MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPERS

Frequency of the Methylenetetrahydrofolate REDUCTASE 677CT and 1298AC mutations in an Iranian Turkish female population

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ABSTRACT

Introduction: *Gene-environmental interactions in the pathway of folate metabolism influence greatly the embryonic development. Individual specific MTHFR 677C/T and 1298A/C mutations are known as risk factors for predisposition to human disorders. Therefore, we studied the frequencies of the MTHFR 677CT and 1298AC mutations in a female general population from Iranian Azeri Turkish.*

Material and methods: *We studied 108 unrelated women from Iranian Azeri Turkish general population. Genomic DNA was extracted using standard procedure. The MTHFR 677CT and 1298AC mutations determined by PCR-RFLP method.*

Outcomes: *The frequencies (percent) at position 677 for C and T alleles were 159(74%), 57(26%), and for CC, CT, and TT genotypes were 59(54.6%), 41(38%), and 8(7.41%) respectively. The frequencies (percent) at position 1298 for A and C alleles were 136(63%), 80(37%), and for AA, AC, and CC genotypes were 43(39.8%), 50(46.3%), and 15(13.9%) respectively.*

Conclusions: *The frequency of MTHFR 677 C and T alleles were 0.74 and 0.26 while that of MTHFR 1298 A and C alleles were 0.63 and 0.37 in present study, respectively. This is the first report in its own kind in Iranian Azeri Turkish women.*

Keywords: MTHFR, C677T, A1298C, Turkish female

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INTRODUCTION

Methylenetetrahydrofolate reductase (MTHFR) plays a wide range of roles in different cellular functions such as reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, DNA methylation, repair and synthesis (1). It has been demonstrated that mutations within MTHFR gene result in human disorders including neural tube defects (2-7), male infertility (8-10), Type II Diabetes Mellitus (11), cardiovascular diseases (12-17) and cancer (18-20).

Approximately 4% of the cytosine nucleotides in the human genes are modified to 5-methylcytosine by methylation (21,22). Aberrant gene methylation leads to silencing of important genes that is one of the main causes of enzyme deficiencies (23,24). Several MTHFR gene mutations including 677CT (C to T) and 1298AC (A to C) have been identified that decrease the MTHFR enzyme activity (2, 25).

The 1298AC mutation in MTHFR gene is described recently. The activity of MTHFR enzyme in combined heterozygote for 677CT/1298AC mutations is lower than homozygote 677CT or homozygote 1298AC genotypes (2,25).

Further analysis showed that deficiency in folate metabolism regarding maternal MTHFR 677CT and 1298AC gene mutations, is risk factors for Down's syndrome predisposition due to non-disjunction of chromosome 21 (26-28). Santos et al. (2006) showed an increase rate in the MTHFR 677TT homozygote genotype in individuals with Turner syndrome (29). Therefore, we studied the frequencies of the MTHFR C677T and A1298C mutations in a general population from Iranian Azeri Turkish women. ❑

MATERIALS AND METHODS

The present study was approved by the ethic committee of Urmia University of Medical Sciences.

We studied 108 unrelated women, who were in good health condition, from Iranian Azeri Turkish general population. Individuals with any known disorders such as cardiovascular disease, diabetes mellitus, infertility, cancer, or a history of having a child with neural tube defects were excluded from the study. An informed consent has been taken from all participants. EDTA-anticoagulated blood sample (3-5 ml) was collected from each person and genomic DNA was isolated using standard procedure (30).

The MTHFR 677CT and 1298AC mutations determined as described by A et al, 2007 and Donnelly, 2000 (31, 32). The set of forward "5'-CAT CCC TAT TGG CAG GTT AC-3'" and reverse "5'-GAC GGT GCG GTG AGA GTG-3'" primers were used for amplification of a fragment of 265 base pairs, and then the amplified fragments were digested with HinfI enzyme. The PCR profile was: initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 30 sec, annealing at 59 °C for 30 sec, extension at 72 °C for 30 sec for 35 cycles and followed at 72 °C for 10 min (31).

