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Genetic Polymorphisms F2 and F13A1and Coagulation Profile and Their Association with the Risk of Recurrent Spontaneous Abortion in Iraqi Women

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Abstract

Background. Recurrent spontaneous abortion is defined as three or more consecutive clinically recognized spontaneous pregnancy loss. Many studies indicated that genetic variations have been proposed to cause recurrent spontaneous abortion (RSA). **Aim**.The proposal target investigating the genetic association between the polymorphism of rs3136520 SNP in *F2* gene and rs1050782 SNP in *F13A1* gene and RSA**. Method**. This case-control study was conducted on 50 women with RSA as a case group and 50 apparently healthy women without any history of abortion with at least one healthy child as the control group. Blood samples were collected in tubes containing EDTA to obtain DNA. Platelets count; prothrombin time and partial thromboplastin time were determined. The rs3136520 SNPs was genotyped using Tetra-ARMS-PCR, while rs1050782 SNP was analyzed using High resolution melting (HRM) technique. Data was analyzed using the SPSS software. **Result.** There were no significant differences between study groups in terms of mean age and body mass index (BMI). No significant differences between the study groups as related to the mean platelets count. Prothrombin time mean was significantly ($p<0.05$) increased in patients with recurrent spontaneous abortion when compared with controls (14.6 ± 0.18 *versus* 13.9 ± 0.24 seconds, respectively; $p<0.05$). Also, the mean of partial thromboplastin time was significantly ($p<0.01$) increased in patients with recurrent spontaneous abortion when compared with controls (34.9 ± 0.65 versus 32.0 ± 0.58 seconds, respectively; $p<0.01$). As related with rs3136520 SNP in *F2* gene, there was an association between the incidence of recurrent spontaneous abortion and the CT genotype of rs3136520 in *F2* gene in Iraqi patients from Al-Najaf city. As related with rs1050782, the GG genotype may represent as a risk factor for RSA susceptibility in Iraqi women from Al-Najaf city. Also, GA and AA genotypes and A allele of rs1050782 were significantly ($p<0.01$) lower in women with RSA than in controls and may act as a protective factor to decrease susceptibility RSA (42%, 28% and 69% of control group *versus* 20% , 18% and 28% of RSA group, for GA, AA genotypes and A allele, respectively). no significant differences were noted between the study groups as related with the percentages of each genotype and allele frequency in Iraqi women from Al-Najaf city. **Conclusion.** The results indicate that rs1050782 represents as a risk factor for RSA incidence while A allele represents as a protective factor for RSA. in Iraqi women from Al-Najaf city.

Keywords: platelets, prothrombin time, partial thromboplastin time, F2 and F13A1 genes, recurrent spontaneous abortion.

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Introduction

Recurrent spontaneous abortion (RSA), defined as the loss of two or more consecutive pregnancies, represents as a significant health and psychological challenge for many couples worldwide (1). Despite advances in reproductive medicine, the underlying causes of this condition remain poorly understood in many cases (2). Genetic factors play a crucial role in

this context, with studies showing an association between certain genetic mutations and an increased risk of recurrent miscarriage (3). Among these mutations, those in the F13A1 and FII genes, which encode coagulation factors XIII and II (4). These mutations affect the function of coagulation factors, potentially disrupting the clotting process during pregnancy and increasing the risk of blood clots that obstruct blood flow to the fetus, thereby threatening its survival (5). However, scientific organizations around the world, including the American Society of Reproductive Medicine (ASRM) and the European Society of Human Reproduction (ESRE), have recently improved the definition of RSA to incorporate two or more cases of pregnancy loss in response to the growing problem of infertility (3). Autoimmune diseases (20%), endocrinological disorders (17–20%), uterine alterations (10–15%), hereditary factors such parental chromosome abnormalities (2–5%), and infections (0.5–5%) are among the causes of the condition [4]. Nonetheless, more than 50% of RSA cases are categorized as idiopathic and cannot be explained [2]. A study conducted by Xu *et al*. (2018) on Han Chinese women showed that the mutations rs3136520 in the FII gene and rs1050782 in the F13A1 gene exhibited statistically significant differences between the control and patient groups for both genes. The study confirmed that these two genes play a key role in the risk of recurrent spontaneous abortion (6).

