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Allelic and Genotypic Frequencies and Haplotype Analysis of C677T and A1298C Polymorphisms in the MTHFR Gene in Khuzestan Province, Iran

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Abstract

Background: *The enzyme methylenetetrahydrofolate reductase (MTHFR) is essential for folate metabolism and homocysteine regulation. Genetic polymorphisms in MTHFR vary among populations, and their distribution may influence susceptibility to complex diseases. This research primarily aimed to determine the allelic, genotypic, and haplotypic frequencies of the A1298C and C677T variants of the MTHFR gene in a cohort of healthy individuals from Khuzestan province, Iran.*

Methods: *Peripheral blood samples were collected from 100 unrelated healthy individuals. Genomic DNA was extracted, and genotype determination of A1298C and C677T polymorphisms was performed using the ARMS-PCR technique.*

Results: *For both variants, the heterozygote genotype was the most frequent. 677T allele frequency was 32%, and the 1298C allele frequency was 43%. Analysis of linkage disequilibrium (LD) showed a moderate degree of LD between the C677T and A1298C variants with a weak correlation. Exploratory analyses suggested potential associations between the variants and some medical conditions; however, Results were constrained by the limited sample size, with no significant associations persisting after covariate adjustment.*

Conclusion: *This study provides the first data on allelic, genotypic, and haplotypic frequencies of A1298C and C677T variants in the Khuzestan population. While exploratory analyses hinted at possible disease associations, these results should be interpreted cautiously and warrant confirmation in larger studies. Additionally, the weak LD observed between the two variants suggests they may act independently in contributing to disease susceptibility.*

Keywords: MTHFR gene, Polymorphisms, ARMS-PCR, Linkage disequilibrium.

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Introduction

The MTHFR gene (NM_005957.5), located on 1p36.3, encodes 5, 10-methylenetetrahydrofolate reductase, a dimeric 77-kilodalton enzyme that is essential for folate metabolism and homocysteine regulation. It converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the key methyl donor required for the remethylation of homocysteine into methionine [1, 2].

The C677T MTHFR variant (rs1801133) is considered the most prevalent cause of elevated homocysteine levels [3]. This genetic variant is believed to contribute to elevated plasma homocysteine (Hyperhomocysteinemia), decreased folate concentration, and various cardiovascular diseases (CVDs) [4]. The C677T variant impairs enzyme function, with enzyme activity in homozygous individuals (TT) being 35-70% lower [5]. Another frequently observed polymorphism of the MTHFR gene is the A1298C (rs1801131) variant. This mutation also results in decreased activity of the enzyme, yet not as severely as observed in C677T [3]. The MTHFR gene mutations that affect enzyme activity can, in turn, alter DNA methylation levels. A1298C affects the extent of methylation, while C677T disrupts its maintenance [6]. Additionally, the combination of heterozygous C677T and A1298C leads to approximately a 50% decrease in enzyme activity [5].

Given the essential function of MTHFR in cellular metabolism, understanding the distribution of its common polymorphisms across populations is critical for assessing both disease susceptibility and genetic diversity [1]. Hyperhomocysteinemia is a known risk factor for high blood pressure (hypertension). While previous research has investigated the links between MTHFR variants, homocysteine levels, and high blood pressure, the findings have been controversial and contradictory [3, 7-9]. Hyperhomocysteinemia is considered a potential risk factor for coronary artery disease. Multiple previous investigations have revealed that the C677T variant raises the risk of developing coronary artery disease [10-14]. Folic acid is vital for DNA synthesis, protein metabolism, fetal growth, and various physiological processes during pregnancy. Disruptions in folic acid metabolism can result in adverse pregnancy outcomes, affecting both maternal and fetal health. The C677T variant has been linked to a higher likelihood of spontaneous abortion, gestational diabetes, pregnancy-induced hypertension, and pre-eclampsia. It has also been associated with hereditary thrombophilia, a major risk factor for recurrent pregnancy loss [15-18].

Despite the importance of these polymorphisms, allele and genotype frequencies of MTHFR variants have not been well established in many Iranian subpopulations. Khuzestan province, located in southwest Iran, is characterized by high ethnic diversity, including Arab, Lur, Bakhtiari, and Persian groups, making it a unique setting for genetic studies [19]. However, to date, no comprehensive data on the occurrence of

A1298C and C677T variants in this population are available. Given the significant role of these mutations in regulating homocysteine and folate levels, as well as their association with various health conditions, this research aimed to estimate the allelic and genotypic frequencies, analyze haplotypes, and assess linkage disequilibrium (LD) of the A1298C and C677T variants in a general population from Khuzestan province. As an exploratory objective, we also evaluated the relationship between these genetic variations and reported underlying diseases, acknowledging the limitations of the small sample size.

