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Frequency and coexistence of the C677T and A1298C polymorphisms of the MTHFR gene in Aures region of Algeria

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Abstract. *Hayat D, Mouloud Y, Abdelali B. 2018. Frequency and coexistence of the C677T and A1298C polymorphisms of the MTHFR gene in Aures region of Algeria. Biodiversitas 19: 1169-1175.* The aim of this study was to assess the frequency of the two most common polymorphisms C677T and A 1298C of the methylenetetrahydrofolate reductase (MTHFR) gene, as well as the coexistence of both these genetic variants in healthy subjects from part of the Algerian population (Aures Region). A total of 94 apparently healthy subjects were enrolled in the study group. The frequency of the both investigated genotypes of the both MTHFR gene polymorphisms (C677T and A1298C) was determined by using the Real-Time Polymerase Chain Reaction-Fluorescence Resonance Energy Transfer (Real-Time PCR-FRET) technic. The frequencies of C and T alleles of C677T polymorphism were 127 (67.55%), 61 (32.45%), and for CC, CT, and TT genotypes were 44 (46.48%), 39 (41.8%) and 11 (11.70%) respectively. Regarding the frequencies at position 1298, for A and C alleles were 147 (78.19%), 41 (21.81%), and for AA, AC, and CC genotypes were 60 (63.82%), 27 (28.72%) and 7 (7.44%) respectively. Also, our results indicated that no significant differences in the percentage distributions of the C677T (P=0.518) and A1298C (P=0.514) polymorphisms between males and females carriers. As noted in the findings, the most frequent coexistence of genotypes were 677CT/1298AA (29.78%), 677CC/1298AA (22.34%) and 677CC/1298AC (17.02%%). The coexistence of 677TT/1298AA (11.70%), 677CT/1298 AC (11.70%) and 677CC/1298 CC (7.44%) genotypes was observed less frequently and for 677TT/1298AC, 677CT/1298CC, 677TT/1298CC genotypes, it has been no observed in the studied population. The frequency of MTHFR 677 C and T alleles were 0.66 and 0.31, whereas that of MTHFR1298 A and C alleles were 0.77 and 0.21, respectively. The allelic distributions of the C677T polymorphism remain intermediate in the Aures region (Northeast of Algeria); that support the idea of a north-south gradient. For the A1298C SNP, our funding appears to be lower compared across populations. In addition, the frequency and coexistence of genotypes of the C677T and A 1298C MTHFR gene polymorphisms in the region studied are similar to other ethnic group populations. □

Keywords: 5,10-Methylenetetrahydrofolate reductase gene, C677T, A1298C SNPs, coexisting genotypes, Real-Time PCR-FRET

INTRODUCTION

Proper function of the folate cycle is directly linked to optimal human functioning. Plasma folate and vitamin B_{12} influence homocysteine (Hcy) metabolism as co-substrate and cofactor, respectively (Lim and Heo 2002). Homocysteine is a sulfur-containing amino acid derived from the metabolism of methionine (Malinow 1994).Total homocysteine levels (tHcy) are controlled by remethylation or transsulfuration pathways of methionine, related to folate and vitamin $B_{-6,\ 12}$ (Taguchi et al. 2012). In addition, Plasma levels of homocysteine are influenced by both environmental and genetic factors.

The 5, 10-methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes of homocysteine metabolism (Frosst et al. 1995; Klerk et al. 2002), that catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which acts as methyl donor for methionine synthesis from homocysteine (remethylation) (Goyette et al. 1998), also, mutations impairing this key enzyme are reported as causes of hyperhomocysteinemia (HHcyt) (Lievers et al. 2003), that is associated with increased risk for many disorders, including vascular and neurodegenerative diseases, pregnancy complications, cancers, etc. (Brustolin et al. 2010; Eloualid et al. 2012).

