that a considerable number of B-ALL patients displayed genomic imbalances that affect the genome to varying extents, including hyperdiploid, the presence of chromosome 9p deletion that affect genes such as CDKN2A/B, genomic instability and duplications (2q, 12p and 1q) (5). In addition, structural alterations in transcriptional regulators of differentiation and B-lymphoid development, such as the deletion of IKZF1 that accelerate the onset of ALL in murine models of BCR–ABL1–ALL, PAX5 and EBF1, and lymphoid signaling, cooperate in leukemogenesis (6). Interestingly,

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an intronic single nucleotide polymorphism (SNP) in p63 conferred susceptibility to ETV6–RUNX1–positive ALL (7), and SNPs in GATA3 conferred susceptibility to BCR–ABL1–like ALL and its underlying somatic lesions (IKZF1 deletions, CRLF2 rearrangements and JAK mutations) (8). Several genome–wide association studies (GWAS) have identified polymorphisms in ARID5B, IKZF1, CEBPE, CDKN2A and PIP4K2A–BMI1 in patients with ALL. Each of these variants confers an increase in the risk of developing ALL and independently and cumulatively contributes to genetic susceptibility to ALL (9–11).

MTHFR is an enzyme that catalyzes 5,10-methyl-tetrahydrofolate (THF) to 5-methyl-THF, which acts as the methyl donor for the conversion of homocysteine to methionine (12,13). One of the polymorphisms described in the MTHFR gene is the 5,10-MTHFR 677C>T (C677T, rs1801133), in which there is a C to T transition at nucleotide 677, causing the substitution of alanine with valine at codon 222, which decreases the enzymatic activity (4,13,14). The frequencies of the 5,10-MTHFR 677C>T polymorphism vary by ethnicity (15–17). Several studies have found an association between the 5,10-MTHFR 677C>T polymorphism and the increased risk of susceptibility to pediatric ALL (18–21).

RFC1 or SLC19A1 is a membrane protein, which maintains the transport system for folates (22). RFC1 also plays a critical role in the transport of antifolate chemotherapeutic agents into cells (23). The RFC1 80G>A polymorphism (rs1051266), in which there is a substitution of guanine to adenine at nucleotide 80, leads to the substitution of arginine with histidine at residue 27 of the protein (24) and causes a decrease in the transport of the antifolate chemotherapeutic agents (25). Several studies have reported the association of the RFC1 80G>A polymorphism and the risk to pediatric ALL (26–28).

In Mexico, data between the association of the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms and pediatric ALL are limited, indicating the need for the description of the genetic alterations to understand the pathogenesis of this neoplasia and to find better prognostic markers with clinical significance. The present study aimed to identify the possible association among 5,10-MTHFR 677C>T (rs1801133) and RFC1 80G>A (rs1051266) polymorphisms and ALL risk in Mexican population.

Materials and Methods

Population

Biological samples were obtained from 60 patients diagnosed with ALL at Pediatric Oncology Service of the Instituto Estatal de Cancerología, Acapulco city, Guerrero, Mexico, between August 2013 and February 2015. The diagnosis was made according to what was reported by Organista-Nava J, et al. (29). The controls were 60 healthy individuals, according to the previously reported data (29). The study was approved by the Ethics Committee of State Cancer Institute and according to the Helsinki Declaration.

Specimen and Total DNA Extraction

Bone marrow (ALL patients) and/or a blood sample (Control group) from the 120 participants were taken according the procedures described in a previous report (29). The bone marrow samples of the patients included in this study were part of the samples taken for clinical diagnostic tests in the hospital. Leukocytes were purified by a selective osmotic lysis, according to the procedure reported by Chomczynski & Sacchi, 2006 (30). DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Genotyping of Genetic Polymorphism

The 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms were determined using the PCR-RFLP method reported by Matsuo K, et al. 2001 and Dervieux T, et al. 2004 (31,32). The primers used for both polymorphisms are described in Table 1. For 5,10-MTHFR 677C>T polymorphism, the PCR products (198 bp) were digested with HinfI (New England Biolabs, Beverly, MA, USA) as described by Matsuo K, et al. 2001 (31). Individuals with the C allele had one fragment (198 bp), and individuals with the T allele had two fragments (175 and 23 bp) (Figure 1A). For the RFC1 80G>A polymorphism, the PCR products (230 bp) were digested with HhaI (New England Biolabs, Beverly, MA, USA) as described by Dervieux T, et al. 2004 (32,33). Individuals with the G allele had three fragments (126, 68 and 37 bp), whereas individuals with the A allele had two fragments (162 and 68 bp), according to the data reported in a previous study (32,33) (Figure 1B).

