© IJHG 2022 Int J Hum Genet, 22(3): 138-145 (2022)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330 DOI: 10.31901/24566330.2022/22.03.815

MTHFR C677T Polymorphism and Risk of Acute Lymphoblastic Leukaemia in Mexican Population

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KEYWORDS Adults. Children. Dominant Inheritance Model. Susceptibility. T Allele. TT Genotype

ABSTRACT The C677T polymorphism of the *MTHFR* gene has been frequently associated with acute lymphoblastic leukaemia (ALL) around the world. Therefore, the objective of this study was to determine whether the *MTHFR* C677T polymorphism is associated with ALL in Mexican children and adults. A case-control study was conducted on 243 patients with ALL (136 children and 107 adults) and 379 healthy controls. Genotyping of the C677T polymorphism was performed by the polymerase chain reaction-restriction fragment length polymorphism method. The frequency of the T allele was forty-three percent, fifty-two percent, and forty-six percent in children, adults, and controls, respectively. No risk of ALL was found for T allele carriers in children [OR=0.81 (0.52-1.25), P=0.33], but an increased risk was detected in adults [OR=4.98 (2.24-11.10), P<0.001]. The findings in children agree with most of the studies but are discordant with those reported in adults. These differences could be due to ethnicity, genetic background, sampling, and methodology.

INTRODUCTION

The methylenetetrahydrofolate reductase gene (*MTHFR*), located on 1p36.22, spans a region of 2.2 kilobases, is composed of 11 exons, and encodes the 5,10-methylenetetrahydrofolate reductase enzyme (MTHFR) (Goyette et al. 1998; Online Mendelian Inheritance in Man (OMIM) 2022). This enzyme is crucial for the metabolism of folate since it catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate and carbon donor for the re-methylation of homocysteine to methionine, in a reaction catalysed by the methionine synthase enzyme (Frosst et al. 1995; Petrone et al. 2021). Further, methionine is a precursor to S-adenosylmethionine, the principal methyl group donor for the recycling of homocysteine (Goyette et al. 1998; Petrone et al. 2021). The substrate 5,10-methylenetetrahydrofolate can also be used as a donor of methyl groups for the synthesis of deoxythymidine monophosphate from deoxyuridine monophosphate, a reaction catalysed by the thymidylate synthase enzyme (Catala et al. 2019; Petrone et al. 2021). In turn, the sub-product 5,10-formyltetrahydrofolate contributes to the production of adenosine and guanosine. All these molecules are essential for deoxynucleic acid (DNA) synthesis and repair. Then, deficiency or disturbance in the metabolism of folate may drop the DNA synthesis and DNA methylation and thus alter gene regulation leading to mutagenesis and malignant transformation (Catala et al. 2019). The presence of single nucleotide polymorphisms (SNP) in the *MTHFR* gene can be a negative effect on the activity of the enzyme. There are more than 30 SNPs in this

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gene (Genome Data Viewer MTHFR Gene 2022), of which the most common is the variant rs1801133, which is a C→T transition at the nucleotide 677 in the exon 4 (C677T), that results in a change of alanine for valine in the position 222 of the protein (A222V) (Online Mendelian Inheritance in Man, OMIM 2022). Homozygous individuals 222VV have about thirty percent of the expected enzymatic activity, while the heterozygotes have about sixty-five percent activity (Frosst et al. 1995). So, decreased activity of MTHFR hampers the synthesis of 5-methyltetrahydrofolate, which leads to the accumulation of 5,10-methylenetetrahydrofolate and, consequently, to rising of homocysteine (Petrone et al. 2021). High levels of this latter have been associated with cancer, including acute lymphoblastic leukaemia (ALL) (Petrone et al. 2021), which represents the most common malignancy in children, accounting for nearly eighty percent of childhood leukaemia. Although it is most common in children, it occurs also in adults, especially after age 50 (Kaatsch 2010; Katz et al. 2015). Even though the aetiology of leukaemia is complex and unknown, it is understood that variation in DNA synthesis or DNA methylation could be the first step toward hematopoietic malignant transformation (Rasmussen and Helin 2016). The *MTHFR* C677T variant has been widely associated with ALL, however the findings have been inconsistent. Some studies have shown risk of the T allele for ALL (Reddy and Jamil 2006; Lv et al. 2010; Bahari et al. 2016; Gómez-Gómez et al. 2019; Frikha et al. 2021), while others have not (Wiemels et al. 2001; Balta et al. 2003; Chiusolo et al. 2004; Thirumaran et al. 2005; Zanrosso et al. 2006; Alcasabas et al. 2008; Kim et al. 2009; Lightfoot et al. 2010; Tong et al. 2010; Chan et al. 2011; Metayer et al. 2011; Yang et al. 2011; Amigou et al. 2012; Nikbakht et al. 2012; Bellampalli et al. 2015; Goricar et al. 2015; Mosaad et al. 2015; Liu et al. 2016; Akin et al. 2017; Kaluzna et al. 2017). Even, other studies have shown significant protection of the T allele against the development of ALL (Skibola et al. 1999; Gemmati et al. 2004; Oh et al. 2007; de Jonge et al. 2009; Yeoh et al. 2010; Silva et al. 2013; Moulik et al. 2014; El-Masry et al. 2015; Pei et al. 2015; Xia et al. 2017).

