



## Ethnic variation of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate-reductase (*MTHFR*) gene in southwestern Mexico

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**ABSTRACT.** In this study, we examined the distribution of genotype and allele frequencies of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate-reductase gene (*MTHFR*) in two ethnic groups in the State of Guerrero, Mexico, which were compared with those of the Mestizo population of the region. A comparative study was conducted on 455 women from two ethnic groups and a group of Mestizo women of the State of Guerrero, Mexico: 135 Nahuas, 124 Mixtecas, and 196 Mestizas. Genotyping of both polymorphisms were performed by using polymerase chain reaction-restriction fragment length polymorphism methods. We found that the 677TT genotype was more frequent in Nahua and Mixteca women compared to Mestiza women ( $P = 0.008$ ), and the most prevalent genotype in both ethnic groups was the 1298AA genotype ( $P < 0.001$ ). We also compared

the 677T allele frequency obtained from the groups studied with the frequencies reported in other ethnic groups of Mexico (Huichol, Tarahumara, and Purepecha). There were significant differences between the three ethnic groups compared to Nahuas (Huicholes,  $P = 0.004$ ; Tarahumaras,  $P < 0.001$ ; Purepechas,  $P = 0.042$ ). Our results indicated significant differences in the frequencies of the C677T and A1298C polymorphisms between the two ethnic groups and the Mestizo population of the State of Guerrero. In addition, we found strong differences with other ethnic groups in Mexico. These results could be useful for future studies investigating diseases related to folate metabolism, and could help the government to design specific nutrition programs for different ethnic groups.

**Key words:** A1298C polymorphism; C677T polymorphism; Ethnic groups; Mixteca; *MTHFR*; Nahua

## INTRODUCTION

The gene encoding the enzyme 5,10-methylenetetrahydrofolate-reductase (*MTHFR*) is highly polymorphic in the general population (Goyette et al., 1994, 1995). The most common functional polymorphisms are C677T (rs1801133) and A1298C (rs1801131). The C677T polymorphism is located in exon 4 and causes a change of an alanine for a valine at codon 222, which is associated with increased thermolability and decreased *MTHFR* enzyme activity of up to 70%, resulting in moderate hyperhomocysteinemia in TT homozygous individuals (Frosst et al., 1995; Weisberg et al., 1998; Song et al., 2001b). The A1298C polymorphism is located in exon 7 and results in a substitution of glutamate by alanine at codon 429, which also decreases the *MTHFR* enzyme activity, although to a lesser degree than the C677T polymorphism (Weisberg et al., 1998; Lievers et al., 2001; Chen et al., 2002). Individuals heterozygous for both polymorphisms have *MTHFR* enzyme activity comparable to that observed in individuals homozygous for the 677TT genotype, resulting in an increase of homocysteine and a decrease in plasma folate levels (van der Put et al., 1998; Weisberg et al., 1998; Friedman et al., 1999; Friso et al., 2002).

Studies of ethnic groups around the world show high variability in the allelic and genotypic distributions of the C677T and A1298C polymorphisms (Wilcken et al., 2003; Guéant-Rodriguez et al., 2006). Mexico has one of the world's highest frequencies for the C677T polymorphism, which varies from 36 to 58%; however, important differences are observed among ethnic groups in Mexico (Mutchinick et al., 1999; Dávalos et al., 2000; González-Herrera et al., 2002). Moreover, the distribution of the A1298C polymorphism in ethnic groups of Mexico is unknown, and has only been reported for the Mestizo population with a frequency of 14 to 21% for heterozygous carriers (Guéant-Rodriguez et al., 2006; Gonzalez-Herrera et al., 2007; Pilsner et al., 2010).

Mexico has a great diversity of ethnic groups (INEGI, 2010); therefore, it is important to determine the frequencies of the C677T and A1298C polymorphisms, especially in ethnically related groups that have been isolated geographically and socially, as those living in the State of Guerrero, where there are no reports on the frequency of these polymorphisms. In this study, we examined the distribution of the genotype and allele frequencies of the C677T and

A1298C polymorphisms in the *MTHFR* gene in two ethnic groups in the State of Guerrero, Mexico, which were compared with those of the Mestizo population of the region.

