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Methylenetetrahydrofolate reductase *(MTHFR)* C677T and A1298C polymorphisms are associated with major depressive disorder in the Saudi patients attending Erada complex for mental health and Erada services – Jeddah, Saudi Arabia

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Background: Major Depressive Disorder (MDD) is a prevalent and debilitating mental disorder that has been linked to hyperhomocysteinemia and folate deficiency. These conditions are influenced by the methylenetetrahydrofolate reductase (*MTHFR*) gene, which plays a crucial role in converting homocysteine to methionine and is essential for folate metabolism and neurotransmitter synthesis, including serotonin. *Study aim*: This study explored the association between *MTHFR C677T* and *A1298C* polymorphisms among Saudi MDD patients attending the Erada Complex for Mental Health and Erada Services outpatient clinic in Jeddah, Saudi Arabia. *Methods*: The study involved 87 MDD patients and 87 control subjects. Saliva samples were collected, and genomic DNA was extracted. Polymerase chain reaction-restriction fragment length polymorphism was used to detect *MTHFR* gene polymorphisms. *Results*: A significant difference was observed in the distribution of genotype frequencies for *MTHFR C677T* and *A1298C* polymorphisms between MDD patients and controls in the Saudi cohort (*C677T*: $P = 0.001$; *A1298C*: $P = 0.01$) Risk analysis indicated that individuals with the mutant TT genotype of the *C677T* polymorphism (Odd Ratio (OR) = 6.80, CI 95% = 1.47–31.36, $P = 0.01$) and the mutant CC genotype of the *A1298C* polymorphism (OR = 2.64, CI 95% = 1.36–5.13, $P = 0.004$) are more common in MDD patients, suggesting a higher risk for depression. Gender-specific analyses showed that the *MTHFR C677T* TT genotype significantly increases the risk of MDD in males compared to females. *Conclusion:* These findings underscore the significant impact of genetic factors, particularly the association of *MTHFR* polymorphisms with MDD. The results highlight the importance of personalized treatment approaches considering individual genetic profiles.

1. Introduction

Major Depressive Disorder (MDD) is a prevalent and debilitating mental disorder characterized by persistent depressed mood, anhedonia, and recurrent thoughts of death (Marx et al. 2023; Otte et al. 2016). Major Depressive Disorder impacts individuals across all age groups, genders, and ethnic backgrounds (Salari et al. 2020). Globally, approximately 5% of adults are estimated to experience depression, with women being more predisposed to depression compared to men (World Health Organization 2023). The overall prevalence of MDD in Saudi Arabia is 3.8%, with a lifetime prevalence of 6.0% (Al-Qadhi et al. 2014; Al-Subaie et al. 2020). However, this figure likely underestimates the prevalence due to cultural stigmatization and associated underreporting of psychological illnesses in the Saudi population (Alamri et al. 2020). Inadequate detection and treatment of MDD can result in chronicity, relapse, and increased risk of suicide in severe cases (Colizzi et al. 2020).

The development of MDD is multifaceted, encompassing genetic, environmental, and neurobiological influences (Caspi et al. 2003; Flint 2023; Remes et al. 2021). Identifying specific genetic variants associated with MDD can provide valuable insights into the underlying molecular mechanisms and potential targets for intervention. The genetics of depression have been thoroughly explored, with López-León et al. (2008) identifying over 393 polymorphisms across 102 genes that have been examined for their potential association with depression. One gene that has received considerable attention in MDD research is the methylenetetrahydrofolate reductase (*MTHFR*) gene, which encodes the MTHFR enzyme (Goyette et al. 1998). The MTHFR enzyme is involved in a critical pathway for the synthesis of DNA, methylation reactions, and the production of neurotransmitters, including serotonin. It is a crucial enzyme for the one-carbon metabolism cycle, which encompasses processes related to homocysteine metabolism, folate utilization, and DNA methylation (Miner et al. 1997).