The 5, 10-MTHFR gene is located on chromosome 1 at 1p36.3. the complementary DNA sequence is 2.2 kilobases long and consists of 11 exons (Goyette et al. 1998), its several mutations including C677T (C to T) and A1298C (A to C) have been identified that decrease the MTHFR enzyme activity (Van der Put et al. 1995; Chen et al. 2005). These two single-nucleotide polymorphisms (SNPs) are the most frequent genetic cause for mild hyperhomo-cysteinemia (Frosst et al. 1995; Van der Put et al. 1995; Van der Put et al. 1998; Weisberg et al. 1998).

The C677T SNP results in a missense mutation, in exon 4, that converts a cytosine (C) into thymine (T) which is leading to substitution of valine for alanine at position 222 (Kang et al. 1991; Frosst et al. 1995), renders the MTHFR enzyme thermolabile and less active (Frosst et al. 1995). A second polymorphism in the MTHFR gene is A1298C polymorphism, results from an adenosine to cytosine transversion, at nucleotide 1298, in exon (Weisberg et al. 2001; Lievers et al. 2003), which leads to substitution of Glutamate-429 by alanine (Frosst et al. 1995; Van der Put

et al. 1998), this mutation can reduce the enzyme activity, although to lesser extent than the C677T polymorphism (Weisberg et al. 1998) and it does not affect the thermolability in the MTHFR gene (Van der Put et al. 1998). The coexistence of the double heterozygous (677CT/1298AC) individuals displays lower MTHFR activity than subjects heterozygous for either SNP (Van der Put et al.1998). Studies of ethnic groups around the world showed high variability in the allelic and genotypic distributions of the C677T and A1298C polymorphisms (Wilcken et al. 2003; Guéant-Rodriguez et al. 2006).

There are few studies and reports on the frequency of these two previous SNPs in Algerian population. The aim of this first study in Aures region, was to investigate the frequency of the MTHFR gene polymorphisms (C677T, rs=1801133/A1298C, rs=1801131), as well as their coexistence in healthy subjects from this area (Northeast of Algeria), and compare the results with data reported from other ethnic populations.

MATERIALS AND METHODS

Subjects

A total of ninety-four healthy unrelated subjects, were volunteered to participate in this study (64 males with mean age 29.46±15.68 and 30 females with mean age 43.03±15.28 living in Aures region), and were recruited, after obtaining their informed consent. The mean age of the subjects studied was 33.79±16.72 years, range [18-83 years].

Blood collection and laboratory analysis

Venous blood samples were collected in EDTA anticoagulant tube (5ml) and centrifuged immediately to isolate the cell pellets and plasma in separate tubes, the aliquots were stored at-20 °C until analyzed. This first step serves for the isolation of DNA from leukocytes.

DNA extraction

Genetic analyzes was performed at the CHU of Nancy (France), in the Laboratory of Nutrition-Genetics and Environmental Risk Exposure. Genomic DNA was extracted from blood leukocytes using the Kit BACC3 NUCLEON® marketed by Amersham Biosciences (Little Chalfont, UK). DNA extraction was performed according to the manufacturer's instructions.

Molecular genetic analysis

The MTHFR C677T, and A1298C and genotypes were determined using the Real-Time Polymerase Chain Reaction-fluorescence resonance energy transfer (Real-Time PCR-FRET), it was carried out with a Light Cycler ® 480 II Instrument (Roche Diagnostic, Meylan, France). (Ririe et al. 1997; Vossen et al. 2009).

The PCR primers and probes used in the assay and probe are: (i) For the C677T polymorphism: Sequence of primers Forward (F): 5'TGGCAGGTTACCCCAAAGG 3', Reverse primer (R): 5'TGATGCCCATGTCGGTGC3'. Probe Flu: TGAGGCTGACCTGAAGCACTTGAAGGAGAAGGTG TCTX. Probe Red (LC Red): CGGGAGCCGATTTCATCAT p. (ii) For the A1298C polymorphism: Sequence of primers Forward (F): 5'CTTTTGGGAGCTGAAGGACTACTAC3'. Reverse primer (R): 5'CACTTTGTGACCATTCCGGTTTG 3'. Prob Flu: AAGGAGGAGCTGCTGAAGATGTGGGG GGAGGAGC TX. Prob Red (LC Red): ACCAGTGAAGAAAGTGTCTTTGAp.