## MATERIAL AND METHODS

### Subjects

A comparative study was conducted on 455 women from two ethnic groups and a group of Mexican-Mestizo women representing the State of Guerrero, Mexico: 135 Nahuas, 124 Mixtecas, and 196 Mestizas. Participants were recruited from a previous study for the detection of human papilloma virus and cytological screenings, and were included after providing informed consent based on the recommendations of the Declaration of Helsinki (1964). The protocol was approved by the Ethics Committee of the University of Guerrero. Exfoliated cervical cells were obtained from each participant and were treated with proteinase K (Sigma, St. Louis, MO, USA) and phenol-chloroform-isoamyl alcohol for extraction of DNA (Strauss, 2001), which was stored at -20°C until analysis.

### Genotyping

Genotyping of the *MTHFR* C677T and A1298C polymorphisms were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), following methods adapted from Frosst et al. (1995) and Weisberg et al. (1998). The regions of the genome containing the two polymorphisms were amplified separately. The PCRs were performed in a GenAmp PCR System 2400 thermocycler (Applied Biosystems).

The C677T polymorphism was identified using 1 µM each of the following primers to amplify a 198-bp region from genomic DNA: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' (forward) and 5'-AGGACGGTGCGGTGAGAGTG-3' (reverse). Each amplification reaction was performed in a total volume of 50 µL, containing 1X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 1.25 U Ampli Taq Gold™ (Applied Biosystems), 0.2 mM dNTP (Invitrogen Life Technologies, Carlsbad, CA, USA), and 1.0 µg DNA. Processing started with 94°C for 10 min, and 40 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by a final extension step at 72°C for 7 min. The amplification products were resolved by electrophoresis on 3% agarose gels stained with 0.5 µg/µL ethidium bromide, and visualized under ultraviolet light using the GeneGenius BioImaging System (Syngene; Synoptics Ltd., Cambridge, UK).

The C→T substitution creates a *Hinf*I restriction site. The digestion was performed by preparing a mixture of 1X buffer, 3 µg RNase, 3 U *Hinf*I (Invitrogen Life Technologies), and 8 µL PCR product, which was incubated at 37°C for 2 h. Digestion products were analyzed on 10% acrylamide gels stained with ethidium bromide. Wild homozygous genotypes (677CC) generated fragments of 198 bp, the heterozygote (677CT) generated fragments of 198, 175, and 23 bp, and the homozygote variants (677TT) generated fragments of 175 and 23 bp.

Genotyping of the *MTHFR* A1298C polymorphism was determined using 1 µM each of the following primers to amplify a 163-bp region from genomic DNA: 5'-CCTTGGGGAGCTG AAGGACTACTAC-3' (forward) and 5'-CACTTTGTGACCATTCCGGTTTG-3' (reverse). The mixture preparation was the same as that used for the C677T polymorphism. The amplification conditions were as follows: denaturing period at 94°C for 10 min and 35 cycles of 92°C for 1 min, 60°C for 1 min, and 72°C for 30 s, followed by a final extension step at 72°C for 7 min.

The A→C substitution eliminates the restriction site *Mbo*II. The amplification products were digested with 3 U restriction enzyme *Mbo*II (Amersham Biosciences, São Paulo, SP, Brazil), followed by electrophoresis on 10% acrylamide gels stained with ethidium bromide. Wild homozygous genotypes (1298AA) generated five fragments of 56, 31, 30, 28, and 18 bp, the heterozygote (1298AC) generated six fragments of 84, 56, 31, 30, 28, and 18 bp, and the homozygote variants (1298CC) generated fragments of 84, 31, 30, and 18 bp.

### Statistical analysis

We determined the genotype and allele frequencies of the C677T and A1298C polymorphisms, which are reported as absolute and relative numbers, and were used to compare ethnic groups. Departures from Hardy-Weinberg equilibrium (HWE) were verified using the chi-square test with one degree of freedom. We calculated Lewontin's *D'* statistic for linkage disequilibrium between loci. The frequency of the C677T polymorphism obtained in this study was compared with frequencies reported in other ethnic groups of Mexico using the Fisher exact test. Statistical analysis was performed using the STATA software (v.11.2) and  $P < 0.05$  was reported as statistically significant.

### RESULTS

The average ages of the participants were 38 years for Nahua women, 43 years for the Mixteca women, and 37 years for the Mestiza women. In analyzing the distribution of the C677T polymorphism, it was found that the TT genotype was more frequent in Nahua and Mixteca women compared to Mestiza women ( $P = 0.008$ ). The most prevalent genotype in both ethnic groups was the AA genotype ( $P < 0.001$ ). The two polymorphisms were in linkage disequilibrium ( $D' = 0.839$ ), and HWE was present in the three study groups (Table 1). In the haplotype analysis,  $T_{C677T}A_{A1298C}$  was found to be more common in Mixteca and Nahua women compared with Mestiza women (Table 2).

