



GENETICS AND CYTOLOGY

INTERNATIONAL JOURNAL DEVOTED TO GENETICAL
AND CYTOLOGICAL SCIENCES

Published by

THE EGYPTIAN SOCIETY OF GENETICS

Volume 55

July 2026

No. 2

ASSOCIATION BETWEEN SPECIFIC THROMBOPHILIA- RELATED GENE POLYMORPHISMS AND RECURRENT PREGNANCY LOSS IN EGYPTIAN COHORT

ABDELRAHMAN A. ABDELRAHMAN^{1*}, ASHRAF M. KONSOUH²,
MANAL EL-HAMSHARY¹

¹ Molecular Diagnostics and Therapeutics Department, Faculty of Biotechnology, University of Sadat City, Egypt.

² Obstetrics & Gynecology Department - Shebin El-Kom Teaching Hospital, Shebin El-Kom, Egypt.

***Corresponding author:** Abdelrahman A. Abdelrahman, PhD, Genetic Engineering and Biotechnology Research Institute, Department of Molecular Diagnostics and Therapeutics, University of Sadat City, Egypt. Tel: +2-01010733444 Email: abdelrahman.abdelhalim@gebri.usc.edu.eg ORCID: 0000-0003-3835-3082

Keywords: Gene polymorphisms; recurrent pregnancy loss; FVL 1691AA; FV H1299R; FII 20210A polymorphisms; thrombophilia.

Recurrent pregnancy loss (RPL) has historically been defined as three or more miscarriages before 24 weeks of gestation, also known as recurrent miscarriage (RCOG, 2011). The European

Society of Human Reproduction and Embryology (ESHRE) updated this definition in 2018, following modifications by the American Society for Reproductive Medicine in 2013 and

again in 2018. RPL is now defined as two or more unsuccessful pregnancies before 24 weeks of gestation.

This revised definition is anticipated to facilitate research, collaborative decision-making, and psychological support for couples. Additionally, following two losses, testing for antiphospholipid syndrome (APS), the most prevalent treatable cause of RPL, may be beneficial (Practice Committee of the American Society for Reproductive Medicine, 2013 & ESHRE Guideline Group on RPL, 2018).

Worldwide, the prevalence of RPL in married couples is estimated at 1%–5%. The etiology involves multiple elements, such as chromosomal abnormalities in the parents, uterine structural abnormalities, immunological issues, and endocrine disorders. Routine gynecological and laboratory tests, however, frequently fall short of identifying the root cause (RCOG, 2011; Practice Committee of the American Society for Reproductive Medicine, 2013 and ESHRE Guideline Group on RPL, 2018).

In up to 40% of cases, especially in early RPL, thrombophilia is the primary cause of the disorder (Brenner *et al.*, 1999). There is an ongoing debate over the role of specific thrombophilic genes in the pathogenesis of RPL. A class of hereditary blood coagulation disorders known as hereditary thrombophilias causes hypercoagulability, which can lead to improper placentation. This may

manifest as spontaneous pregnancy loss in the early stages of pregnancy (Younis *et al.*, 1997 and Brown, 1991). Recent years have seen several studies looking into the frequency of particular thrombophilic gene polymorphisms in women experiencing unexplained pregnancy loss. While some of these investigations found evidence of a correlation between RPL and polymorphisms in thrombophilic genes (Sarig *et al.*, 2002 & Rey *et al.*, 2003), others did not (Carp *et al.*, 2002).

Early RPL risk is increased by the presence of the FII 20210A or FVL 1691A gene polymorphism; the odds ratios (ORs) for FII G20210A and for homozygous and heterozygous carriers of FVL gene are 2.49, 2.71, and 1.68, respectively (Robertson *et al.*, 2006). According to the EPCOT study, women with combined thrombophilic abnormalities have the highest chance of stillbirth (but not miscarriage) (Girolami *et al.*, 2005). However, research into even minor thrombophilic polymorphisms in search of evidence of a connection to recurrent miscarriages has produced inconsistent findings (Hohlagschwandtner *et al.*, 2003). Furthermore, because these conditions confer a minor absolute risk of clinically significant thrombosis, the majority of polymorphism carriers will not exhibit clinical signs and will go undetected. However, the chance of potentially fatal thrombotic events increases dramatically. Clinical signs may appear in carriers who are exposed to other risk factors, such as pregnancy or

oral contraceptives (Martinelli *et al.*, 1998 and Rosendaal *et al.*, 1995). The majority of carriers are asymptomatic; hence, the diagnosis is typically overlooked. Crucially, early research on thromboprophylaxis in pregnant carriers indicates that medication may considerably improve pregnancy outcomes (Brenner *et al.*, 2000 and Younis *et al.*, 2000).