The amplification conditions were as follows: PCR comprise an initial denaturation cycle at 95 °C for 10 seconds, followed by 40 cycles of PCR, each comprising denaturation at 95 °C, hybridization at 55 °C and elongation at 72 °C for 10 seconds, 5 seconds, 5 seconds respectively, and finally cooling to 40 ° C for 30 seconds. (i) The melting temperatures for the three genotypes of MTHFR1 C677T are respectively: Wild genotype (CC): 63°C; homozygous mutated (TT): 55°C; heterozygous (CT): 55°C+64°C. (ii) The melting temperatures for the three genotypes of MTHFR2 A1298C are respectively: Wild genotype (AA): 63°C; homozygous mutated (CC): 60°C; heterozygous (AC): 61°C.

The data were expressed as a percentage for both allelic and genotypic frequencies. Pearson Chi-square test was used to compare different genotypes regarding gender (sex) characteristics. Data were analyzed using Statistical Package for the Social Sciences (SPSS) (Version 23.0.0.0-2015 for Windows, SPSS Inc., Chicago, IL). tics. $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Allelic and genotypic frequencies of MTHFR C677T polymorphism

We recruited ninety-four apparently healthy subjects, representing the Aures region in Algeria. Their mean age was 33.79 ±16.72 years. This population was tested for C677T and A1298C MTHFR polymorphisms.

After Genotyping the 94 subjects, we found the wild type genotypes CC in 32 (50%) males , and 12 females (40%) among carriers of C677T MTHFR polymorphism, 24 (37.5%) of males and 15 (50%) of females were found to be heterozygous subjects, 8 (12.5%) of males and 3 (10%) of females were homozygous mutated, as shown in Table 1, the allelic frequency of the mutated 677T allele was 40 (31.25%) for males, and 21 (35%) for females, and for C allele was 88 (68.75%) for males, and 39 (65%) for females. The most frequent genotype observed in men was the heterozygous CC, followed by the homozygous CT, the homozygous genotype TT had the lowest frequency, but in women, the first genotype was CT, then CC genotype and at last the TT genotype.

We compared the genotype and allelic frequencies of MTHFR C677T between the men subjects and women subjects, as reported in Table1. We did not find statistically significant differences in the percentage distributions of C677T MTHFR polymorphism between males and females carriers in this population (P=0.518).

Table 1. The frequencies of genotypes and C, T alleles among men and women carriers of C677T MTHFR polymorphism

	Men (n= 64)	Women (n= 30)	Total	P value
Frequency	N (%)	N (%)	N (%)	
Wild genotype CC	32 (50)	12 (40)	44 (46.48)	
Heterozygous CT	24 (37.5)	15 (50)	39 (41.8)	0.518a
Homozygous TT	8 (12.5)	3 (10)	11 (11.70)	
Allele C	88 (68.75)	39 (65)	127 (67.55)	
Allele T	40 (31.25)	21 (35)	61 (32.45)	

Note: [a]Pearson Chi-square test. $P < 0.05$ is statistically significant.

There are functional polymorphisms in genes encoding enzymes that may be associated with disease susceptibility. The MTHFR gene polymorphisms are among the most studied, however, two main variants from the functional point of view are the most studied: A1298C and C677T.

Although over the last few years C677T MTHFR polymorphism has been widely investigated, this is the first study that investigated the prevalence and the coexistence of MTHFR C677T and A1298C alleles and genotypes distribution in the Aures region population (Northeast Algeria).

