

EFFECT OF SLC19A1 GENE POLYMORPHISM AND HAPLOTYPES ON IDIOPATHIC RECURRENT PREGNANCY LOSS AMONG WOMEN IN DUHOK CITY.

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ABSTRACT:

Recurrent pregnancy loss (RPL) affects 1-2% of women of reproductive age and is defined as two or more consecutive losses before 20 weeks of gestation. Idiopathic RPL, which has no known etiology, is increasingly linked to genetic factors, particularly genes involved in folate metabolism. The *SLC19A1* gene encodes the reduced folate carrier (RFC1), which is necessary for cellular folate transport. Polymorphisms in this gene may impair folate absorption and homocysteine regulation, potentially leading to poor pregnancy outcomes.

This study investigated the relationship between *SLC19A1* gene polymorphisms (rs1131596, rs1051266, rs12659) and haplotypes with idiopathic RPL in women from Duhok, Iraq. A case-control study included 70 women with idiopathic RPL and 70 age-matched healthy controls. Genotyping was performed using ARMS-PCR and RFLP-PCR, and statistical analysis was carried out using chi-square, t-tests, and logistic regression.

There was no significant association between RPL and the -43T>C or 80G>A polymorphisms. The 696C>T variant showed a marginal association ($p = 0.041$), with the TT genotype more frequent among RPL cases. Although this suggests a potential link, the result should be interpreted cautiously. Haplotype analysis revealed minimal linkage disequilibrium and no significant associations.

KEYWORDS: recurrent pregnancy loss, folate metabolism, *SLC19A1*, single nucleotide polymorphisms, gene variants

1. INTRODUCTION

Recurrent pregnancy loss (RPL) also termed recurrent miscarriage (RM) is defined as the loss of two or more pregnancies before 20 weeks of gestation. This condition affects about 0.5–2% of women of reproductive age and causes a significant clinical and psychological burden for affected couples (Ford & Schust, 2009; Mohammed *et al.*, 2018). RPL is a multifactorial condition involving genetic, anatomical, immunological, hormonal, infectious, and environmental contributors. However, in nearly half of the cases, no clear underlying cause can be identified, and such cases are termed idiopathic RPL. Understanding the molecular and genetic components contributing to these unexplained cases is important to improve prevention, diagnosis, and management strategies (Ford & Schust, 2009; Luo *et al.*, 2015; Turesheva *et al.*, 2023). In the pathophysiology of idiopathic RPL, genetic factors have become increasingly important in recent years. Among these, polymorphisms influencing genes related to metabolism of folate and homocysteine regulation have attracted considerable attention. One such gene is *SLC19A1*, also known as the reduced folate carrier 1 (*RFC1*) gene and encodes the RFC1 protein, which serves as the main transporter of folate into cells (Luo *et al.*, 2015). Folate is a B-vitamin necessary for DNA synthesis,

repair, methylation, and cell division. Neural tube abnormalities, intrauterine growth limitation, preeclampsia, and early pregnancy loss have all been linked to folate deficiency or impaired folate transport (Scholl & Johnson, 2000).

The RFC1 protein affects homocysteine metabolism because it is essential for preserving intracellular folate levels. Hyperhomocysteinemia or elevated homocysteine levels are toxic to the endothelium, encourage thrombosis, and have been linked to unfavorable pregnancy outcomes, including RPL (Afaq *et al.*, 2023; Eissa *et al.*, 2019; Govindaiah *et al.*, 2009). Polymorphisms in the *SLC19A1* gene, particularly the c.80G>A (rs1051266) variant, have been linked to altered RFC1 activity and reduced folate absorption. This single nucleotide polymorphism (SNP) leads to an amino acid substitution (Arg27His), which may affect the protein's shape and function (Chango & Emery-Fillon, 2000). In women with folate transport deficiencies, early placental development may be compromised due to increased oxidative stress and thrombosis at the maternal-fetal interface, potentially resulting in early pregnancy loss (Sato *et al.*, 2011).

Furthermore, folate deficiency can affect DNA methylation patterns, which are essential for embryogenesis and epigenetic regulation of gene expression. In this context, polymorphisms in the *SLC19A1* gene may not only alter folate uptake but also contribute to aberrant methylation and gene silencing, thereby

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influencing pregnancy outcomes (Saeed *et al.*, 2019; Souza *et al.*, 2025).

