

Recurrent Pregnancy Loss in a Subject with Heterozygote Factor V Leiden Mutation; a Case Report

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Abstract

Recurrent pregnancy loss is usually defined as the loss of two or more consecutive pregnancies before 20 weeks of gestation, which occurs in approximately 5% of reproductive-aged women. It has been suggested that women with thrombophilia have an increased risk of pregnancy loss and other adverse pregnancy outcomes. Thrombophilia is an important predisposition to blood clot formation and is considered as a significant risk factor for recurrent pregnancy loss. The inherited predisposition to thrombophilia is most often associated with factor V Leiden mutation, prothrombin G20210A mutation, and methylenetetrahydrofolate reductase C677T and A1298C gene variants. The net effect is an increased cleavage of prothrombin to thrombin and excessive blood coagulation.

Keywords: Hereditary thrombophilia, Factor V Leiden mutation, Recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL) is defined as 2 or more spontaneous abortions before the 20th week of gestation, which affects approximately 5% of women of reproductive age (1). Thrombophilia has been suggested as one putative etiology of RPL (2). Hereditary thrombophilia is a genetic disorder of blood coagulation resulting in an unusual hypercoagulation state, which in turn can result in abnormal implantation and may manifest as spontaneous loss (3). Numerous polymorphisms are associated with coagulation disorders, such as polymorphisms in factor V Leiden, factor II G20210A, or methylene tetrahydrofolate reductase (4). Factor V Leiden thrombophilia is characterized by a poor anticoagulant response to activated protein C (APC) and an increased risk for venous thromboembolism

(VTE). Deep venous thrombosis (DVT) is the most common form of venous thromboembolism (5). Heterozygosity for factor V Leiden is inherited as an autosomal dominant trait. A specific G-to-A point mutation at nucleotide 1691 in the factor V gene, which leads to the single amino-acid replacement Arg506Gln, causes resistance to cleavage and inactivation by activated protein C and increased susceptibility to clotting (6, 7). Activated protein C (APC) is a natural anticoagulant protein that cleaves and inactivates active forms of factors V and VIII, thereby down-regulating more clot formation (8, 9). Thrombophilia due to factor V Leiden mutation should be suspected in someone with a history of venous thromboembolism (VTE) and pulmonary embolism, particularly in pregnant women with a

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histories of VTE during pregnancy and family histories of recurrent thrombosis (10, 11).

Table 1. Primers used to amplify variant polymorphisms of MTHFR C677T, A1298C, and factor V Leiden G1691A

Polymorphism	Primer	Size (bp)
MTHFR C677T	Forward GGCAGAAGCATATCAGTCATGAG	494
	Reverse CTGGGAAGAAGCTACGGAACTCAG	
MTHFR A1298C	Forward AAGGAGGAGCTGCTGAAGATG	237
	Reverse CTTTGCCATGTCACAGCATG	
Factor V Leiden G1691A	Forward CATACTACAGTGCAGTGGAC	206
	Reverse TGTTCTCITGAAGGAAATGC	

Materials and Methods

The subject was a 42-year-old woman with a history of three first trimester spontaneous abortions. Five ml of peripheral blood from this patient were collected in an EDTA-anticoagulant tube and genomic DNA was extracted using the salting-out method as described previously (12). The genomic DNA was amplified with specific forward and reverse primers to detect MTHFR C677T, MTHFR A1298C, and factor V Leiden G1691A gene variants by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) (Table 1). The PCR amplification conditions for both MTHFR gene variants were 5 minutes at 95 °C followed by 30 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 30 seconds, and final extension at 72 °C for 10 minutes. The PCR conditions for amplification of the factor V Leiden mutation were 5 minutes at 95 °C followed by 30 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and final extension at 72 °C for 10 minutes. Each PCR was performed in a final reaction volume of 25 µl. The amplified PCR products for MTHFR C677T, MTHFR A1298C, and factor V Leiden G1691A variants were digested with *HinfI*, *MboII*, and *MnlI* (Fermentas) restriction enzymes, respectively (Table 2). Digested PCR products were separated by electrophoresis on 2% agarose gels containing ethidium bromide and visualized under ultraviolet light. The presence of the factor V Leiden G1691A nucleotide mutation was confirmed by direct PCR product sequencing (ABI Prism 3700 automatic sequencer).

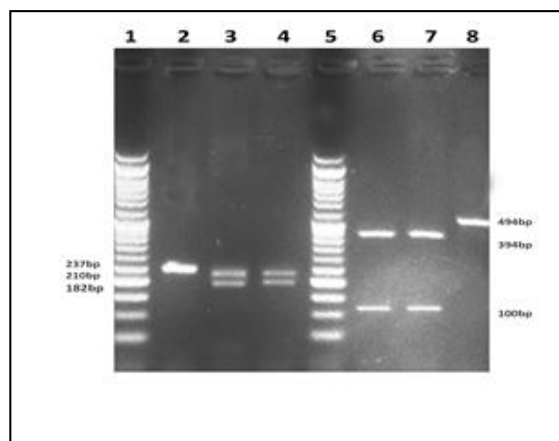


Fig. 1. The PCR-RFLP products for polymorphisms MTHFR C677T and MTHFR A1298C were electrophoresed on a 2% agarose gel. The patient was shown to have homozygous wild type alleles for MTHFR C677T and heterozygous alleles for MTHFR A1298C. Lanes 1 and 5: 50 bp DNA marker; Lane 2: MTHFR A1298C PCR product; Lanes 3 and 4: *MboII*-digested MTHFR A1298C PCR product; Lanes 6 and 7: *HinfI*-digested MTHFR C677T PCR product; Lane 8: MTHFR C677T PCR product.