The allele frequency of the C677T polymorphism varies across different geographical regions and ethnic groups (Wang et al. 2016). In this study, there were no significant differences in either the genotype distribution or allele between men and women (P= 0.518), (Table 1). Our results provide further support that this frequency is not associated with gender, similar results were obtained in mainland China, a total of 88255 samples with reported C677T polymorphisms, based on all these samples, the authors did not find any difference between the males [19% (12-25%)] and females [21% (19-24%)] in terms of 677TTgenotype frequency (Wang et al. 2016). No difference in the MTHFR mutation frequency was also seen with respect to gender in Lebanese population (AL-Habboubi et al. 2003). A study of the C677T polymorphism frequency in 207 healthy subjects of Bosnian population, found that the Allele and genotype frequency of MTHFR C677T did not differ between males and females carriers (P=0.35) (Amela et al. 2013). In contrast, another study about Lebanese healthy subjects, showed statistical significance correlated to gender (P = 0.012) (Amira et al. 2008).

There was some evidence for geographic gradients in Europe (north to south increase) in the prevalence of the TT homozygous genotype across the study areas, for example, the prevalence of the TT genotype was 4% in Finland and 7% in Russia, intermediate values (8-10%) were noted in France and Hungary and higher values in southern Europe (12-15% in Spain and northern Italy), peaking in southern Italy (20-26% in Campania and Sicily) (Wilcken et al. 2003). Also, Nishio et al. (1996) reported that the prevalence of the TT genotype found in Germany was (10.4%) and in Greece was (10.0%).

A similar gradient has been observed in North America, where the frequency of TT genotype increases from western Canada (Alberta) to the south-eastern United States (Atlanta), peaking in Mexico (Wilcken et al. 2003).

Another comparative study between Mexican, West African, and European populations, about the prevalence of the MTHFR C677T and A1298C alleles and folate status, found that the T677 allele frequency was highest in Mexico City, intermediate in eastern France and Sicily, and lowest in West Africa. The frequencies of the 677TT genotype and the 677T allele observed were as follows: in West Africa (0.8-9.0%), in French (14.2-36.1%), Italy (Sicily) (19.9-47.3%) and Mexico City (35.7-58.0%), an adequate folic acid intake has presumed enabled increase in the MTHFR 677T frequency in these populations (Guéant-Rodriguez et al. 2006).

It is worth mentioning that in South-East Asian communities the prevalence of TT genotype was; (12.0%) in South Korea, (11.0%) in Japan (Nishio et al. 1996), and (6.9%) in Taiwan (Wassim et al. 2004).

Across all 23 of the studied provinces, Wang et al. (2016) observed increases in the 677T allele and 677TT genotype frequencies along the geographical gradient, in the southern-central-northern direction across Mainland, from lower values, to intermediate, to higher values respectively. The frequencies of the 677TT genotype and the 677T allele were: 7% (5-8%) and 25% (23-27%) in southern, 19% (16-21%) and 41% (36-45%) in central, and 28% (25-31%) and 53% (51-55%) in northern China (all P values ≤ 0.001). One such nutritional investigation revealed that the geometric mean of the blood folate concentration is lower in the northern populations than the southern populations (Hao et al. 2003).

In Indian study results showed wide variations, with the highest frequency of the 677T allele among the Sindhi population (23.8%). In contrast, the 677T allele is absent in the Kom, Thadou and Munda populations, and its average frequency is 10.1% across all 23 populations in India (Saraswathy et al. 2012).The lower frequency of the 677T allele among the tribal groups (the Kom, Thadou and Munda populations) may have been influenced by folate deficiencies because the majority of the population in India were vegetarians with low intake of vitamin B_{12} (Wang et al. 2016).

In another evaluation of Colombian individuals, healthy subjects were included, different genotype frequencies were found relative to the present report: the most frequently observed genotype was the heterozygous C/T (80 individuals, 52.6%), followed by the homozygous C/C with 52 individuals (34.2%) and T/T had the lowest frequency in the population studied (20 individuals, 13.2%) (Romero-Sánchez et al. 2015).