In the position 677 of MTHFR gene, transition of "C" base, which is the wild base, to "T" base, produces a cut site for HinfI enzyme, which cut the amplicons into two fragments of 171 and 94 bp. Then, CC genotype would be reflected by a single band of 265 bp (uncut), CT genotype by three bands of 265, 171 and 94 bp, and TT genotypes by two bands of 171 and 94 bp.

The set of forward "5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3" and reverse "5'-CAC TTT GTG ACC ATT CCG GTT TG-3" primers were used for amplification of a fragment of 241 base pairs and then the amplified fragment was digested with MboII enzyme. The PCR profile was: initial denaturation at 95 °C for 5 min, denaturation at 94 °C for 30 sec, annealing at 51 °C for 30 sec, extension at 72 °C for 30 sec for 35 cycles and followed at 72 °C for 10 min (32). In the position 1298 of MTHFR gene, transversion of "A" base, which is the wild base, to "C" base produces a cut site for MboII enzyme, which cut the amplicons into two fragments of 211 and 30 bp. Then, AA genotype results in a single band of 241 bp (uncut), AC genotypes produces three bands of 241, 211 and 30 bp, and CC genotype produces two bands of 211 and 30 bp. Digestion of 10 μl PCR products carried out with 1.5 U HinfI and MboII restriction enzyme in 37 °C for two hours for MTHFR 677CT and 1298AC genotyping, respectively.

Then, products were electrophoresed on 3% agarose gel. Gel was stained with ethidium bromide and presence or absent of products were observed by UV transilluminator. The

MTHFR genotype and alleles were computed by direct counting. The fit to the Hardy-Weinberg equilibrium was analyzed. The distributions of MTHFR alleles and genotypes in studied group were checked by $\chi 2$ test or Fisher's exact test.

Differences were considered to be statistically significant when p-value was less than 0.05. Statistical analysis was performed by SPSS ver. 16.0 and Microsoft Excel 2003. ❑

OUTCOMES

Study population checked for MTHFR 677CT and 1298AC genotypes based on the cleavage profile of the HinfI and MboII enzymes.

The Hardy-Weinberg equilibrium analysis of study group implied that the frequencies were in agreement with the expected equilibrium and showed that MTHFR 677CT and 1298AC polymorphisms were randomly distributed (MTHFR 677CT: p-value= 0.97 > 0.05; df=2; $\chi 2 = 0.056$ and MTHFR 1298AC: p-value= 0.99 > 0.05; df=2; $\chi 2 = 0.006$). The frequencies (percent) at position 677 for C and T alleles were 159(74%), 57(26%), and for CC, CT, and TT genotypes were 59(54.6%), 41(38%), and 8(7.41%) respectively.

The frequencies (percent) at position 1298 for A and C alleles were 136(63%), 80(37%), and for AA, AC, and CC genotypes were 43(39.8%), 50(46.3%), and 15(13.9%) respectively.

The frequency of MTHFR 677 C and T alleles were 0.74 and 0.26 and that of MTHFR 1298 A and C alleles were 0.63 and 0.37 respectively.

The different combined genotypes of MTHFR 677CT and 1298AC genotypes and the frequencies (percent) in the present study were summarized in Table 1. ❑

DISCUSSION

DNA polymorphisms may play a role in the non-disjunction and production of aneuploid gametes with consequent increased risk for trisomy 21 predisposition (54,55).

MTHFR enzyme has important role in metabolic pathway of folate and nucleotide methylation (56). Presence of T allele at position 677 of MTHFR gene leads to reduction of MTHFR activity and DNA hypomethylation. It has been understood that hypomethylation of DNA has been related to chromosomal changes and instability.