In Iraqi women, many variants were found to be risk factor for RSA incidence such as HLA-G*0104/0104 genotype (7), homozygosity of HLA-E*0101 (8), TT genotype of rs1042838 in progesterone receptor gene (9), GG genotype of rs6152 in androgen receptor gene (10), while, AA genotype of rs2275913 in IL-17A gene was found to be a protective factor (11). Jassim *et al*. (12) found an association between both heterozygous and homozygous mutants genotypes at rs37389 of prolactin receptor gene with the risk of the recurrent miscarriage susceptibility. No association between some variants with RSA such as rs1801133 in MTHFR gene (13) and rs1264459 in HLA-E gene (14).

The purpose of the present study was to investigate the association of some genetic variants in F2 and F13A1 genes with the susceptibility of recurrent spontaneous abortion in Iraqi women from Al-Najaf city.

Materials and Methods

Sampling

This case – control study was carried out in the obstetrics and gynecology department of Al-Zahraa teaching hospital, Al-Najaf city, Iraq and in the institute of genetic engineering and biotechnology for higher studies, university of Baghdad, Iraq during June 2022 – April 2023 with two groups. The control group (n=50) consisted of 20-45 years old women with no history of miscarriage or infertility and with at least one successful pregnancy and a delivery without any complications. The case-control group (n=50) consisted of 17-46 years old women with at least two consecutive idiopathic miscarriage and no successful pregnancies. It should also be considered that all the women in both groups were Iraqis from Al-Najaf city.

All women with recurrent spontaneous abortion (RSA) due to infections, uterine conformational abnormalities, immune disorders, hormonal abnormalities including

thyroid and prolactin disorders were excluded from the study.

Every single blood sample was split into two tubes: (1) sodium citrate tubes for plasma collection, the tube was centrifuged for 10 minutes at 3000 rpm. The plasma samples were used for prothrombin time and partial thromboplastin time determination using Human device, Huma-clot junior, Germany (2) EDTA tubes for measurement of platelets count in whole blood using BC-500 device, Mindray, China and DNA extraction for use in genotyping. Five ml of peripheral blood samples were taken from each individual. Genomic DNA was extracted from a one ml ethylenediamine tetra acetic acid-anticoagulated peripheral blood sample using a DNA extraction kit (Genomic DNA mini kit, Favorgen, China) according to the manufacturers' instructions. The concentration and purity of the extracted DNA was estimated using a nanodrop.

Genotyping

Rs3136520 of F2 gene is a C>T intron variant which causes alternative splicing that can alter coagulation factor II function. The location of this SNP in intron 4 of F2 gene (g.7490 C>T, NG_008953.1), chromosome 11. R1050782 is a A>G 3' UTR variant. The location of this SNP in F13A1 gene (g.180199A>G, NG_008107.1), Chromosome 6.

F2 gene

Genetic polymorphisms of each participant for rs3136520 in F2 gene were determined by Tetra-ARM primer amplification refractory mutation system PCR (T-ARMS-PCR). Detailed information about the primers is listed in figures 1.

C allele

TGAACACTTTCCTATCCTTCAA
GGACTGCTTCAAATGTCACCAC
TTTTGCTGAGACTTCAGGGAGC
ACCCTCCCTCCTGCACTGTGTT
CTGAAGGCACCTTTAGCACGAC
AAAAATGGAACTCTTTGTTTAT
TTATAAGAGACAGGGTCTCCCT
TTTTTGCCCAGGCTGATCTTGA
ACTCCTGGGCTCAGGCAATTCT
CCCATCTCAGTCTCCCAAAGGA
GTAGGATTATAAGTGTGAGCCA
CCATGCCT**GGCTGCCATACTTT**
CATTTTTTTTTTT

Forward	5' – TGAACACTTTCCTATCCTTCAAGGACTG-3'	134 bp
C allele	5' – AAATAAACAAAGAGTTCCATTTTTGGCG – 3'	

T allele

TGAACACTTTCCTATCCTTCAA
GGACTGCTTCAAATGTCACCAC
TTTTGCTGAGACTTCAGGGAGC
ACCCTCCCTCCTGCACTGTGTT
CTGAAGGCACCTTTAGCACGAC
AAAAATGGAACTCTTTGTTTAT
TTATAAGAGACAGGGTCTCCCT
TTTTTGCCCAGGCTGATCTTGA
ACTCCTGGGCTCAGGCAATTCT
CCCATCTCAGTCTCCCAAAGGA
GTAGGATTATAAGTGTGAGCCA
CCATGCCT**GGCTGCCATACTTT**
CATTTTTTTTTTT

T allele	5' – CACTGTGTTCTGAAGGCACCTTTAGAAT – 3'	200 bp
Reverse	5' - AAAAAAAAAAAATGAAAGTATGGCAGCC– 3'	

