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Factor V Leiden G1691A, *Prothrombin* G20210A, and *MTHFR* C677T Mutations among Sudanese Women with Recurrent Pregnancy Loss

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Abstract

Background: Various factors, such as genetic causes, anatomic abnormalities of the uterus, infectious diseases, coagulative disorders, and endocrinological and immunological diseases, might influence recurrent pregnancy loss (RLP). This study aimed to evaluate the prevalence and frequency of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms in Sudanese women with RPL.

Methods and Results: This descriptive cross-sectional study involved 100 women with a history of 3 or more RPLs (the case group) and 94 healthy multiparous women without pregnancy complications (the control group). DNA was extracted from peripheral blood samples. The study of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms was performed by PCR and RFLP analysis. For the *FII* G20210A, the genotype distribution in the case group and control group was as follows: GG=97.0%, GA=3.0%, AA=0% and GG=94.0%, GA=0%, AA=0%, respectively. In the case group, the allelic distribution was as follows: G=98.5%, A=1.5%. In the control group, the A allele was absent, and the frequency of the G allele was 100%. For the *MTHFR* C677T, the genotypic and allelic frequencies in the case group were 97%, 3%, and 0%, respectively, for the CC, CT, and TT genotypes, and 98.5% and 1.5%, respectively, for the C and T alleles. In the control group, the genotype distribution was as follows: CC-100% CT-0%, TT-0%; the T allele was absent, and the frequency of the C allele was 100%. For the *FVL* G1691A, the genotype distribution in the case group and control group was as follows: GG=92.0%, GA=8.0%, AA=0% and GG=93.6%, GA=6.4%, AA=0%, respectively. For G and A alleles, the frequencies were 96.0% and 4.0%, respectively, for the case group, and 96.8% and 3.2%, respectively, for the control group. Our analysis did not reveal a significant positive association between the *MTHFR* C677T, *FII* G20210A, and *FVL* G1691A polymorphisms and the risk of RPL across the dominant model, multiplicative model, and a comparison of the frequencies of the heterozygous and homozygous dominant genotypes.

Conclusion: The research findings suggest that the *MTHFR* C677T, *FVL* G1691A, and *FII* G20210A variants do not significantly contribute to the increased susceptibility to RPL in this specific population of Sudanese women. **(International Journal of Biomedicine. 2024;14(1):59-65.)**

Keywords: recurrent pregnancy loss • Factor V Leiden • methylenetetrahydrofolate reductase • prothrombin • Sudanese women

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Abbreviations

APC, activated protein C; **FVL**, Factor V Leiden; **MTHFR**, methylenetetrahydrofolate reductase; **RPL**, recurrent pregnancy loss; **RFLP**, restriction fragment length polymorphism.

Introduction

Recurrent pregnancy loss (RPL), also referred to as recurrent miscarriage or habitual abortion, is historically defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period.[1] The Practice Committee of the American Society for Reproductive Medicine has defined RPL as 2 or more failed pregnancies before the 20th week of pregnancy.[2,3] Various factors, such as genetic causes, anatomic abnormalities of the uterus, infectious diseases, coagulative disorders, and endocrinological and immunological diseases, might influence RLP.[4-9] The association between thrombophilia and RPL has become an undisputed fact. The *FVL* G1619A mutation, prothrombin or factor II (*FII*) G20210A, and *MTHFR* gene polymorphisms are believed to play a key role in the pathogenesis of RPL. These genetic conditions have been linked to a range of obstetric complexities, including venous thromboembolism, recurrent miscarriage, abruption of placentae, preeclampsia, and the delivery of a fetus that is small for its gestational age.[10]

The *FVL* G1619A mutation occurs by substituting guanine with adenine at the nucleotide 1691 in exon 10. As a result of this missense mutation, arginine (Arg) at amino acid 506 is substituted with glutamine (Gln), leading to the generation of FVL resistant to the APC. APC is a natural anticoagulant that, in normal situations, cleaves activated factor V at amino acid 506 and makes it inactive.[11-17] This results in a hypercoagulable state with a 5- to 10-fold risk of thrombosis in heterozygotes and an 80-fold risk in homozygotes.[18] Studies investigating the relationship between *FVL* mutation and RPL found an association, with odds ratios ranging from 0.5 to 18.[19-22]

