

Prevalence of *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms in Bosnian women with pregnancy loss

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ABSTRACT

The relationship between genetic risk factors of thrombophilia and pregnancy loss (PL) is being discussed. The focus has been on *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms that may predispose women to microthrombosis during the stages of embryo implantation and placentation. Although, the frequencies of these polymorphisms were reported in different populations, such studies have not yet been performed in Bosnian population. In this study, we determined the prevalence of *F5* G>A (rs6025), *F2* G>A (rs1799963) and *MTHFR* C>T (rs1801133) polymorphisms in Bosnian women. A total of 154 women with PL, mean age 33 (± 5.4) years, were enrolled in the study. As a control group, 154 mothers [mean age 31.4 (± 6.7) years] with at least one live-born child were included. We used real-time polymerase chain reaction (PCR) to determine the frequencies of *F5* G>A and *F2* G>A genotypes, and PCR-restriction fragment length polymorphism (RFLP) for analyzing *MTHFR* C>T genotypes. The frequency of heterozygotes for *F5* and *F2* was significantly higher in women with venous thrombosis (VT) compared to women without VT ($p = 0.047$ and $p = 0.001$, respectively). There was no significant difference in the distribution of *MTHFR* genotypes and alleles between these two groups. In addition, we observed no significant differences in the genotype and allele frequencies between the group with PL and control group, for all investigated polymorphisms. The allele frequencies for 1691A (*F5*), 20210A (*F2*), and 677T (*MTHFR*) reported in this study are consistent with the data obtained for other European countries, however, we were not able to confirm the association between the three polymorphisms and PL in Bosnian women.

KEY WORDS: Pregnancy loss; risk factors; polymorphisms; thrombophilia; *F5*; *F2*; *MTHFR*

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INTRODUCTION

There is a growing evidence that women with thrombophilia are at increased risk, not only for pregnancy-related venous thromboembolism (VTE), but also for other vascular complications in pregnancy, such as fetal loss, pre-eclampsia (PE) and intrauterine growth restriction (IUGR) [1].

According to the definition, pregnancy loss (PL) is the spontaneous abortion of an embryo or fetus before the

20th week of pregnancy or when the fetus weighs <500 g or measures <25 cm [2].

The *F5* gene mutation 1691G>A (rs6025) results in an altered variant of factor V, namely Factor V Leiden, which cannot be easily cleaved by activated protein C (aPC). The 1691G>A mutation increases the risk of venous thrombosis (VT) up to 50-100 fold in adult homozygous [3]. In European populations, the frequency of *F5* 1691A allele is higher in Bosnians (6.0%) than in the Czechs (2.6%), Ukrainians (1.5%), and Croats (1.4%) [4]. Numerous studies indicated that the *F5* 1691G>A is responsible for 3-42% of PL [5].

The *F2* 20210G>A (rs1799963) mutation, results in increased prothrombin levels and is considered as another risk factor for VT [6]. The heterozygotes have 2-5-fold increased risk of thrombosis which may also increase the risk for PL. The

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prevalence of *F2* 20210G>A in European Caucasians is from 1% to 8%, and from 1% to 12% in Mediterranean populations. Furthermore, in European Caucasians with VTE, the prevalence of *F2* 20210G>A is from 3% to 17%, and in Mediterranean populations with VTE this figure is from 3% to 24% [7-9]. The *F2* 20210A allele frequency in Bosnians is 6.0%, and is higher than in Poles and Ukrainians, 2.5% and 1.7%, respectively [4].