Table 1. PCR primer sequences and RFLP conditions for the identification of 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms

	Primer sequence (5′ → 3′)	PCR product length (bp)	SNP	Restriction enzyme	Restriction fragment length (bp)
5,10-MTHFR 677C>T					
F	TGAAGGAGAAGGTGTCTGCGGGA	198	rs1801133	HinfI	C: 198
R	AGGACGGTGCGGTGAGAGTG				T: 175 and 23
RFC1 80G>A					
F	AGTGTCACCTTCGTCCCCTC	230	rs1051266	HhaI	A: 162 and 68
R	CTCCCGCGTGAAGTTCTT				G: 126, 68 and 37

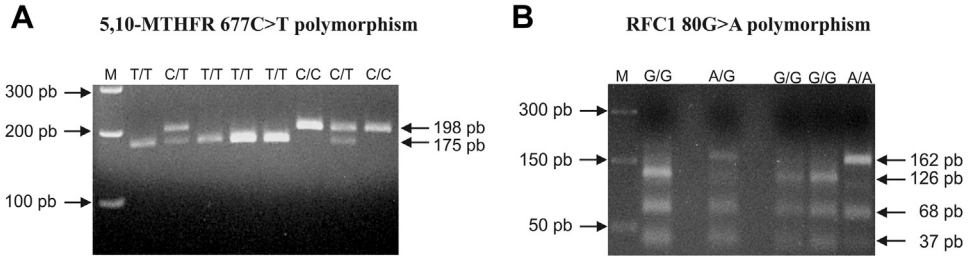

Figure 1. Analysis of the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms. (A) Line 1: Molecular weight marker (M); lines 2, 4, 5 and 6: 5,10-MTHFR 677T/T genotype (with two fragments 175 and 23 bp [not seen]); lines 3 and 8: 5,10-MTHFR 677C/T genotype (with three fragments 198, 175 and 23 bp [not seen]); lines 7 and 9: 5,10-MTHFR 677C/C genotype (with one fragment 198 bp). (B) Line 1: Molecular weight marker (M); lines 2, 6 and 7: RFC1 80 G/G genotype (with three fragments 126, 68 and 37 bp); line 4: RFC1 80A/G genotype (with four fragments 162, 126, 68 and 37 bp); line 8: RFC1 80A/A genotype (with two fragments 162 and 68 bp).

Statistical Analysis

The results are presented as the mean ± standard deviation (SD) or median, 25th and 75th interquartile. For each of the polymorphisms, Hardy Weinberg equilibrium (HWE) was used to determine the genetic equilibrium. Frequencies from 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms were compared using χ^2 or Fisher's exact test. The association between 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms and the risk of ALL was analyzed by computing the ORs and 95% CI from logistic regression models. $p < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed by using SPSS version 20.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Study Population

The study group included 60 patients (age: 5.9 ± 3.8 years), of whom 55% were male, 45% were female, and 60 were control children (age: 9.9 ± 5.0 years), of which, 43.3% were male, and 56.7% were female. Twenty-seven patients (45.0%) were in the low-risk age group (1−10 years and < 50,000/mm^3 leucocytes). Thirty-three patients (55.0%) were <1 and >10 years of age and had >50,000/mm^3 leucocytes at the time of the initial diagnosis (high risk). Twenty-two (53.3%) of the patients with ALL did not respond to treatment (Table 2). In this study, we did not observe an association between the risk of ALL, sex and age (data not shown).

Genotypic Distribution of 5,10-MTHFR 677C>T and RFC1 80G>A Polymorphisms

The genotype frequencies of the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are shown in Table 3. The most frequent genotype in both groups was the heterozygous 5,10-MTHFR 677C/T, which was observed in 71.7% of the patients with ALL and 60.0% of the control group. The genotype distributions in the control children were found to be in genetic equilibrium ($p = 0.07$). The observed frequency of the T allele from the 5,10-MTHFR 677C>T polymorphism was 55.8% in patients with ALL and 41.7% in the control group. When the genotype frequencies of the 5,10-MTHFR 677C>T polymorphism were compared between groups, a statistically significant difference was observed ($p = 0.01$) (Table 3).

The genotype heterozygous RFC1 G80A was observed in 71.7% of the patients with ALL, while the genotype homozygous RFC1 G80G was observed in 51.7% of the control group. The genotype distributions to RFC1 80G>A polymorphism in control children were in genetic equilibrium ($p = 0.40$).