A single missense mutation on the *FII* gene, leading to the substitution of guanine by adenine at nucleotide position 20210, was recently identified as a genetic risk factor for thrombosis. The *FII* G20210A polymorphism is associated with increased plasma prothrombin levels, and its carriers present a 2 to 3-fold increased risk for developing venous thromboembolism.[23] The *FII* gene mutation was found in 4%–9% of women with RPL, compared with 1%–2% of those with uncomplicated pregnancies, with odds ratios ranging from 2 to 9.[24,25]

Mutations in the *MTHFR* gene lead to decreased enzyme activity and hyperhomocysteinemia, which induces platelet aggregation.[26] The *MTHFR* C677T is a missense mutation in exon 4 of this gene, which converts an alanine to a valine residue in the N-terminal catalytic domain of the protein, resulting in decreased enzymatic activity and hyperhomocysteinemia, which induces platelet aggregation.[27,28] Homozygous C677T mutations and the *MTHFR* 677T allele have been associated with elevated levels of homocysteine and are identified as risk factors for thrombosis.[29,30]

Earlier research has demonstrated variations in the presence of the *FVL*, *FII*, and *MTHFR* mutations across different geographical regions and racial and ethnic backgrounds.[31,32]

This study aimed to evaluate the prevalence and frequency of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms in Sudanese women with RPL.

Material and Methods

This descriptive cross-sectional study involved 100 women (mean age of 25±4.0 years) with a history of 3 or more RPLs (the case group) and 94 healthy multiparous women (mean age of 30±4.0 years) without pregnancy complications (the control group).

DNA was extracted from peripheral blood samples using a Master Pure DNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) according to the manufacturer's standard protocol. The study of the *FII* G20210A, *FVL* G1691A, and *MTHFR* C677T polymorphisms was performed by PCR and RFLP analysis. For *FII* G20210A polymorphism, a 345-bp genomic DNA segment, including the mutation site, was amplified using forward and reverse primers.[33] Digestion of the PCR products containing the wild-type heterozygous and homozygous allele with the restriction enzyme Hind III results in 345 bp, 322 bp, 23 bp, and 322 bp, 23 bp fragments, respectively. For *FVL* G1691A polymorphism, a 267-bp genomic DNA segment was amplified as described by Bertina et al.[34] The 267 bp amplification product was digested with Mnl I for 60 minutes at 37°C, and the resulting fragments were separated by electrophoresis in a 3% agarose gel. The presence of the mutant allele was indicated by a 200-bp product and the normal allele by a 163-bp product, the heterozygotes having both. The *MTHFR* C677T polymorphism was detected according to the method described by Frosst et al.[35] A length of 198bp in exon 4 of the *MTHFR* gene was amplified using the special primers 5′ TGAAGGAGAAGGTGTCTGCGGGA3′ and 5′AGGACGGTGCGGTGAGAGTG3′, followed by restriction digestion using the HinfI enzyme. A single band of 198bp characterized the wild-type C allele for codon 677, while the presence of 3 bands at 198 bp, 175 bp, and 23 bp or 175 bp and 23 bp characterized the heterozygous (CT) and homozygous (TT) variant status, respectively.

Statistical analysis was performed using the statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). Genetic markers for HWE were tested (Table 1). Differences in the allele and genotype distribution between the groups were assessed by χ2-test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. Two inheritance models were analyzed (the dominant model and the multiplicative model), and a comparison of the frequencies of the heterozygous and homozygous dominant genotypes of the studied gene polymorphisms. The recessive and additive models were not calculable due to the homozygous recessive genotype frequency of zero in both cases and controls. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Ethics Committee at the Omdurman Maternity Hospital.

Table 1.

The distribution of polymorphic markers of the MTHFR C677T, FII G20210A and FVL G1691A polymorphisms in RPL women (cases) and non-RPL women (control).