Materials and Methods

This study included 100 unrelated individuals from ethnic groups native to Khuzestan, recruited as volunteers from multiple sources, including attendees of several medical laboratories, university staff, and students who responded to announcements. Participants provided written informed consent before sample collection. The inclusion criteria required individuals to be free from any acute illnesses at the time of sampling. No strict exclusion criteria based on past medical history were applied to better represent the general population. Information on chronic conditions, medication use, and other underlying conditions was collected through self-report and used only for exploratory analyses, as some participants reported a history of diabetes, hypertension, or other conditions. The study protocol and procedures were conducted in accordance with the Declaration of Helsinki's ethical guidelines and were approved by the ethics committee of Dezful University of Medical Sciences.

Peripheral blood (2 mL) was drawn from each participant using EDTA as an anticoagulant. leukocytes DNA was extracted by the salting-out method. The quality and yield of the isolated DNA were assessed using a UV spectrophotometer at 260 and 280 nm. The isolated DNA was subsequently stored at −20 °C until further analysis. MTHFR gene A1298C and C677T polymorphisms were genotyped using the ARMS-PCR method. Details of the primers used for polymerase chain reaction are presented in Table 1.

Table 1. List of primers used for DNA amplification and genotyping

Primer		Primer sequence
C677T	677RC	5′-GCGTGATGATGAAATCGG-3′
	677FC	5′-TGCTGTTGGAAGGTGCAAGAT-3′
	677RM	5′-GCGTGATGATGAAATCGA-3′
	677RN	5′-GCGTGATGATGAAATCGG-3′
A1298C	1298FIN	5′-GGTAAAGAACGAAGACTTCAAAGACACATT-3′
	1298RO	5′-GAAGAAGTTTGCATGCTTGTGGTTG-3′
	1298RIM	5′-GAGGAGCTGACCAGTGATGC-3′
	1298FO	5′-CAGGCAAGTCACCTGGGAGAGA-3′

ARMS-PCR for the C677T polymorphism was performed using two separate reactions per allele using different primer sets (766RC/677FC/677RM for detection of the T allele and 677RC/677FC/677RN for detection of the C allele). The 15 µL reaction contained Taq DNA Polymerase Master Mix (Ampliqon, Denmark) and was run in a Veriti™ Thermal Cycler (Applied Biosystems, US). The cycling conditions included an initial denaturation step (96°C for 2 min), followed by 35 cycles of 96 °C for 20 s, 58 °C for 30 s, and 72 °C for 30 s, and concluded with a final extension step (72 °C for 5 min). The A1298C variant was genotyped using a tetra ARMS-PCR protocol with the four primers in a 15 µL reaction. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min. PCR products were analyzed on a 1.5% agarose gel.

To ensure accuracy, 10% of samples were genotyped in duplicate, NTCs (no template controls) were included in every PCR run to detect contamination, and PCR products were validated against reference samples obtained from commercial genotyping kits for C677T and A1298C.

Haplotype analysis and LD estimation were performed using the SNPAnalyzer 2 software. The LD parameters, including D' and r^2, were calculated to assess the extent of linkage between SNPs. The frequencies of the observed haplotypes were inferred using the expectation-maximization (EM) algorithm. To perform the statistical analyses, SPSS version 16 was used. The chi-square test was employed to evaluate the Hardy-Weinberg equilibrium (HWE). Fisher's exact test was used to assess associations in cases where expected frequencies were low, ensuring the accuracy of results. Logistic regression was utilized to adjust for possible confounders such as age and sex.

Results

A total of 100 participants (58 females and 42 males) were included. The mean age was 44.97 years overall (42.48 years for females and 48.4 years for males). Information on pre-existing underlying conditions was collected and is summarized in Table 2.

Genotype determination of both polymorphisms was conducted using the ARMS-PCR. Representative gel electrophoresis results are shown in Figure 1.

The allelic and genotypic frequency of the polymorphisms were examined for HWE in the studied population (Table 3).

Table 2. Demographic and clinical characteristics of the study participants

Characteristic	Overall (n=100)	Female (n=58)	Male (n=42)
Age, mean±SD (years)	44.97±13.5	42.48±12.1	48.4±14.1
Hypertension, n (%)	24 (24%)	14 (24.1%)	10 (23.8%)
Diabetes, n (%)	17 (17%)	10 (17.2%)	7 (16.7%)
Cardiovascular diseases (CVDs), n (%)	9 (9%)	5 (8.6%)	4 (9.5%)
Cataract, n (%)	5 (5%)	3 (5.2%)	2 (4.8%)
History of miscarriage*, n (%)	5 (8.6%)	5 (8.6%)	-