The β -fibrinogen gene (FGB) is a thrombophilic gene located at 4q31.3 and encodes the β -chain of fibrinogen, a coagulation factor. Thrombin breaks down fibrinogen, leading to the formation of fibrin and blood clots in response to tissue injury. Additionally, it plays a role in the formation of new blood vessels and the narrowing of blood vessels. Plasma fibrinogen levels may be increased by 7% to 10% in individuals with the common polymorphism 455G/A (rs1800790) of the β -fibrinogen gene, due to a G/A change in the promoter region (Tybjaerg-Hansen *et al.*, 1997 and Ticconi *et al.*, 2011). Gene expression is influenced by two allelic polymorphisms containing either four or five guanine repeats (4G/5G), which result from a polymorphism in the promoter region of the PAI-1 gene. The 4G polymorphism increases the risk of thrombosis by increasing PAI-1 expression and decreasing fibrinolysis (Djordjevic *et al.*, 2013; Zhao *et al.*, 2013 and Chen *et al.*, 2015).

Recent studies have examined gene polymorphisms in factor XIII (FXIII) in connection with recurrent

pregnancy loss (RPL). FXIII is an integral part of the fibrinolytic system and forms covalent bonds between the alpha and gamma chains of fibrin (Board *et al.*, 1993; Mikkola *et al.*, 1996 and Balogh *et al.*, 2000). The Val34Leu variation in the FXIII gene (V34L) is commonly associated with thrombotic events (Balogh *et al.*, 2000). This variation alters the structure of blood clots, making them more resistant to fibrinolysis due to enhanced dimerization, polymerization, and FXIII activation (Balogh *et al.*, 2000; Kreutz *et al.*, 2014 and Dossenbach-Glaninger *et al.*, 2013).

The present study aims to investigate the association between unexplained recurrent pregnancy loss (RPL) and common polymorphisms in the FVL, PAI-1 4G/5G, and β -fibrinogen genes.

PATIENTS AND METHODS

Study design and populations

Between April 2026 and May 2026, 200 participants were enrolled at Shebin El-Kom Teaching Hospital and divided into two groups. The control group included 100 pregnant women who had no prior history of abortion and parenting children by natural pregnancy. The second group, referred to as the patient group (RPL group), consisted of 100 women who had experienced two or more recurrent miscarriages and did not have any anatomical, infectious, or endocrine disorders, and had unremarkable hormonal, immunological,

hematological, and chromosomal findings. Informed consent was obtained from all participants included in this study.

Methods

DNA extraction:

Genomic DNA was extracted using the GeneJET™ Genomic DNA Purification Kit (Cat # K0721, K0722-Thermoscientific-Thermo Fisher) according to the manufacturer's instructions. The concentration of the extracted DNA was measured using Thermo Scientific NanoDrop 2000c Spectrophotometer | GMI, at wavelengths of 260 as well as 280 nm and the concentration was ranged from 20-30 ng/μL of DNA. CVD StripAssay®-CE/IVD-Cat#: 4-240) kit- from VienneLab® was used for the molecular detection of FV Leiden (G1691A), β-fibrinogen -455 G>A, and PAI-1 4G/5G gene polymorphisms, following the manufacturer's instructions.

Multiplex PCR Amplification:

The reaction master mix included multiplex biotinylated primers, and amplification was performed using a thermal cycler (Techne 5Prime, UK). The reaction components were subjected to an initial denaturation at 94°C and 35 cycles of denaturation, at 95°C for 15 s., annealing at 58°C for 45 s. and extension for 60 s.

Reverse hybridization:

The amplified products were hybridized to the StripAssay kit (Vienna Lab Diagnostics, Austria), which comprised allele-specific oligo-nucleotide probes (wild-type and mutant-specific) fixed on a strip of parallel lines (Fig. 1). The bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and chromogenic substrates. The observed enzymatic color reaction identified the mutant and wild-type alleles.