Gene	SNP/ mutations	Genotype	Cases	HWE	χ^2	*P*	Control	HWE	χ^2	*P*	Allele	Frequency of alleles	
												Cases	Control
FII	rs1799963 G20210A	GG	0.970	0.970	0.00	1	1.000	1.000	0.00	1	G	0.985	1.000
		GA	0.030	0.030			0.000	0.000			A	0.015	0.000
		AA	0.000	0.000			0.000	0.000					
FVL	G1619A	GG	0.920	0.922	0.00	1	0.936	0.937	0.00	1	G	0.960	0.968
		GA	0.080	0.077			0.064	0.062			A	0.040	0.032
		AA	0.000	0.002			0.000	0.001					
MTHFR	rs1801133 C677T	CC	0.970	0.970	0.00	1	1.000	1.000	0.00	1	C	0.985	1.000
		CT	0.030	0.030			0.000	0.000			T	0.015	0.000
		TT	0.000	0.000			0.000	0.000					

Results

The distribution of polymorphic markers of the *MTHFR* C677T, *FII* G20210A and the *FVL* G1691A polymorphisms in the case group and control group was in HWE (Table 1).

For the *FII* G20210A, the genotype distribution in the case group and control group was as follows: GG=97.0%, GA=3.0%, AA=0% and GG=94.0%, GA=0%, AA=0%, respectively. In the case group, the allelic distribution was as follows: G=98.5%, A=1.5%. In the control group, the A allele was absent, and the frequency of the G allele was 100%.

For the *MTHFR* C677T, the genotypic and allelic frequencies in the case group were 97%, 3%, and 0%, respectively, for the CC, CT, and TT genotypes, and 98.5% and 1.5%, respectively, for the C and T alleles. In the control group, the genotype distribution was as follows: CC-100% CT-0%, TT-0%; the T allele was absent, and the frequency of the C allele was 100%.

For the *FVL* G1691A, the genotype distribution in the case group and control group was as follows: GG=92.0%, GA=8.0%, AA=0% and GG=93.6%, GA=6.4%, AA=0%, respectively. For G and A alleles, the frequencies were 96.0% and 4.0%, respectively, for the case group, and 96.8% and 3.2%, respectively, for the control group.

Our analysis did not reveal a significant positive association between the *MTHFR* C677T, *FII* G20210A, and *FVL* G1691A polymorphisms and the risk of RPL across the dominant model (C677T: OR=6.78, 95% CI = 0.35 – 133.14, *P*=0.09; G20210A: OR=6.78, 95% CI = 0.35 – 133.14, *P*=0.09; G1619A: OR=1.28, 95% CI = 0.43 – 3.82, *P*=0.66), multiplicative model (C677T: OR=6.68, 95% CI = 0.34 – 130.22, *P*=0.09; G20210A: OR =6.68, 95% CI = 0.34 – 130.22, *P*=0.09; G1619A: OR =1.26, 95% CI = 0.43 – 3.71, *P*=0.67), and a comparison of the frequencies of the heterozygous and homozygous dominant genotypes (C677T: OR =6.78, 95% CI = 0.34 – 133.14, *P*=0.207; G20210A: OR=6.78, 95% CI = 0.34 – 133.14, *P*=0.207; G1619A: OR=1.28, 95% CI = 0.42 – 3.82, *P*=0.664) (Tables 2 and 3).

Discussion

The present study continued previously published research on the prevalence of genetic polymorphisms among Sudanese women with RPL.[5] The findings align with prior research outcomes, indicating no association between the *MTHFR* C677T, *FVL* G1619A and *FII* G20210A, and RPL. In a study by Serrano et al.,[36] which involved 100 participants with RPL, it was concluded that neither *FII* G20210A nor *FVL* G1619A is linked to recurrent miscarriage before the 10th week of pregnancy. However, in a study by Ahmed et al.,[37] in Sudanese women with preeclampsia, the *FVL* G1619A mutation was found in 9.6% of the cases, compared with 0.6% of the controls (*P*<0.001; OR=18.60, 95% CI = 2.38-136.1), and homozygous AA genotype was found in 2.2% of patients with severe preeclampsia and was not detected in the controls.

Contradictory results were reported by other extensive prospective studies, reporting that thrombophilia-associated mutations associated with hypercoagulability are not elevated in women experiencing RPL.[38,39]

Cardona et al.[40] evaluated whether inherited thrombophilia is associated with RPL in a Colombian subpopulation. The frequency of thrombophilia-associated SNPs (*FII* G20210A and *FVL* G1691A), APC resistance, and anticoagulant protein deficiencies were low overall, except for the *MTHFR* C677T. The differences between patients with RPL and healthy multiparous women (controls) had no statistical significance. This study also confirmed the low prevalence of inherited thrombophilia in non-Caucasian populations. These findings concurred with other research by Abu-Asab et al.[41] The authors failed to find a significant

Table 2.