*** Only applicable to female participants.**

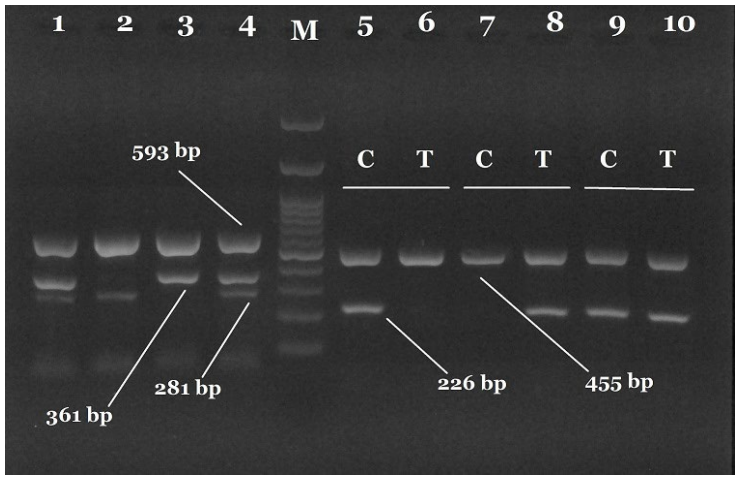

Figure 1. Agarose gel electrophoresis results for genotyping A1298C and C677T variants: Lane M: 100 bp DNA ladder. A1298C (lanes 1–4): Lane 1 and 4, heterozygous genotype (AC; bands at 281 bp, 361 bp, and 593 bp as internal control); Lane 2, homozygous wild-type (AA; bands at 281 bp and 593 bp); Lane 3, homozygous mutant (CC; bands at 361 bp and 593 bp). C677T (lanes 5–10): Lane 5–6, homozygous wild-type (CC; bands at 226 bp and 455 bp as internal control); Lane 7–8, homozygous mutant (TT; bands at 226 bp and 455 bp); Lane 9–10, heterozygous (CT; bands at 226 bp, 455 bp, and internal control)

Table 3. Genotypic and allelic frequencies of C677T and A1298C polymorphisms with HWE analysis

Polymorphism	Frequency (%)	HWE P-value
C677T		0.051
CC	42	
CT	52	
TT	6	
C	68	
T	32	
A1298C		0.152
AA	36	
AC	42	
CC	22	
A	57	
C	43	

The analysis showed that both polymorphisms follow the HWE.

In an exploratory analysis, we examined possible links between C677T and A1298C, and several self-reported clinical conditions among the participants, including a history of hypertension, diabetes, CVDs, cataracts, and abortion. Cross-tabulation analysis for the A1298C polymorphism and CVDs showed that the CC genotype was not present among individuals with CVDs, whereas the AC genotype had the highest frequency among affected individuals (8 out of 9 cases). The AA genotype was observed in only one individual with CVDs, while it was more common among unaffected individuals. Fisher's exact test indicated a significant association between the A1298C polymorphism and CVDs, but logistic regression revealed no significance following adjustment for sex and age (P-value>0.05). Age was found to be an independent risk factor, while sex showed no significant association (P-value=0.159) (Table 4).

Table 4. Association of A1298C and C677T polymorphisms with medical conditions and risk factors

Polymorphism	Condition	Fisher's exact test (P-value)	Logistic regression (P-value)	Independent risk factor
A1298C	CVDs	0.015	>0.05	Age (P-value=0.011)
C677T	Cataracts	0.037	>0.05	Age (P-value=0.065, OR=1.098)
C677T	Abortion	0.028	>0.05	-

Cross-tabulation analysis for the C677T variant revealed that the CC genotype was the most common among individuals without cataract (41 out of 95 cases) and was present in only one affected individual. The CT genotype had the highest frequency among affected individuals (2 out of 5 cases), whereas the TT genotype was found in two cataract cases. Similarly, Fisher's exact test indicated a significant association between the C677T variant and cataracts, but after adjusting for age and sex, this association became non-significant. Cataract risk was not significantly related to participant sex (P-value=0.194, OR=0.194), and age showed a marginal effect. For abortion, Fisher's exact tests showed an association with C677T, showing that the homozygous TT genotype was over-represented among women reporting a history of abortion, but logistic regression found no significance after controlling for age, diabetes, and hypertension (Table 4). None of the studied MTHFR variants showed statistically significant associations with other diseases, such as hypertension and diabetes.

Haplotype analysis was performed using SNPAnalyzer 2, identifying four major haplotypes. The most and least frequent haplotypes were CC and CT, respectively. LD analysis was conducted for SNPs within a 500 kb region on chromosome 1. The D' value for LD between the two markers was 0.56458, indicating a moderate level of linkage. The r^2 value was 0.11316, suggesting that while the two SNPs are in LD, they do not exhibit strong correlation in allele inheritance. The LOD score for this LD block was 12.63 (P-value=0.00038), and the chi-square test yielded a significant result, confirming that these SNPs are not in complete equilibrium and may have some degree of co-segregation (Table 5).

Table 5. Haplotype frequencies and linkage disequilibrium of MTHFR C677T and A1298C polymorphisms

Haplotype	Frequency (%)	D'	r^2	LOD Score	X^2 (P-value)
CC	37.6	0.565	0.113	12.63	22.63 (<0.0001)
AC	30.4				
AT	26.6				
CT	5.4				

Discussion

Deficiency in the MTHFR gene is the most frequent genetic factor associated with increased plasma homocysteine levels. Genetic variations in the MTHFR gene can impair or deactivate this enzyme, leading to mildly elevated homocysteine levels, particularly in individuals with folate deficiency [20]. The C677T variant results in production of a thermolabile form of the enzyme with diminished activity at higher temperatures, leading to elevated homocysteine and lower serum folate levels in TT homozygous individuals [20]. This genotype is found in over 25% of Hispanics, 10-15% of North American Caucasians, and only 6% of individuals of African descent [21, 22]. The A1298C variant alone causes only a slight reduction in MTHFR enzyme activity and does not significantly increase homocysteine levels. Still, when combined in a heterozygous state with C677T (677CT/1298AC), it results in a marked decrease in enzyme activity comparable to the TT homozygous genotype [23].