**Table 1.** Genotype and allele frequencies of the C677T and A1298C polymorphisms in the *MTHFR* gene by ethnic group in the State of Guerrero, Mexico.

SNP	Nahua (N = 135) [N (%)]	Mixteca (N = 124) [N (%)]	Mestiza (N = 196) [N (%)]	P value*
<b>C677T</b>				
CC	7 (5.2)	12 (9.7)	33 (16.8)	0.008
CT	58 (43.0)	50 (40.3)	87 (44.4)	
TT	70 (51.9)	62 (50.0)	76 (38.8)	
CT+TT	128 (94.8)	112 (90.3)	163 (83.2)	
C	72 (26.7)	74 (29.8)	153 (39.0)	0.004
T	198 (70.3)	174 (70.2)	239 (61.0)	
HWE (P value)	(0.253)	(0.681)	(0.346)	
<b>A1298C</b>				
AA	127 (94.1)	115 (92.7)	151 (77.0)	<0.001
AC	8 (5.9)	9 (7.3)	44 (22.5)	
CC	0	0	1 (0.5)	
AC+CC	8 (5.9)	9 (7.3)	45 (23.0)	<0.001
A	262 (97.0)	239 (96.4)	346 (88.3)	
C	8 (3.0)	9 (3.6)	46 (11.7)	
HWE (P value)	(0.723)	(0.675)	(0.241)	

HWE = Hardy-Weinberg equilibrium. \* $\chi^2$  or Fisher exact tests.

**Table 2.** Haplotype frequencies in ethnic groups in the State of Guerrero, Mexico

	C677T	A1298C	All (%)	Nahua (%)	Mixteca (%)	Mestiza (%)
1	T	A	66.4	72.5	70.2	59.9
2	A	A	26.7	24.6	26.2	28.4
3	C	C	6.2	2.1	3.6	10.6
4	C	C	0.8	0.9	0	1.1

We compared the 677T allele frequency obtained in the study groups investigated (70.3% for Nahuas and 70.2% for Mixtecas) with the frequencies reported in other ethnic groups of Mexico, including Huichol, Tarahumara, and Purepecha (56, 36, and 57%, respectively) (Dávalos et al., 2000). There were significant differences between the three ethnic groups compared with Nahuas (Huicholes,  $P = 0.004$ ; Tarahumaras,  $P < 0.001$ ; and Purepechas,  $P = 0.042$ ). Whereas, with respect to the Mixtecas, significant differences were only found with two of the ethnic groups (Huichol,  $P = 0.028$  and Tarahumara,  $P < 0.001$ ). Interestingly, the 677T allele frequency obtained in the two ethnic groups in the State of Guerrero was higher than that reported for the other three ethnic groups of Mexico (Dávalos et al., 2000) (Table 3).

**Table 3.** Comparison of genotype and allele frequencies of the C677T polymorphism among ethnic groups reported in Mexico (Dávalos et al., 2000) with the groups studied in the State of Guerrero, Mexico.

Ethnic group	N (%)	CC	CT	TT	C	T	p*	p*	p*	p*	p*
Huichol	50	8 (16)	28 (56)	14 (28)	44 (28)	56 (56)	Reference				
Tarahumara	38	17 (45)	15 (39)	6 (16)	6 (16)	27 (36)	0.012	Reference			
Purepecha	21	4 (19)	10 (48)	7 (33)	7 (33)	24 (57)	0.776	0.100	Reference		
Nahua	135	7 (5.2)	58 (43.0)	70 (51.9)	72 (26.7)	198 (70.3)	0.004	<0.001	0.042	Reference	
Mixteca	124	12 (9.7)	50 (40.3)	62 (50.0)	74 (29.8)	174 (70.2)	0.028	<0.001	0.251	0.381	Reference

\* $\chi^2$  or Fisher exact tests.

## DISCUSSION

In this study, we examined the genotype and allele frequencies of the C677T and A1298C polymorphisms in two ethnic groups in the State of Guerrero, Mexico, which were compared with those of the Mestizo population of the region. Significant differences were found in the frequency of the 677TT genotype among the ethnic groups Nahua and Mixteco with Mestiza women ( $P = 0.008$ ). We detected a very low frequency of the 1298CC genotype in Mestiza women (0.5%). In the Nahua and Mixteca groups, we did not detect this genotype, and the polymorphism was found only in the heterozygous state (1298AC). Furthermore, we found significant differences in the frequency of the C allele between the two ethnic groups studied and Mestiza women ( $P < 0.001$ ). The most prevalent genotype in the three studied groups was 1298AA. Our results are consistent with the results obtained in other populations of Mexico, indicating that the 1298CC genotype frequency reported in the Mestizo population is one of the lowest in the world (Guéant-Rodriguez et al., 2006; Gonzalez-Herrera et al., 2007; Pilsner et al., 2010), and this is the first report of the genotype frequencies of the A1298C polymorphism in ethnic groups in southern Mexico.

