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Hereditary thrombophilia and recurrent pregnancy loss: is it time to reassess routine genetic testing?

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ABSTRACT

Aims: Hereditary thrombophilias are genetic conditions that increase thromboembolic risk, with heightened thrombotic potential during pregnancy due to the associated hypercoagulable state. These conditions are recognized as significant risk factors for recurrent pregnancy loss (RPL), defined as the loss of two or more consecutive pregnancies before the 20th gestational week, affecting approximately 1-2% of women of reproductive age. RPL is multifactorial, influenced by genetic, environmental, and hormonal factors, with advanced maternal age and previous pregnancy loss being prominent risk factors. While the role of hereditary thrombophilias in adverse pregnancy outcomes remains controversial, studies have reported inconsistent findings regarding their association with RPL.

Methods: This retrospective cohort study included 736 women with a history of RPL and aimed to investigate the relationship between genetic thrombophilia variants and RPL, focusing on their impact on miscarriage risk. Genetic testing using PCR panels identified common mutations such as factor V Leiden, MTHFR, PAI-1, and prothrombin G20210A.

Results: Statistical analyses revealed no significant associations between these variants and RPL.

Conclusion: The findings suggest that, despite the prevalence of certain mutations, their contribution to miscarriage risk may be limited, underscoring the importance of selective genetic testing. This study highlights the need to optimize genetic screening practices to avoid unnecessary testing and reduce healthcare costs, supporting a more targeted approach in managing patients with RPL.

Keywords: Thrombophilia, miscarriage, FV Leiden, prothrombin G20210a

INTRODUCTION

Hereditary thrombophilias are genetic conditions that increase the risk of thromboembolic diseases, and their thrombotic potential further increases during pregnancy due to the hypercoagulable state associated with normal gestation.[1] Hereditary thrombophilia has been recognized as a significant risk factor for recurrent pregnancy loss (RPL), defined as two or more consecutive pregnancy losses before the 20th week of gestation.[2] RPL affects approximately 1-2% of women of reproductive age.[3] Early pregnancy loss occurs before the 12th week of gestation, while late pregnancy loss refers to losses occurring after this period.[4]

RPL is a multifactorial condition influenced by environmental, hormonal, and genetic factors. Common risk factors include advanced maternal age, a history of previous miscarriages, endocrine disorders, structural uterine abnormalities, chromosomal abnormalities in the parents, antiphospholipid syndrome, and hereditary thrombophilia. Among these, advanced maternal age and previous pregnancy losses are the most prevalent.[5]

The role of hereditary thrombophilias in adverse pregnancy outcomes is controversial. Studies investigating the association between hereditary thrombophilia and RPL have produced inconsistent findings. While prospective cohort studies have not demonstrated a significant association, meta-analyses and retrospective cohort studies have suggested potential links between specific genetic mutations and RPL. The prevalence of hereditary thrombophilia in women with recurrent first-trimester miscarriages has been

found to be similar to that in the general population, leading to recommendations that routine thrombophilia screening may not be necessary in these cases.[1,6-9]

This study aims to investigate the relationship between genetic variants associated with hereditary thrombophilia and RPL, with a particular focus on the impact of these variants on miscarriage risk. The findings aim to contribute to the literature on the potential role of thrombophilia in predicting or understanding recurrent miscarriages.

METHODS

The study was carried out with the permission of the Gazi Yaşargil Training and Research Hospital Scientific Researches Evaluation and Ethics Committee (Date:20.12.2022, Decision No: 295). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This retrospective cohort study included 736 women with a history of pregnancy loss who presented to our center between 2019 and 2023. These women were referred to us following gynecological evaluations in obstetrics clinics, where no other cause of abortion was identified, to determine mutations associated with hereditary thrombophilia. The exclusion criteria for the study included patients with known risk factors for RPL other than thrombophilia. RPL was defined as the occurrence of two or more consecutive miscarriages. Clinical assessments of patients included investigating the number of pregnancy losses, the trimester during which losses occurred, and family history of RPL. Additionally, patients with three or more consecutive miscarriages were grouped separately. The relationship between genetic thrombophilia mutations and RPL was examined based on the number of abortions.