This study was primarily designed to determine the allelic and genotypic frequencies of the MTHFR A1298C and C677T variants. The allelic frequency of the 677C allele was 68%, while that of the 677T allele was 32%. For the A1298C polymorphism, the frequency of the 1298A allele was 57% and that of the 1298C allele was 43%. Genotypic analysis revealed that the most common genotype for C677T was CT (52%), followed by CC (42%) and TT (6%). For A1298C, the most common genotype was AC (42%), followed by AA (36%) and CC (22%). Both polymorphisms were consistent with the HWE, indicating no significant deviation from the expected genetic distribution in the studied population. This suggests that the sample is representative of the broader population in terms of these genetic variants.

Comparison with previous studies highlights considerable inter-population heterogeneity in the prevalence of MTHFR polymorphisms throughout Iran. For the C677T variant, the highest reported frequency of the TT was found in Mashhad (13.9%), whereas the lowest frequency belonged to Kerman province (1.1%) [24, 25]. Likewise, the heterozygous CT genotype showed the highest frequency in Rasht (44%) and the lowest in Kerman (11.6%) [25, 26]. Regarding allele frequency, the T allele was most prevalent in the Mashhad population (34.2%) and least common in Kerman (6.9%) [24, 25].

In the case of the A1298C polymorphism, Tabriz had the highest frequency of the homozygous mutant genotype (CC) (28.3%), while Yazd had the lowest frequency (0%) [27, 28]. The highest allele frequency for the mutated C allele was observed in a study conducted in Tehran (41.8%) [29], followed by Ahvaz

(40.5%) [30]. Conversely, the lowest C allele frequency belonged to the Mazandaran population (11%) [31]. These variations in genotype and allele frequencies across different populations underscore the role of genetic diversity in shaping the distribution of MTHFR polymorphisms. Such differences should be considered when evaluating the potential health implications of these genetic variants in different ethnic groups.

When compared to international data, the T allele frequency of 32% for C677T in the current study is consistent with the rates reported in European populations such as Germany (32.08–35.05%) [32, 33], the UK (31.92%) [34], and the USA (36.35%) [35], suggesting a moderate to high frequency of the T allele in our study. Even though the frequency of the TT genotype (6%) in our sample is somewhat lower than that observed in most European populations, the overall allelic and genotypic distributions are comparable. The C allele frequency of 43% observed for the A1298C polymorphism in our population ranks among the highest reported globally, closely matching findings from Turkey (43.96%) [36] and Russia (32.5%) [37], and considerably exceeding those reported in East Asian populations such as South Korea (16.29%) [38] and India (29.09%) [39]. These findings underscore substantial regional and ethnic variability in the prevalence of MTHFR variants and highlight the importance of population-specific genetic profiling to evaluate disease susceptibility and inform public health strategies.

Studies investigating the relationship between different MTHFR variants and various health conditions have yielded varying conclusions. One of the main contributing factors to this discrepancy is ethnic diversity, which influences the prevalence of these polymorphisms and their potential associations with diseases. Researchers have emphasized the need for further investigations in different ethnic groups and larger populations, particularly in regions where the distribution of MTHFR polymorphisms has not been well studied [40]. In the current study, we carried out an exploratory examination of the potential associations between the A1298C and C677T variants and various medical conditions. The findings yield preliminary insights and generate hypotheses, particularly regarding the potential role of demographic factors like age in modulating the relationship between genetic variations and disease risk, which warrant further investigation. Previous research on the relationship between cardiovascular diseases and MTHFR deficiency has shown contradictory outcomes. While some meta-analysis studies suggest that hyperhomocysteinemia due to MTHFR deficiency increases the risk of CVDs, others have failed to confirm a significant link [13, 41]. Our data indicated a significant association between A1298C and CVDs, according to Fisher's exact test. However, after adjustment for confounding factors, including sex and age, through logistic regression analysis, the associations were no longer statistically significant. However, the study was underpowered to detect modest genetic effects. Rather than indicating a definitive lack of effect, this attenuation suggests that the observed association may be influenced by confounding factors—particularly age, which emerged as an independent risk factor for CVDs. Although sex did not show a significant association with any of the diseases in our analysis, it remains possible that other unmeasured factors—such as lifestyle habits, dietary folate intake, or gene–gene interactions—could modify disease susceptibility. The conflicting results reported in previous studies further emphasize the complexity of this relationship and the necessity for more stratified and mechanistic investigations. Klerk and colleagues [42] reported a higher risk of coronary heart disease in individuals with the TT genotype of the C677T polymorphism, whereas Frosst et al. [43] suggested that the homozygous 677T variant may contribute to vascular disease. Furthermore, Kluijtmans et al. [44] observed an approximately threefold higher risk of premature cardiovascular disease in homozygous 677T carriers—a finding that contrasts with the results of the current study. The discrepancies may be attributable to variations in population characteristics, sample size, or study design, which could influence the observed associations.