Non-disjunction of chromosome 21 in mothers of patients with Down syndrome is known as the etiology of trisomy 21 (57-60). In agreement with these studies, Rai et al (2006) reported that individuals with MTHFR 677TT genotype have a risk of non-disjunction, but in the case of 1298AC genotypes, there was no

Combined Genotype	United States	Turkey	Indian	Present Study
CC/AA	148(11.95)	178(10.46)	18(25.71)	16(14.81)
CC/AC	235(18.98)	401(23.56)	31(44.28)	29(26.85)
CC/CC	129(10.42)	164(9.636)	5(7.14)	13(12.04)
CT/AA	298(24.07)	411(24.15)	10(14.28)	20(18.52)
CT/AC	285(23.02)	363(21.33)	6(8.57)	19(17.59)
CT/CC	2(0.162)	17(0.999)	0(0)	2(1.852)
TT/AA	138(11.15)	146(8.578)	0(0)	6(5.556)
TT/AC	3(0.242)	22(1.293)	0(0)	2(1.852)
TT/CC	0(0)	0(0)	0(0)	1(0.926)

TABLE 1a. Combined MTHFR 677CT and 1298AC genotypes frequencies (percent) in different populations and the present study

Combined Genotype	United States Versus Present Study			Turkey Versus Present Study			Indian Versus Present Study		
	OR (95% C.I.)	χ^2	P-Value	OR (95% C.I.)	χ^2	P-Value	OR (95% C.I.)	χ^2	P-Value
CC/AA	0.781(0.447-1.364)	0.759	0.384	0.672(0.386-1.168)	2.014	0.156	1.99(0.936-4.232)	3.26	0.07
CC/AC	0.638(0.408-1)	3.902	0.048	0.84(0.541-1.304)	0.607	0.436	2.16(1.147-4.087)	5.77	0.01
CC/CC	0.85(0.463-1.561)	0.275	0.6	0.779(0.427-1.422)	0.664	0.415	0.56(0.191-1.652)	1.11	0.29
CT/AA	1.395(0.844-2.306)	1.697	0.193	1.401(0.851-2.305)	1.774	0.183	0.73(0.320-1.676)	0.54	0.46
CT/AC	1.401(0.839-2.339)	1.674	0.196	1.27(0.764-2.112)	0.851	0.356	0.43(0.166-1.161)	2.86	0.09
CT/CC	0.086(0.012-0.615)	9.579	0.002	0.535(0.122-2.345)	0.711	0.399	0	1.31	0.25
TT/AA	2.133(0.919-4.951)	3.251	0.071	1.595(0.688-3.697)	1.206	0.272	0	4.02	0.04
TT/AC	0.129(0.021-0.779)	6.953	0.008	0.694(0.161-2.991)	0.243	0.622	0	1.31	0.25
TT/CC	0	11.47	7E-04	0	15.77	7E-05	0	0.65	0.41

TABLE 1b. OR (95% C.I.), $\chi 2$ and P-Value were calculated for comparing of data from United States, Turkey, and Indian versus the present study

correlation (61). In this regard, the finding of *Meguid et al* (2008) indicate that MTHFR 677T allele, 677CT and 677TT genotypes, also, 1298C allele and 1298CC genotype were more frequent among mothers of Down syndrome kids (62).

To the best of our knowledge, this is the first study that investigated the prevalence of MTHFR 677CT and 1298AC alleles and genotype distribution in the Iranian Azeri Turkish females. Our finding revealed that 1) 57 out of 216 (26.38%) alleles at position 677 MTHFR were T allele. 2) 80 out of 216 (37.03%) alleles at position 1298 of MTHFR gene were C allele 3) 65 (60.185%) and 43(39.814%) out of 108 (100%) individuals had 1298 CC +1298 AC and 1298 AA genotypes, respectively. 4) 49 (45.370%) and 59(54.629%) out of 108 (100%) individuals had TT677+CT677 and CC677 genotypes, respectively.

The distribution of 677 T and 1298 C alleles in the present study indicated that the rate of 677 CT and 1298 AC mutations in hetero- or homo-zygote genotype were high. Several reports are collected in tables 1-4 and imply that the MTHFR CC/AC, CT/CC, TT/AC and TT/CC genotypes frequencies were higher in our population in comparison with that of United States.

The distribution of combined MTHFR 677CT and 1298AC genotypes frequencies (percent) in the present study was similar to Turkey population, but the MTHFR TT/CC genotype frequency differs significantly in the present study versus Turkey [0(0%) vs. 1(0.926%)].

