An Association Between Thrombophilias and Pregnancy Loss: Should We Test?

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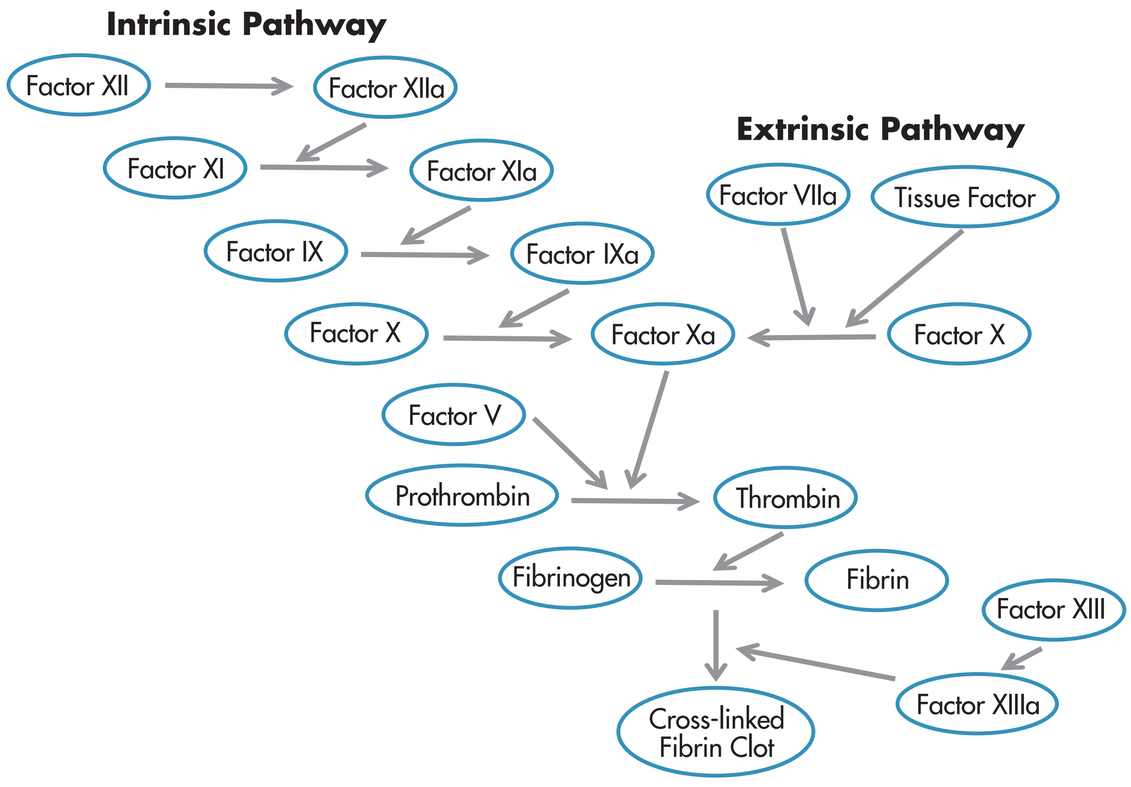
Introduction

- Sporadic miscarriages prior to 20 weeks gestation occur in 15–25% of pregnancies.[1] Chromosomal aneuploidies, such as trisomy or monosomy, account for over 50% of these losses.[2] While sporadic miscarriages are relatively common, only 1–5% of women will experience multiple miscarriages, also referred to as recurrent pregnancy loss (RPL).[3] RPL is defined as 2 consecutive, or 3 non-consecutive, pregnancy losses prior to 20 weeks gestation. Compared to sporadic miscarriage, chromosomal aneuploidies are rarely the cause of RPL; balanced translocations carried by one parent, accounting for only 2–5% of RPL cases, can affect chromosomal stability in the embryo.[1] Additional genetic causes of RPL have been extensively studied. Such causes include endocrine factors, anatomic abnormalities, and immunological factors.[1,4] However, nearly 50% of RPL cases are diagnosed as idiopathic.[4]
- Inherited thrombophilias are another potential cause of RPL. Thrombophilias are those genes that are a part of the coagulation cascade (**Figure 1**). Mutations within thrombophilias genetically predispose individuals to a hypercoagulable state, altering the fine-tuned balance between coagulation and fibrinolysis. Pregnancy also induces a hypercoagulable state. Combining this physiological change with inherited thrombophilias may further exacerbate the stasis between coagulation and fibrinolysis, increasing the risk for pregnancy loss.
- The contribution of thrombophilias as a cause of RPL remains controversial. While a number of studies have implicated a number of thrombophilia-related genes in RPL, other studies have found no association between thrombophilias and RPL.[5] Further, several prospective studies investigating the effect of treating women with low molecular weight heparin (LMWH) or low dose aspirin (LDA) have demonstrated conflicting results on pregnancy outcomes.[6,7] As a result of these conflicting findings, recommendations by professional societies vary. For example, the American Society for Reproductive Medicine (ASRM) and the American Congress of Obstetricians and Gynecologists (ACOG) do not currently recommend screening for these genetic variants.[1,8] Conversely, the Royal College of Obstetricians and Gynecologists (RCOG) do recommend screening for these genetic variants in women diagnosed with RPL.[9] The European Society of Human Reproduction and Embryology (ESHRE) is currently drafting guidelines. This highlights the importance of continuing to investigate the association of thrombophilias to RPL.