TGAACACTTTCCTATCCTTCAA
GGACTGCTTCAAATGTCACCAC
TTTTGCTGAGACTTCAGGGAGC
ACCCTCCCTCCTGCACTGTGTT
CTGAAGGCACCTTTAGCACGAC
AAAAATGGAACTCTTTGTTTAT
TTATAAGAGACAGGGTCTCCCT
TTTTTGCCCAGGCTGATCTTGA
ACTCCTGGGCTCAGGCAATTCT
CCCATCTCAGTCTCCCAAAGGA
GTAGGATTATAAGTGTGAGCCA
CCATGCCT**GGCTGCCATACTTT**
CATTTTTTTTTTTT

Targeted fragment, 278bp.

Outer forward	**TGAACACTTTCCTATCCTTCAAGGACTG**	**278bp**
Outer revers	**GGCTGCCATACTTTCATTTTTTTTTTTT**	

After their design, all the primers were evaluated with the BLAST-NCBI database and analyzed using the oligo analyzer software to ensure the validation of each primer. To increase the specificity of each of the two inner primers in T-ARMS-PCR, an extra mismatch was designed in the second nucleotide for ARMS-PCR and the third nucleotide for T-ARMS-PCR from the 3' end.

Amplifications were carried out in a thermal cycler (Analytic jena, Germany) with one tube for each sample using T-ARMS-PCR with 4 primers in each tube. Each tube contained 25 µl : 12.5 µl of 2x easy Taq® PCR Super Mix (Promega, USA), 1 µl of each primer, 5 µl of genomic DNA and up to 25 µl final volume of deionized water. The Tetra ARMS-PCR technique is an extension of the original concept and has been applied to improve the detection of specific genetic mutations in a fast and cost-effective manner. The system relies on designing four primers in a single reaction: two external primers to amplify a large target region, and two internal primers specifically designed to amplify alleles or specific mutation (15). The (Rs3136520 F2gene) are shown in table 1.

Table 1. Tetra-ARMS-PCR for the *F2* gene in the present study.

	(Rs3136520 F2gene)		
	Temperature C°	**Time sec.**	**cycle**
Initial denaturation	95	120	1
Denaturation	95	40	35
Annealing	56	30	
Extension	72	45	
Final extension	72	300	1

The PCR products were analyzed in 2% agarose gel stained with ethidium bromide and with a 100-base pair (bp) DNA ladder as the template of measurement. The sizes of the PCR products as related to rs3136520 SNP in F2 gene were 134 bp + 278 bp for CC genotype, 200 bp + 278 bp for TT genotype and 134 bp + 200 bp + 278 bp for CT genotype. The PCR products are shown in figure 1.

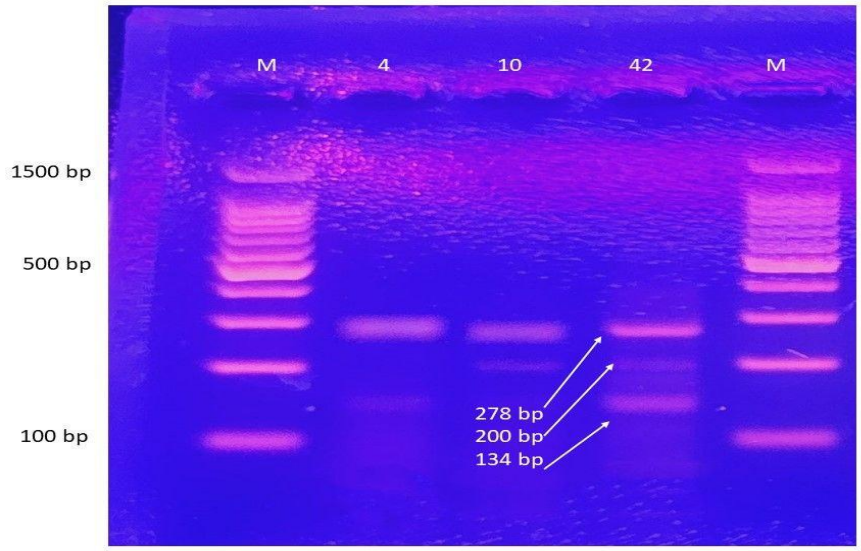

Figure 1. ARMS-PCR products of rs3136520 SNP in F2 gene, CC genotype (wild-type) : 134 bp+278 bp (sample 4), CT genotype (heterozygous mutant) : 134 bp+ 200 bp + 278 bp (sample 42) and TT genotype (homozygous mutant) : 200 bp + 278 bp (sample 10). M: 100 bp DNA ladder.