Two prevalent functional single nucleotide polymorphisms (SNPs) of the *MTHFR* gene, namely *C677T* (NCBI SNP ID: rs1801133) and *A1298C* (NCBI SNP ID: rs1801131), have been extensively scrutinized across a spectrum of health conditions, including MDD (Bousman et al. 2014; Norkeviciene et al. 2022). The *C677T* polymorphism involves a cytosine-to-thymine substitution at nucleotide 677 in exon 4, resulting in an amino acid change from alanine to valine at position 222 of the MTHFR protein (Goyette et al. 1998). This genetic mutation reduces MTHFR enzyme activity, as evidenced by a 50–60% reduction in MTHFR enzyme activity in *C677T* homozygous (TT) patients, impairing folate metabolism and disrupting the one-carbon metabolism cycle (Rozen, 1997).

This leads to increased homocysteine levels (hyperhomocysteinemia) associated with MDD (Nabi et al. 2013; Zhou et al. 2020). Reduced activity also hinders the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, affecting neurotransmitter synthesis, including serotonin, which is crucial for mood regulation and implicated in MDD (Shao and Zhu 2020).

The A1298C polymorphism, on the other hand, involves an adenine-to-cytosine substitution at nucleotide 1298 in exon 7, resulting in a glutamate-to-alanine amino acid change at position 429 of the MTHFR protein (Goyette et al. 1998). This mutation leads to a milder reduction in MTHFR enzyme activity compared to the *C677T* polymorphism. While the exact functional consequences of the A1298C polymorphism are not yet fully elucidated, it is thought to impact folate metabolism and potentially influence MDD susceptibility and severity (Cho et al. 2017; Shao and Zhu 2020).

Various studies have investigated the association between the *MTHFR C677T* and *A1298C* polymorphisms and MDD, yet findings have been inconsistent. Several meta-analyses reported a positive correlation between the *MTHFR C766T* and *A1298C* polymorphisms and depression risk (Gilbody et al. 2007; Wu et al. 2013; Zhang et al. 2022). However, conflicting results were observed in meta-analyses, which found no significant association (Gaysina et al. 2008; Zintzaras, 2006). These discrepancies may stem from differences in sample sizes and ethnic and geographic variations across populations, highlighting the complexity of genetic influences on depression susceptibility.

Ethnic subgroup analysis in the Asian population revealed a significant correlation between MDD and *MTHFR C677T* and *A1298C* polymorphisms. In contrast, this association was not observed in Caucasians (Zhang et al. 2022), despite existing research on *MTHFR C677T* and *A1298C* polymorphisms and MDD in various populations. Little is known about their impact in Saudi Arabia. This gap highlights the need for targeted studies, particularly given the unique cultural, ethnic, and environmental factors in Saudi Arabia that may influence MDD severity (Bailey et al. 2019; Remes et al. 2021). Furthermore, the high prevalence of these polymorphisms in the Saudi population suggests potentially significant effects (Bagher et al. 2021). Our study aims to explore this association in MDD patients attending the Erada Complex for Mental Health and Erada Services outpatient clinic in Jeddah, Saudi Arabia. Jeddah is the biggest city on the Red Sea in the west of Saudi Arabia, located in the Southwest of Asia.

Table 1: Distribution of genotype frequencies for the *MTHFR C677T* and *A1298C* polymorphisms among individuals with MDD and controls

Polymorphisms	Genotype	Control	MDD	χ2, df, p
MTHFR C677T	CC	70 (80.45%)	49 (56.32%)	χ2= 14 df=2 *P*= 0.001*
	CT	15 (17.24%)	26 (29.88%)	
	TT	2 (2.29%)	12 (13.79%)	
MTHFR A1298C	AA	8 (9.1%)	6 (6.8%)	χ2= 8.5 df=2 *P*= 0.01*
	AC	60 (68.9%)	44 (50.5%)	
	CC	19 (21.8%)	37 (42.5%)	