Due to the involvement of the polymorphisms C677T and A1298C in MTHFR enzyme activity (van der Put et al., 1998; Weisberg et al., 1998; Friedman et al., 1999; Ulrich

et al., 1999), we determined the distribution of haplotypes in the study groups and found that they differed significantly between the two ethnic groups and Mestiza women. This finding suggests that genetic differences may have implications for folate metabolism, since it has been reported that combined heterozygosity for both *MTHFR* polymorphisms reduces the *MTHFR*-specific activity, leading to higher homocysteine and lower plasma folate levels (van der Put et al., 1998).

Different studies have been conducted to determine the frequency of the C677T and A1298C polymorphisms in various populations around the world, and the results are as varied as the populations studied (Rosenberg et al., 2002; Rady et al., 2002; Esfahani et al., 2003). For the 677TT genotype, the highest frequency (40%) was found in Mexico and the lowest (10%) was observed in African populations (Wilcken et al., 2003; Guéant-Rodriguez et al., 2006). However, there are few reports of the 1298CC genotype frequencies (approximately 0.8 to 12%) (Keku et al., 2002; Guéant-Rodriguez et al., 2006; Bagheri and Abdi, 2010), and all of them have been conducted in Mestizo populations. Therefore, it is important to pay special attention to ethnic groups that have remained partially isolated from the rest of the population that have retained their own cultural characteristics such as language, lifestyle, traditions, and religion.

Mexico is a multicultural country with over 60 officially recognized ethnic groups, four of which are represented in Guerrero: Nahuua, Mixteco, Amuzgo, and Tlapaneco (CDI, 2013). Despite the enormous ethnic diversity of the country, most studies have focused on the distribution of the C677T polymorphism in the Mestizo population, and to date, there is only one report of the three ethnic groups of northern and central Mexico (Huichol, Tarahumara, and Purepecha) (Dávalos et al., 2000), while in the southern part of the country, where the State of Guerrero is located, there are no such reports. Moreover, there is no data on the A1298C polymorphism of indigenous Mexicans, and only frequencies have been reported from 0.84 to 2.3% for the 1298CC genotype in the Mestizo population (Guéant-Rodriguez et al., 2006; Gonzalez-Herrera et al., 2007; Pilsner et al., 2010).

In order to investigate whether variation in the genotype and allele frequencies of the C677T polymorphism observed in the Mestizo population of different regions of Mexico (Mutchinick et al., 1999; Martínez de Villarreal et al., 2001; Dávalos et al., 2000) also occurs among ethnic groups in the rest of the country, we compared our results with previous reports, and found that the differences were more significant when Nahuas and Mixtecos were compared with the Tarahumaras group ( $P < 0.001$ ). There was virtually no difference when the comparison was made with the Purepecha group. These results are important because they can serve as a basis for future studies of the association of the 677T allele with various diseases in ethnic groups in southern Mexico.

The high frequency of the 677T allele found in the two ethnic groups in the State of Guerrero is an interesting finding because other studies have shown that this allele is associated with the risk of developing various diseases such as defects of neural tube closure, cardiovascular illnesses, and some cancers (Shaw et al., 1998; Volcik et al., 2000; Shen et al., 2001; Song et al., 2001a; Ueland et al., 2001; Esfahani et al., 2003; Izmirlı, 2013; Trimmer, 2013). Although this study did not assess any biochemical markers that show the effect of polymorphisms on folate metabolism, the frequency of these polymorphisms may serve as a basis for future studies evaluating the association with these diseases in southern Mexico. In conclusion, our results indicate that there are significant differences in the frequencies of the

C677T and A1298C polymorphisms between the two ethnic groups and the Mestizo population of the State of Guerrero. In addition, we found strong differences with other ethnic groups in Mexico; however, additional studies are needed in other ethnic groups in the region to determine the consistency of these results. This study provides valuable information for future investigations about diseases related to folate metabolism, and it will be very useful for designing appropriate nutrition programs in a country with a great diversity of ethnic groups such as Mexico.

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