Despite growing international literature on the role of folate metabolism genes in RPL, there are limited genetic studies that are specific to a given region, especially in Kurdistan and broader Iraqi populations. The Duhok population is predominantly of Kurdish ethnic background, with additional minority groups, contributing to its genetic diversity. Given the ethnic and genetic diversity of the Duhok City population and potential nutritional or environmental factors that could modify gene expression, it is essential to investigate population-specific associations. Moreover, the incidence of consanguinity in the region may amplify the effects of certain recessive or homozygous polymorphisms. Therefore, understanding the frequency and significance of *SLC19A1* polymorphisms and haplotypes in this population could provide key insights into the genetic foundations of idiopathic RPL and guide the development of focused preventive strategies, such as folic acid supplementation or personalized genetic counseling.

2. METHODS

Study Design and Patient Selection

This case-control study was conducted between August, 2024 and April, 2025 in Duhok City, Kurdistan- Region of Iraq. The

study included a total of 70 women diagnosed with idiopathic RPL and 70 age-matched healthy women with no history of miscarriage and at least one successful full-term pregnancy. Patients with known causes of miscarriage such as uterine anomalies, hormonal disorders, antiphospholipid syndrome, chromosomal abnormalities, infectious diseases, or thrombophilias were excluded from the study. All participants were recruited from gynecology and obstetrics outpatient clinics at Duhok Maternity Hospital, and detailed medical and obstetric histories were obtained using a structured questionnaire.

Sample Collection and Molecular Analysis:

Five milliliters of peripheral venous blood were collected from each participant and placed in sterile EDTA-containing tubes. Samples were coded and stored at -20°C until DNA extraction. Genomic DNA was isolated from whole blood using a modified salting-out method (Iranpur-Mubarekeh & Esmailzadeh, 2010; Kashmoola *et al.*, 2015). The quality and quantity of DNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific) at 260/280 nm ratios. To amplify the regions containing the *SLC19A1* (*RFC1*) rs1131596 (-43T>C), rs1051266 (80G>A), rs12659 (696C>T) polymorphisms, specific primers were designed (Table 1).

Table 1: primers and possible products sizes following amplification of the studied polymorphisms.

SLC19A1 SNP (rsID)	Technique	Primer sequences	Product size
rs1131596 (-43T>C)	ARMS-PCR	F(A): CGGAGGGGACGAAGGTGGCA F(G): GGAGGGGACGAAGGTGGCG F(control): GACCCATCCTCACCCCGTA R: CTGACCGCCACAGGTACTGC	416 bp 416 bp 257 bp (Control band) (Mohtaram <i>et al.</i> , 2016)
rs1051266 (80G>A)	RFLP-PCR	F: 5-AGTGTCACCTTCGTCCCTC-3' R: 5-CTCCCGCGTGAAGTTCTT-3' Enzyme: <i>HaeII</i>	230 bp (Nomair <i>et al.</i> , 2024)
rs12659 (696C>T)	ARMS-PCR	F(A): CAGCTTCCCGCCTGGGTCA F(G): CAGCTTCCCGCCTGGGTCTG F(control): GTTGTAGACCCGCGCACTG R: CTCCACGCTCAACTACATCTCG	169 bp 169 bp 373 bp (Control band) (Mohtaram <i>et al.</i> , 2016)

A final volume of 20 μL was used for the PCR, with the following thermal cycling conditions: an initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 25 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds; and a final extension at 72°C for 5 minutes.

For both rs1131596 (-43T>C) and rs12659 (696C>T) polymorphisms, the ARMS-PCR technique was used. In addition to amplification of the target allele, an internal control band was also amplified to confirm reaction validity as shown in Figure (1) and Figure (2).