Genetic predisposition to RPL (the genetic models)

Inheritance model	Allele, Genotype	Cases	Control	χ^2	*P*	OR (95%CI)	
		n=100	n=94			OR	95%CI
MTHFR C677T							
Multiplicative model (χ^2 test, df=1)	C	0.985	1.000	2.84	0.09	0.15	0.01 – 2.92
	T	0.015	0.000			6.68	0.34 – 130.22
Dominant model (χ^2 test, df=1)	CC	0.970	1.000	2.86	0.09	0.15	0.01 – 2.89
	CT + TT	0.030	0.000			6.78	0.35 – 133.14
FVL G1619A							
Inheritance model	Allele, Genotype	Cases	Control	χ^2	*P*	OR (95%CI)	
		n=100	n=94			OR	95%CI
Multiplicative model (χ^2 test, df=1)	G	0.960	0.968	0.18	0.67	0.79	0.27 – 2.32
	A	0.040	0.032			1.26	0.43 – 3.71
Dominant model (χ^2 test, df=1)	GG	0.920	0.936	0.19	0.66	0.78	0.26 – 2.35
	GA + AA	0.080	0.064			1.28	0.43 – 3.82
FII G20210A							
Inheritance model	Allele, Genotype	Cases	Control	χ^2	*P*	OR (95%CI)	
		n=100	n=94			OR	95%CI
Multiplicative model (χ^2 test, df=1)	G	0.985	1.000	2.84	0.09	0.15	0.01 – 2.92
	A	0.015	0.000			6.68	0.34 – 130.22
Dominant model (χ^2 test, df=1)	GG	0.970	1.000	2.86	0.09	0.15	0.01 – 2.89
	GA + AA	0.030	0.000			6.78	0.35 – 133.14

Table 3.

Comparison of the frequencies of the heterozygous and homozygous dominant genotypes of the studied gene polymorphisms.

Gene	Heterozygous genotype	Homozygous dominant genotype	*P*-value	OR (95%CI)	
				OR	95%CI
FII G20210A	GA	GG	0.207	6.78	0.34 to 133.14
Case	3	97			
Control	0	94			
FVL G1619A	GA	GG	0.664	1.28	0.42 to 3.82
Case	8	92			
Control	6	88			
MTHFR C677T	CT	CC	0.207	6.78	0.34 to 133.14
Case	3	97			
Control	0	94			

association between the *FVL* G1691A, *FII* G20210 and *MTHFR* C677T polymorphisms, and RPL in either the first or second trimester in 329 Palestinian women with RPL. In contrast, Abdelsalam et al.,[42] reported a significant increase in the prevalence of *FVL* G1691A and *MTHFR* C677T mutations in the RPL patients, compared to controls without

involvement of the *FII* gene. A study by Al-Achkar et al.[43] involving Syrian women showed that RPL women with homozygous TT genotype of *MTHFR* C677T had a high risk of RPL.

Eldeen et al.[44] investigated the distribution of the analyzed polymorphic markers in Saudi women in the Northern area of Saudi Arabia. They found a significantly higher frequency of the *FVL* G1691A AA genotype and the *FII* G20210A GA genotype in RPL women than in healthy controls. For the *MTHFR* C677T, there was no significant difference in the distribution of genotypes and alleles among the RPL patients and controls.

In general, the complex interaction of genetic factors in the context of RPL requires continued research into the genetic predisposition of individual populations to reproductive problems. Variations observed across populations and studies highlight the multifaceted nature of genetic influence on pregnancy outcomes.[45,46]

Conclusion

The research findings suggest that the *MTHFR* C677T, *FVL* G1691A, and *FII* G20210A variants do not significantly contribute to the increased susceptibility to RPL in this specific population of Sudanese women. Continued scientific inquiry is crucial for developing more nuanced and personalized strategies for the diagnosis and prevention of RPL, ultimately improving women's reproductive health.

Competing Interests

The authors declare that they have no competing interests.

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