Statistical Analysis: Statistical analysis was performed using the IBM SPSS software package version 29.0 (IBM Corp., Armonk, NY, USA) and SNPStats software. The age and body mass index of the studied groups were presented as mean ± standard deviation. The frequencies of qualitative variables were expressed as percentages. Hardy-Weinberg equilibrium was assessed by the chi-square test. The prevalence of gene polymorphisms was compared between study groups using Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using logistic regression. Haplotype frequencies and association with outcome were determined using SNPStats software. P-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

In recent years, there has been increasing interest in identifying the possible role of thrombophilic and

hypofibrinolytic genes in susceptibility to RPL in different ethnic groups and populations (McNamee *et al.*, 2012 and Farahmand *et al.*, 2016). This may help reduce the widespread use of therapeutic anticoagulants as a prophylactic regimen in cases of RPL with an unidentified cause. Based on these findings, we were encouraged to investigate the common polymorphisms of the FV Leiden 1691A (rs6025), β -fibrinogen 455G/A (rs1800790), and PAI-1 4G/5G (rs1799889) genes and their association with RPL. Genotype frequencies of included polymorphisms conformed to Hardy-Weinberg equilibrium in the control group, supporting population stability and the validity of the association.

Two hundred women were enrolled in the current study and divided into two groups: group 1 included 100 women with RPL, and group 2 included 100 healthy women with no history of abortion. There was a non-significant difference between the two groups regarding age, BMI, and number of live births. Among the RPL group, 64 (64.0%) showed two miscarriages, while 36 (36.0%) had three or more miscarriages. Table (1) shows the demographic data of the women population enrolled in the study.

The FVL 1691G>A gene polymorphism is a G-to-A point mutation at nucleotide 1691 in the factor V gene, which results in the single amino-acid replacement Arg506Gln, leading to

resistance to cleavage and, therefore, to inactivation by APC, thereby increasing susceptibility to clotting (Rosendaal *et al.*, 1995 and Ridker *et al.*, 1995). This polymorphism enhances the risk of venous thrombosis up to 50-100 times in homozygous carriers (Rodeghiero and Tosetto, 1999).

Regarding FV Leiden 1691A gene polymorphism, the GG genotype frequency was higher in women with RPL than in the control group (47% vs. 31%, $P = 0.048$), with a significant increase in G allele frequency in the RPL group compared with the control group (0.66 vs. 0.54, $P = 0.011$). In the current sample, the risk for RPL was insignificantly associated with the recessive model of FV Leiden 1691A gene polymorphism ($P = 0.101$), and it was 0.51-fold lower with the dominant model, which was statistically significant ($P = 0.02$) as shown in Table (2). These findings indicated that FV Leiden had a substantial correlation with RPL. Case-control studies and meta-analyses have demonstrated that women with RPL have a high prevalence of FVL gene (Rey *et al.*, 2003; Reznikoff-Etiévan *et al.*, 2001 and Grandone *et al.*, 1997).

A systematic analysis of the literature indicated that RPL and FVL gene had an OR of 2 (Ivanov *et al.*, 2009 and Yousefian *et al.*, 2014). Rey and colleagues (2003) found that FVL gene was associated with both late nonrecurrent fetal loss and early as well as late RPL in a meta-analysis. According

to the report by Reznikoff-Etiévant *et al.*, (2001), 10.3% of patients (260 Caucasian women) who had two or more pregnancy losses before the 10th week of gestation were positive for FVL gene polymorphism, whereas 4.6% of controls showed the same polymorphism, indicating a significant association between FVL gene and RPL. Moreover, Grandone *et al.*'s study (1997) also supported this finding. In contrast, Ivanov *et al.*'s study (2009) included 94 women who had experienced embryonic loss before 10 weeks of gestation and 59 women who had lost a pregnancy between 10 and 14 weeks of gestation. These women were compared with 100 healthy women who had previously experienced at least one uncomplicated full-term pregnancy. The results showed that there was no significant correlation between FVL gene prevalence and pregnancy loss before 10 weeks of gestation (9.6%) compared with the control group (7%). However, there was evidence of an association in women who had experienced postembryonic loss (10-14 weeks of gestation), with an FVL gene polymorphism prevalence of 18.6%. Such contradictory results may be attributed to ethnic differences and the prevalence of this polymorphism in different populations. Other factors that cannot be ignored were related to this study design, the definition of recurrent pregnancy loss, whether based on two or three losses before the 24th week of gestation, and selection criteria for the studied cases. Other genetic factors and/or epigenetic modulations may have had an impact.

Regarding β -fibrinogen 455G/A gene polymorphism, the AA genotype frequency was higher in women with RPL than in the control group (5% vs. 4%, $P = 0.88$), with a non-significant increase in A allele frequency in the control group compared with the RPL group (0.25 vs. 0.24, $P = 0.907$). In the current sample, the risk for RPL was non-significantly low with the recessive model of β -fibrinogen 455G/A gene polymorphism ($P = 0.733$), and it was 0.92-fold with the dominant model, which was statistically insignificant ($P = 0.776$) as shown in Table (3).