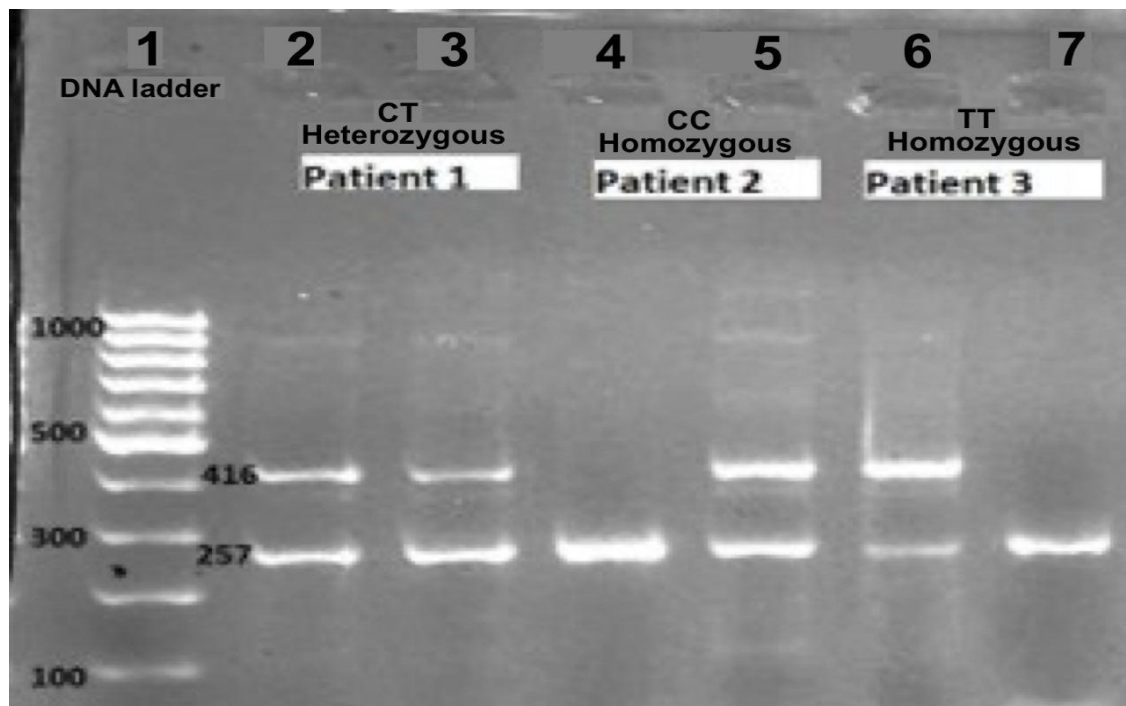


Figure 1: ARMS-PCR genotyping of rs1131596 (-43T>C). Lane 1: 100 bp ladder; Lanes 2–3: heterozygous (CT); Lanes 4–5: homozygous mutant (CC); Lanes 6–7: homozygous wild-type (TT). A 416 bp allele-specific band and a 257 bp internal control band were detected.