Regarding the C677T polymorphism and cataracts, a significant association was initially observed; however, this relationship did not persist after adjustment for age and sex, which may reflect the limited sample size. This attenuation indicates that the association may be confounded by demographic factors rather than representing a direct genetic effect. Age showed a borderline influence on cataract occurrence, suggesting that while genetic variation in MTHFR could potentially contribute, its role should currently be considered exploratory and hypothesis-generating rather than conclusive. Environmental and age-related processes may therefore play a more dominant role in cataract formation. Cataract development is often linked to oxidative stress, which is more prevalent with aging, thus potentially overshadowing the genetic influence. A study by Tan et al. [45] found that while the C677T allele, along with raised circulating homocysteine, emerged as independent predictors of cortical cataract, the association with the polymorphism was weakened after controlling for factors like sex and age. In contrast, Wu and colleagues [46] showed that C677T was linked to a higher risk of age-related cataract in a Chinese population, with specific genotypes showing either protective or increased risk for certain subtypes.

Inherited thrombophilia, a condition that elevates the chance of developing venous or arterial thromboembolism, is considered a potential factor in females experiencing unexplained recurrent pregnancy loss. Proper fetal growth and development rely on sufficient blood flow within the placental circulation. However, in women with inherited thrombophilias, a persistent hypercoagulable state combined with the natural shift toward increased coagulation during pregnancy creates a heightened tendency for blood clot formation. This can compromise the placental blood supply, ultimately disrupting embryo development and growth [16]. As for abortion, we observed a significant relationship with C677T in Fisher's test. However, logistic regression analysis did not find a significant relationship after adjusting for potential confounders. Considering the complex and multifactorial nature of miscarriage, it is reasonable to assume that the impact of a single genetic polymorphism is limited and may be overshadowed by other biological or environmental influences. Consequently, this observation should be viewed as an early signal that warrants further exploration rather than a definitive causal link. Further investigation with additional variables and larger sample sizes might be needed to fully understand the genetic underpinnings of abortion risk.

Finally, no significant links were observed between either polymorphism and hypertension or diabetes. These findings imply that the A1298C and C677T polymorphisms are unlikely to have a substantial standalone effect on these conditions. Instead, their influence—if present—may be subtle or overshadowed by other genetic or environmental factors. More refined study designs may be required to capture such effects. There are both similar and contradictory findings in the literature. In a systematic review and meta-analysis, Zhong et al. [47] showed that there was no evidence of an association between C677T and diabetes across different populations, which is consistent with our results. Similarly, Nishio et al. [48] observed no significant relationship between hypertension and the C677T variant in a Japanese population, supporting our findings. However, Qian et al. [49] reported an association between hypertension and C677T in their meta-analysis, which contradicts our results. Similarly, in a case–control study, Kuras et al. reported that the T allele of C677T may contribute to a higher chance of developing hypertension [3]. Our study has some limitations that should be acknowledged when interpreting the results obtained from the analysis performed on the association of the polymorphisms with health conditions. The modest sample size limits the power to detect associations, particularly in subgroup analyses. Medical history and underlying conditions were based on self-reports, which may introduce some reporting bias. Therefore, associations observed between MTHFR variants and chronic conditions are exploratory and should be interpreted cautiously as hypothesis-generating rather than definitive conclusions. Although we conducted multiple statistical tests, no correction for multiple comparisons (e.g., Bonferroni) was applied, since no robust associations were found.

In this study, we also conducted a LD and haplotype analysis to examine the genetic architecture of the region on chromosome 1. Our results revealed a moderate level of haplotype diversity, with four distinct haplotypes identified in the studied population. The most common haplotype, CC, was observed at a frequency of 37.6%, while the least frequent haplotype, CT, had a frequency of just 5.4%. This suggests that the region may harbor a range of genetic variations, which could be influenced by historical selection pressures or recombination events within the population. Furthermore, the LD analysis between markers in this region showed a moderate D' value, consistent with the presence of multiple haplotypes. However, the corresponding r^2 value (0.11316) was very low, indicating a weak correlation and suggesting that these SNPs are largely independent, with one SNP being a poor predictor of the other. The high LOD score (12.63091) and very low p-value provide statistical support for true linkage, but the low r^2 emphasizes that the predictive relationship between these markers is limited. These results imply that, although some co-segregation may occur in certain populations, the SNPs are not tightly linked. This highlights the importance of considering multiple SNPs in genetic association studies, as their combined effects may contribute to phenotypic variation, while individual SNPs may act largely independently. The low r^2 also reflects the complex genetic interactions and multiple recombination events in this region, resulting in diverse allele combinations. Although haplotype frequencies provide useful descriptive information, their clinical relevance remains limited in the absence of significant associations with disease outcomes.