The frequency of the A allele of the RFC1 80G>A polymorphism was observed in 42.5% of patients with ALL and in 26.7% of the control group. In addition, the genotype frequencies of the RFC1 80G>A polymorphism showed a statistically significant difference between the groups ($p = 0.003$) (Table 3).

Table 2. Population characteristics in this study

Variable	All 60 (100.0)	Controls 60 (100.0)
Age at diagnosis (years, mean ± SD)	5.9 ± 3.8	9.9 ± 5.0
No. of leukocytes/mm^3 at diagnosis	8600 (4,100-40,025)[a]	8000 (7,000-9,000)[a]
Gender		
Female	27 (45.0)	34 (56.7)
Male	33 (55.0)	26 (43.3)
Inmunophenotype		
B lineage	52 (86.7)	
T lineage	8 (13.3)	
Risk by age and leukocytes at diagnosis		
Low (1−10 years and 1−10 years and <50,000/mm^3 leucocytes)	27 (45.0)	0
High (<1 and >10 years and >50,000/mm^3)	33 (55.0)	0
Response to treatment		
With response	28 (46.7)	0
No response	32 (53.3)	0

Data indicate: *n* (%)
[a]Median (25−75 percentiles).

Table 3. Genotypic and allele distribution of the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms in ALL and control group

	ALL 60 (100.0)	Controls 60 (100.0)	p[a]	HWE
5,10-MTHFR 677C>T				
Genotypes				
C/C	5 (8.3)	17 (28.3)		
C/T	43 (71.7)	36 (60.0)	0.01[b]	0.07
T/T	12 (20.0)	7 (11.7)		
Alleles				
C	53 (44.2)	70 (58.3)	0.039[b]	
T	67 (55.8)	50 (41.7)		
RFC1 80G>A				
Genotypes				
G/G	13 (21.7)	31 (51.7)		
G/A	43 (71.7)	26 (43.3)	0.003[b]	0.40
A/A	4 (6.7)	3 (5.0)		
Alleles				
G	69 (57.5)	88 (73.3)	0.014[b]	
A	51 (42.5)	32 (26.7)		

HWE, Hardy–Weinberg equilibrium.
Data indicate: n (%).
[a] p-value between ALL and controls.
[b] $p < 0.05$.

Risk of Acute Lymphoblastic Leukemia Based on the Genotypes from 5,10-MTHFR 677C>T and RFC1 80G>A Polymorphisms

The genotype distributions for the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are shown in Tables 3 and 4. In this study, an association between the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms was observed with susceptibility to ALL ($p < 0.05$). Carrier patients of the 5,10-MTHFR 677T/T genotype have a high risk of susceptibility to ALL (OR = 2.89, 95% CI 1.49–22.82, $p = 0.011$). Carrier patients of the 5,10-MTHFR 677C/T genotype also have a high risk of susceptibility to ALL (OR = 1.9, 95% CI 1.36–12.09). Likewise, those carrier patients of the RFC1 80G/A genotype had a 3.3 risk for ALL (OR = 3.3, 95% CI 1.75–8.87, $p = 0.002$).

Discussion

Several studies have reported that polymorphisms in genes involved in the folate pathway are risk factors that influence the susceptibility to acute leukemia (18–21,23,26–28,34). However, little is known about the relationship between the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms and the risk for ALL in Mexican population (35). In this study, we investigated whether the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are involved in the susceptibility to pediatric ALL in Mexican population. Moreover, a high frequency of the 5,10-MTHFR 677 C/T and RFC1 80 G/A genotypes in ALL patients and a high susceptibility to develop ALL by the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms were observed; therefore, we concluded that the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are associated with susceptibility to developing ALL.

Our results demonstrated that the heterozygous 5,10-MTHFR 677 C/T genotype is linked to a 1.9 fold increased risk of developing ALL and that the homozygous 5,10-MTHFR 677 T/T genotype is linked to a 2.8 fold increased risk. This finding corroborates the data reported by Kim et al. (2009), who showed that the 5,10-MTHFR 677T/T genotype increased the risk of ALL (OR = 1.77, 95% CI = 1.02–3.09, $p = 0.044$) in Korean population (12). Likewise, Sood et al. (2010) found that patients had

Table 4. Association of the 5,10-MTHFR 1298A > C and 5,10-MTHFR 677C>T polymorphisms with the susceptibility of acute lymphoblastic leukemia