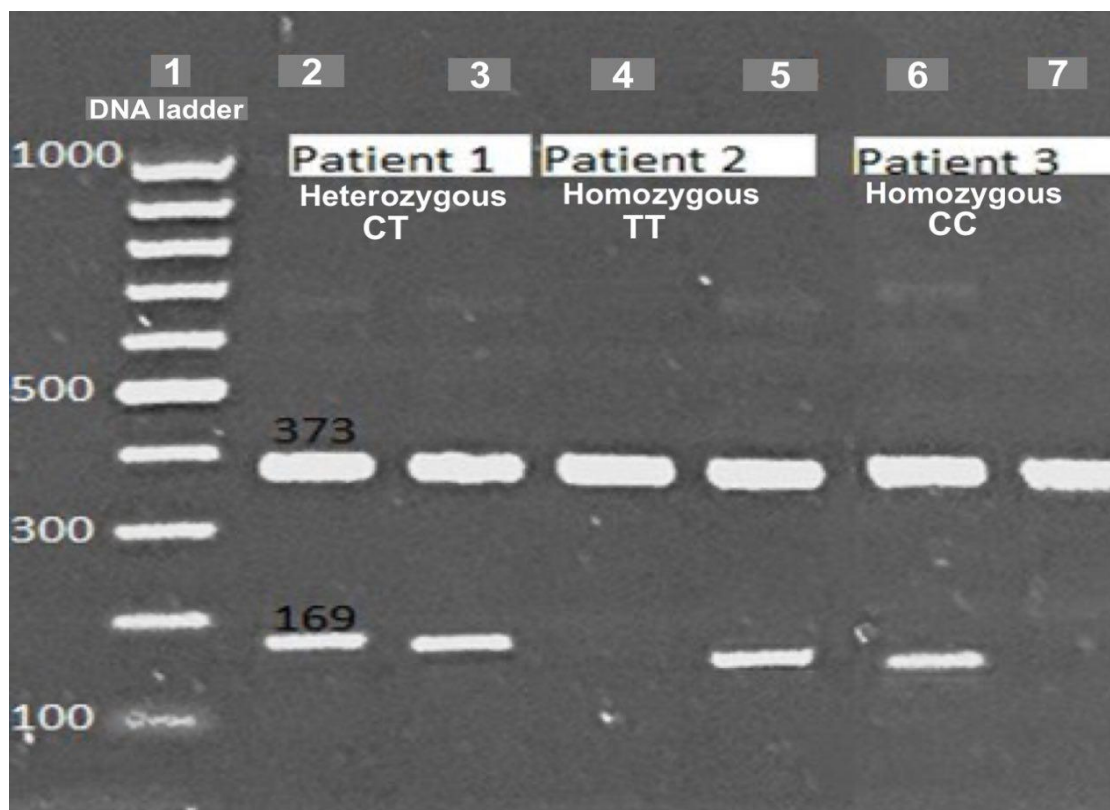


Figure 2: ARMS-PCR genotyping of rs12659 (696C>T). Lane 1: 100 bp ladder; Lanes 2–3 heterozygous (CT); Lanes 4–5 homozygous mutant (TT) and Lanes 6–7 homozygous wild-type (CC). A 169 bp allele-specific band and a 373 bp internal control band were detected.

For rs1051266 (SLC19A1 80G>A), a RFLP-PCR technique was used, the amplified products (230 bp) were subjected to

enzyme digestion with *HaeII* enzyme, the 80A allele remained uncut (230 bp), whereas the 80G allele was cut into 2 fragments

of 126 and 104 bp, shown in Figure (3). Finally, the PCR products for all 3 polymorphisms were visualized on 2% agarose

gel with safe dye (maestroSafe, Maestrogen, Taiwan) under UV transillumination.