In comparison, Shi et al. (2003) reported higher LD levels (D' up to 1.0) in certain populations, such as Pakistan and Brazil, suggesting stronger allelic co-segregation in those groups [50]. Similarly, Stegmann et al. (1999) observed complete LD in a German population, with no TC haplotype detected, indicating that the two variants rarely occur on the same chromosome. Although the 677C/T–1298A/C haplotypes were observed, no significant relationship with NTD was found, indicating limited clinical relevance in this population [51]. In another study on Chinese women, the three most frequent haplotype combinations at these two loci were 677T/1298A, 677C/1298A, and 677C/1298C, while the 677T-1298C haplotype was observed at very low frequency. LD analysis showed high D' (0.883) but low r^2 (0.143), indicating a weak correlation between the two variants [52].

Conclusion: Our study provides baseline data on the allelic, genotypic, and haplotype frequencies of the A1298C and C677T variants of the MTHFR gene in a representative sample of the Khuzestan population. Observed associations with medical conditions were exploratory and did not remain significant after adjustment for confounding factors, highlighting the need for caution in interpreting these findings. Haplotype and LD analysis revealed moderate genetic variation within the population, providing insight into the genetic structure of MTHFR polymorphisms. These data can serve as a foundation for future larger-scale, case–control or cohort studies, ideally incorporating biochemical measurements such as homocysteine and folate levels, to further investigate the potential health implications of MTHFR variants. Additionally, our findings contribute valuable information for population genetics and public health research in Iran.

Ethical Considerations

This study was approved by the Ethics Committee of Dezful University of Medical Sciences, Dezful, Iran (Approval Code: IR.DUMS.REC.1401.065).

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Conflict of Interest

None of the authors has any conflict of interest to disclose.

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References

1. Raghubeer S, Matsha TE. Methylenetetrahydrofolate (MTHFR), the One-Carbon Cycle, and Cardiovascular Risks. Nutrients. 2021;13(12). doi: 10.3390/nu13124562

2. Ye M, Xu G, Zhang L, Kong Z, Qiu Z. Meta Analysis of Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphism and its association with folate and colorectal cancer. BMC Cancer. 2025;25(1):169. doi: 10.1186/s12885-025-13546-w

3. Kuras S, Pence ME, Arac E, Yildiz A, Erdogan B, Pence S. Investigation of the effects of MTHFR gene variations and homocysteine levels in hypertensive

patients. Northern Clinics of Istanbul. 2025;12(1):55-61. doi: 10.14744/nci.2023.84770

4. Rosenberg N, Murata M, Ikeda Y, et al. The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in whites, Japanese, and Africans. American Journal of Human Genetics. 2002;70(3):758-62. doi: 10.1086/338932

5. Li Y, Qiu S, Shi J, et al. Association between MTHFR C677T/A1298C and susceptibility to autism spectrum disorders: a meta-analysis. BMC Pediatrics. 2020; 20:1-11. doi: 10.1186/s12887-020-02330-3

6. You J, Huang Y, Shen X, Chen Y, Ding X. Associations between MTHFR gene polymorphisms (C677T and A1298C) and genetic susceptibility to prostate cancer: a systematic review and meta-analysis. Frontiers in Genetics. 2024; 15:1343687. doi: 10.3389/fgene.2024.1343687

7. Meng H, Huang S, Yang Y, He X, Fei L, Xing Y. Association Between MTHFR Polymorphisms and the Risk of Essential Hypertension: An Updated Meta-analysis. Frontiers in Genetics. 2021; 12:698590. doi: 10.3389/fgene.2021.698590

8. Fu L, Li Yn, Luo D, Deng S, Wu B, Hu YQ. Evidence on the causal link between homocysteine and hypertension from a meta-analysis of 40 173 individuals implementing Mendelian randomization. The Journal of Clinical Hypertension. 2019;21(12):1879-1894. doi: 10.1111/jch.13737

9. Chiu MH, Chang CH, Tantoh DM, et al. Susceptibility to hypertension based on MTHFR rs1801133 single nucleotide polymorphism and MTHFR promoter methylation. Frontiers in Cardiovascular Medicine. 2023; 10:1159764. doi: 10.3389/fcvm.2023.1159764

10. Luo Z, Tang K, Huang G, et al. Homocysteine concentration in coronary artery disease and severity of coronary lesions. Journal of Cellular and Molecular Medicine. 2024;28(12):e18474. doi: 10.1111/jcmm.18474

11. Luo Z, Lu Z, Muhammad I, et al. Associations of the MTHFR rs1801133 polymorphism with coronary artery disease and lipid levels: a systematic review and updated meta-analysis. Lipids in Health and Disease. 2018;17(1):191. doi: 10.1186/s12944-018-0837-y

12. Li L, Yu H, Zhang H, Wang J, Hu W. Association between MTHFR C677T polymorphism and risk of coronary artery disease in the Chinese population: meta-analysis. Herz. 2022;47(6):553-563. doi: 10.1007/s00059-021-05087-2