These results mean that there is no correlation between RPL and the genotypes or alleles of the β -fibrinogen 455G/A gene polymorphism. A survey of the literature revealed a dearth of research on the relationship between the β -fibrinogen gene polymorphism and RPL risk, particularly in Arab nations. In general, the majority of research has not discovered any significant correlation between various genetic models of the β -fibrinogen 455G/A polymorphism and RPL, particularly in Asian and Caucasian subgroups (Attili *et al.*, 2019; Al-Astal *et al.*, 2014 and Li *et al.*, 2015). This could be due to gene-gene and gene-environment interactions or other polymorphisms of β -fibrinogen that might obscure the role of the β -fibrinogen gene. These findings contradicted findings from other studies (Torabi *et al.*, 2012 and Karami *et al.*, 2018).

Regarding PAI-1 4G/5G gene polymorphism, the 4G/4G genotype frequency was higher in women with RPL than in the control group (49% vs. 31%, $P = 0.030$), with a significant difference in 4G allele frequency between the control group and the RPL group (0.56 vs. 0.68, $P = 0.009$), as shown in Table (4).

These findings revealed a strong correlation between RPL and PAI-1 4G/5G gene polymorphisms, which concurred with the findings of earlier meta-analyses. For instance, while patients with homozygous 4G polymorphisms were much more likely to experience RPL compared with healthy controls, Khosravi *et al.*, (2014) found a link between PAI-1 gene and RPL and implantation failure (IF) in the Iranian population. A systematic review and meta-analysis reported that subgroup analysis showed a significantly elevated susceptibility to RPL in Asians, Caucasians, and Africans (Huang *et al.*, 2017). Consistent with this, our study showed that the PAI-1 4G/5G gene polymorphism probably provides a genetic contribution to the emergence of RPL. On the other hand, Su *et al.* published a systematic review and meta-analysis of 11 studies showing that there was high clinical heterogeneity among studies of PAI-1 4G/5G, and the aggregated data failed to show higher susceptibility to idiopathic RPL in Caucasian and non-Caucasian patients (Su *et al.*, 2013). A prior systematic review and meta-analysis found a significant

association between the PAI-1 4G/5G polymorphism and the risk of RPL under the recessive model ($OR = 1.70$, 95% $CI = 1.21-2.38$). However, in contrast to our findings, no significant association between the PAI-1 4G/5G polymorphism and RPL was reported in this study (Shi *et al.*, 2017). The link between PAI-1 4G/5G gene polymorphisms and RPL could be explained by a hypofibrinolytic state, which results from the PAI-1-675 4G polymorphism, which is linked to gene overexpression. PAI-1 is upregulated throughout implantation to alter trophoblast cells and stop hemorrhage during placentation. The most plausible explanation is that 4G alleles (including 4G/5G and 4G/4G) can increase PAI-1 gene expression, preventing fibrin lysis that causes placental thrombosis and limiting trophoblastic migration by regulating proteolysis and maternal tissue modification, ultimately leading to abortion (Levin *et al.*, 2020).

List of abbreviations: RPL: Recurrent pregnancy loss; FVL: Factor V Leiden; PAI-1: Plasminogen activator inhibitor-1; FGB: β -fibrinogen gene; APS: antiphospholipid syndrome

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Shebin El-Kom Teaching Hospital (HSH000128) and was performed in accordance with the relevant guidelines and regulations of the Shebin El-Kom Teaching Hospital. Informed

consent was obtained from all individual participants for inclusion in this study.

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Conflicts of interest: The authors declare no conflicts of interest.

Financial support and sponsorship: None.

Authors' contributions: Manal El-Hamshary designed the study. Ashraf M. Konsouh and Abdelrahman A. Abdelrahman collected and extracted data. Abdelrahman A. Abdelrahman performed the *in vitro* and *in vivo* experiments. Abdelrahman A. Abdelrahman and Manal El-Hamshary analyzed and interpreted the data. Manal El-Hamshary and Abdelrahman A. Abdelrahman critically revised the manuscript. All authors read and approved the final manuscript.

. In the final conclusion There was significant variation in the genotype frequencies of FV Leiden 1691A and PAI-1 4G/5G polymorphisms between the patient and control groups. Additionally, our Egyptian cohort did not exhibit any indication of the involvement of β -fibrinogen -455 polymorphisms in RPL. It is crucial to screen women with a history of RPL for hereditary thrombophilia to provide high-risk couples with appropriate treatment and genetic counseling.manuscript.