Figure 1. Thrombophilia Genes in the Coagulation Cascade



Objective

- The objective of this study was to calculate the frequency of thrombophilia-related genetic variants in three populations: (1) females seeking treatment at fertility centers, (2) females self-reporting RPL who are seeking treatment at fertility centers, and (3) the general population. The frequencies of these thrombophilic genetic variants for the general population were compared to the females seeking fertility treatment and to the females who self-reported RPL.

Materials and Methods

Study Population

- A total of 2,201 females referred for carrier screening from fertility centers across the United States were included in the study. A history of RPL, defined as ≥2 miscarriages before 20 weeks gestation, was determined based on information provided during genetic counseling sessions. Of the 2,201 study subjects, 69.9% received genetic counseling sessions that included intake of pregnancy history. A total of 118 participants who received genetic counseling self-reported a history of RPL, accounting for 7.7% of the study population. This is a larger proportion of individuals with a history of RPL than is reported in the general population.
- Documented informed consent was obtained to utilize genetic data generated from carrier screening and clinical information provided during genetic counseling sessions in a de-identified manner. Patients were enrolled between January 2013 and October 2014. Samples from the 1000 Genomes Project served as the general population control for comparisons.[10,11]

Genotyping Platform

- Illumina's Infinium HD Custom Genotyping Platform was utilized to genotype thrombophilia genetic variants. Six genetic variants were genotyped across 5 genes (**Table 1**).

Table 1. Genetic variants studied in present investigation

Gene	rsID	cDNA	gDNA	Protein Name
Factor V Leiden	rs6025	c.G1601A	g.G36576A	p.R534Q
Factor II Prothrombin	rs1799963	c.1869+97G>A	g.G20270A	N/A
Factor XII	rs1801020	c.-4T>C	c.-4T>C	N/A
Factor XIIIA1	rs5985	c.G103T	g.G103T	p.V35L
Factor XIIIA1	rs3024477	c.A614T	g.A67778T	p.Y205F
FGB	rs1800790	c.-463G>A	g.-463G>A	N/A

Statistical Analysis

- Fisher's Exact Test was used to calculate significant differences in genotype frequencies between 1 case group and the general population control. A p-value of $p \leq 0.05$ was considered significant. For each genetic variant included in the study, two comparisons were made:
  1. Females seeking treatment at fertility centers versus the general population
  2. Females with a self-reported history of RPL seeking treatment at fertility centers versus the general population

Results

- A statistically significant difference was found for all 6 studied genetic variants when comparing females seeking treatment at fertility centers to the general population control (**Table 2**).