Table 2: Association between *MTHFR C677T* and *A1298C* polymorphisms and depression

Polymorphisms	Genotype	OR	CI 95%	*P*
MTHFR C677T	CC	0.31	0.15 – 0.61	0.0008*
	CT	2.04	0.99 – 4.20	0.05*
	TT	6.80	1.47 – 31.36	0.01*
MTHFR A1298C	AA	0.73	0.24 – 2.20	0.56
	AC	0.46	0.24 – 0.85	0.01*
	CC	2.64	1.36 – 5.13	0.004*

Table 3: Distribution of genotype frequencies for the *MTHFR C677T* and *A1298C* polymorphisms among male individuals with MDD and controls

Polymorphisms	Genotype	Control	MDD	χ2, df, p
MTHFR C677T	CC	36 (90%)	26 (56.53%)	χ2= 13.26 df=2 *P*= 0.001*
	CT	4 (10%)	12 (26.08%)	
	TT	0 (0%)	8 (17.39%)	
MTHFR A1298C	AA	4 (10%)	3 (6.52%)	χ2= 5.38 df=2 *P*= 0.06
	AC	28 (70%)	23 (50%)	
	CC	8 (20%)	20 (43.37%)	

2. Investigations and results

The present study involved 174 participants, evenly divided into 87 controls and 87 patients diagnosed with MDD. Gender distribution was nearly equivalent between the groups, with 46 males (52.87%) and 41 females (47.13%) in the MDD group and 40 males (45.97%) and 47 females (54.03%) in the control group, showing no significant statistical difference (P=0.36) Notable age disparities were evident; the control group averaged younger at 26.8 years (SD = 7.25), compared to the MDD group's average of 44.02 years (SD = 11.32) MDD prevalence was significantly higher in older adults (ages >30 to <50 years) and the elderly (ages >50 years) compared to younger adults (ages 18-30 years), with p-values <0.0001.

Significant genetic associations between *MTHFR* polymorphisms and MDD were evident in our findings (Tables 1 and 2) Expressly, the genotype frequencies for *MTHFR C677T* polymorphism indicated a shift from the wild-type CC genotype in controls (80.45%) to increased heterozygous CT (29.88%) and homozygous mutant TT (13.79%) genotypes in MDD patients, with significant divergence ($\chi2 = 14$, df = 2, P= 0.001) (Table 1) A similar pattern emerged for the *A1298C* polymorphism, with higher frequencies of heterozygous AC (50.5%) and homozygous mutant CC (42.5%) genotypes in MDD patients, again with statistical significance ($\chi2 = 8.5$, df = 2, P = 0.01) (Table 1) The odds ratios confirmed these associations, notably increasing MDD risk with the *C677T* TT genotype (OR = 6.80, 95% CI = 1.47-31.36, P= 0.01) and the *A1298C* CC genotype (OR = 2.64, 95% CI = 1.36-5.13, P = 0.004) (Table 2)

Gender-specific genetic associations were observed, particularly among male participants (Tables 3 and 4) For the *MTHFR C677T* polymorphism, significant differences were noted between controls and MDD patients, with the TT genotype appearing more frequently in MDD patients (17.39%, $\chi2 = 13.26$, df = 2, P = 0.001) compared to predominantly CC genotype in controls (90%) (Table 3) The odds ratios indicated a protective effect for the CC genotype against MDD (OR = 0.14, 95% CI = 0.04-0.47, P = 0.001) and a marked increase in MDD risk associated with the TT genotype (OR = 17.43, CI = 0.99-320.53, P = 0.04) However, no significant associations were found with the *A1298C* polymorphism in male participants (P = 0.06) (Table 3), except for a higher risk with the CC genotype (OR = 3.47, CI = 1.32-9.06, P = 0.01), underscoring a pronounced impact of the *C677T* polymorphism on MDD susceptibility among the male Saudi population (Table 4)