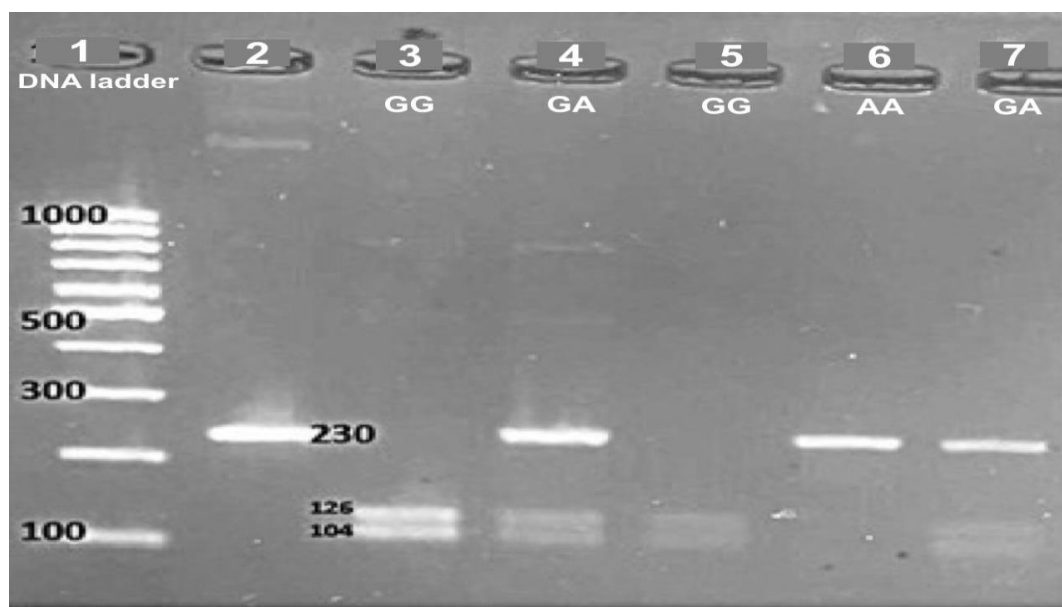


Figure 3: RFLP-PCR genotyping of rs1051266 (80G>A) using specific primers and enzyme digestion. Lane 1: ladder 100 bp; Lane 2 amplified sample without digestion used as a control (230bp); Lane 3 and 5 homozygous (GG) variant that shows complete digestion of 230bp with production of 2 bands of 126 and 104 bp. Lane 4 and 7 heterozygous (GA) variant that show persistence of 230 bp band in addition to the formation of 126 and 104 bp. Lane 6 homozygous (AA) variant that shows persistence of 230bp band indicating no enzyme digestion.

Statistical Analysis:

All data were entered and analyzed using SPSS software (version 26). Allele and genotype frequencies between the case and control groups were compared using the Chi-square (χ^2) test and Student's t-test, where appropriate. A p-value of < 0.05 was considered statistically significant. Allele and genotype distributions were calculated for both study groups. If multiple SNPs within the *SLC19A1* gene were genotyped, haplotype analysis was performed using software such as SHEsis or Haploview. The Hardy-Weinberg equilibrium (HWE) was assessed in the control group to ensure the validity of genotype distribution. Logistic regression analysis was employed to adjust

for potential confounding variables, including age, body mass index (BMI), and folic acid supplementation.

3. RESULTS

A total of 140 women were enrolled in this study, comprising 70 patients diagnosed with idiopathic recurrent pregnancy loss (RPL) and 70 age-matched healthy controls who had never experienced a loss and had at least one full-term successful pregnancy. The demographic and clinical characteristics of the two groups were compared to ensure baseline comparability (Table 2).

Table 2: Demographic characteristics of all enrolled patients and controls.

Variables	Patients (70)	Controls (70)	t-test (p value)
Age	18-44 (33.34 $\pm$ 0.75)	19-45 (32.33 $\pm$ 6.73)	0.922 (0.358)
BMI	20.2-38.06 (26.98 $\pm$ 0.46)	19.38-37.66 (27.45 $\pm$ 0.45)	0.726 (0.468)
Consanguinity	20/70	19/70	0.59 (0.43)
Parity	0-6 (2.18 $\pm$ 0.19)	1-10 (2.84 $\pm$ 0.21)	2.25(0.026)
Gravida	2-10 (5.58 $\pm$ 0.24)	1-10 (2.88 $\pm$ 0.21)	8.804(0.000)
No. of abortion	3-10 (3.4 $\pm$ 0.19)	0	19.7 (0.000)
Comorbidities	21	20	
Smoking	1/70	7/70	

There were no statistically significant differences between RPL cases and controls in terms of age (mean age: 33.34 ± 0.75 years in RPL vs. 32.33 ± 6.73 years in controls; $p = 0.358$), body mass index (BMI; 26.98 ± 0.46 vs. 27.45 ± 0.45 ; $p = 0.468$), or rates of consanguinity (20/70 vs. 19/70; $p = 0.43$). These findings confirm that the groups were matched in key demographic variables, minimizing potential confounding influences.

However, reproductive history parameters showed statistically significant differences. The mean parity was significantly lower in the RPL group (2.18 ± 0.19) compared to the control group (2.84 ± 0.21 ; $p = 0.026$). In contrast, the mean number of pregnancies (gravida) was significantly higher in RPL cases (5.58 ± 0.24) compared to controls (2.88 ± 0.21 ; $p < 0.001$). As expected, the mean number of abortions was also significantly higher in the RPL group (3.4 ± 0.19), whereas no abortions were reported among the control group ($p < 0.001$). Comorbidities refers to one or more additional medical condition that is not the main reason for the participant's reproductive disorder. In this study, the list of comorbidities was: diabetes mellitus, hypertension, liver disease (including alcoholic), any other known chronic illness, any medication or supplement taken to treat or relieve the above, which was asked under "Previous medical history" in the questionnaire. These were coded together under the name "comorbidities" for the purpose of analysis. There were no significant differences of comorbidities between RPL cases and controls. Smoking prevalence was low in both groups but was higher among controls (7/70) compared to RPL patients (1/70), though statistical testing was not feasible due to low event counts.