F13A1 gene

Genetic polymorphisms of each participant for rs1050782 in F13A1 gene were determined by high resolution melting (HRM). HRM is a new, post-PCR analysis method used for identifying genetic variation in nucleic acid sequences and can discriminate DNA sequences based on their composition, length, GC content, or strand complementarities. A Rotor gene (Qiagen) was employed to perform qPCR-HRM, following a program in table 3 , followed by an HRM analysis with ramping by 0.2 °C from 65 to 95 °C. The cycling protocol was programmed for the following optimized cycles and according to the thermal profile shown in table 2.

Table 2. Thermal profile of HRM genotyping.

Step	Temperature (ºC)	Duration	Cycles
Enzyme activation	95	5 min	1
Denaturation	95	15 sec	40
Annealing	60	15 sec	40
Extension	72	30 sec	1
HRM	65-95	0.2 degree for 1 sec	1

Statistical analysis

The statistical analyses were performed using IBM SPSS Packages for social sciences – version 29. The comparison of genotypes and allele frequencies between RSA patients and

controls were calculated by X^2 test or Fisher's exact test. To show the effects of the studied polymorphisms on RSA, odds ratios (OR) with a 95% confidence interval were calculated.

Results

Means of age and body mass index (BMI) for recurrent spontaneous abortion (RSA) patients *versus* apparently healthy women from Al-Najaf city in Iraq are presented in table 3.

Table 3. Mean age and BMI values in recurrent spontaneous abortion women *versus* apparently healthy women in the present study.

Parameters	Control [1]	RSA[2] patients	p-value
Age (year)	32.84± 1.00	33.06 ± 1.06	0.88 NS
BMI	28.00 ± 0.49	27.93 ± 0.66	0.93 NS

[1] apparently healthy subject. [2] recurrent spontaneous abortion

The means of platelets count, prothrombin time and partial thromboplastin time for patients with recurrent spontaneous abortion *versus* apparently healthy subjects are presented in table 4.

Table 4. Mean platelets count (PT)and (PTT) in (RSA)women *versus* apparently healthy women in the present study

Parameters	Control [1]	RSA [2] patients	p- value
Platelets count (x 1000)	269.3 ± 7.9	260.7 ± 10.2	0.51 NS
Prothrombin time (sec.)	13.9± 0.24	14.6 ± 0.18	0.022 *
Partial thromboplastin time (sec.)	32.0 ± 0.58	34.9 ± 0.65	0.001 **

[1] apparently healthy subject. [2] recurrent spontaneous abortion

Table 5. Genotypes and allele frequencies for coagulation factor II (F2) gene at rs3136520 SNP in recurrent spontaneous abortion patients *versus* apparently healthy subjects.

Genotypes Rs3136520	Control[1] n (%)	RSA[2] Patients n(%)	*Chi square* (X^2)	Odd Ratio (95% CI)	*p*- value
CC	19 (38%)	20 (40%)	0.026	Ref.	-
CT	28 (56%)	30 (60%)	0.069	1.01 (0.45-2.29)	0.96
TT	3 (6%)	0 (0%)	1.80	0.13 (0.00 – 2.80)	0.19
Allele frequency					
C	0.66	0.70	0.118	Ref.	-
T	0.34	0.30	0.250	0.83 (0.45- 1.50)	0.54

[1] apparently healthy subjects. [2] recurrent spontaneous abortion.