In contrast, the genetic associations between *MTHFR* polymorphisms and MDD among female participants were less pronounced (Tables 5 and 6) There were no statistically significant differences in genotype frequencies for both *MTHFR C677T* and *A1298C* polymorphisms (C677T: $\chi2 = 2.75$, df = 2, p = 0.25; A1298C: $\chi2 = 3.31$, df = 2, P = 0.19) (Table 5) The odds ratios also indicated no significant associations between *C677T* genotypes (CC, CT, TT) and MDD in females, with P-values above the threshold for statistical significance (Table 6) Similarly, while a trend suggested an increased risk for MDD among females with the CC genotype of the *A1298C* polymorphism (OR = 2.31, CI = 0.92-5.80, P = 0.07), it did not reach statistical significance (Table 6) These findings highlight potential gender differences in the genetic basis of MDD, suggesting a more nuanced role of *MTHFR* polymorphisms within the female segment of the Saudi population.

Table 4: Association between *MTHFR C677T* and *A1298C* polymorphisms and depression polymorphisms in males

Polymorphisms	Genotype	OR	CI 95%	*P*
MTHFR C677T	CC	0.14	0.04 – 0.47	0.001*
	CT	3.08	0.90 – 10.48	0.07
	TT	17.88	0.99 – 320.53	0.04*
MTHFR A1298C	AA	0.62	0.13 – 2.99	0.58
	AC	0.42	0.17 – 1.04	0.06
	CC	3.47	1.32 – 9.06	0.01*

Table 5: Distribution of genotype frequencies for the *MTHFR C677T* and *A1298C* polymorphisms among female individuals with MDD and controls

Polymorphisms	Genotype	Control	MDD	χ2, df, p
MTHFR C677T	1	34 (72.34%)	23 (56.09%)	χ2= 2.75 df=2 *P*= 0.25
	CT	11 (23.40%)	14 (34.14%)	
	TT	2 (4.25%)	4 (9.75%)	
MTHFR A1298C	AA	4 (8.51%)	3 (7.31%)	χ2= 3.31 df=2 *P*= 0.19
	AC	32 (68.08%)	21 (51.21%)	
	CC	11 (23.40%)	17 (41.46%)	

Table 6: Association between *MTHFR C677T* and *A1298C* polymorphisms and depression in females

Polymorphisms	Genotype	OR	CI 95%	*P*
MTHFR C677T	CC	0.48	0.20 – 1.18	0.11
	CT	1.69	0.66 – 4.31	0.26
	TT	2.43	0.42 – 14.00	0.32
MTHFR A1298C	AA	0.84	0.17 – 4.03	0.83
	AC	0.45	0.19 – 1.04	0.08
	CC	2.31	0.92 – 5.80	0.07

3. Discussion

Our study at the Erada Complex for Mental Health and Erada Services in Jeddah, located in the Western region of Saudi Arabia, demonstrates significant associations between *MTHFR C677T* and *A1298C* polymorphisms and MDD. These associations are particularly marked in Saudi males, enriching our understanding of the genetic foundations of MDD across diverse demographics.

Identifying the TT genotype of the *C677T* polymorphism and the CC genotype of the *A1298C* polymorphism as significant risk factors echoes findings from regional research in Taif, pointing to the possible influence of environmental or regional factors on genetic expression (Alhomrani et al. 2022). The significant impact of the TT genotype of the *C677T* polymorphism, as documented by Arinami et al. (1997) was the first who focus on *MTHFR* gene polymorphism, with an odds ratio of 2.8, highlights a substantial genetic risk associated with MDD. This finding is consistent with a range of studies and is further validated by a comprehensive meta-analysis conducted by Zhang et al. (2022), which analyzed data from 81 studies. This analysis confirmed a significant correlation between *MTHFR C677T* and *A1298C* polymorphisms and major depression, particularly within the Asian population. This evidence of consistency is bolstered by additional meta-analyses, all reinforcing the vital link between this genotype and an increased risk of MDD (Gilbody et al. 2007; Lok et al. 2013; López-León et al. 2008; Peerbooms et al. 2011; Wu et al. 2013). Nevertheless, contrasting meta-analysis studies by Gaysina et al. and Zintzaras et al. which did not establish significant links, may reflect methodological differences, genetic diversity, or the complex nature of depression, emphasizing the necessity for further research into genetic and environmental interactions (Gaysina et al. 2008; Zintzaras, 2006).