Genotypic and Allelic Distribution:

Three single nucleotide polymorphisms (SNPs) within the SLC19A1 gene were investigated (Table 3): rs1131596 (43T>C), rs1051266 (80G>A), and rs12659 (696C>T). Genotype and allele frequencies were compared between RPL patients and controls using chi-square tests.

Table 3: Genotype and Allele frequencies of SLC19A1 SNP among patients and controls.

SNP (rsID)	Group	Genotype (n)	t-test (p value)	Allele	p-value (Allele)
rs1131596 (43T>C)	RPL	TT:18, CT:32, CC:20	0.78 (0.431)	T:18, C:20	(0.667)
	Control	TT:23, CT:33, CC:16		T:23, C:16	
rs1051266 (80G>A)	RPL	GG:15, GA:31, AA:24	0.992 (0.323)	G:15, A:24	(0.334)
	Control	GG:21, GA:30, AA:19		G:21, A:19	
rs12659 (696C>T)	RPL	CC:18, CT:32, TT:20	1.40 (0.164)	C:18, T:20	2.01 (0.041)
	Control	CC:28, CT:28, TT:14		C:28, T:14	

- **rs1131596 (43T>C):** The distribution of genotypes (TT, CT, CC) did not differ significantly between RPL cases and controls ($p = 0.431$). The allele frequencies were also similar (T allele: 48.6% in cases vs. 54.9% in controls; C allele: 51.4% in cases vs. 45.1% in controls).

- **rs1051266 (80G>A):** Similarly, genotype distribution (GG, GA, AA) showed no significant difference between the two groups ($p = 0.323$). Allelic analysis revealed the G allele occurred in 43.6% of cases and 51.4% of controls, while the A allele was found in 56.4% of cases and 48.6% of controls.

- **rs12659 (696C>T):** Notably, this SNP showed a borderline significant difference in genotype distribution between cases and controls ($p = 0.041$). The CC genotype was more frequent in controls (28/70, 40%) than in RPL patients (18/70, 25.7%), while

the TT genotype was more frequent in RPL patients (20/70, 28.6%) than controls (14/70, 20%). Allelic frequencies indicated that the T allele was more frequent in RPL patients (51.4%) than in controls (40.0%), which could be an indication of a potential tendency to being associated with greater RPL risk. This was however a marginal level of significance. Hence, this result is considered to be restricted and needs to be confirmed in large-scale researches.

Hardy-Weinberg Equilibrium and Haplotype Analysis:

All three SNPs were found to be in Hardy-Weinberg equilibrium in both the control and patient groups, indicating a stable genetic distribution in the studied population and supporting the reliability of the genotyping data (Table 4).

Table 4: Hardy-Weinberg Equilibrium (HWE) Analysis of SLC19A1 SNPs in Patients and Controls.

SNP (rs ID)	Group	Observed Genotypes (n)	Allele Frequencies (p/q)	Expected Genotypes (n)	χ^2 (HWE)	HWE Status
rs1131596	Controls	TT: 23,	T = 0.549, C = 0.451	TT:21.07,	0.25	In equilibrium
		CT: 33,		CT:34.65,		
		CC: 16		CC:14.28		
	Patients	TT: 18, CT: 32, CC: 20	T = 0.486, C = 0.514	TT:16.52, CT:34.93, CC:18.48	0.51	In equilibrium
rs1051266	Controls	GG: 21, GA: 30, AA: 19	G = 0.514, A = 0.486	GG:18.51, GA:34.94, AA:16.47	1.44	In equilibrium

rs12659	Patients	GG: 15,	G = 0.436,	GG:13.3,	0.69	In equilibrium
		GA: 31,		GA:34.4,		
		AA: 24	A = 0.564	AA: 22.3		
	Controls	CC: 28,	C = 0.600,	CC: 25.2,	1.52	In equilibrium
		CT: 28,		CT: 33.6,		
		TT: 14	T = 0.400	TT: 11.2		
	Patients	CC: 18,	C = 0.486,	CC:16.52,	0.51	In equilibrium
		CT: 32,		CT:34.93,		
		TT: 20	T = 0.514	TT:18.48		

- χ^2 values were calculated using the formula: $\Sigma ((\text{Observed} - \text{Expected})^2 / \text{Expected})$.