SUMMARY

Recurrent pregnancy loss (RPL) affects an estimated 1–5% of couples worldwide, and inherited thrombophilia is implicated in up to 40% of unexplained cases. This study investigated the association between unexplained RPL and three thrombophilia-related gene polymorphisms — Factor V Leiden (FVL) 1691G>A, β -fibrinogen -455G>A, and plasminogen activator inhibitor-1 (PAI-1) 4G/5G — in an Egyptian cohort.

This case-control study enrolled 200 women at Shebin El-Kom Teaching Hospital: 100 with unexplained RPL (two or more miscarriages) and 100 controls with no history of pregnancy loss. Genotyping was performed by multiplex PCR and reverse-hybridization strip assay (CVD StripAssay®). Genotype and allele frequencies were compared between groups using Fisher's exact test and logistic regression, with Hardy-Weinberg equilibrium assessed in controls. The GG genotype and G allele of FVL 1691G>A were significantly more frequent in the RPL group than in controls (47% vs. 31%, $P = 0.048$; 66.5% vs. 54%, $P = 0.011$). The 4G/4G genotype and 4G allele of PAI-1 4G/5G were also significantly more frequent among women with RPL (49% vs. 31%, $P = 0.030$; 68.5% vs. 56%, $P = 0.009$). No significant association was found between the β -fibrinogen -455G>A polymorphism and RPL ($P > 0.05$ for all comparisons). FVL 1691G>A and PAI-1 4G/5G polymorphisms are significantly

associated with unexplained RPL in this Egyptian cohort, while β -fibrinogen -455G>A is not. These findings support incorporating FVL and PAI-1 genotyping into an expanded thrombophilia screening panel for women with unexplained RPL.

ACKNOWLEDGMENTS

We thank all the women who participated in this study.

REFERENCES

- Al-Astal M. G. and Sharif F. A.(2014). Beta-fibrinogen (-455 G/A) and integrin beta-3 (PLA1/A2) polymorphisms and recurrent pregnancy loss in Gaza Strip-Palestine. *Int. J. Reprod Contracept Obstet Gynecol* 3:134-8.
- Attili R., Hussein A., Odeh H. and Hejaz H. (2019). Prevalence of thrombophilia in Palestine and the association of thrombophilic gene polymorphisms with recurrent pregnancy loss. *Res. J. Obstet Gynecol*.12:6-10.
- Balogh I., Szôke G., Kárpáti L., Wartiovaara U., Katona E., Komáromi I., Haramura G., Pfliegler G., Mikkola H. and Muszbek L. (2000). Val34Leu polymorphism of plasma factor XIII:biochemistry and epidemiology in familial thrombophilia. *Blood*, 96(7): 2479-86.
- Board P. G., Losowsky M. S. and Miloszewski K. J. (1993). Factor XIII: inherited and acquired deficiency. *Blood Rev.* 7: 229-42.
- Brenner B., Hoffman R., Blumenfeld Z., Weiner Z. and Younis J. S. (2000). Gestational outcome in thrombophilic women with recurrent pregnancy loss treated by enoxaparin. *Thromb Haemost*; 83:693-7.
- Brenner B., Sarig G., Weiner Z., Younis J., Blumenfeld Z. and Lanir N. (1999). Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. *Thromb Haemost*; 82:69.
- Brown H. L. (1991). Antiphospholipid antibodies and recurrent pregnancy loss. *Clin. Obstet Gynecol*; 34:17-26.
- Carp H., Salomon O., Seidman D., Dardik R., Rosenberg N. and Inbal A. (2002). Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. *Hum. Reprod*; 17:1633-7.
- Chen H., Nio S. and Lu M. (2015). Association between Plasminogen Activator Inhibitor-1 Gene Polymorphisms and Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis. *Am. J. Reprod Immun.*; 73: 292-300.
- Djordjevic V., Gvozdenov M., Pruner I., Kovac M. and Tomic B. et al.