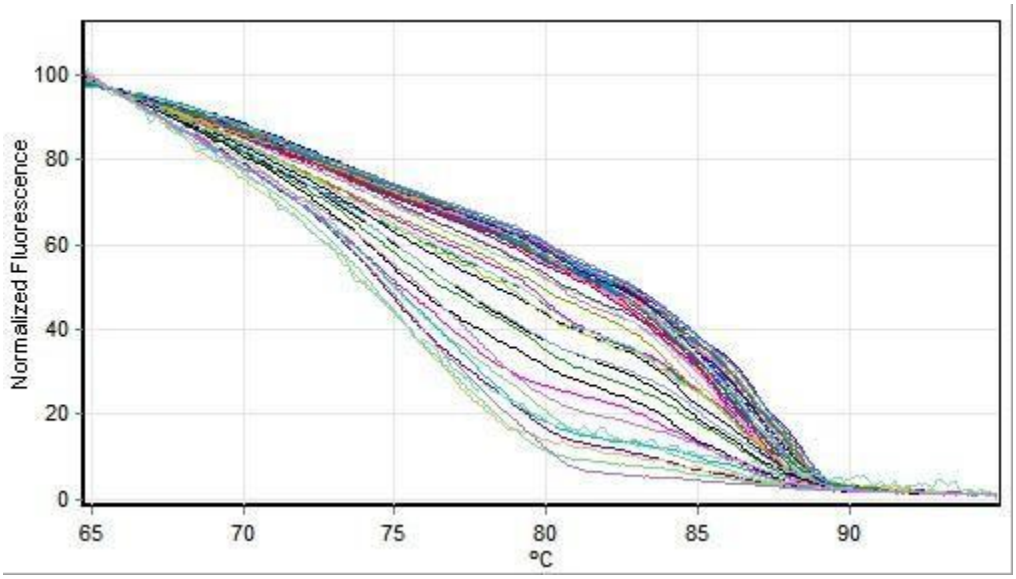

Figure 2. Normalized flourecence intensity curves generated by HRM for determination of *F13A1* gene genotypes at rs1050782 SNP.

Table 6. Genotypes and allele frequencies for coagulation factor F13A1 gene at rs1050782 SNP in recurrent spontaneous abortion patients *versus* apparently healthy subjects.

Genotypes Rs1050782	Control[1] n (%)	RSA[2] Patients n (%)	*Chi square* (X^2)	Odd Ratio (95% CI)	*p*- value
GG	5 (10%)	31 (62%)	18.77	Ref.	-
GA	21 (42%)	10 (20%)	3.90	0.076 (0.02-0.26)	0.0001
AA	24 (28%)	9 (18%)	6.81	0.60 (0.02- 0.20)	0.0001
Allele frequency					
G	0.31	0.72	16.32	Ref.	-
A	0.69	0.28	17.33	0.17 (0.10-0.32)	0.0001

[1] apparently healthy subject. [2] recurrent spontaneous abortion.

Discussion

No significant differences in age and BMI were noted between RSA patients and apparently healthy women in the present study which were 33.06 and 32.84 years old, whereas for BMI, were 27.93 and 28 kg/m^2 , respectively.

The means of platelets count, prothrombin time and partial thromboplastin time for patients with recurrent spontaneous abortion *versus* apparently healthy subjects are presented in table 4.

No significant differences between the study groups as related to the mean platelets count. Prothrombin time mean was significantly ($p<0.05$) increased in patients with recurrent spontaneous abortion when compared with controls (14.6 ± 0.18 *versus* 13.9 ± 0.24 seconds, respectively; $p<0.05$). Also, the mean of partial thromboplastin time was

significantly ($p<0.01$) increased in patients with recurrent spontaneous abortion when compared with controls (34.9 ± 0.65 versus 32.0 ± 0.58 seconds, respectively; $p<0.01$).

Platelets are a component of blood whose function along with the coagulation factors is to react to bleeding from blood vessel injury by clumping, thereby initiating a blood clot. They are activated rapidly to provide the initial response to the vascular injury (16).

Platelet counts represent the quantity of platelets in the bloodstream, which are crucial for the formation of blood clots and the healing of wounds (18).

Deviation from normal values in these parameters can indicate different diseases such as coagulation disorders, thrombocytopenia, or other abnormalities related to blood, which may necessitate specialized treatment to ensure positive results during pregnancy (19). The present study stated that there was no statistically significant difference for Pletelate count count between the two groups. This agrees with (.20) found that there is no significant difference in platelets count in women with recurrent miscarriage. Also, (21) conducted a prospective study based on the comparison of 74 patients with unexplained recurrent first-trimester pregnancy loss with 208 control subjects matched for age. The two groups were compared in terms of platelet indices and found that there was no significant difference for platelets count between the two groups. In contrast, a study conducted on 208 patients who experienced 2 or more first trimester spontaneous abortions and 95 controls who had no abortions showed that the platelets count was significantly higher in patients with recurrent pregnancy loss than in controls. Also, (23) study which was conducted on 45 women with a history of RPL and 45 women who gave birth without recurrent pregnancy loss disagrees with our study and found that there was significant difference in platelets count in women with recurrent pregnancy loss.