- Degrees of freedom = 1; HWE is considered valid if $\chi^2 < 3.84$ ($p > 0.05$).

- All SNPs are in Hardy-Weinberg equilibrium in both study groups, supporting genotype data quality.

Haplotype analysis, which combined the three SNPs to identify possible gene-gene interactions or cumulative effects, did not yield any statistically significant associations. This may be attributed to the low linkage disequilibrium observed between the SNPs and the relatively small sample size, which might have inhibited the statistical power to identify actual associations. Otherwise, in an event of insufficient power, false-negative results can be obtained. Though the formal power analysis was not carried out, this is a limitation that should be taken into account. Nonetheless, haplotype construction remains a useful exploratory tool in genetic epidemiology.

In summary, while rs1131596 and rs1051266 did not show significant differences between groups, the rs12659 (696C>T) polymorphism showed a weak but statistically notable association with RPL. This finding warrants further validation in larger studies and functional analyses to determine its biological relevance in folate transport and early pregnancy outcomes.

4. DISCUSSION

This study investigated three polymorphisms in the SLC19A1 gene (rs1131596, rs1051266, rs12659) and their relationship with idiopathic recurrent pregnancy loss in women from Duhok, Iraq. While two SNPs (rs1131596 and rs1051266) did not show any significant association, the 696C>T variant (rs12659) was marginally associated with increased RPL risk. Although rs12659 is a synonymous variant, it is located in a non-coding or regulatory area of the SLC19A1 gene, suggesting that it still has some effect on the expression of the gene through mechanisms such as regulation of mRNA destruction, secondary structure, or pre-mRNA splicing. The non-coding SNPs have also been known to regulate as synonymous variants are increasingly identified, and these SNPs tend to interfere with RNA splicing process or transcript stability (Baralle & Giudice, 2017; Sauna & Kimchi-Sarfaty, 2011). More functional analyses are required to determine the possibility of alternative effects of rs12659 that influences the expression or activity of SLC19A1 at the transcriptional level.

Our findings on rs1051266 (80G>A) align with several studies conducted in Iranian, Turkish, and Korean populations, which similarly reported no significant association between this SNP and RPL or recurrent miscarriage (Chango & Emery-Fillon, 2000; Rah *et al.*, 2012; Nomair *et al.*, 2024). In a study from China, polymorphisms in genes related to folate metabolism, including SLC19A1, significantly impact the risk of unexplained

RPL in Chinese women. In their study, the 80G>A (rs1051266) variant showed a borderline association with RPL, particularly when combined with MTHFR polymorphisms, suggesting a possible synergistic effect between multiple folate pathway genes (Luo *et al.*, 2015). Being unable to find such significance in the current study may be due to compensatory mechanisms in folate metabolism or gene-environment interactions that modulate its effect.

Similarly, a study from Iran assessed the SLC19A1 80G>A polymorphism among women with RPL and reported a non-significant difference in genotype distribution between cases and controls, in agreement with the findings arrived at in this study. However, when stratified by folate intake, women with the AA genotype and low folate levels had a significantly higher RPL risk, highlighting the importance of gene-nutrient interactions (Mohtaram *et al.*, 2016).

However, the association of rs12659 (696C>T) with RPL is noteworthy and consistent with limited evidence in the literature. While the functional consequences of this SNP remain unclear, it may reside in a regulatory region influencing SLC19A1 mRNA stability or splicing efficiency. Rah *et al.* (2012) found this SNP to be potentially associated with idiopathic recurrent spontaneous abortion in Korean women, particularly in TT homozygotes, suggesting possible functional effects such as altered mRNA stability or splicing (Rah *et al.*, 2012). Moreover, studies have indicated that RFC1 gene variants may influence folate uptake and hence methylation status critical for placental function (Luo *et al.*, 2015; Souza *et al.*, 2025). Variants in non-coding regions of folate transport genes can modulate homocysteine levels and folate uptake efficiency, which are critical during placental development (Chango & Emery-Fillon, 2000).