Objectives

This study aims at determining whether the *MTHFR* C677T polymorphism is associated with ALL in Mexican children and adults.

MATERIAL AND METHODS

Ethical Declaration

This research was approved by the ethics committee of each participating institute and written informed consent was obtained from the patients or their parents before enrolment. Moreover, this study was conducted following the Helsinki Declaration and the Official Mexican Norm NOM-012-SSA3-2012 guidelines.

Study Population

The researched included a total of 243 patients diagnosed with ALL, which were divided into two groups, that is, one composed of children (136 patients: 85 boys and 51 girls) and the other formed by adults (107 subjects: 70 men and 37 women). The children were enrolled at the Paediatric Hospital of the Centro Médico Nacional de Occidente in Guadalajara, Jalisco, and their mean age was of 7 years at the time of the analysis. The adult patients were recruited from the Hospital, Dr. José María Cantú in Reynosa, Tamaulipas. Their mean age was of 43 years. The researchers also analysed a control group, which consisted of 379 healthy young students with no history of hematologic malignancies or other types of cancer, registered from the Unidad Académica Multidisciplinaria Reynosa-Aztlán, in Reynosa, Tamaulipas, and the average age of this group was 19 years.

Genotyping

Genomic DNA was isolated from peripheral blood by the phenol-chloroform procedure. Genotyping of the *MTHFR* C677T polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with the following primers, that is, forward 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and reverse 5'-AGGACGGTGCGGTGAGAGTG-3'. DNA amplification was conducted using 100 ng of genomic DNA, PCR buffer containing 3 mM $MgCl_2$, 0.25 mM deoxynucleotide triphosphates (dNTPs) (Bioline), 5 pmol of each primer, and 1 U *Taq* DNA polymerase (Invitrogen, USA). PCR was carried out in a Thermal Cycler C1000 System during 35 cycles, consisting of initial denaturation at 94°C for 4 minutes, followed by 94°C for 30 seconds,

61°C for 30 seconds, and 72°C for 30 seconds. The final extension was performed at 72°C for 7 minutes (Bazzaz et al. 2010). A fragment of 198 base pairs (bp) was obtained, and 10 µl of the PCR product was digested for 4 hours at 37°C with the enzyme *Hinf* I (BioLabs, New England, USA). The resulting fragments were analysed in polyacrylamide gels stained with silver nitrate. The C variant remained uncut (198 bp), whereas the T allele was cut in fragments of 175 bp and 23 bp (data not shown).

Statistical Analysis

The allele and genotype frequencies were calculated in every group, and these were contrasted between patients and controls using the chi-square test (χ^2). Association was estimated by odds ratio (OR) and ninety-five percent confidence interval (CI) (available at https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). For the analysis between the alleles, the C allele was taken as reference, while for the comparison of genotypes, the CC homozygotes were taken as reference and contrasted *versus* the sum of the CT heterozygote and the TT homozygote individuals (dominant inheritance model). A $P \leq 0.05$ was considered significant.

RESULTS

The purpose of this study was to analyse the relationship between the *MTHFR* C677T polymorphism and ALL in Mexican children and adults. So, in children, the allele frequencies were fifty-seven percent and forty-three percent for C and T respectively, while these were forty-eight percent and fifty-two percent respectively, in the adults. Then, the allele and genotype frequencies of the C677T polymorphism were contrasted independently between the controls and the children, and between the controls and the adult patients. No significant differences between controls and children were found in the analysis [OR=0.81 (0.52-1.25), P=0.33]. But an increased risk of ALL for T allele carrier adults [OR=4.98 (2.24-11.10), P<0.001] was detected under a dominant inheritance model (CC *vs* CT+TT) (Table 1). The gender did not show any association with this polymorphism on the risk of ALL in any group (data not shown).