13. Samii A, Aslani S, Imani D, et al. MTHFR gene polymorphisms and susceptibility to myocardial infarction: Evidence from meta-analysis and trial sequential analysis. International Journal of Cardiology Heart and Vasculature. 2023; 49:101293. doi: 10.1016/j.ijcha.2023.101293

14. Badea IA, Daba LC, Leopa N, Parepa IR, Ispas S, Botnarciuc M. Association of MTHFR C677T and A1298C Polymorphisms with First-Episode Myocardial Ischemia: A Case-Control Study. Genes (Basel). 2025;16(8) doi: 10.3390/genes16080858

15. Du B, Shi X, Yin C, Feng X. Polymorphisms of methalenetetrahydrofolate reductase in recurrent pregnancy loss: an overview of systematic reviews and meta-analyses. Journal of Assisted Reproduction and Genetics. 2019;36(7):1315-1328. doi: 10.1007/s10815-019-01473-2

16. Wen Y, He H, Zhao K. Thrombophilic gene polymorphisms and recurrent pregnancy loss: a systematic review and meta-analysis. Journal of Assisted Reproduction and Genetics. 2023;40(7):1533-1558. doi: 10.1007/s10815-023-02823-x

17. Huang LL, Tong JR, Huang Y, et al. Association of MTHFR gene C677T polymorphism with pregnancy outcome. European Review for Medical and Pharmacological Sciences. 2023;27(1):166-171.

18. Rao J, Chen Y, Chen X, et al. The gene polymorphisms of eNOS and MTHFR modulates the development of preeclampsia in Han population. Heliyon. 2023;9(12): e22223. doi: 10.1016/j.heliyon.2023.e22223

19. Tahmasebi P, Nezhad SRK. An overview of hereditary diseases in Khuzestan Province, Southwest Iran. Gene, Cell and Tissue. 2015;2(2) doi: 10.17795/gct-29272

20. Dean L. Methylenetetrahydrofolate Reductase Deficiency. In: Pratt VM, Scott SA, Pirmohamed M, Esquivel B, Kattman BL, Malheiro AJ, eds. Medical Genetics Summaries. National Center for Biotechnology Information (US); 2012.

21. Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. Genetics in Medicine: Official Journal of the American College of Medical Genetics. 2013;15(2):153-6. doi: 10.1038/gim.2012.165

22. Wilcken B, Bamforth F, Li Z, et al. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas worldwide. Journal of Medical Genetics. 2003;40(8):619-25. doi: 10.1136/jmg.40.8.619

23. He Q, Wei Y, Zhu H, et al. The combined effect of MTHFR C677T and A1298C polymorphisms on the risk of digestive system cancer among a hypertensive population. Discover Oncology. 2024;15(1):97. doi: 10.1007/s12672-024-00960-y

24. Jahanbin A, Hasanzadeh N, Abdolhoseinpour F, et al. Analysis of MTHFR Gene C.677C>T and C.1298A>C Polymorphisms in Iranian Patients with Non-Syndromic Cleft Lip and Palate. Iranian Journal of Public Health. 2014;43(6):821-7.

25. Safdarian L, Najmi Z, Aleyasin A, Aghahosseini M, Rashidi M, Asadollah S. Recurrent IVF failure and hereditary thrombophilia. Iranian Journal of Reproductive Medicine. 2014;12(7):467-70.

26. Delshadpour M, Mashayekhi F, Bidabadi E, Shahangian S, Salehi Z. MTHFR rs1801133 gene polymorphism and autism susceptibility. Caspian Journal of Neurological Sciences. 2017;3(1):39-45. doi: 10.18869/acadpub.cjns.3.8.39

27. Ghadirkhomi E, Angaji SA, Khosravi M, Mashayekhi MR. Association of Novel Single Nucleotide Polymorphisms of Genes Involved in Cell Functions with Male Infertility: A Study of Male Cases in Northwest Iran. Journal of Reproduction and Infertility. 2021;22(4):258-266. doi: 10.18502/jri.v22i4.7651

28. Sheikhha M, Kalantar S, Ghasemi N, Soleimanian S. Association between MTHFR 1298A>C Polymorphism with RSA and IVF Failure. Research. Iranian Journal of Pediatric Hematology and Oncology. 2012;2(3):109-115.

29. Najmabadi H hV, Imanian V. Genetic investigation of MTHFR A1298C polymorphism as a cause of cardiovascular diseases in Iranian population. Genetics in the Third Millennium. 2010;8th (1):1962-.

30. Foroughmand AM, Galehdari H, Pooryasin A, Ajam T, Kazemi-Nezhad SR. Additive effect of MTHFR and GRIN1 genetic polymorphisms on the risk of schizophrenia. Molecular Biology Research Communications. 2015;4(1):33-42.

31. Golchin S, Hedayatizadeh-Omran A, Alizadeh-Navaei R, et al. Association between MTHFR Gene Polymorphism (C677T) and coronary artery disease. Journal of Mazandaran University of Medical Sciences. 2019;28(168):50-58.