The 677C>T mutation (rs1801133) of the *MTHFR* gene leads to a defective methylene tetrahydrofolate reductase (*MTHFR*) enzyme that has only 50% of the normal activity and is a common cause of elevated levels of homocysteine, previously identified as a risk factor for VTE. The frequency of *MTHFR* 677T allele in Bosnians is 37.5% and is higher than those reported for the Czechs, Ukrainians, Norwegians, and Swedes (25.0%, 29.1%, 29.0%, and 25.0%, respectively), as well as for Austrians and the Dutch (each 29.3%) [4]. The American College of Obstetricians and Gynecologists recommends against screening for *MTHFR* variants to determine the presence of thrombophilia in pregnant women [10]. On the other hand, the co-inheritance of the alleles linked to thrombophilia, such as the *F5* 1691A, *F2* 20210A, and *MTHFR* 677T, may increase the risk for PL.

The prevalence of the genetic risk factors for thrombophilia is higher in Bosnian population compared to several European populations [4]. Therefore, the aim of our study was to investigate the prevalence of *F5* G>A (rs6025), *F2* G>A (rs1799963) and *MTHFR* C>T (rs1801133) polymorphisms in Bosnian women with miscarriages and to determine the correlation with the risk of PL.

MATERIALS AND METHODS

The medical history and buccal swabs were collected at the Institution of Health Protection of Women and Motherhood (Sarajevo, Bosnia and Herzegovina) from 2013 to 2015. The study was conducted according to the standards of the Declaration of Helsinki (1975, revised 2000), and the protocol was approved by the local Bioethical Committee (decision reference numbers 10-1285-03-14, KB-0012/46/15, and KB-0012/133/13). A total of 154 women with PL were enrolled in the study, and 121 (78.6%) of these women had at least one live born child. In this group, all pregnancies were anembryonic, and miscarriage took place between 6 and 28 weeks gestation. As a control group, 154 mothers without PL and with at least one live-born child were included. All 308 women were of Bosnian origin and came from the general population of Sarajevo (an estimated population of 369,534 inhabitants [http://fzs.ba/index.php/popis-stanovnistva/popis-stanovnistva-2013/konacni-rezultati-popisa-2013/]). In our study ethnicity refers to linguistic, cultural, religious, and political aspects. Clinical and lab data were recorded for each

women in the StatView computer software version 5.0 (SAS Institute Inc. Cary, NC, USA).

Data on obstetric history, body weight, number of successful pregnancies, and history of VT are shown in Table 1.

DNA extraction and amplification

Genomic DNA from buccal swabs was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The extraction was performed according to the manufacturer's instructions. DNA samples were stored at +4°C for further analyses.

Following DNA extraction from buccal swabs, polymerase chain reaction (PCR) was performed. The *F5* G>A and *F2* G>A genotypes were determined by real-time PCR on a Roche LightCycler® 2.0 (Roche, Bazylea, Switzerland) and using Gene Proof PCR kits (Imogena, Brno, Czech Republic). The *MTHFR* C>T genotypes were analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) using the following primers: forward 5'-CAAAGGCCACCCCGAAGC-3' and reverse 5'-AGGACGGTGCGGTGAGAGTG-3' (TIB MolBiol, Poznań, Poland). The PCR reactions were performed on a Hightech Thermocycler Cycler-Technology for Life (SensoQuest, Gottingen, Germany) according to previously described techniques by Schmitz et al. [11]. After digestion, the genotypes were determined by electrophoresis in 2-3% agarose gels, stained with DNA-star dye (Lonza, Inc., Rockland, ME, USA).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.2. (IBM Corp, Armonk, NY). Deviation of Hardy-Weinberg equilibrium (HWE) was assessed by Student's *t*-test for independent samples. Difference in genotype distribution between women with and without PL was determined using Fisher's exact test. Odds ratio (OR) and 95% confidence interval (CI) were calculated. A value of $p < 0.05$ was considered statistically significant.

RESULTS

In our study group, 9.7% ($n = 15$) women with PL and 7.8% ($n = 12$) without PL had VT, but there was no statistically significant difference between the two groups ($\chi^2 = 0.688$; $p = 0.344$).