DISCUSSION

The frequency of the *MTHFR* 677T allele in the control group was forty-six percent, agreeing with the forty-nine percent of another analysis conducted in the Mexican population (Gutiérrez-Álvarez et al. 2016), but different from most other countries. Thus, the T allele frequency ranges from ten percent in Filipinos (Alcasabas et al. 2008) and Indians (Bellampalli et al. 2015) up to fifty-four percent in Chinese people (Yang et al. 2011) (Table 2). The results showed no association of the T allele under a dominant inheritance model with childhood ALL, even displaying a trend towards leukaemia protection (non-significant OR<1.0) in agreement with most studies, in which ORs lesser than 1.0 and P>0.05 were also detected (Wiemels et al. 2001; Balta et al. 2003; Chiusolo et al. 2004; Thirumaran et al. 2005; Zanrosso et al. 2006; Tong et al. 2010; Chan et al. 2011; Metayer et al. 2011; Yang et al. 2011; Nikbakht et al. 2012; Bellampalli et al. 2015; Goricar et al. 2015; Akin et al. 2017). In this regard, some series have reported significant protection of the T allele against susceptibility to ALL in paediatric population. Namely, de Jonge et al. (2009) in the Netherlands, Yeoh et al. (2010) and Xia et al. (2017) in China, Silva et al. (2013) in Brazil, Moulik et al. (2014) in north India, and Pei et al. (2015) in Taiwan, all them under a dominant model (Table 2).

Table 1: Analysis of allele and genotype frequencies of the MTHFR C677T polymorphism between controls and children with ALL, and between controls and adults with ALL of the Mexican population

Genotype/ Allele	*Controls (n=379)*	*ALL children (n=136)*	*OR (95% CI)*	*P-value*	*Controls (n=379)*	*ALL adults (n=107)*	*OR (95% CI)*	*P-value*
CC (n)	98	41	1.0 Reference		98	7	1.0 Reference	
CT+TT (n)	281	95	0.81 (0.52-1.25)	0.33*	281	100	4.98 (2.24-11.10)	<0.001*
C (%)	54	57	1.0 Reference		54	48	1.0 Reference	
T (%)	46	43	0.89 (0.67-1.18)	0.41	46	52	1.29 (0.96-1.75)	0.10

*Analysis under a dominant model (CC vs CT+TT) through the χ^2 test using the CC genotype as reference.

Table 2: Frequency of the 677T allele and distribution of genotypes in controls and ALL children from several populations

Country	*Controls/ Cases*	**T allele (%)*	*Genotype of Controls*		*Genotype of Cases*		*OR (95% CI)*	*[a]P-value*	*Reference*
			CC	*CT+TT*	*CC*	*CT+TT*			
UK	200/216	35.8	89	111	98	118	0.97 (0.66-1.42)	0.85	Wiemels et al. 2001
UK	760/805	31.9	359	401	374	431	1.03 (0.85-1.26)	0.76	Lightfoot et al. 2010
Turkey	185/142	27.8	90	95	71	71	0.95 (0.61-1.47)	0.81	Balta et al. 2003
Turkey	296/180	31	141	155	86	94	0.99 (0.69-1.44)	0.96	Akin et al. 2017
Italy	110/174	43.2	35	75	65	109	0.78 (0.47-1.30)	0.34	Chiuosolo et al. 2004
Germany	1448/453	35	600	848	199	254	0.90 (0.73-1.12)	0.35	Thirumaran et al. 2005
Brazil	198/165	30.1	96	102	96	69	0.68 (0.45-1.03)	0.07	Zanrosso et al. 2006
Brazil	224/144	33	95	129	82	62	**0.56 (0.37-0.85)**	**0.007**	Silva et al. 2013
India	142/135	23.9	79	63	51	84	**2.07 (1.28-3.34)**	**0.003**	Reddy and Jamil, 2006
India	100/125	35.5	40	60	54	71	0.88 (0.51-1.50)	0.63	Nikbakht et al. 2012
India	300/150	20.7	192	108	113	37	**0.58 (0.38-0.90)**	**0.015**	Moulik et al. 2014
India	191/129	10	156	35	107	22	0.92 (0.51-1.65)	0.77	Bellampalli et al. 2015
Philippines	394/189	9.9	322	72	145	44	1.36 (0.89-2.07)	0.16	Alcasabas et al. 2008
Korea	1700/107	42.9	540	1160	29	78	1.25 (0.81-1.94)	0.31	[b]Kim et al. 2009
Netherlands	496/245	33.4	219	277	130	115	**0.70 (0.51-0.95)**	**0.022**	De Jonge et al. 2009
China	345/318	31	163	182	184	134	**0.65 (0.48-0.89)**	**0.006**	Yeoh et al. 2010
China	508/361	40.6	173	335	135	226	0.87 (0.65-1.15)	0.31	Tong et al. 2010
China	367/231	54.2	84	283	54	177	0.97 (0.66-1.44)	0.89	Yang et al. 2011
China	423/210	29.8	212	211	134	76	**0.57 (0.41-0.80)**	**0.001**	Xia et al. 2017
USA[c]	177/154	40.9	59	118	62	92	0.74 (0.47-1.16)	0.19	Metayer et al. 2011
USA[d]	269/221	29.9	127	142	107	114	0.95 (0.67-1.36)	0.79	Metayer et al. 2011
Indonesia	177/185	16.7	122	55	140	45	0.71 (0.45-1.13)	0.15	Chan et al. 2011
France	427/434	35.7	178	249	172	262	1.09 (0.83-1.43)	0.54	Amigou et al. 2012
Taiwan	266/266	29.1	134	132	169	97	**0.58 (0.41-0.82)**	**0.002**	Pei et al. 2015
Slovenia	184/121	ND	72	112	57	64	0.72 (0.45–1.15)	0.17	Goricar et al. 2015
Egypt	100/100	21	61	39	53	47	1.39 (0.79-2.43)	0.25	Mosaad et al. 2015
Iran	120/100	12.1	92	28	64	36	**1.85 (1.03-3.33)**	**0.04**	Bahari et al. 2016
Poland	404/117	33	178	226	54	63	1.10 (0.61-1.99)	0.75	Kaluzna et al. 2017