High-quality genomic DNA was isolated from peripheral blood samples of all participants using standard extraction protocols. Genetic testing was conducted using a polymerase chain reaction (PCR) panel to screen for mutations commonly associated with thrombophilia. The PCR panels included the Prothrombin G20210A, Factor V Leiden (G1691A), MTHFR C677T, MTHFR A1298C variants, PAI-1 gene 4G/5G polymorphism, FXIII V34L variant, and FV H1299R variant.

Statistical Analysis

Data analysis was performed using IBM SPSS version 25. Summary statistics for quantitative data are presented as mean±standard deviation and median (minimum-maximum). Summary statistics for qualitative variables are presented as frequency and percentage. Relationships between categorical variables were evaluated using Chi-square tests (Pearson, Yates, Fisher Exact). A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of the 736 women in our study was 30 years (range: 16-52). The average number of abortions was 2.43±1.15. The number of patients who miscarried at different trimesters and the details of recurrent miscarriages are presented in Table 1. The study included 510 women who had only first-trimester miscarriages, 54 who experienced only second-trimester miscarriages, and 9 who had only third-trimester miscarriages.

Table 1. Distribution of recurrent miscarriages according to trimesters

Trimester	Number of patients with abortion	Number of patients with recurrent miscarriage	Number of patients with recurrent miscarriage in only one trimester
1st trimester	660	462	458
2nd trimester	185	43	40
3rd trimester	58	11	10

A family history of RPL was assessed in 158 cases. Among patients with fewer than two miscarriages, 18.5% had a positive family history, while the rate was 22.9% in those with two or more miscarriages; however, this difference was not statistically significant (p=0.807).

The distribution of the MTHFR c.677C>T mutation was similar between groups with ≥2 abortions and <2 abortions, showing no statistically significant difference (p=0.438). In fact, the wild-type genotype was slightly more common in the group with ≥2 miscarriages (53.8% vs. 47.8%). Similarly, the MTHFR c.1298A>C mutation did not show a significant difference between groups (p=0.877). Results for patients with three or more miscarriages are shown in Table 2.

Table 2. Distribution of thrombophilia-related genetic variants in patients with less than three abortions versus three or more abortions

Mutation		Number of abortions		p
		<3	≥3	
PAI	5g5g	116 (26.7%)	89 (29.7%)	0.17
	4g5g	215 (49.5%)	157 (52.3%)	
	4g4g	103 (23.7%)	54 (18%)	
MTHFR c.677C>T	Normal	227 (52.1%)	161 (53.7%)	0.561
	Heterozigot	170 (39%)	107 (35.7%)	
	Homozigot	39 (8.9%)	32 (10.7%)	
MTHFR c.1298A>C	Normal	164 (37.6%)	111 (37%)	0.981
	Heterozigot	194 (44.5%)	134 (44.7%)	
	Homozigot	78 (17.9%)	55 (18.3%)	
FXIII p.V34L	Normal	191 (73.7%)	124 (77.5%)	0.657
	Heterozigot	60 (23.2%)	31 (19.4%)	
	Homozigot	8 (3.1%)	5 (3.1%)	
Protrombin G.20210G>A	Normal	423 (97%)	293 (97.7%)	0.34
	Heterozigot	13 (3%)	6 (2%)	
	Homozigot	0 (0%)	1 (0.3%)	
FV (Leiden) C.1691G>A	Normal	416 (95.4%)	285 (95%)	0.934
	Heterozigot	20 (4.6%)	15 (5%)	
	Homozigot	0	0	
FV H1299R	Normal	158 (89.3%)	120 (85.7%)	0.433
	Heterozigot	19 (10.7%)	20 (14.3%)	
	Homozigot	0	0	

For the PAI-1 mutation, the most common genotype observed in both groups was heterozygous 4G/5G. Its prevalence was higher in patients with two or more miscarriages (52.3%) compared to those with fewer than two (43.7%). Comparisons

of RPL for PAI 5G5G, 4G5G, and 4G4G genotypes in patients with ≥2 and ≥3 miscarriages are presented in Table 2.