Furthermore, the interplay between folate transport and DNA methylation pathways is well-documented. Aberrant methylation due to inadequate intracellular folate levels can affect the expression of key developmental genes and lead to embryonic loss. As demonstrated by (Stanisławska-Sachadyn *et al.*, 2009), parental hyperhomocysteinemia and folate-pathway polymorphisms, including SLC19A1, significantly contributed to RPL through impaired methylation and vascular dysfunction.

Interestingly, no significant haplotypes emerged in the present study, which may be attributed to the low linkage disequilibrium observed and the relatively modest sample size. This aligns with findings that emphasized how single polymorphisms may not fully explain genetic susceptibility and that multi-locus and gene-environment interaction models are more informative. Since the sample included 70 cases and 70 controls, the study was potentially underpowered to identify haplotype-level associations. A formal power calculation was not performed; therefore, future studies should adopt a multi-gene haplotype approach, along with quantification of serum folate

and homocysteine levels, to provide a clearer mechanistic link (Luo *et al.*, 2015).

The lack of strong associations for some SNPs could reflect environmental modifiers such as folate supplementation, which is widespread in prenatal care in Iraq (Lateef & Al-Hashimi, 2016). Moreover, the high prevalence of consanguineous marriages in the region may lead to genomic homozygosity, which can obscure the effect of dominant alleles and highlight recessive genetic traits (Hawi *et al.*, 2024). Taken together, the data support a modest but likely contribution of the 696C>T variation to RPL risk, particularly in genetically different populations.

To the best of the researchers' knowledge, this is the first study in the Duhok Governorate—and among the first in Iraq—to investigate the relationship between SLC19A1 gene polymorphisms and idiopathic recurrent pregnancy loss (RPL). By focusing on three specific single nucleotide polymorphisms (rs1131596, rs1051266, and rs12659) and conducting haplotype analysis in a genetically and environmentally unique population. This study has provided novel perspectives into the potential role of folate transporter gene variants in unexplained pregnancy loss. Importantly, the majority of research sample was composed of Kurdish women, a relatively understudied ethnic group that may have different genetic patterns. The identification of a marginally significant correlation between the 696C>T polymorphism and RPL risk provides a valuable genetic clue that warrants further research and could support population-specific diagnostic or preventive strategies.

CONCLUSION

This study indicated that the SLC19A1 696C>T (rs12659) polymorphism may be linked to a higher risk of idiopathic recurrent pregnancy loss in Duhok women, while rs1131596 and rs1051266 showed no significant associations. Considering the Kurdish majority of the cohort studied, possible results can be discussed as population-specific genetic trends, and it is important to pay attention to them with the planned continuation of the research in other ethnicities. These findings highlight the importance of evaluating folate transporter gene variants in the context of population-specific genetic backgrounds. Future studies with larger sample sizes, functional assays, and evaluation of environmental modifiers such as folate intake and consanguinity are required to validate these preliminary results and explore potential preventative strategies.

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Author Contributions:

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Adil Abozaid Eissa

Data collection and practical work: Hozheen Abdulrahman Darweesh

Data analysis and interpretation: Adil Abozaid Eissa, Hozheen Abdulrahman Darweesh

Drafting of the manuscript: Hozheen Abdulrahman Darweesh, with critical revision by Adil Abozaid Eissa.

Ethical Considerations and Consent:

Ethical approval was obtained for the study from the Scientific and Ethics Committee of the Duhok Directorate of health (Approval No: 31072024-6-30, dated July 31, 2024) in

conformity with the Declaration of Helsinki which guarantees compliance with medical research standards, including patient privacy and moral management of clinical data. Written informed consent was obtained from all participants prior to sample collection, after explaining the purpose, procedures, benefits, and risks of the study in their native language.

Declaration:

Ethical approval was obtained from the Scientific and Ethics Committee of the Duhok Directorate of Health (Approval No: 31072024-6-30).

Written informed consent was obtained from all participants prior to data and sample collection.

Conflict of interest: The authors declare no conflict of interest.

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