Just case-control studies with at least 100 patients and 100 controls were included (series with a smaller number were omitted due to insufficient statistical power); meta-analysis and systematic reviews were discarded. Significant analyses are highlighted in boldface. *T allele frequency determined from the group control. [a]Analysis under a dominant model (CC vs CT+TT) through the χ^2 test using the CC genotype as reference. [b]These authors reported an increased risk, adjusted for sex and age, for the TT genotype compared to the CC genotype [OR=1.77 (1.02-3.09), P=0.044] (Kim et al. 2009). [c]Hispanic children. [d]Non-Hispanic children. ND. Not determined.

However, it is relevant to note that these observations are not universal and differ from the findings by Reddy and Jamil (2006), Bahari et al. (2016), and Gómez-Gómez et al. (2019) whose results showed association of the T allele with ALL risk in children from India [OR=2.07 (1.28-3.34), P=0.003], Iran [OR=1.85 (1.03-3.33), P=0.04], and Mexico [OR=4.35 (1.49-12.73), P<0.005], respectively. Moreover, other investigations have revealed a trend of the T allele with susceptibility to childhood ALL (non-significant ORs>1.0) (Alcasabas et al. 2008; Kim et al. 2009; Lightfoot et al. 2010; Amigou et al. 2012; Mosaad et al. 2015; Kaluzna et al. 2017). The discrepancy between the findings by Gómez-Gómez et al. (2019) and this study could be explained, as apart from the sample size (they analysed 60 patients), through the origin of the patients, this study analysed children from the state of Jalisco, while they studied children from the state of Guerrero.

As for adult patients, the study showed an increased risk of ALL for T allele carriers under a dominant model [OR=4.98 (2.24-11.10), P<0.001]. Studies on this polymorphism performed in adult ALL patients are scarce, and as in children, the results are inconsistent. Only research, conducted in a sample of Chinese patients with B-cells ALL, showed a trend toward ALL risk for carriers of the T allele [OR=1.53 (0.92-2.56), P=0.08, dominant

Table 3: Frequency of the 677T allele and distribution of genotypes in controls and adult patients from some populations

Country	*Controls/ Cases*	**T allele (%)*	*Genotype of Controls*		*Genotype of Cases*		*OR (95% CI)*	*[a]P-value*	*Reference*
			CC	*CT+TT*	*CC*	*CT+TT*			
Italy	257/114	44.7	78	179	52	62	**0.52 (0.33-0.82)**	**0.005**	Gemmati et al. 2004
Korea	427/118	40.9	138	289	49	69	0.67 (0.44-1.02)	0.06	Oh et al. 2007
China	182/127	37.6	72	110	38	89	1.53 (0.95-2.48)	0.082	Lv et al. 2010
China	367/130	54.2	84	283	31	99	0.95 (0.59-1.52)	0.82	Yang et al. 2011