A positive family history of VT was more frequent in the group of women with PL, 24.7% ($n = 38$) compared to the control group, 16.2% ($n = 25$), and this difference was statistically significant ($\chi^2 = 3.372$; $p = 0.045$). Both groups were in HWE, and we observed no significant differences in the genotype and allele frequencies between the group with PL and control group, for all investigated polymorphisms ($p > 0.05$).

The distribution of genotypes and alleles in women with PL and control group is presented in Table 2. The distributions of genotypes and alleles in women with PL and control group in relation to the number of PL and the presence of VT are shown in Tables 3 and 4, respectively.

The *F5* GA genotype was more frequent in women with multiple miscarriages (the borderline statistical significance, $p = 0.051$, $\chi^2 = 7.791$). No statistically significant correlation between the *F2* and *MTHFR* genotype and allele distribution and the number of PL was observed.

Both *F5* and *F2* heterozygotes were significantly more frequent in women with VT compared to women without VT ($p = 0.047$ and 0.001 , respectively). There was no statistically significant difference in the distribution of *MTHFR* genotypes and alleles between women with VT and those without VT.

DISCUSSION

Pregnancy is a physiological state linked to increased clotting, decreased anticoagulant activity, and decreased fibrinolysis [12].

Although the allele A of the *F5* gene (1691A) is a risk factor for thrombosis, the results on the association between *F5* A allele and PL are contradictory. In our study, the frequency of 1691A allele was 7.8% in both groups (i.e., women with and without PL), and the differences in the genotype and allele distributions between the two groups were not significant ($p > 0.05$). Similarly, Polish, German, and Turkish studies showed no significant differences in the frequency of *F5* 1691A allele between women with PL and those without PL ($p > 0.05$). In the above-mentioned studies, the following frequencies of the 1691A allele were reported in women with PL versus women without PL: 3.2% versus 3.0% in the Polish study, 10.9% versus 7.4% in the German study, and 7.9% versus 7.0% in the Turkish group [13-15]. In Serbian women with and without PL, the frequency of the 1691A allele was 4.0% versus 1.5%, respectively ($p = 0.05$) [16]. Similarly, studies on Lebanese and Palestinian women with PL confirmed that the 1691A allele was significantly more frequent in PL group compared to controls ($p < 0.001$ and $p < 0.0001$, respectively) [6,17]. Surprisingly, in an Israeli study, the frequency of

TABLE 1. Characteristics of the study group

Characteristics	Women with PL (mean±SD)	Women without PL (mean±SD)	<i>p</i>
Age	32.9±5.1	31.7±6.6	0.069
Weight	73.9±9.3	74.7±9.2	0.484
Number of pregnancies	1.4±1.1	1.2±0.8	0.066
VT, n (%)	15 (9.7)	12 (7.8)	0.344
VT in the family, n (%)	38 (24.7)	25 (16.2)	0.045

PL: Pregnancy loss; VT: Venous thrombosis; SD: Standard deviation

TABLE 2. Distribution of genotypes and alleles of *F5*, *F2*, and *MTHFR* polymorphisms in women with and without PL

Gene polymorphism	Women with PL n=154	Women without PL n=154	Compared genotypes	<i>p</i> value	OR (95% CI)
<i>F5</i> 1691G>A					
Genotypes, n (%)					
GG	142 (92.2)	142 (92.2)	GA versus GG	1	
GA	12 (7.8)	12 (7.8)			
AA	0	0			
Allele frequency, n (%)					
G	296 (96.1)	296 (96.1)			
A	12 (3.9)	12 (3.9)			
<i>F2</i> 20210G>A					
Genotypes, n (%)					
GG	148 (96.1)	149 (96.8)	GA versus GG	0.759	1.208 (0.36-4.06)
GA	6 (3.9)	5 (3.2)			
AA	0	0			
Allele frequency, n (%)					
G	302 (98.1)	303 (98.4)			
A	6 (1.9)	5 (1.6)			
<i>MTHFR</i> 677C>T					
Genotypes, n (%)					
CC	61 (39.6)	71 (46.1)	CC versus CT+TT	0.215	0.767 (0.49-1.21)
CT	76 (49.4)	74 (48.1)	CT versus CC+TT	0.819	1.053 (0.67-1.65)
TT	17 (11.0)	9 (5.8)	TT versus CC+CT	0.106	1.999 (0.86-4.64)
Allele frequency, n (%)					
C	198 (64.3)	216 (70.1)			
T	110 (35.7)	92 (29.9)			