Prothrombin time and platelet counts are essential factors for assessing the state of coagulation. Prothrombin time is a measurement of the duration it takes for plasma to form a clot after the introduction of tissue factor. It is utilized to assess the extrinsic and common pathways of coagulation (24). In the present study, the prothrombin time was significantly ($p<0.05$) increased in patients with recurrent spontaneous abortion compared with controls (14.6 ± 0.8 versus 13.9 ± 0.24, respectively). This result is in agreement with Nasr *et al.* 25(2021) found that values of prothrombin time revealed highly significant increase in patients with recurrent miscarriage compared to control (19.9 ± 6.1 *versus* 14.0 ± 2.1, for recurrent miscarriage patients and contr differences in prothrombin time values ols, respectively). Whereas, in Japan, (26) found no significant between patients with recurrent fetal loss and controls. In the present study, the partial thromboplastin time was significantly ($p<0.01$) increased in patients with recurrent spontaneous abortion compared with controls (34.9 ± 0.65 *versus* 32.0 ± 0.58, respectively). This result is in agreement with Nasr *et al.*25 (2021) reported partial thromboplastin time values significantly higher in patients with recurrent miscarriage compared with control. (27) in Turkey found prolonged activated partial thromboplastin time in recurrent miscarriage patients. Our results opposite to study done by (28) in Indian

population which revealed that significant shortened in mean activated partial thromboplastin time values of patients with recurrent abortions (27.01 and 31.01 for patients and control, respectively). While, (26) in Japan, observed a shortened APTT before conception is associated with further miscarriages.

DNA was extracted from the fresh blood samples of RSA patients and apparently healthy subjects. DNA Concentration (ng/µl) =40-60, purity (1.7- 1.9) .

The present study investigates the genetic variation of *F2* gene (rs3136520) on recurrent spontaneous abortion risk in a group of women from Al-Najaf city, Iraq. Our finding shows no significant difference between RSA patients and apparently healthy women in term of allelic frequencies and genotyping distribution for all analyzed polymorphisms as shown in table 8. There was an association between the incidence of recurrent spontaneous abortion and the CT genotype of rs3136520 in *F2* gene in Iraqi patients from Al-Najaf city (Odd ratio = 1.01).

The F2 gene provides instructions for making a protein called prothrombin (also called coagulation factor II). Coagulation factors are a group of related proteins that are essential for normal blood clotting (hemostasis). After an injury, clots protect the body by sealing off damaged blood vessels and preventing further blood loss. Prothrombin is made chiefly by cells in the liver. The protein circulates in the bloodstream in an inactive form until an injury occurs that damages blood vessels. In response to injury, prothrombin is converted to its active form, thrombin. Thrombin then converts a protein called fibrinogen into fibrin, the primary protein that makes up blood clots (6)

The results related with rs3136520 of F2 gene in the present study are in agreement with the results of (29) who found no relationship between rs3136520 of F2 gene and recurrent pregnancy loss in Iranian Azeri women. Also, the results of the present study are in accordance with the results (6) who showed that TT genotype and T allele of rs3136520 were significantly lower in women with RSA than in controls and may act as the protective factor to decrease susceptibility to RSA, but the results were no significant in this study. In contrast (6) suggested that rs3136520 may have a significant association with the genetic.

susceptibility of RSA in Chinese Han women. The results of the present study that related to the role of rs3136520 in RSA susceptibility required further large-scale studies to be confirmed.

The detection of rs1050782 SNP in *F13A1* gene was achieved by using HRM real-time PCR. High-resolution melting (HRM) analysis isa method for genotyping, sequence and mutation scanning in DNA samples (**30**). Normalized flourecence intensity curves generated by HRM analysis for determination of *F13A1* gene genotype at rs1050782 SNP are shown in figure 2.

The results of genotypes and allele frequencies for coagulation factor F13A1 gene at rs1050782 SNP in recurrent spontaneous abortion patients *versus* apparently healthy subjects are presented in table 8.

The percentage of GG genotype was significantly ($p<0.01$) higher in RSA patients than in apparently healthy subjects (62% *versus* 10%, respectively), therefore, GG genotype

may represent as a risk factor for RSA susceptibility in Iraqi women from Al-Najaf city. Also, GA and AA genotypes and A allele of rs1050782 in *F13A1* gene were significantly ($p<0.01$) lower in women with RSA than in controls and may act as a protective factor to decrease susceptibility RSA (42%, 28% and 69% of control group *versus* 20% , 18% and 28% of RSA group, for GA, AA genotypes and A allele, respectively). In contrast, **(6)** found tin Chinese Han women that AG and GG genotypes and G allele of rs1050782 may be associated with decreased risk of RSA.