Just case-control studies with at least 100 patients and 100 controls were included (series with a smaller number were omitted due to insufficient statistical power); meta-analysis and systematic reviews were discarded. Significant analysis is depicted in boldface. *T allele frequency determined from the controls. [a]Analysis under a dominant model (CC vs CT+TT) through the χ^2 test using the CC genotype as reference.

model] (Lv et al. 2010). However, other studies found a decreased risk of ALL in T allele carriers (Gemmati et al. 2004; Oh et al. 2007) (Table 3). Moreover, two studies, conducted with small sample size, reported protection of the TT genotype against ALL. Specifically, in Caucasian population from England was documented a 4.3-fold decrease [OR=0.23 (0.06-0.81), P<0.05] (Skibola et al. 1999), while in Egyptians was reported a 2.1-fold reduction [OR=0.48 (0.28-0.80), P=0.005] (El-Masry et al. 2015).

The researchers do not have a precise explanation for the findings in adults. Perhaps, the differences among the diverse studies, whether in children or adult patients, could be due to ethnicity, genetic background, sample size, sample heterogeneity, and methodological designs used. However, the results of the analysis are not entirely consistent, since the 677T polymorphism only showed a risk of ALL in adults, despite both examined groups being of the same ethnicity, although the children were enrolled in the state of Jalisco and the adults in the state of Tamaulipas. As in other multifactorial diseases, the interaction between genetic and environmental factors plays an important role in the risk of developing ALL. So, the presence of the C677T variant in combination with polymorphisms in this and other genes, and environmental factors, including diet, could interact to influence this risk.

The MTHFR enzyme with the 222V variant is known to have decreased activity relative to the 222A variant (Frosst et al. 1995). Since this is essential to produce molecules important for DNA synthesis, methylation, and repair, a reduction in its activity may increase susceptibility to malignant transformation (Rasmussen and Helin 2016; Catala et al. 2019), leading to an increased risk of ALL. Moreover, it has been documented that the MTHFR 222V variant raises the rate of dissociation of its flavin adenine dinucleotide (FAD) cofactor. Because this latter is essential for transferring electrons between reduced nicotinamide adenine dinucleotide (NADH) and folate, its liberation is accompanied by changes in the quaternary structure of the enzyme and consequently, in loss of activity, as folate binding can protect the enzyme from premature loss of FAD (Yamada et al. 2001). Therefore, chronic deficiency of folates in adults could be a possible cause for the 677T variant to have shown a risk of ALL in subjects of this age group in this study's population. However, this is just an assumption, and some limitations of the study must be considered. One of these is the fact that the researchers do not know the intake of folate in these patients, nor if a possible deficiency is greater in adults than in children. Though, it is well known that the diet of the Mexican population is deficient in folates and vitamins (Cuevas-Nasu et al. 2012; Shamah-Levy et al. 2015). It should also be noted that these inconclusive results could be influenced by the presence of other polymorphisms, mutations, and/or chromosomal abnormalities not analysed in the study.

CONCLUSION

In this study, the frequency of the *MTHFR* 677T allele was similar to that reported in the Mexican population but different from that of most other countries. Moreover, the T allele was associated, under a dominant inheritance model, with acute

lymphoblastic leukaemia in adults but not in children in the Mexican population. These inconsistent results could be influenced by several factors, including nutritional deficiencies, mutations, and polymorphisms in other genes, or chromosomal abnormalities.

RECOMMENDATIONS

Further studies based on large-scale sampling and the recruitment of patients from other Mexican states are needed to clarify the role of the *MTHFR* C677T polymorphism on the risk of acute lymphoblastic leukaemia in the population.

ACKNOWLEDGMENTS

The researchers thank the patients for their collaboration to participate in this study.

FUNDING

This work was supported by the UAT-CA-69 P/PFCE-2018-28MSU0010B-13 grant.

ETHICS APPROVAL

This research was approved by the ethics committee of each participating institute and written informed consent was obtained from the patients or their parents before enrolment. Moreover, this study was conducted following the Helsinki Declaration and the Official Mexican Norm NOM-012-SSA3-2012 guidelines.

ABBREVIATIONS

ALL: Acute Lymphoblastic Leukaemia
bp: base pairs
DNA: Deoxynucleic Acid
FAD: Flavin adenine dinucleotide
dNTPs: Deoxynucleotide triphosphates
MTHFR: Methylenetetrahydrofolate reductase
NADH: Reduced nicotinamide adenine dinucleotide
PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism
SNP: Single nucleotide polymorphism
Taq: *Thermus aquaticus*

STATISTICAL SYMBOLS

CI: Confidence interval
OR: Odds ratio
χ^2: Chi-square test

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Paper received for publication in March, 2022
Paper accepted for publication in March, 2022