PL: Pregnancy loss; OR: Odds ratio; CI: Confidence interval

TABLE 3. Distribution of genotypes and alleles of *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms by the number of PL

Gene polymorphism	Number of PL			
	1	2	3	4
<i>F5</i> 1691G>A				
Genotypes, n (%)				
GG	98 (95.1)	32 (88.9)	11 (84.6)	1 (50.0)
GA	5 (4.9)	4 (11.1)	2 (15.4)	1 (50.0)
AA	0	0	0	0
Allele frequency, n (%)				
G	201 (97.6)	68 (94.4)	24 (92.3)	3 (75.0)
A	5 (2.4)	4 (5.6)	2 (7.7)	1 (25.0)
<i>F2</i> 20210G>A				
Genotypes, n (%)				
GG	100 (97.1)	34 (94.4)	12 (92.3)	2 (100.0)
GA	3 (2.9)	2 (5.6)	1 (7.7)	0
AA	0	0	0	0
Allele frequency, n (%)				
G	203 (98.5)	70 (97.2)	25 (96.1)	2 (100)
A	3 (1.5)	2 (2.8)	1 (3.9)	0
<i>MTHFR</i> 677C>T				
Genotypes, n (%)				
CC	42 (40.8)	15 (41.7)	4 (30.8)	0
CT	48 (46.6)	17 (47.2)	9 (69.2)	2 (100.0)
TT	13 (12.6)	4 (11.1)	0	0
Allele frequency, n (%)				
C	132 (64.1)	47 (65.3)	17 (65.4)	2 (50.0)
T	74 (35.9)	25 (34.7)	9 (34.6)	2 (50.0)

$\chi^2=7.791$, $p=0.051$. PL: Pregnancy loss

the mutated *F5* allele was higher in women without PL compared to women with PL ($p > 0.05$) [18].

Rodger et al. [19] performed a meta-analysis on the relationship between mothers, carriers of the *F5* 1691A and *F2* 20210A alleles, and complications of pregnancy. They demonstrated no significant association between the mutated alleles and pregnancy complications, including PL [19]. In the present study, we also showed no significant differences in the frequency of *F2* 20210A allele and obstetric complications between women with and without PL ($p > 0.05$); the frequency of the mutated allele was 1.9% and 1.6%, respectively.

Although Sehirali et al. [20] found the association between the *F2* 20210A allele and PL in Turkish women ($p < 0.05$) [20], these results should be interpreted with caution due to the small sample size; they included 55 women with PL and 49 controls.

Rey et al. [21] conducted a meta-analysis of 31 studies, published between 1975 and 2002. Their conclusion was that the *F5* 1691A allele is linked to early (OR: 2.01, 95% CI: 1.13-3.58), late recurrent fetal loss (OR: 7.83, 95% CI: 2.83-21.67), and late non-recurrent fetal loss (OR: 3.26, 95% CI: 1.82-5.83). The *F2* 20210A allele was linked to PL in the first trimester (OR: 2.32, 95% CI: 1.12-4.79), repeated miscarriages (OR: 2.56, 95% CI: 1.04-6.29), and late non-recurrent fetal loss (OR: 2.3, 95% CI: 1.09-4.87) [21].