	LLA 60 (100.0)	Controls 60 (100.0)	p	OR	CI 95%	p[a]
5,10-MTHFR 677C>T						
Genotypes						
C/C	5 (8.3)	17 (28.3)		1.0		
C/T	43 (71.7)	36 (60.0)	0.01[b]	1.9	1.36–12.09	0.012[b]
T/T	12 (20.0)	7 (11.7)		2.8	1.49–22.82	0.011[b]
C/C	5 (8.3)	17 (28.3)		1.0		
C/T + T/T	55 (91.7)	43 (71.7)	0.008[b]	4.3	1.48–12.72	0.007[b]
RFC1 80G>A						
G/G	13 (21.7)	31 (51.7)		1.0		
G/A	43 (71.7)	26 (43.3)	0.003[b]	3.3	1.75–8.87	0.002[b]
A/A	4 (6.7)	3 (5.0)		1.35	0.29–6.34	0.698
G/G	13 (21.7)	31 (51.7)		1.0		
G/A + A/A	47 (78.3)	29 (48.3)	0.001[b]	3.9	1.74–8.57	0.001[b]

OR, odds ratio; CI, 95% confidence interval.
[a] p obtained by logistic regression analysis, taking the reference to 5,10-MTHFR C677 C and RFC1 G80 G genotypes.
[b] $p < 0.05$.

approximately 1.6 fold (95% CI = 0.96−2.96, p = 0.05) higher susceptibility to ALL than those with the 5,10-MTHFR 677C/C genotype (18). Similarly, Silva RMS, et al. (2013) reported that individuals with the 5,10-MTHFR 677C/T genotype had a 1.6 fold (OR = 1.6, 95% CI = 1.1−2.7) increased risk of developing ALL in a Brazilian population (19). Similarly, Mosaad YM, et al. (2015) found that children in the Egyptian population with the 5,10-MTHFR 677T/T genotype had increased risk of ALL (OR = 2.81, 95% CI = 0.72−10.92). However, Mosaad YM, et al. observed no significant differences (20). Additionally, in a meta-analysis carried out by Zhang B, et al. (2017) an association between the 5,10-MTHFR 677C>T polymorphism and ALL was reported (21). In contrast to our results, no association between the 5,10-MTHFR 677C>T polymorphism and childhood ALL was observed in 2 Iranian populations (4,36) and a Japanese population (31). The discrepancy in the results related to the association between the 5,10-MTHFR 677C>T polymorphism and ALL risk might be due to the different genetic background between populations and sample size, as well as other factors (gene-environment interactions, folate status) (37).

In our study, the RFC1 80G>A polymorphism showed that the heterozygous RFC1 80A/G genotype is linked to the risk of developing ALL. This finding is similar to that observed by Gast A, et al. (2007), in German population, who reported that the RFC1 80A/G genotype increased the risk of ALL (OR = 1.4) (34). Likewise, de Jonge R, et al. (2009) found that individual carriers of RFC1 80A/G or RFC1 80A/A genotypes had an approximately 1.5 fold higher susceptibility to ALL than those with the G/G genotype (26). Similarly, in the Indonesian population, Chan CYS, et al. (2011) reported that individuals with the RFC1 80G/A genotype had 1.9 fold risk, while patients with the RFC1 80A/A genotype had 1.5 fold risk for ALL (28). Additionally, Zhao W, et al. (2011) reported an association between the RFC1 80G>A polymorphism and leukemia in Chinese population (27). Forat-Yazdi M, et al. (2016) reported an association between the RFC1 80 A/G genotype and ALL (23). However, in contrast to our results, Silva RMS, et al. (2013) found no association between the RFC1 80A/G or RFC1 80A/A genotypes and childhood ALL in a Brazilian population (19).

One of the limitations of our study in the relationship to the polymorphisms and the development of leukemia was the lack of data related to folate consumption during pregnancy in mothers, as the concentration of folate in each of the patients could enrich more the association of 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms and ALL found in the studied population. Although the number of patients is small, the association between the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms and the risk of ALL was evident, so the data suggest that the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms could be linked to factors associated with the risk of developing ALL.

In conclusion, our results suggest, as a first approach, that the 5,10-MTHFR 677C>T and RFC1 80G>A polymorphisms are closely related to the development of childhood ALL in a Mexican population. Since ALL is a multifactorial neoplasia, it is necessary to perform a study with a broader population and consider other related risk factors, where different populations are analyzed with unified techniques for the genotyping of polymorphisms, as well as statistical analysis, to try to explain the discrepancies found between the different populations.

Conflict of Interest

The authors declare that there are no conflicts of interest

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