We observed that gender-specific analyses showed that the MTHFR C677T TT genotype significantly increases the risk of MDD in males compared to females. The gender disparities observed in our study align with global research, highlighting distinct genetic profiles in mood disorders across the sexes. Studies from Middle Eastern cohorts, including Lebanon, and research from China support our findings, suggesting a potential universal genetic factor influencing susceptibility to MDD in males, indicative of a broader regional trend (Sabbagh et al. 2008; Shen et al. 2014). The higher prevalence of MDD in males suggests that hormonal factors may modulate genetic susceptibility to MDD. Estrogen, for instance, acts as a protective factor in women by potentially lowering homocysteine levels and thereby reducing the risk of MDD. In contrast, men often exhibit higher plasma homocysteine levels, primarily due to lower estrogen levels, which may increase their risk of developing MDD (Wang et al. 2019). This, combined with the higher prevalence of the *MTHFR C677T* variant in Saudi males and various hormonal and environmental influences, could significantly elevate the risk of MDD in this group.

Contrary to our findings, some studies report that the relationship between MTHFR polymorphisms and MDD is more pronounced in females, as observed in North African Berber populations and among postmenopausal Polish women (Chojnicka et al. 2012; Sayadi et al. 2016). Interestingly, studies within Algerian and Slovak populations do not reflect our findings, showing significant gender differences in the prevalence of MTHFR polymorphisms (Djaara et al. 2018; Evinova et al. 2012). This inconsistency underscores the complex interplay of genetic, hormonal, and environmental factors influencing MDD.

Recent advancements have significantly enhanced our understanding of the biochemical pathways implicated in MDD. For instance, Bottiglieri et al. (2021) explored how *MTHFR* polymorphisms extend their influence beyond homocysteine metabolism, potentially affecting critical neurotransmitter systems such as serotonin and dopamine, vital for mood regulation (Bottiglieri, 2000). This research underscores the potential benefits of adapting treatment strategies, notably the integration of L-methylfolate supplementation. Such approaches have demonstrated efficacy in alleviating depressive symptoms by compensating for the reduced methylation capacity in patients with the *MTHFR C677T* TT genotype (Liwinski and Lang 2023). These insights support the growing relevance of personalized medicine in psychiatry, emphasizing the value of screening for *MTHFR* polymorphisms within clinical settings to tailor treatment strategies effectively (Liwinski and Lang 2023). Furthermore, the differential impact of these polymorphisms on the risk of MDD in males and females highlights the urgent need for gender-specific treatments that account for both hormonal and genetic variations. This approach aligns with the latest research and promises enhanced therapeutic outcomes by addressing the unique biological underpinnings of each patient's condition.

Our study's limited sample size and regional focus may impact the generalizability of our findings across the broader Saudi population. Additionally, the cross-sectional design hinders our ability to establish causal relationships between *MTHFR* polymorphisms and MDD. These issues underscore the necessity for validation through more extensive, more diverse, and longitudinal studies to confirm the associations and their implications for depression.

Future research should employ longitudinal studies to clarify causal links between *MTHFR* polymorphisms and MDD progression. Investigating additional genetic markers that influence folate metabolism and neurotransmitter regulation could deepen our understanding of MDD susceptibility. Furthermore, assessing interventions that modulate folate metabolism, especially for those with high-risk genotypes, is crucial for developing personalized treatment strategies. Exploring the interplay between genetic, environmental, and cultural factors is also vital for crafting culturally sensitive approaches to prevention and treatment.