TABLE 4. Distribution of genotypes and alleles of *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms in relation to the presence of VT

Gene polymorphism	VT	
	Yes	No
<i>F5</i> 1691G>A		
Genotypes, n (%)		
GG	22 (81.4)	262 (93.2)
GA	5 (18.6)	19 (6.8)
AA	0	0
Allele frequency, n (%)		
G	49 (90.7)	543 (96.6)
A	5 (9.3)	19 (3.4)
<i>F2</i> 20210G>A		
Genotypes, n (%)		
GG	22 (81.4)	275 (97.8)
GA	5 (18.6)	6 (2.2)
AA	0	0
Allele frequency, n (%)		
G	49 (90.7)	556 (98.9)
A	5 (9.3)	6 (1.1)
<i>MTHFR</i> 677C>T		
Genotypes, n (%)		
CC	12 (44.4)	120 (42.7)
CT	14 (51.8)	136 (48.4)
TT	1 (3.8)	25 (8.9)
Allele frequency, n (%)		
C	38 (70.4)	376 (66.9)
T	16 (29.6)	186 (33.1)

VT: Venous thrombosis

In Spanish women with three or more unexplained PL, significant differences between the 677T allele of the *MTHFR* gene and PL were not found [22].

In a Greek study, Sotiriadis et al. [23] reported that the *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms were not significantly associated with increased risk for PL ($p = 1.00$, $p = 0.652$, and $p = 0.869$, respectively) [23]. In another Greek study, the association between PL in the second trimester and primary PL with the *F5* 1691G>A and *F2* 20210G>A was found ($p = 0.03$, $p = 0.038$, respectively), but not with the *MTHFR* 677C>T ($p = 0.134$) [24].

In the present study, the frequency of *F5* 1691A and *F2* 20210A alleles was significantly higher in women with VT compared to women without VT, 9.3% versus 3.4% ($\chi^2 = 4.739$; $p = 0.047$) and 9.3% versus 1.1% ($\chi^2 = 19.199$, $p = 0.001$), respectively. In 2006, Robertson et al. [25] reported a relationship between the risk of VTE and adverse pregnancy outcomes and thrombophilia in pregnancy. The authors concluded that the risk of VTE for *F5* 1691A homozygotes was 34.4 (95% CI: 9.86-120.05), while for the heterozygotes the risk was - 8.32 (95% CI: 5.44-12.70) [25].

In a meta-analysis of 63 studies, the authors found that the *F5* 1691G>A mutation was associated with a 4.5- and 8.6-fold increased risk of the first VTE during pregnancy (95% CI:

1.8-10.9; 95% CI: 5.9-12.6, respectively) [26]. Patients with VTE have a high risk of recurrent events. This risk is estimated to be 4.5%/year in the first 2 years after the initial event [27].

A Romanian retrospective study analyzed a possible association between thrombosis and inherited thrombophilia in pregnant women. The risk of thrombosis in women with the *F5* 1691G>A mutation was 2.66 times higher than in women without the mutation (OR = 2.66, 95% CI: 0.96-7.37, $p = 0.059$). The authors did not find any statistical association with the *MTHFR* 677C>T polymorphism [28]. Furthermore, in another Romanian study, homozygous individuals for the *F5* 1691G>A had a 9-fold higher risk of thrombosis compared to individuals without the mutation ($p = 0.015$, 95% CI: 1.080-72.923) [29].

To our knowledge, this is the first report on the association between the *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms and risk of PL in Bosnian women. The allele frequencies reported in this study are consistent with the data obtained for other European countries. The main limitation of our study was the small sample size of women with three and four PL. We suggest further studies with larger sample size and equal samples of women with two, three, and four PLs.