According to a study published on the association between C677T and A1298C polymorphisms and elevated homocysteine, in 117 healthy volunteers in Portugal population (southwestern Europe), that reported the distribution of the genotype and allele frequencies of C677T polymorphism was 33.3% for T allele and 66.7% for C allele. Overall, 10.3% of the subjects carried the 677TT genotype; the heterozygous status was 46.2%, and 43.6% for the homozygous status. Therefore, these results show relative frequencies of the 677T allele and of the 677TT genotype lower than those reported for the other

two South European countries, Spain and Italy, and are not consistent with the idea of a north-south gradient, and confirm the notion that local geographic population studies are necessary (Castro et al. 2003).

An important study comprised of 408 healthy Lebanese and 152 healthy Bahraini subjects, found that the carriers 159 (39.0%) Lebanese and 26 (17.1%) Bahraini carriers were heterozygote, while 10 (2.50%) Lebanese and 4 (2.63%) were in the homozygous state (TT genotype) which are relatively low (Al-Habboubi et al. 2003). Another Lebanese study about healthy subjects, where the authors investigated the prevalence of MTHFR C677T polymorphism. The distribution of CC, CT, and TT genotypes found was, respectively: 65.3%, 30.8%, and 3.9%, with an overall carrier rate of 34.6% and allelic frequency of 0.19 (Saraswathy et al. 2012). In Jordanian population, Eid and Rihani (2004) reported that the TT genotype frequency was 8 and T allele frequency was 0.16. These values are lower than observed in our study.

A few studies in the Arab Maghreb region have examined the MTHFR C677T polymorphism. In Morocco, based on this study that concerned 182 peoples apparently healthy and randomly selected in Arabs and Berbers Moroccan individuals, the following genotypes frequency of C677Tpolymorphism were found: CC genotype in 97 (53.3%), CT genotype in 74 (40.7%), TT genotype in 11 (6%), the T allele was (26.4%) (Paluku They-They et al. 2009). A study in Tunisia, which aims to determine the allelic and genotypic frequencies of the C677T polymorphism among a Tunisian healthy population, showed the following results: CC (69.7%), CT (24.9%), and TT (5.4%). The frequency of the T allele estimated in the sample of 185 individuals was 17.8. These values are intermediate between those observed in Africa and those observed in Western countries (Jerbi et al. 2005). Bourouba et al. (2009) showed in healthy populations in Setif city (Algeria), that MTHFR 677CC genotype was found in 67 (45.6%) individuals; 59 individuals were heterozygous (40.1%), and 21 were homozygous (TT: 14.3%). The frequency of MTHFR 677T was found to be 34.3%. These values remain intermediate between those observed in Africa and those observed in the West and in South America. An evaluation of the genotypic and allelic frequencies of C677T polymorphism on 100 healthy Algerian people, showed the following results: C allele (69.5%), T allele (30.5%), CC (45%), CT (49%), TT (6%). Their study indicates an intermediate allelic frequency that joins the North-South world gradient with a high prevalence of Hyperhomocysteinemia (Hambaba et al. 2008). These last results are closely comparable to our outcomes achieved: T allele (32.45%), TT (11.70%).

In the present study, our findings corroborate those of other worldwide research, and are consistent with the hypothesis of a north-south gradient.

Allelic and genotypic frequencies of MTHFR A1298C polymorphism

The distribution of the MTHFR genotypes for the A1298C mutations is summarized in Table 2, the frequencies of AA, AC and CC genotypes of MTHFR A1298C polymorphism in males and females subgroup found as follows: [AA genotype for males; 39 (60.93%), for females; 21 (70%)], [AC genotype for male; 19 (29.68%), for females: 8 (26.66%)], [CC genotype for males; 6 (9.37%), for females; 1 (3.33%)], one women was detected with homozygous genotype (CC).