Approximately 95% of patients showed the normal genotype for FV (Leiden) c.1691G>A. There was no significant difference in the distribution of the mutation between groups (p=1). The heterozygous mutation was slightly more common in the group with ≥2 miscarriages, though this difference was not statistically significant. In 317 cases, the FV H1299R variant was analyzed, while the FXIII V34L variant was evaluated in the remaining 419 cases. The Factor V H1299R mutation also showed no significant difference between groups (p=0.76).

The normal genotype of FXIII was observed in more than 70% of patients (Table 1, 3). The FXIII p.V34L mutation did not show a significant difference between the groups with ≥2 and <2 miscarriages (p = 0.583). Results for patients with three or more miscarriages are provided in Table 2.

Table 3. Comparison of thrombophilia-related genetic variants between patients with less than two abortions and those with two or more abortions

Mutation		Number of abortion		p
		<2	≥2	
PAI	5g5g	40 (29.6%)	165 (27.5%)	0.141
	4g5g	59 (43.7%)	313 (52.3%)	
	4g4g	36 (26.7%)	121 (20.2%)	
MTHFR c.677C>T	Normal	65 (47.8%)	323 (53.8%)	0.438
	Heterozigot	56 (41.2%)	221 (36.8%)	
	Homozigot	15 (11%)	56 (9.3%)	
MTHFR c.1298A>C	Normal	53 (39%)	222 (37%)	0.877
	Heterozigot	58 (42.6%)	270 (45%)	
	Homozigot	25 (18.4%)	108 (18%)	
FXIII p.V34L	Normal	62 (72.1%)	253 (76%)	0.583
	Heterozigot	22 (25.6%)	69 (20.7%)	
	Homozigot	2 (2.3%)	11 (3.3%)	
Protrombin G.20210G>A	Normal	130 (95.6%)	586 (97.7%)	0.369
	Heterozigot	6 (4.4%)	13 (2.2%)	
	Homozigot	0	1 (0.2%)	
FV (Leiden) C.1691G>A	Normal	130 (95.6%)	571 (95.2%)	1
	Heterozigot	6 (4.4%)	29 (4.8%)	
	Homozigot	0	0	
FV H1299R	Normal	45 (90%)	233 (87.3%)	0.76
	Heterozigot	5 (10%)	34 (12.7%)	
	Homozigot	0	0	

There was no significant difference in the prothrombin G.20210G>A polymorphism between patients with ≥2 miscarriages and those with only one miscarriage (p=0.369). Comparisons for patients with RPL of three or more miscarriages are presented in Table 2.

DISCUSSION

In our study, we assessed the potential association of specific genetic variants with the risk of recurrent miscarriage to determine their clinical significance.

In our study, no significant difference was found between individuals with and without a family history of thromboembolic events, consistent with the literature.[5] It is important to note that, in this context, thromboembolic events also include a family history of miscarriages, as these may be indicative of underlying thrombophilic conditions. A 2022 cohort study showed that the prevalence of hereditary thrombophilia in individuals with RPL was comparable to that of the general population.[5] The ALIFE2 study also supported this, finding that low molecular weight heparin (LMWH) had no effect on RPL. The European Society of Human Reproduction and Embryology recommends against routine hereditary thrombophilia screening, except in women with additional risk factors such as personal or family history of venous thromboembolism.[10]

Certain meta-analyses have shown that mutations such as MTHFR C677T, MTHFR A1298C, and PAI-1 4G/5G may increase the risk of RPL[11-14], though some studies have reported no increase in risk.[15,16] In the study by Barut et al.[17], no significant association was found between MTHFR A1298C polymorphism and RPL (p>0.05). The American College of Obstetricians and Gynecologists (ACOG) recommends screening for Prothrombin G20210A, protein C, protein S, and antithrombin III for hereditary thrombophilia.[18] Over 40% of physicians include MTHFR and homocysteine levels in thrombophilia screening, despite ACOG's recommendation against these tests. In our study, the MTHFR c.677C>T and MTHFR c.1298A>C mutations did not show significant differences between groups, suggesting that screening for these mutations in RPL may not be necessary.