Results

In this patient both MTHFR 677 and MTHFR 1298 loci were genotyped by PCR and RFLP analyses. The genotypes homozygous wild type CC in position 677 and heterozygous AC in position 1298 of the MTHFR gene were determined (Fig. 1). Also, in the factor V gene, heterozygous genotype G1691A was detected by PCR and RFLP analysis (Fig. 2).

The presence of the G1691A point mutation in the factor V gene was confirmed by PCR sequencing (Fig. 3).

Table 2. Restriction enzymes used to digest PCR products, and related product sizes

Restriction enzyme	Polymorphism	Size (bp)
HinfI	MTHFR Wild type allele (677C)	100, 394
	MTHFR Variant allele (677T)	100, 165, 229
MboII	MTHFR Wild type allele (1298A)	27, 28, 182
	MTHFR Variant allele (1298C)	27, 210
MnlI	Factor V Leiden Wild type allele (1691G)	47, 36, 123
	Factor V Leiden Variant allele (1691A)	47, 159

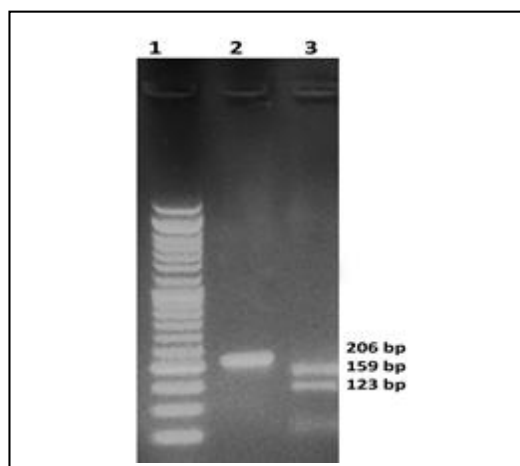


Fig. 2. The PCR-RFLP for the factor V Leiden mutation was electrophoresed on a 2% agarose gel. The patient was shown to have a heterozygous genotype for this mutation. Lane 1: 50 bp DNA marker; Lane 2: PCR product for factor V Leiden; Lane 3: *MnlI*-digested factor V Leiden PCR product.

Discussion

In this report, we present a case of a woman with a history of recurrent pregnancy loss that was complicated with the factor V Leiden mutation and heterozygote methylenetetrahydrofolate reductase A1298C genotypes. An estimated 50% of recurrent pregnancy loss is idiopathic. Most couples with recurrent pregnancy loss should be evaluated for genetic and non-genetic causes. Mutations that promote thrombophilia are often associated with pregnancy failures. Placental thrombosis can cause recurrent miscarriages and pregnancy disorders. It has been postulated that maternal risk factors to thrombophilia may lead to adverse pregnancy outcomes (13). In several studies, the potential clinical significance of hereditary thrombophilia factors, such as the factor V Leiden mutation, prothrombin G20210A mutation, and methylenetetrahydrofolate reductase C677T and A1298C gene variants has been addressed (14-16). Thrombophilia should be considered in patients with RPL. Factor V Leiden is the most common genetic thrombotic risk factor for VTE, found in 20–25% of patients with VTE, and 50% of patients with familial thrombophilia (6). Factor V Leiden thrombophilia may increase the risk for pregnancy loss and other obstetrical complications (14, 17). There is growing evidence that women with thrombophilia are at increased risk of pregnancy-related venous thromboembolism and possibly other pregnancy complications including pregnancy loss

(18). The relative risk for VTE is increased 3- to 8-fold in Factor V Leiden heterozygotes and thrombotic risk is increased 10- to 80-fold in homozygotes (19-20). Kupferminc et al. (1999) identified the factor V Leiden mutation in 22 of 110 women with obstetrical complications and in 7 of 110 women with normal pregnancies (17). Espana F et al. (1999) described a heterozygous genotype for the factor V Leiden mutation in a 39-year-old woman who suffered recurrent fetal loss (21). Sarig et al. (2002) reported an incidence of factor V Leiden in 25% in women with fetal losses vs. 7.6% in controls. (22) Herman M et al. (2006) reported the factor V Leiden mutation in a 39-year-old patient with a history of spontaneous abortion who had been pregnant six times and had delivered one healthy child (23). Rodger et al. (2010) found that the odds ratio of pregnancy loss appears to be 52% higher in women with the factor V Leiden mutation than in pregnant women without the mutation (24). We recommend considering genetic markers of thrombophilia during pregnancy, especially for women with recurrent unexplained first trimester pregnancy losses, unexplained severe preeclampsia, severe intrauterine growth restriction, placental abruption, and stillbirth. The results should be interpreted by a physician experienced in the management of thrombophilia and thrombosis, and an appropriate clinical intervention should be considered during pregnancy to prevent adverse pregnancy outcomes.

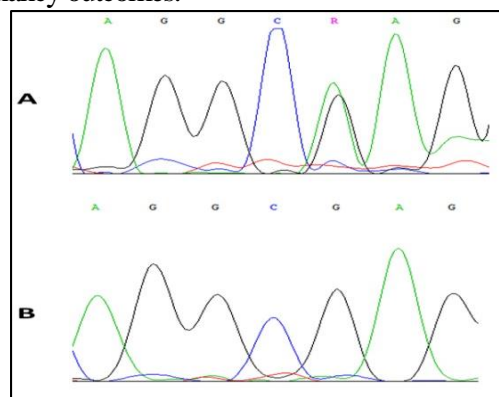


Fig. 3. Panel A shows a chromatogram of the G to A transition at nucleotide position 1691 in the factor V Leiden gene. Panel B shows the normal sequence of the same region.

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