As shown previously in Table 2, the relative frequencies of the 1298A and1298C alleles in both males and females were [A Allele for males: 97 (75.78), for females: 50 (83.33)], [C Allele for males; 31 (24.21), for females: 10 (16.66)]. The same genotype frequency order's distribution was observed for A1298C polymorphism in both males and females; (firstly the AA genotype frequency, followed by AC genotype, and finally CC genotype). The carriers of wild-type allele homozygous (AA) genotype and heterozygous (AC) genotype were most represented in males than females; and the homozygous for the mutant allele (CC) genotype had the lowest frequency in women, 1 (3.33)%.

We also did not find statistically significant differences in the percentage distributions of A1298C MTHFR polymorphism between men and women carriers in our group studied (P=0.514). Overall, in our analysis, 11participants (11.70%) of the subjects carried the 677 TT genotype and 7 participants (7.44%) had the 1298 CC genotype. The heterozygous status for C677T mutations (41.8%) was more frequent than the 1298 AC mutations (28.72%).

The frequency distribution of the A1298C polymorphism is not as widely studied as the C677T polymorphism. Our results showed (Table 2) that no significant difference was found for both genders, in terms of carrier frequency (p=0.514). A study revealed that gender did not affect the distribution of the A1298C mutation since the frequencies of the genotypes are comparable between males and females (p=0.618) (Amira et al. 2008).

Interesting study performed by researchers, showed that the West African countries and Mexico City were the 2 areas with the lowest frequencies of the 1298C allele and the1298CC genotype, and the highest frequency was reported in France. The CC genotype and C allele frequency observed was: in west Africa subjects CC (1.9%), C (13.9%), in Mexico city CC (2.3%), C (14.7%), in France CC (11.5%), C (35.7%), in Italy (Sicily) such frequencies were CC (7.5%) and C (28.1%) (Guéant-Rodriguez et al. 2006). Of the 120 healthy Irish participants,

Table 2. The frequencies of genotypes and C, A alleles among men and women carriers of A1298C MTHFR polymorphism

	Men (n= 64)	**Women (n= 30)**	**Total**	**P value**
Frequency	N (%)	N (%)	N (%)	
Wild genotype AA	39 (60.93)	21 (70)	60 (63.82)	
Heterozygous AC	19 (29.68)	8 (26.66)	27 (28.72)	0.514[a]
Homozygous CC	6 (9.37)	1 (3.33)	7 (7.44)	
Allele A	97 (75.78)	50 (83.33)	147 (78.19)	
Allele C	31 (24.21)	(16.66)	41 (21.81)	

Note: [a] Pearson Chi-square test. P<0.05 is statistically significant.

56 were heterozygous carriers, giving a genotype frequency of 46.7%, whereas 11 (CC: 14.2%) were homozygous for A1298C. The prevalence of 1298CC homozygotes in this Irish study is significantly higher than that reported for most European populations (Rita and Thomas 2004).

The frequencies in Turkey of MTHFR 1298AA, 1298 AC, and 1298 CC genotypes were 43.7%, 46.3%, and 10.0%, respectively (Sazci et al. 2005). Overall, the frequencies of the 1298C allele range from 24% to 40% in Europe, 0% to 15% in South America and 14.7% in North America (Amouzou et al. 2004). Lower frequencies were observed in Asian population CC (1.9%) C (0.16) (Esfahani et al. 2003) and in Chinese population CC (3.8%), C (0.176) (Shrubsole et al. 2004). However, it is crucial to note that in Lebanon which harbors the highest prevalence of the MTHFR A1298C polymorphism among different ethnic populations; the AC genotypic prevalence was 50.2% followed by the wild-type genotype AA, 25.9%, and the homozygote genotype (CC) was (23.9%), with an overall carrier rate of 74.14% and an allelic frequency of C 0.49 (the highest frequency) (Amira et al. 2008). In addition, the genotype frequency of CC and C allele was also higher among Tamilians population CC (10%), C (0.35) (Angeline et al. 2004) and Bahraini Arabs (7.3%), C (0.34) (Al-Habboubi et al. 2004). Indians population CC (3%), C (0.10) (Markan et al. 2007) and Japanese CC (0.16), C (1.3%) (Hiraoka et al. 2004), which scored the lowest values.