- (2013). The prevalence of PAI-1 4G/5G polymorphism in women with fetal loss - first data for a Serbian population. *Journal of Medical Biochemistry*; 203- 7.
- Dossenbach-Glaninger A., van Trotsenburg M., Oberkanins C. and Atamaniuk J. (2013). Risk for Early Pregnancy Loss by Factor XIII Val34Leu: The Impact of Fibrinogen Concentration. *Journal of Clinical Laboratory Analysis* ; 27: 444-9.
- ESHRE (2018). Guideline Group on RPL, Bender Atik R, Christiansen OB, et al. ESHRE guideline: recurrent pregnancy loss. *Hum Reprod Open.*; (2):hoy 004. doi:10.1093/hropen/hoy004.
- Farahmand K., Totonchi M., Hashemi M., Reyhani Sabet F., Kalantari H., Gourabi H., et al. (2016). Thrombophilic gene alterations as risk factor for recurrent pregnancy loss. *J. Matern Fetal Neonatal Med*; 29:1269-1273.
- Girolami A., Scandellari R., Lombardi A. M., Girolami B., Bortoletto E. and Zanon E. (2005). Pregnancy and oral contraceptives in factor V deficiency: a study of 22 patients (five homozygotes and 17 heterozygotes) and review of the literature. *Haemophilia*; 11:26-30.
- Grandone E., Margaglione M., Colaizzo D., d'Addetta M., Cappucci G., Vecchione G., Sciannamé N., Pavone G. and Di Minno G. (1997). Factor V Leiden is associated with repeated and recurrent unexplained fetal losses. *Thromb Haemost*; 77:822-4.
- Hohlgeschwandtner M., Unfried G., Heinze G., Huber J. C., Nagele F. and Tempfer C. (2003). Combined thrombophilic polymorphisms in women with idiopathic recurrent miscarriage. *Fertil Steril*; 79:1141-8.
- Huang Z., Tang W., Liang Z., Chen Q., Li M., Li Y., Lao S., Pan H. and Huang L, et al. (2017). Plasminogen activator inhibitor-1 polymorphism confers a genetic contribution to the risk of recurrent spontaneous abortion: an updated meta-analysis. *Reprod Sci.*;24:1551-60.
- Ivanov P. D., Komsa-Penkova R. S., Konova E. I., Kovacheva K. S., Simeonova M. N. and Popov J. D. (2009). Association of inherited thrombophilia with embryonic and postembryonic recurrent pregnancy loss. *Blood Coagul Fibrinolysis*; 2:134-40.
- Karami F., Askari M. and Modarressi M. H. (2018). Investigating Association of rs5918 Human Platelets Antigen 1 and rs1800790 Fibrinogen β Chain as Critical Players with Recurrent Pregnancy Loss. *Med. Sci. (Basel).*;6:98.

- Khosravi F., Zarei S., Ahmadvand N., Akbarzadeh-Pasha Z., Savadi E., Zarnani A. H., Sadeghi M. R. and Jeddi-Tehrani M. (2014). Association between plasminogen activator inhibitor-1 gene polymorphism and different subgroups of recurrent miscarriage and implantation failure. *J. Assist Reprod Genet.*; 31:121-4.
- Kreutz R. P., Bitar A., Owens J., Desta Z., Breall J. A., von der Lohe E., Sinha A., Vatta M., Nystrom P., Jin Y. and Flockhart D. A. (2014). Factor XIII Val34Leu polymorphism and recurrent myocardial infarction in patients with coronary artery disease. *J. Thromb Thrombolysis* 38: 380-7.
- Levin, Veronika, Rachel Booth and Shahab (2020). Minassian. Bleeding Disorders and ART. In: Textbook of Assisted Reproduction. Springer;
- Li J, Wu H., Chen Y., Wu H., Xu H. and Li L. (2015) Genetic association between FXIII and β -fibrinogen genes and women with recurrent spontaneous abortion: A meta-analysis. *J. Assist Reprod Genet.*;32:817-25.
- Martinelli I., Sacchi E., Landi G., Taioli E., Duc F. and Mannucci P. M. (1998). High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J. Med*; 338:1793-7.
- McNamee K., Dawood F., Farquharson R., (2012). Recurrent miscarriage and thrombophilia: An update. *Curr Opin Obstet Gynecol*; 24:229-34.
- Mikkola H. and Palotie A. (1996). Gene defects in congenital factor XIII deficiency. *Semin Thromb Hemost* 1996; 22: 393-8.
- Practice Committee of the American Society for Reproductive Medicine. (2013). Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril.*;99:63.
- RCOG. (2019). Recurrent Miscarriage, Investigation and Treatment of Couples (Green-top Guideline No. 17) 19/05/2011. Available from: <https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg17/>. [last accessed 2019 Dec 5].
- Rey E., Kahn S. R., David M. and Shrier I. (2003). Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet*; 361:901-8.
- Reznikoff-Etiévan M. F., Cayol V., Carbonne B., Robert A, Coulet F. and Milliez J. (2001). Factor V Leiden and G20210A prothrombin mutations are risk factors for very