CONCLUSION

In summary, we were not able to confirm the association between the *F5* 1691G>A, *F2* 20210G>A, and *MTHFR* 677C>T polymorphisms and PL in Bosnian women. The significant differences in the frequency of mutated alleles were found only between women with VT and those without VT.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

- [1] Walker ID. Thrombophilia in pregnancy. *J Clin Pathol* 2000;53(8):573-80. <https://doi.org/10.1136/jcp.53.8.573>.
- [2] WHO: Recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October. *Acta Obstet Gynecol Scand* 1977;56(3):247-53.
- [3] Rodeghiero F, Tosetto A. Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. *Ann Intern Med* 1999;130(8):643-50. <https://doi.org/10.7326/0003-4819-130-8-199904200-00004>.

- [4] Adler G, Agnieszka G, Valjevac A, Czarska E, Kiseljakovic E, Salkic NN. Prevalence of genetic prothrombotic risk factors: 1691G > A FV, 20210G > A PT and 677C > T MTHFR mutations in the Bosnian population. *Ann Hum Biol* 2015;42(6):576-80. <https://doi.org/10.3109/03014460.2014.968618>.
- [5] Inbal A, Carp H. Defects in coagulation factor leading to recurrent pregnancy loss. In: Carp H, editor. *Recurrent Pregnancy Loss. Causes Controversies and Treatment*. UK: Informa Healthcare; 2007. p. 127-39. <https://doi.org/10.3109/9780203931677.019>.
- [6] Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY. Prevalence of factor V G1691A (factor V-Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population. *Am J Hematol* 2002;71(4):300-5. <https://doi.org/10.1002/ajh.10223>.
- [7] Franco RF, Reitsma PH. Genetic risk factors of venous thrombosis. *Hum Genet* 2001;109(4):369-84. <https://doi.org/10.1007/s004390100593>.
- [8] Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996;88(10):3698-703.
- [9] Jadaon MM. Epidemiology of prothrombin G20210A mutation in the Mediterranean region. *Mediterr J Hematol Infect Dis* 2011;3(1):e2011054. <https://doi.org/10.4084/mjhid.2011.037>.
- [10] American College of Obstetricians and Gynecologists Women's Health Care Physicians. ACOG Practice Bulletin No. 138: Inherited thrombophilias in pregnancy. *Obstet Gynecol* 2013;122(3):706-17. <https://doi.org/10.1097/01.AOG.0000433981.36184.4e>.
- [11] Schmitz C, Lindpaintner K, Verhoef P, Gaziano JM, Buring J. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. A case-control study. *Circulation* 1996;94(8):1812-4. <https://doi.org/10.1161/01.CIR.94.8.1812>.
- [12] Hellgren M. Hemostasis during normal pregnancy and puerperium. *Semin Thromb Hemost* 2003;29(2):125-30. <https://doi.org/10.1055/s-2003-38897>.
- [13] Slezak R, Laczanski L, Karpinski P, Reszczynska-Slezak D. The role of 1691G>A (Leiden) mutation in Factor V gene, 20210G>A in prothrombin gene and 677C>T in MTHFR gene in etiology of early pregnancy loss. [Article in Polish]. *Ginekol Pol* 2011;82(6):446-50.
- [14] Reznikoff-Etievan MF, Cayol V, Carbonne B, Robert A, Coulet F, Milliez J. Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage. *BJOG* 2001;108(12):1251-4. [https://doi.org/10.1016/S0306-5456\(01\)00298-4](https://doi.org/10.1016/S0306-5456(01)00298-4).
- [15] Altintas A, Pasa S, Akdeniz N, Cil T, Yurt M, Ayyildiz O, et al. Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss: Data from the southeast of Turkey. *Ann Hematol* 2007;86(10):727-31. <https://doi.org/10.1007/s00277-007-0327-1>.
- [16] Djurovic J, Stojkovic O, Todorovic J, Brajic A, Obradovic S, Stankovic S, et al. Genetics of suspected thrombophilia in Serbian females with infertility, including three cases, homozygous for FII 20210A or FV 1691A mutations. *Hum Fertil (Camb)* 2017;20(2):132-9. <https://doi.org/10.1080/14647273.2016.1255785>.
- [17] Hussein AS, Darwish H, Shelbayeh K. Association between factor V Leiden mutation and poor pregnancy outcomes among Palestinian women. *Thromb Res* 2010;126(2):e78-82. <https://doi.org/10.1016/j.thromres.2010.04.017>.
- [18] Carp H, Salomon O, Seidman D, Dardik R, Rosenberg N, Inbal A. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. *Hum Reprod* 2002;17(6):1633-7. <https://doi.org/10.1093/humrep/17.6.1633>.
- [19] Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, et al. The association of factor V Leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: A systematic review and meta-analysis of prospective