Table 2. A comparison of females seeking fertility treatment and a general population

Gene (cDNA)	Seeking Fertility Treatment		1000 Genomes Control		Fisher's Exact p-value
	% Heterozygous	% Homozygous Mutant	% Heterozygous	% Homozygous Mutant	
Factor V Leiden (c.G1601A)	4.2%	0%	1.9%	0.066%	3.56×10^{-5}
Factor II Prothrombin (c.1869+97G>A)	2.5%	0.091%	0.64%	0.040%	1.14×10^{-7}
Factor XII (c.-4T>C)	37.7%	13.9%	25.6%	31.1%	2.20×10^{-16}
Factor XIIIA1 (c.G103T)	29.4%	4.0%	23.1%	3.2%	6.56×10^{-7}
Factor XIIIA1 (c.A614T)	3.8%	0.045%	1.6%	0%	3.43×10^{-6}
FGB (c.-463G>A)	31.3%	3.8%	22.0%	3.2%	4.96×10^{-13}

- A statistically significant difference was found for 2 of the 6 genetic variants when comparing females seeking treatment at fertility centers who self-reported a history of RPL to the general population control (**Table 3**).

Table 3. A comparison of females seeking fertility treatment who self-reported a history of RPL and a general population

Gene (cDNA)	Self-Reported History of RPL and Seeking Fertility Treatment		1000 Genomes Control		Fisher's Exact p-value
	% Heterozygous	% Homozygous Mutant	% Heterozygous	% Homozygous Mutant	
Factor V Leiden (c.G1601A)	5.1%	0%	1.9%	0.066%	0.103
Factor II Prothrombin (c.1869+97G>A)	0.85%	0%	0.64%	0.040%	0.565
Factor XII (c.-4T>C)	44.1%	13.6	25.6%	31.1%	1.82×10^{-6}
Factor XIIIA1 (c.G103T)	26.3%	3.4%	23.1%	3.2%	0.646
Factor XIIIA1 (c.A614T)	5.9%	0%	1.6%	0%	0.00496
FGB (c.-463G>A)	28.0%	5.1%	22.0%	3.2%	0.121

Discussion

- We find that the genotype frequencies of all six studied thrombophilia genetic variants significantly differ between females seeking treatment at fertility centers compared to the general population control (**Table 2**). While individuals and couples seek treatment at fertility centers for many reasons, a common cause of visits is the inability to conceive naturally for a period of time. Among the women in our study population, 42% reported that they had never been pregnant. It is possible that these couples do achieve pregnancy yet experience an early-stage miscarriage without any knowledge of either event. Therefore, it may be that these women's infertility is affected by these thrombophilia-related genetic variants.
- Genotype frequencies of genetic variants in Factor XII (c.-4T>C) and Factor XIIIA1 (p.Y205F) differed significantly between women seeking treatment at fertility centers who self-reported a history of RPL and the general population control (**Table 3**). Interestingly, Factor XII has been shown to be a phospholipid-binding protein implicated in antiphospholipid syndrome (APS).[12] APS is an autoimmune condition that also induces a hypercoagulable state and is an example of an acquired thrombophilia. While the mechanism of causing a hypercoagulable state is different for inherited thrombophilias (genetic variants) versus APS (development of antibodies), it is possible that individuals carrying this specific Factor XII variant could also develop APS, which is a known risk factor for RPL.
- The findings from this study suggest that women diagnosed with idiopathic infertility may benefit from screening for these thrombophilia genetic variants to guide reproductive treatment and pregnancy monitoring. It is possible that women trying to conceive may in fact be able to conceive, but implantation is not successful due to a hypercoagulable state that is further affected by inherited thrombophilias. It has been suggested that anticoagulants such as LMWH and LDA may aid in reducing the risk of miscarriage. Similar to the conflicting results from genetic association studies, there has yet to be consensus on the best practices in offering these treatments. Further studies should be designed to investigate the impact of treatment with anticoagulants on miscarriage risk with multiple thrombophilia genetic variants.
- The findings from previous studies investigating the link between inherited thrombophilias and RPL have produced conflicting results. In cases where the significant association has been identified, the risk of multiple miscarriages is increased only slightly. While individually weak, the combination of two (or more) of these variants is additive and the collective risk of RPL becomes more substantial. Further studies investigating the complex genetic interactions related to thrombophilias and RPL may shed additional light onto the causes of RPL.
- The field could benefit from a study of similar focus with an increased sample size, thereby increasing the statistical power. Our population of participants with a reported history of RPL was small, and may thus have affected the statistical analysis. Further, larger sample sizes will allow for the study of the complex genetic contributions of multiple thrombophilia genetic variants. Additionally, the study may be limited by the self-reported nature of participants' pregnancy histories. A large scale study with access to medical records would allow for greater confidence in participant categorization.

References

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