In our group, MTHFR 677TT and 1298AA genotypes were higher and MTHFR 677CC and 1298AC genotypes were lower compared to Indian population. In the rest of combined

Population (refrence)	CC	TC	TT	T allele frequency (%)
Sri Lanka (33)	61	6	0	4.5
PNG Highlanders (33)	77	8	0	4.7
Kenya (33)	55	6	0	4.9
Gambia (33)	21	3	0	6.25
Sub-Sahara (34,35)	263	38	0	6.3
Madagascar (33)	84	13	0	6.7
Indonesia (34)	57	11	0	8.1
South Africa(34,36)	85	22	0	10.3
African American (34,35)	363	127	6	14
Yemen (33)	31	14	1	17.4
United Kingdom (33)	45	42	7	18.6
Nu-Chah-Nulth (33)	25	10	2	18.9
Present Study	59	41	8	26
Holland (34,35)	224	234	45	32.2
Ireland (34,35)	600	568	141	32.5
England (34,37)	96	97	29	34.9
Australia(34,38)	88	113	24	35.8
China(34, 39)	51	53	17	35.9
Canada(34,40)	172	183	59	36.3
Japan(34)	96	116	32	36.9
Korea(34,41)	33	82	9	40.3
Italia N.(34,44)	42	71	17	40.4
Hispanic(34, 43)	63	71	35	41.7
Italia S.(34,44)	130	223	78	44
Brazilian Amerindians(33)	12	19	8	44.9
Mexico(34,45)	44	119	87	58.6

TABLE 2. CC, CT, and TT genotype distribution and T allele frequency (%) at position 677 of MTHFR gene in different populations and the present study

Population	AA	AC	CC	A	C	A allele frequency	C allele frequency
Mexico (46)	94	25	1	213	27	0.89	0.11
Tunis (47)	130	62	8	322	78	0.805	0.195
USA (48)	29	19	2	77	23	0.77	0.23
USA (48)	27	21	2	75	25	0.75	0.25
Ashkenazi Jewish (49)	80	57	12	217	81	0.73	0.27
Portugal (50)	58	52	7	168	66	0.72	0.28
United States (51)	164	139	26	467	191	0.71	0.29
Turkey (52)	736	779	169	2251	1117	0.67	0.33
Ashkenazi Jewish (49)	77	89	21	243	131	0.65	0.35
present study	43	50	15	136	80	0.63	0.37
Iran (53)	35	50	15	120	80	0.6	0.4

TABLE 3. AA, AC, and CC genotype distribution, A and C allele frequency and A and C allele frequency (%) at position 1298 of MTHFR gene in different populations and the present study

genotypes, significant differences were not found in different populations.

Figure 1 shows the combined MTHFR 677 and 1298 genotypes in United States, Turkey, India and the present study. ❑

CONCLUSION

The frequency of MTHFR 677 C and T alleles were 0.74 and 0.26 while that of MTHFR 1298 A and C alleles were 0.63 and 0.37 the in present study, respectively. Study in a large cohort would be valuable to evaluate the more precise frequency of the MTHFR mutations.

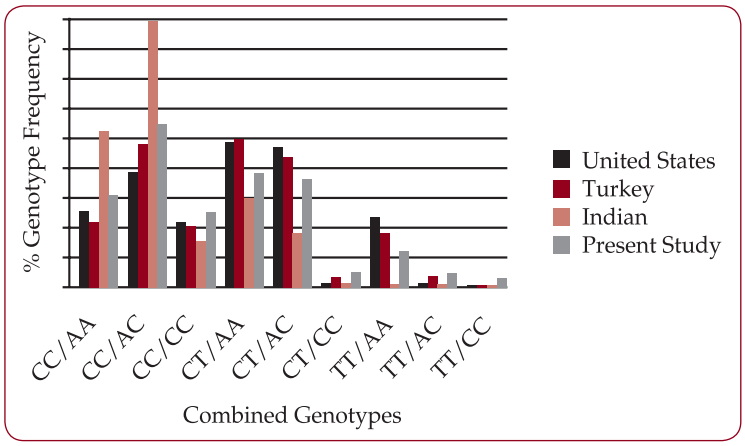

FIGURE 1. Combined MTHFR 677CT and 1298AC genotypes (%) in different ethnic populations

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