This discrepancy in results may be attributed to differences in sample size and ethnicity. Coagulation factor XIII is known as the last zymogen and formerly named fibrin-stabilizing factor that could stabilize the fibrin clot after series of actions (31). Factor XIII consists of 2 A subunits and 2 B subunits. The A subunits were encoded by theF13A1 gene and possess catalytic ability, the B subunits may only act as carriers (32). (6) suggested that rs1050782 may have a significant association with the genetic susceptibility of RSA in Chinese Han women. In addition, (6) reported that rs1050782 located in the 3' UTR of F13A1 gene , may act as a functional site influence the expression of F13A1.

Conclusion

This study suggests that the GG genotype of rs1050782 in the F13A1 gene may be associated with an increased susceptibility to recurrent spontaneous abortion (RSA), while the A allele may have a protective role in Iraqi women. In addition, prolonged prothrombin time (PT) and partial thromboplastin time (PTT) were observed in RSA patients. Further studies with larger sample sizes are recommended to validate these findings.

References

1. Pandey D, Gupta S. (2019). Current update on recurrent pregnancy loss. Journal of Basic and Clinical Reproductive Sciences.; 8(1):1-6.
2. Homer HA. (2019). Modern management of recurrent miscarriage. Australian and New Zealand Journal of Obstetrics and Gynaecology.; 59(1):36-44. https://doi.org/10.1111/ajo.12920
3. Lefta, S.F. and Kadhim, A. (2022). Association of Pregnancy Loss with Breast Cancer in Babil Governorate's Women. Medical Journal of Babylon, 19(1), pp.15-20.
4. Alireza Isazadeh*, Saba Haj Azimian, Nazila Tariverdi, Seyed Ali Rahmani, Maryam Esmaeili, Samaneh Karimkhanilouei and Milad Mohammadoo-Khorasani. (2017). Effects of coagulation factor XIII (Val34Leu) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Received January 28; accepte March 17, 2017.
5. MERVAT EL ANSARY, M.D.*; RASHA SAYED, M.D.*; MARIAM DAWOUD, M.D.**; HEND TAMIM, M.D.* and IRENE BISHAI, M.DMed. J. Cairo Univ., Vol. 90, No. 7, December: 2325-2332, 2022 www.medicaljournalofcairouniversity.net Thrombophilic Gene Mutation and Correlation to Recurrent Miscarriage: An Egyptian Study.
6. Zuying Xu, MD, Ying Zhang, MD,Wei Liu, MD, Yunyun Liu, MD,Yezhou Su, MD, Qiong Xing, PhD, Xiaojin He, PhD,Zhaolian Wei, PhD, Yunxia Cao, PhD and Huifen Xiang, PhD,. 2018.Polymorphisms of F2, PROC, PROZ, and F13A1 Genes are Associated With Recurrent Spontaneous Abortion in Chinese Han Women.
7. Abd, R.M.; Al-Azzawie, H.F. and Al-Kazzaz, K. (2016).Genetic polymorphisms of HLA-G antigen in Iraqi women with unexplained recurrent miscarriage. Int. J. Advan. Res., 4(11): 1654-1663.
8. Abd, R.M. (2017). HLA-E polymorphism in Iraqi women with unexplained recurrent

spontaneous miscarriage (URSM). Current Res. Microbiol. Biotech., 5(6): 1345-1349.