- early recurrent miscarriage. *BJOG*; 108:1251-4.
- Ridker, C. H., Klaus Lindpaintner, Stampfer, Eisenberg P. R. and Joseph P. Miletich (1995). Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N. Engl. J. Med.*; 332(14):912-7.
- Robertson L., Wu. O., Langhorne P., Twaddle S., Clark P., Lowe G. D., Walker I. D., Greaves M., Brenkel I., Regan L. and Greer I. A. (2006). Thrombophilia in pregnancy: a systematic review. *Br J Haematol*; 132:171-96.
- Rodeghiero F. and Tosetto A. (1990). Activated protein C resistance and factor V Leiden mutation are independent risk factors for venous thromboembolism. *Ann. Intern. Med.*;130(8):643-50.
- Rosendaal F. R., Koster T., Vandenbroucke J. P. and Reitsma P. H. (1995). High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 1995; 85:1504-8.
- Sarig G., Younis J. S., Hoffman R., Lanir R., Blumenfeld Z. and Brenner B. (2002). Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. *Fertil Steril*; 77:342-7.
- Shi X., Xie X., Jia Y. and Li S. (2017). Maternal genetic polymorphisms and unexplained recurrent miscarriage: a systematic review and meta-analysis. *Clin. Genet.*; 91:265-84.
- Su M. T., Lin S. H., Chen Y. C. and Kuo P. L. (2013). Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss. *Thromb Haemost.*;109(8):15.
- Ticconi C., Mancinelli F., Gravina P., Federici G., Piccione E. and Bernardini S. (2011). Beta-fibrinogen G-455A polymorphisms and recurrent miscarriage. *Gynecol Obstet Invest.*; 71:198-201.
- Torabi R., Zarei S., Zeraati H., Zarnani A. H., Akhondi M. M. and Hadavi R. (2012). Combination of thrombophilic gene polymorphisms as a cause of an increased risk of recurrent pregnancy loss. *J. Reprod Infertil*;13:89-94.
- Tybjærg-Hansen A., Agerholm-Larsen B., Humphries S. E., Abildgaard S., Schnohr P. and Nordestgaard B. G. A. (1997). Common polymorphism (G-455-->A) in the beta-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic

- heart disease. A study of 9,127 individuals based on the Copenhagen City Heart Study. J. Clin Invest.;99:3034-9. doi: 10.1172/JCI119499.
- Younis J. S., Ohel G., Brenner B. and Ben-Ami M. (1997). Familial thrombophilia - the scientific rationale for thromboprophylaxis in recurrent pregnancy loss? Hum. Reprod; 12:1389-90.
- Younis J. S., Ohel G., Brenner B., Haddad S., Lanir N. and Ben-Ami M. (2000.). The effect of thromboprophylaxis on pregnancy outcome in patients with recurrent pregnancy loss associated with factor V Leiden polymorphism. BJOG; 107:415-9.
- Yousefian E., Kardi M. T. and Allahveisi A. (2014). Methylenetetrahydrofolate reductase C677T and A1298C polymorphism in Iranian women with idiopathic recurrent pregnancy losses. Iran Red Crescent Med J.; 16:e16763.
- Zhao L., Bracken M., DeWan A. and Chen A. (2013). Association between the SERPINE1(PAI-1) 4G/5G insertion/deletion promoter polymorphism (rs1799889) and pre-eclampsia: a systematic review and meta-analysis. Molecular Human Reproduction; 19(3): 136–43.

Table (1): Demographic and pregnancy data of the study groups.

	RPL (n = 100)	Control (n = 100)	p-value
Age (years), mean ± SD	28±5.07	29±5.43	0.237
BMI (kg/m2), mean ± SD	25.6±5.4	24.9±5.5	0.417
Number of live births, median (range)	1.4 (1-2)	2 (1-4)	0.188
Number of abortions, median (range)	5 (3-9)	0	< 0.01
Type of abortion (%)			
Two miscarriages	64	0	< 0.01
Three or more miscarriages	36	0	< 0.01

*Statistically significant at P<0.05. BMI = Body mass index, SD = Standard deviation, RPL = Recurrent pregnancy loss

Table (2): Distribution of FV Leiden 1691A gene polymorphism (rs6025) genotypes among women with recurrent pregnancy loss and controls.