In conclusion, our research confirms that *MTHFR* gene polymorphisms, mainly the *C677T* and *A1298C* variants, are significantly linked to MDD within the Saudi population, especially among males. Individuals with the TT genotype of the *C677T* polymorphism and the CC genotype of the *A1298C* polymorphism demonstrate a higher prevalence of MDD, with gender-specific analyses revealing a notably higher risk in males. These findings underscore the importance of genetic factors in understanding MDD's etiology and tailoring prevention and treatment strategies. Ongoing research into the longitudinal effects of these polymorphisms and their interaction with environmental and cultural aspects is crucial for advancing personalized medicine and improving outcomes in mental health care.

4. Experimental

4.1. Subjects and study design

This cross-sectional study was conducted at the Erada Complex for Mental Health and Erada Services outpatient clinic in Jeddah, Kingdom of Saudi Arabia, from December 2022 to September 2023. The study received ethical approval from the Local Committee for Research Ethics in Jeddah Health, under Institutional Review Board (IRB) number A01473.

The case-control study comprised 174 participants, segmented into two distinct groups: 87 healthy Saudi controls and 87 participants diagnosed with MDD, including both adult genders. The control group consisted of healthy Saudi adults aged 18 years or older without any history of psychiatric illness, confirmed by scores below nine on the Beck Depression Inventory (BDI) Exclusion criteria for the control group included any history of psychiatric illness.

In contrast, the MDD group comprised Saudi adults aged 18 years or older diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) criteria. Exclusion criteria for the MDD group included vulnerable populations such as children, pregnant or breastfeeding individuals, and those with significant neurological disorders, substance abuse disorders, or current treatment with mood stabilizers or antipsychotic medications.

All study participants were thoroughly informed about the study's objectives and procedures. Informed consent, provided in Arabic, was obtained before participation. Detailed demographic information, family history of MTHFR enzyme polymorphisms, and MDD were collected for each participant. A single investigator collected saliva samples using the Oragene™ DNA Sample Collection Kits (OGR-250, DNA Genotek Inc., Canada)

4.2. Detecting MTHFR C677T and A1298C polymorphism

The genomic DNA extraction from these saliva samples was conducted per the manufacturer's guidelines (Nunes et al. 2012). Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis were utilized to assess *C677T and* A1298C *MTHFR* polymorphisms (Bagher et al. 2021). The following primers, forward primer 5′-CCTTGAACAGGTGGAGGCCAG-3′ and reverse primer 5′-GCGGTGAGTGGGGTGGAG-3′ (Macrogen, South Korea), were used to amplify a 294-base pair (bp) fragment of the *MTHFR* gene. The PCR mixture consisted of 10 µL of Solarbio 2×Taq PCR Master Mix (Beijing Solarbio Science and Technology Co., Ltd.), 100 ng of genomic DNA, and 0.8 µL of each primer, bringing the total volume to 20 µL with nuclease-free water (Beijing Solarbio Science and Technology Co., Ltd.) The PCR process began with an initial denaturation at t 95°C for 10 minutes, succeeded by 35 cycles of denaturation at 95°C for 1 minute, primer annealing at 65°C for 1 minute, and extension at 72°C for 1 minute. Subsequently, a final extension step was performed at 72°C for 10 minutes. Subsequently, a 10 µl aliquot of the PCR product was digested in a 20 µl reaction, incorporating 0.5 µl of *HinfI* restriction enzyme and a 1× concentration of Cutsmart buffer (New England Biolabs, USA) Electrophoresis was performed on a 3% agarose gel at 80 V for 120 minutes to separate the digested PCR products. A single band at 294 bp indicated the homozygous wild-type variant (*C677C*), while the homozygous variant (*T677T*) was identified by two bands at 168 bp and 126 bp. The heterozygous genotype (*C677T*) displayed three bands at 294, 168, and 126 bp (Fig. 1A)