According to the British Society for Haematology guidelines, PAI-1 plasma levels have no role in predicting thrombosis risk in women with pregnancy-related morbidities.[4] Although Wen et al.'s[11] meta-analysis initially indicated a strong association between PAI-1 mutation and RPL, subgroup analyses later revealed that this association was influenced by ethnicity and gestational age rather than the number of miscarriages. Consistent with the literature, our study did not find a significant association between PAI-1 mutation and recurrent miscarriage; thus, we do not recommend its inclusion in thrombophilia screening. According to UpToDate, both MTHFR polymorphism (C677T, A1298C) and PAI-1 polymorphism testing are not recommended in pregnancy.[19]

The factor V Leiden mutation is one of the most well-known genetic causes of thrombophilia, leading to resistance to activated protein C and an increased risk of venous thromboembolism.[20] Several meta-analyses have identified a significant association between factor V Leiden H1299R and RPL.[21] In the study by Barut et al.[17], a significant difference was found in the rate of heterozygous factor V Leiden H1299R mutation between patients with two miscarriages and those with three or more miscarriages. In our study, the mutation frequency was 2.4%, consistent with the literature. However, no significant difference was found between patients with fewer than three miscarriages and those with three or more miscarriages (p=0.934), suggesting that while factor V Leiden is a known risk factor for thrombosis, it may not contribute significantly to RPL in this cohort. We found no patients with the FV (Leiden) c.1691G>A or FV H1299R homozygous mutation, and no statistically significant difference was observed among those with heterozygous H1299R mutation.

The FXIII p.V34L mutation affects clot stability and has been studied for its potential role in RPL.[20] In our study, no significant difference was found between patients with two or more miscarriages and those with fewer miscarriages. However, the FXIII homozygous mutation was slightly more prevalent in patients with recurrent miscarriages, particularly among those with three or more miscarriages. Although this finding was not statistically significant, it suggests that FXIII mutation may contribute to RPL in certain patient groups; further studies with larger populations are warranted. Research has proposed that FXIII mutation may increase thrombotic risk during pregnancy by affecting clot stability, as seen in a study by Dossenbach-Glaninger et al.[22], which found that FXIII mutation in homozygous form could increase the risk of recurrent miscarriage. Conversely, other studies have not confirmed this association.[23]

Meta-analyses examining the Prothrombin gene mutation and RPL have generally shown no significant association.[7,8,24] Our findings align with these meta-analyses. However, in a study conducted on Turkish patients, Barut et al.[17] did report a relationship between this mutation and RPL. As hereditary thrombophilia may vary across ethnicities, the differing results in our study with a larger Turkish patient cohort may be of particular relevance.

Limitations

Differences in the prevalence and impact of genetic mutations across different ethnic groups could affect the applicability of our findings to other populations. Given that certain mutations are more prevalent in specific populations, the results may not be generalizable to other ethnic groups.

CONCLUSION

Our findings suggest that, despite the prevalence of certain thrombophilia mutations, their impact on pregnancy loss may be limited, prompting questions about the efficacy of routine screening in miscarriage prevention. This is important to prevent unnecessary genetic testing and optimize resource allocation. Considering the high cost and potential overuse of genetic testing, the results of this study emphasize the need for a more selective testing policy. Thus, our findings support a more targeted approach to risk assessment in pregnancy loss. The data presented here contribute to clinical practice and underscore the need for larger, prospective studies to further clarify the relationship between thrombophilia and pregnancy loss.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Gazi Yaşargil Training and Research Hospital Scientific Researches Evaluation and Ethics Committee (Date:20.12.2022, Decision No: 295).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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