32. Thirumaran RK, Gast A, Flohr T, et al. MTHFR genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Blood. 2005;106(7):2590-1; author reply 2591-2. doi: 10.1182/blood-2005-04-1719

33. Kurzwelly D, Knop S, Guenther M, et al. Genetic variants of folate and methionine metabolism and PCNSL incidence in a German patient population. Journal of Neuro-Oncology. 2010;100(2):187-92. doi: 10.1007/s11060-010-0154-4

34. Lightfoot TJ, Skibola CF, Willett EV, et al. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005;14(12):2999-3003. doi: 10.1158/1055-9965.EPI-05-0515

35. Li Q, Lan Q, Zhang Y, et al. Role of one-carbon metabolizing pathway genes and gene-nutrient interaction in the risk of non-Hodgkin lymphoma. Cancer Causes and Control: 2013;24(10):1875-84. doi: 10.1007/s10552-013-0264-3

36. Boduroğlu K, Alanay Y, Koldan B, Tunçbilek E. Methylenetetrahydrofolate reductase enzyme polymorphisms as maternal risk for Down syndrome among Turkish women. American Journal of Medical Genetics Part A. 2004;127a (1):5-10. doi: 10.1002/ajmg.a.20432

37. Weiner AS, Beresina OV, Voronina EN, et al. Polymorphisms in folate-metabolizing genes and risk of non-Hodgkin's lymphoma. Leukemia Research. 2011;35(4):508-15. doi: 10.1016/j.leukres.2010.10.004

38. Kim JK, Kim S, Han JH, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of stomach cancer in a Korean population. Anticancer Research. 2005;25(3b):2249-52.

39. Muthuswamy S, Agarwal S. Do the MTHFR gene polymorphism and Down syndrome pregnancy association stands true? A case-control study of Indian population and meta-analysis. Egyptian Journal of Medical Human Genetics. 2016;17(1):87-97. doi: 10.1016/j.ejmhg.2015.08.003

40. Gao M, Feng N, Zhang M, Ti X, Zuo X. Meta-analysis of the relationship between methylenetetrahydrofolate reductase C677T and A1298C polymorphism and venous thromboembolism in the Caucasian and Asian. Bioscience Reports. 2020;40(7). doi: 10.1042/BSR20200860

41. Clarke R, Bennett DA, Parish S, et al. Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication

bias. PLoS Medicine. 2012;9(2): e1001177. doi: 10.1371/journal.pmed.1001177

42. Klerk M, Verhoef P, Clarke R, et al. MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA. 2002;288(16):2023-31. doi: 10.1001/jama.288.16.2023

43. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genetics. 1995;10(1):111-3. doi: 10.1038/ng0595-111

44. Kluijtmans L, Van den Heuvel L, Boers G, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. American Journal of Human Genetics. 1996;58(1):35.

45. Tan AG, Kifley A, Mitchell P, et al. Associations Between Methylenetetrahydrofolate Reductase Polymorphisms, Serum Homocysteine Levels, and Incident Cortical Cataract. JAMA Ophthalmology. 2016;134(5):522-528. doi: 10.1001/jamaophthalmol.2016.0167

46. Wu X, Lai W, Lin H, Liu Y. Association of OGG1 and MTHFR polymorphisms with age-related cataract: A systematic review and meta-analysis. PloS One. 2017;12(3):e0172092. doi: 10.1371/journal.pone.0172092

47. Zhong J-H, Rodríguez AC, Yang N-N, Li L-Q. Methylenetetrahydrofolate reductase gene polymorphism and risk of type 2 diabetes mellitus. PloS One. 2013;8(9): e74521. doi: 10.1371/journal.pone.0074521

48. Nishio H, Lee MJ, Fujii M, et al. A common mutation in methylenetetrahydrofolate reductase gene among the Japanese population. Japanese Journal of Human Genetics. 1996;41(2):247-251. doi: 10.1007/BF01875985

49. Qian X, Lu Z, Tan M, Liu H, Lu D. A meta-analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. European Journal of Human Genetics. 2007;15(12):1239-1245. doi: 10.1038/sj.ejhg.5201914

50. Shi M, Caprau D, Romitti P, Christensen K, Murray JC. Genotype frequencies and linkage disequilibrium in the CEPH human diversity panel for variants in folate pathway genes MTHFR, MTHFD, MTRR, RFC1, and GCP2. Birth defects research Part A, Clinical and Molecular Teratology. 2003;67(8):545-9. doi: 10.1002/bdra.10076

51. Stegmann K, Ziegler A, Ngo ET, et al. Linkage disequilibrium of MTHFR genotypes 677C/T-1298A/C in the German population and association studies in probands with neural tube defects (NTD). American Journal of Medical Genetics. 1999;87(1):23-9. doi: 10.1002/(SICI)1096-8628(19991105)87:1<23::AID-AJMG5>3.0.CO;2-E

52. Fan S, Yang B, Zhi X, Wang Y, Zheng Q, Sun G. Combined genotype and haplotype distributions of MTHFR C677T and A1298C polymorphisms: A cross-sectional descriptive study of 13,473 Chinese adult women. Medicine (Baltimore). 2016;95(48): e5355. doi: 10.1097/MD.0000000000005355