To determine the genotype of the *MTHFR A1298C* polymorphism, we amplified a 163-bp segment of the *MTHFR* gene using customized primers (forward: 5′-CCTTGAACAGGTGGAGGCCAG-3′; reverse: 5′-GCGGTGAGAGTGGGGTGG -3′ (Macrogen, Korea) The PCR protocol and thermal cycling parameters replicated those utilized to amplify *C677T*, except for the annealing temperature, which was adjusted to 62°C. After amplification, the PCR products were processed with the *MboII* enzyme (Thermo Fisher Scientific, USA) and separated on a 4% agarose gel. The homozygous *A1298A* genotype was indicated by five bands measuring 56, 31, 30, 28, and 18 bp. The heterozygous *A1298C* genotype displayed three bands measuring 84, 56, and 30 bp. Detection of the homozygous *C1298C* genotype involved identifying four bands measuring 84, 31, 30, and 18 bp (Fig. 1A) To ensure the accuracy of the subject genotypes, random patient samples were chosen for direct sequencing.

4.3. Statistical analysis

GraphPad Prism version 9.4.1 (GraphPad Software, San Diego, USA) was utilized for statistical analyses. Qualitative variables were compared using the Chi-squared ($\chi 2$) test, while quantitative variables were presented as mean ± SD and analyzed using the Student's t-test. Additionally, Odds Ratios (ORs) were calculated using the Odds Ratio Calculator from MedCalc Software Ltd. (https://www.medcalc.org/calc/odds_ratio.php, Version 22.021; accessed March 8, 2024) The threshold for statistical significance was established at $p \leq 0.05$.

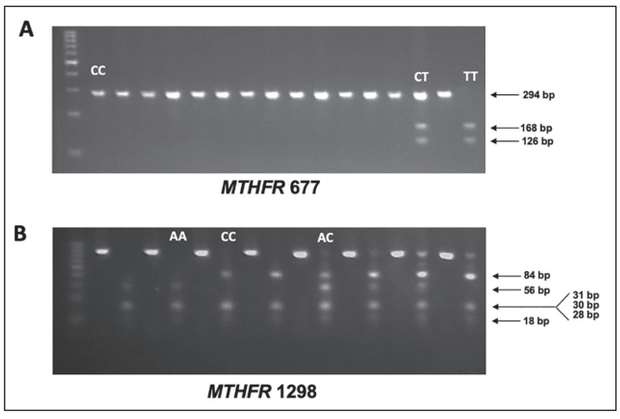

Fig. 1: (A) PCR-RFLP findings for the MTHFR C677T polymorphism. The agarose gel electrophoresis depicts the results of Hinf digestion. The homozygous wild-type CC genotype produces a single 294 bp band, while the heterozygous CT genotype exhibits three bands at 294, 168, and 126 bp. The homozygous TT genotype displays two bands at 168 bp and 126 bp. (B) The MTHFR A1298A polymorphism was assessed through PCR-based Restriction Fragment Length Polymorphism (RFLP) Subsequently, agarose gel electrophoresis was employed to analyze the A1298C polymorphism following digestion with MboII. The digestion of the homozygous AA genotype resulted in five discernible bands at 56, 31, 30, 28, and 18 base pairs (bp) In contrast, the heterozygous AC genotype exhibited three bands measuring 84, 56, and 30 bp, while the homozygous CC genotype displayed four bands at 84, 31, 30, and 18 bp. The close similarity in molecular weights (31, 30, and 28 bp) rendered them indistinguishable on the gel. Predominant bands observed were at 84 bp and 56 bp. Lane 1 contained a 25 bp DNA marker.

Conflicts of interest: None declared.

Availability of data and materials: All data are available without restriction.

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