9. Farhan, S.H.; Abdulhassan, I.A. and Abdul-Rahman, R. (2020). Gene expression and single-nucleotide polymorphism (rs1042838) of progesterone receptor gene in Iraqi females with recurrent spontaneous abortion (RSA). Int. J. Psychosocial Rehab., 24(10): 3043-3050.
10. Farhan, S.H. and Abdulhassan, I.A. (2021). The association of gene expression and single nucleotide polymorphism (rs6152 SNP) in androgen receptor gene with recurrent spontaneous abortion (RSA) in Iraqi women. Medico-Legal Update, 22(2): 1126-1132.
11. Baqer, N.N.; Saheb, E.J. and Ahmed, N.S. (2021). Genetic polymorphism of IL-17A (rs2275913) in Iraqi women with recurrent abortion and its relationship with susceptibility to toxoplasmosis. Meta gene,29: 100939.
12. Jassim, H.A.; Kadhim, S.J. and Alhubbobi, H.M. (2021). The association of prolactin receptor gene polymorphism with recurrent miscarriage in a sample of Iraqi women.Sys. Rev. Pharm. , 12(1): 176-178.
13. Alfaisal, A.H.M.; Nada, S.M. and Mahood, R.A. (2015). Evaluation of homocysteine, folic acid and methylenetetrahydrofolate reductase C677T gene polymorphism associated with recurrent spontaneous miscarriage. J. Wassit Sci. Med., 8(3): 38-47.
14. Farhan, S.H.; Abdulhassan, I.A. and Mohammed, N. (2020). Association of polymorphism rs1264459 in the HLA-E gen promoter with incidence of RSA andits consequences to steroid hormones in Iraqi women. Iraqi J. Biotech., 19(3): 9-15.
15. Ye, Sahar Dhillon, Xiayi Ke, Andrew R. Collins, Ian N. M. Day Nucleic Acids Research, Volume 29, Issue 17, 1 September (2001). An efficient procedure for genotyping single nucleotide polymorphisms Page e88, https://doi.org/10.1093/nar/29.17. Published: 01 September 2001.
16. Jurk K, Kehrel BE. (2022). Platelets and Their Role in Hemostasis and Thrombosis—From Physiology to Pathophysiology and Therapeutic Implications. Biomolecules.; 12(11):1590.
17. Machlus KR, Thon JN and Italiano JE. (2014). Interpreting the developmental dance of the megakaryocyte: a review of the cellular and molecular processes mediating platelet formation. British Journal of Haematology.,165 (2): 227–36.
18. Gonsoulin WJ Jr, Silver RM, eds. (2018). Early Pregnancy Loss: Mechanisms and Treatment. Springer.
19. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller AB, Daniels J, et al. (2014). Global causes of maternal death: a WHO systematic analysis. Lancet Glob Health. ; 2(6):e323-e333.
20. Dundar o. (2014). Mıne Kanat Pektas, Serkan Bodur, Lale Vuslat Bakır and Ahmet Cetin. Recurrent pregnancy loss is associated with increased red cell distribution width and platelet distribution width. J. Obstet. Gynaecol. Res.
21. Ural Mete Ü, Bayoğlu TY, Balik G, Kir ŞF and Çolak S. (2014). Could platelet distribution width be a predictive marker for unexplained recurrent miscarriage? Archives of Gynecology and Obstetrics.; 290 (2): 233–236.
22. Aynıoglu O, Isık H, Sahbaz A, Harma M I, Isık M, Kokturk F. (2014). Can Plateletcrit be a Marker for Recurrent Pregnancy Loss? Clinical and Applied Thrombosis/ Hemostasis.; 22 (5), 447–452.
23. Al Aghbary A, Mohammed AA, Dikra WM, Waiel MA. (2018). Platelet indices in evaluation of patients with recurrent pregnancy loss. Asian Pacific Journal of Reproduction.; 7: 15-18.
24. ACOG Practice Bulletin No. 128. (2012). Diagnosis of abnormal uterine bleeding in reproductiveaged women. Obstet Gynecol.; 120(1): 19 7-206.
25. Nasr MGM, Merghani MM, Babiker NE. (2021). Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) Level among Sudanese Women with Recurrent Miscarriage. Journal of Drug Delivery and Therapeutics.;11(6-S):128–131. doi:10.22270/jddt.v11i6-S.5257.
26. Ogasawara M, Aoki K, Katano K, Aoyama T, Ozaki Y, Suzumori K. (1998). Activated partial thromboplastin time is a predictive parameter for further

miscarriages in cases of ecurrent fetal loss. Fertility and Sterility.; 70(6):1081-1084.

27. Karaca N, Aktün LH. (2018). Evaluation of Factor XII Activity in Women with Recurrent Miscarriages.Bezmialem Science.; 6(4):279-82.
28. Ali A, Mohan P, Kareem H, Muhammed MK. . (2016). Elevated Factor VIII Levels and Shortened APTT in Recurrent Abortions. Journal of Clinical and Diagnostic Research.; 10(1): EC04-EC06.
29. Yousefian, Mahsa ; Seyed Abdolhamid Angaji ;, Elham Siasi ;Seyed Ali Rahmani ;Shamci Abbasalizadeh Khiaban (2021). Association of Polymorphisms of F2 (rs3136520) and PAI1(rs6090) Genes with Recurrent Pregnancy Loss in Iranian Azeri Women. Avicenna Journal of Clinical Medicine, 28: 118-125.
30. Wittwer, C. T., Reed, G. H., Gundry, C. N., Vandersteen, J. G., & Pryor, R. J. (2003). High-resolution genotyping by amplicon melting analysis using LCGreen. Clinical chemistry, 49(6), 853-860.
31. Rijken DC, Uitte de Willige S. (2017). Inhibition of fibrinolysis by coagulation factor XIII. Biomed Res Int.; 2017:1209676.
32. Lorand L. (1986). Activation of blood coagulation factor XIII. Ann N Y Acad Sci.; 485:144-158.