In the present study, our results appear lower CC (7.44%), C (0.21), compared to the Arab's (Lebanese, Bahrain's Arabs) and Tamilians population. The A1298C allele frequency, although less documented, seems more uniform in the majority of the studied populations (Lievers et al. 2001; Peng et al. 2001; Weisberg et al. 2001). The association of the A1298C mutation with decreased MTHFR specific activity is agreed (Lievers et al. 2001). Different from C677T, the biochemical effect of the A1298C mutation on MTHFR function is still controversial (Van der Put et al. 1998; Friso et al. 2002), indeed the relationship between the gene polymorphisms MTHFR A1298C and Hcy levels remains controversial (Wang et al. 2017).

The frequency of coexistence of MTHFR C677T and A 1298C polymorphisms

The coexistence of genotypes of both mutations was examined and given in Table 3, our data showed that the most frequent coexistence genotypes were: 677CC/ 1298AA (22.34%), 677CT/1298AA (29.78%), 677CC/ 1298AC (17.02%) and the genotypes less frequent were: 677TT/1298AA (11.70%), 677CT/1298AC (11.70%), 677CC/1298 CC (7.44%), the 677TT genotype was associated with the wild type homozygous 1298AA genotype in all individuals, it's same case as 1298CC which always was associated with the wild type homozygous 677CC genotype.

In our study, the following genotypes: 677TT/1298AC, 677CT/1298CC, 677TT/1298CC (the homozygous double mutant) has been no observed.

Table 3. The frequency of coexistence of C677T and A 1298C polymorphisms

Genotypes		C677T MTHFR			
		CC	CT	TT	Total
A1298C	AA	21	21	11	60
MTHFR		(22.34)	(29.78)	(11.70)	(63.82)
	AC	16	11	00	27
		(17.02)	(11.70)	(00.00)	(28.72)
	CC	07	00	00	7
		(7.44)	(00.00)	(00.00)	(7.44)
	Total	44	39	11	94
		(46.80)	(41.48)	(11.70)	(100.00)

Table 4. MTHFR activity (%) according to the presence of C677T and A1298C polymorphisms (Van der Put et al. 1998; Weisberg et al. 1998)

Genotypes		C677T MTHFR		
		CC (%)	CT (%)	TT (%)
A1298C	AA (%)	100	60-70	30-40
MTHFR	AC (%)	79-80	50-60	-
	CC (%)	50-60	-	-

Few studies have anchored their analyses in examination of the coexistence of C677T and A1298C variants in different populations, and their correlation with MTHFR activity and folate level change (Guéant-Rodriguez et al. 2006). One of the first studies was performed on the Dutch population (Van der Put et al.1998), the study did not found the coexistence of the 677CT/1298CC, 677TT/1298AC, 677TT/1298CC genotypes, similar results obtained in our studied group (Table 3), and in several European studies. A less frequent coexistence genotypes 677TT/1298AA (9%), 677CC/1298CC (9.4%) was found in the Dutch population, comparable to our results: 677TT/1298AA (11.70%), 677CC/1298 CC (7.44%). The most frequent coexistence genotypes 677CT/1298AA (20.1%), as well as found in our group 677CT/1298AA (29.78%), whereas 677CT/1298AC (20.1%), was detected low common in our study (11.70%), equally the 677TT/1298AA genotypes were noted low in our population (11.70%).

An exploration of MTHFR activity's according to the presence of C677T and A1298C polymorphisms are shown in Table 4 above (Van der Put et al.1998; Weisberg et al.1998). The authors revealed a significant decrease of the MTHFR activity in cases of coexistence of the genotypes MTHFR 677TT/1298AA (30-40%, approximately 70% less active), 50% decrease of MTHFR activity was observed in 677CT/1298AC genotypes. Nearly 40% reduction in enzyme activity was observed in the other genotypes (677CC/1298CC, 677CT/1298AA).