Genotype/allele	Patients (n = 100) n (%)	Controls (n = 100) n (%)	OR (95% CI)	p-value
G/G	47 (47%)	31 (31%)	Reference	0.048
G/A	39 (39%)	46 (46%)	0.56 (0.3 - 1.04)	
A/A	14 (14%)	23 (23%)	0.4 (0.18 - 0.89)	
G	133 (66.5%)	108 (54%)	Reference	0.011
A	67 (33.5%)	92 (46%)	1.69 (1.13 - 2.53)	
Dominant model (GG+GA vs AA)			0.51 (0.29 - 0.91)	0.02
Recessive model (GA+AA vs GG)			0.54 (0.26 - 1.12)	0.101
HWE				0.459

*Statistically significant at $P < 0.05$. HWE = Hardy-Weinberg equilibrium, OR = Odds ratio, CI = Confidence interval, RPL = Recurrent pregnancy loss.

Table (3): Distribution of β -fibrinogen -455 gene polymorphism (rs1800790) genotypes among women with recurrent pregnancy loss and controls.

Genotype/allele	Patients (n = 100) n (%)	Controls (n = 100) n (%)	OR (95% CI)	p-value
G/G	56 (56%)	54 (54%)	Reference	0.88
G/A	39 (39%)	42 (42%)	0.9 (0.51 - 1.6)	
A/A	5 (5%)	4 (4%)	1.21 (0.31 - 4.75)	
G	151 (75.5%)	150 (75%)	Reference	0.907
A	49 (24.5%)	50 (25%)	1.03 (0.65 - 1.62)	
Dominant model (GG+GA vs AA)			0.92 (0.53 - 1.61)	0.776
Recessive model (GA+AA vs GG)			1.26 (0.33 - 4.84)	0.733
HWE				0.23

*Statistically significant at $P < 0.05$. HWE = Hardy-Weinberg equilibrium, OR = Odds ratio, CI = Confidence interval, RPL = Recurrent pregnancy loss.

Table (4): Distribution of PAI-1 4G/5G gene polymorphism (rs1799889) genotypes among women with recurrent pregnancy loss and controls.

Genotype/allele	Patients (n = 100) n (%)	Controls (n = 100) n (%)	OR (95% CI)	p-value
4G/4G	49 (49%)	31 (31%)	Reference	0.030
4G/5G	39 (39%)	50 (50%)	0.49 (0.27 - 0.91)	
5G/5G	12 (12%)	19 (19%)	0.4 (0.17 - 0.94)	
4G	137 (68.5%)	112 (56%)	Reference	0.009
5G	63 (31.5%)	88 (44%)	1.71 (1.14 - 2.57)	
Dominant model (4G/4G+4G/5G vs 5G/5G)			0.47 (0.26 - 0.84)	0.009
Recessive model (4G/5G + 5G/5G vs 4G/4G)			0.58 (0.27 - 1.27)	0.171
HWE				0.885

*Statistically significant at P<0.05. HWE = Hardy-Weinberg equilibrium, OR = Odds ratio, CI = Confidence interval, RPL = Recurrent pregnancy loss.

\

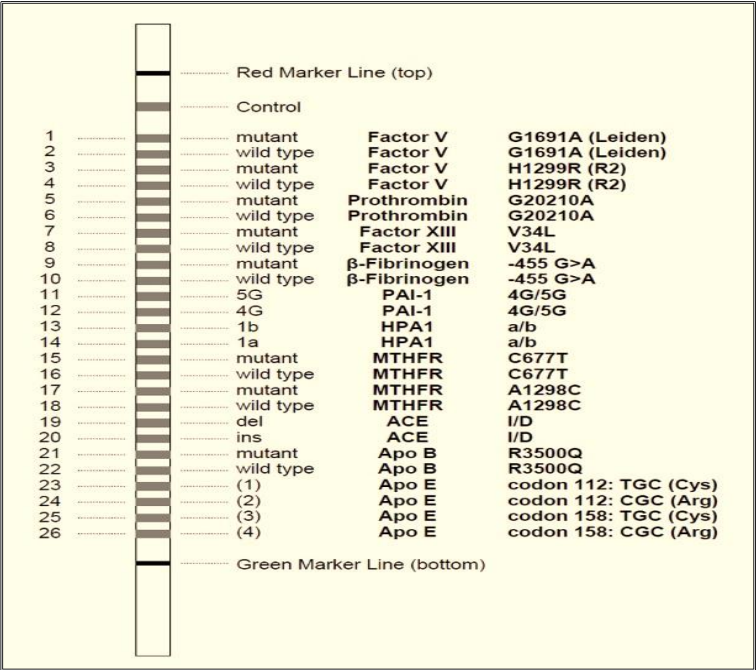


Fig. (1): Thrombotic gene polymorphism strip assay.