- cohort studies. *PLoS Med* 2010;7(6):e1000292.
<https://doi.org/10.1371/journal.pmed.1000292>.
- [20] Sehirali S, Inal MM, Yildirim Y, Balim Z, Kosova B, Karamizrak T, et al. Prothrombin G20210A mutation in cases with recurrent miscarriage: A study of the mediterranean population. *Arch Gynecol Obstet* 2005;273(3):170-3.
<https://doi.org/10.1007/s00404-005-0061-7>.
- [21] Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: A meta-analysis. *Lancet* 2003;361(9361):901-8.
[https://doi.org/10.1016/S0140-6736\(03\)12771-7](https://doi.org/10.1016/S0140-6736(03)12771-7).
- [22] Creus M, Deulofeu R, Peñarrubia J, Carmona F, Balasch J. Plasma homocysteine and vitamin B12 serum levels, red blood cell folate concentrations, C677T methylenetetrahydrofolate reductase gene mutation and risk of recurrent miscarriage: A case-control study in Spain. *Clin Chem Lab Med* 2013;51(3):693-9.
<https://doi.org/10.1515/cclm-2012-0452>.
- [23] Sotiriadis A, Vartholomatos G, Pavlou M, Kolaitis N, Dova L, Stefanos T, et al. Combined thrombophilic mutations in women with unexplained recurrent miscarriage. *Am J Reprod Immunol* 2007;57(2):133-41.
<https://doi.org/10.1111/j.1600-0897.2006.00454.x>.
- [24] Foka ZJ, Lambropoulos AF, Saravelos H, Karas GB, Karavida A, Agorastos T, et al. Factor V Leiden and prothrombin G20210A mutations, but not methylenetetrahydrofolate reductase C677T, are associated with recurrent miscarriages. *Hum Reprod* 2000;15(2):458-62.
<https://doi.org/10.1093/humrep/15.2.458>.
- [25] Robertson L, Wu O, Langhorne P, Twaddle S, Clark P, Lowe GD, et al. Thrombophilia in pregnancy: A systematic review. *Br J Haematol* 2006;132(2):171-96.
<https://doi.org/10.1111/j.1365-2141.2005.05847.x>.
- [26] Biron-Andreani C, Schved JF, Daures JP. Factor V Leiden mutation and pregnancy-related venous thromboembolism: What is the exact risk? Results from a meta-analysis. *Thromb Haemost* 2006;96(1):14-8.
<https://doi.org/10.1160/tho6-02-0086>.
- [27] Van Dongen CJ, Vink R, Hutten BA, Buller HR, Prins MH. The incidence of recurrent venous thromboembolism after treatment with vitamin K antagonists in relation to time since first event: A meta-analysis. *Arch Intern Med* 2003;163(11):1285-93.
<https://doi.org/10.1001/archinte.163.22.2793-a>.
- [28] Coriu L, Ungureanu R, Talmaci R, Uscatescu V, Cirstoiu M, Coriu D, et al. Hereditary thrombophilia and thrombotic events in pregnancy: Single-center experience. *J Med Life* 2014;7(4):567-71.
- [29] Hotoleanu C, Popp R, Trifa A. Factor V Leiden, prothrombin G20210A and MTHFR C677T mutations in Romanian patients with deep venous thrombosis. *HVM Bioflux* 2014;6(1):15-9.