Contrary to our results, exceptional cases were reported in Turkish population observed a coexistence of 677CT/1298CC, 677TT/1298AC (Ergul et al. 2003; Sazci et al. 2005). In one of these two studies, which involved 1004 women and 680 men (1684), the frequency of double heterozygous genotypes was 677CT/1298AC (21.6%) (Sazci et al. 2005). Interestingly, 677TT/1298AC was

detected also in Hundo population (Kumar Rai et al. 2006), and equally it was detected in Canada by Isotalo et al. (2000) who studied 119 Canadians newborns; TT/AC: 2 (1.70%), for the others coexisting genotypes, they were as follow: CC/AA: 17 (14.30%), CC/AC: 42 (35.30%), CC/CC: 9 (7.60%), CT/AA: 14 (11.70%), CT/AC: 23 (19.30%), TT/AA: 12 (10.10%), TT/CC and CT/CC genotypes were not observed in this study.

De Re et al. 2010 demonstrated the coexistence of the 677CT/1298AC in (30.20%) of 454 Italian subjects (315 men and 139 women). In Irish population study of the MTHFR C677T/A1298C genotype combinations, 28 participants (23.3%) were double heterozygotes (Rita and Thomas 2004), another study population which consisted of 1684 randomized individuals from around Turkey, of whom 1004 were females and 680 were males the frequency of C677T/A1298C compound heterozygosity is highest in Turkey (21.6%), as compared to Canada (15%), the United States (17%) and the Netherlands (20%) (Sazci et al. 2005), this results were most frequent than those obtained in our analysis (11.70%). Also, 677TT/1298AA genotypes were found in (12.60%) by (De Re et al. 2010), results were similar to those achieved in our study (11.70%). A study investigated the frequency of these two MTHFR polymorphisms in a Portuguese population, in 117 healthy volunteers (71 females, 46 males) studied, 21.4% of the subjects were compound heterozygotes for the two MTHFR SNPs (677CT/1298AC), the 1298C allele frequency was 28.2%, demonstrating that the mutation is also common in this population (Castro et al. 2003).

Noteworthy, a higher frequency of the coexistences of genotypes with the mutated allele suggested a significant role of diet rich in folate in the south of Europe (Italy, Turkey) that could affect an individual's ability to survive with mutated genotypes of MTHFR polymorphisms (Hubert et al. 2015). It appears that C677T and A1298C polymorphisms can act synergistically, given that heterozygosity for both polymorphisms causes lower MTHFR activity than heterozygosity alone for either mutation (Van der Put et al. 1998). Also, an association of double heterozygote status with elevated tHcy levels in the presence of low folate status was observed (Van der Put et al. 1998). Previous studies have proved that the A1298C polymorphism in combination with C677T appeared to affect enzyme activity and tHcy (Weisberg et al. 1998; Weisberg et al. 2001). However, other authors failed to find any influence of folate on the distribution of the 677CT/1298AC combined genotype in the 7 areas studied; West Africa, coastal and savannah areas of Togo, coastal Benin, France, Sicily, and Mexico City (Guéant-Rodriguez et al. 2006).

In conclusion, this study is the first to report frequency and the coexistence of C677Tand A1298C MTHFR polymorphisms in healthy Algerian population (Aures region). For the C677T SNP, our intermediate data were agreed with the hypothesis of a North-South gradient. The C allele and CC genotype frequencies of the A1298C polymorphism was found lower; CC (7.44%), C (0.21) compared to ethnic groups; this SNP has not been extensively studied worldwide and in Algeria particularly. The Coexistence of C677T and A1298C MTHFR polymorphisms genotypes observed in this study is consistent with the data from literature with lower frequency of the double heterozygotes (677CT/1298AC: 11.70%). Analysing the combination genotypes of the two SNPs in Algerian population is also an important point should be raised. Furthers studies in MTHFR gene polymorphisms are needed to clarify their possible impact on public health in Aures region (Northeast Algeria).

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