Case Report

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First Korean case of factor V Leiden mutation in pregnant woman with a history of recurrent pregnancy loss

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Thrombophilia refers to inherited or acquired hemostatic disorders that result in a predisposition to blood clot formation. When combined with the hypercoagulable state that is characteristic of pregnancy, there is an increased risk of severe and recurrent pregnancy complications. Activated protein C resistance caused by factor V Leiden (FVL) mutation is known to be the most common cause of inherited thrombophilia in Caucasian population. FVL mutation has been related to pregnancy complications associated with hypercoagulation, e.g. miscarriage, intrauterine fetal demise, placental abruption, and intrauterine growth retardation. Although the FVL mutation is easily detected using molecular DNA techniques, patients who are heterozygous for this disorder often remain asymptomatic until they develop a concurrent prothrombotic condition. Because there are potentially serious effects of FVL mutation for pregnancy, and because effective treatment strategies exist, early detection and treatment of this condition might be considered.

Key words: Factor V Leiden, Mutation, Recurrent pregnancy loss, Thrombophilia, Hereditary.

Introduction

Recurrent pregnancy loss (RPL) has been public health concern, developed 1 in 300 pregnancies and 2-4% of reproductive-aged couples. RPL is defined as 2 or more spontaneous abortions before the 20th week of gestation [1]. An estimated 50% of RPL is idiopathic. Most couples with RPL should be evaluated for genetic and non-genetic causes. Thrombophilia has been suggested as one putative etiology of RPL [2]. Inherited thrombophilia is a genetic disorder of blood coagulation resulting in an unusual hypercoagulable state, which in turn can result in abnormal implantation and may manifest as spontaneous fetal loss [3]. The inherited predisposition to thrombophilia is most often associated with factor V Leiden (FVL) mutation (c.1601G>A), coagulation factor II (*F2*, also known as prothrombin) gene variant (c.20210G>A), and methylenetetrahydrofolate reductase (*MTFHR*) gene variants (c.677C>T and c.1298A>C) [4].

Activated protein C (APC) resistance is known to be the most frequent genetic cause of venous thrombosis, accounting for up to 40% of all thromboses. The FVL mutation, inherited as an autosomal dominant trait, is responsible for 95% of cases of APC resistance [5]. The FVL mutation is caused by a point mutation in the *F5* (OMIM 612309, NM_000130.4), encoding a substitution of arginine for glutamine at position 534 of the factor V

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molecule located on chromosome 1q24, the site at which APC cleaves factor Va. The FVL mutation renders factor Va resistant to the anticoagulant effects of APC, resulting in a genetic predisposition to thrombosis [5]. The distribution of FVL mutation varies greatly in ethnic groups. The FVL mutation is reported in 5% of the healthy white population, 1% of the healthy black population, reaching 10-15% in some European countries [6]. However, it appears that FVL mutation is extremely rare in Africans and Asian countries [7], and until now, there has been no report of FVL mutation positive detected in Korean population [8-11]. Here, we present the first Korean case of FVL mutation in pregnant woman with a history of RPL.

Case

The patient was a 36-year-old Korean woman, gravida 7, para 1, aborta 6, who presented with RPL at her initial obstetrical visit. Her past obstetrical history was significant for 1 ectopic pregnancy and 5 early first trimester miscarriages, followed by 1 full-term delivery by caesarean section. She had no previous history of preeclampsia, placental abruption, or intrauterine growth retardation (IUGR). The results of her physical examination were within normal limits. For evaluation of RPL, she was tested for complete blood count, coagulation profile, thyroid function test (T3/free T4/thyroid stimulating hormone [TSH]), prolactin, natural killer cell proportion, anticardiolipin antibodies immunoglobulin (Ig) M/IgG, lupus anticoagulant screening, anti-β2 glycoprotein-1 antibodies IgM/IgG, thyroid peroxidase antibody, protein C and S functional activity, antithrombin III activity, homocysteine, TSH receptor antibody, thyroglobulin antibody, chromosomal analysis, and genotype for FVL mutation (c.1601G>A), *F2* gene variant (c.20210G>A), and *MTFHR* gene variants (c.677C>T and c.1298A>C).

Her baseline complete blood count, coagulation profile and thyroid function test were within normal limits. Serum prolactin was 10.6 ng/mL (normal range, 2.8 to 29.2 ng/mL). Serum homocysteine was 8.65 μmol/L (normal range, 6.72 to 15.16 μmol/L). Thrombophilia profile of this patient showed within normal range of protein S (62%; normal range, 58.7-119.2%), protein C (115%; normal range, 70-130%) and antithrombin III (104%; normal range, 70-120%). Antiphospholipid antibodies including anticardiolipin IgM/IgG, anti-β2-glycoprotein 1 IgM/IgG, and lupus anticoagulant were within normal limits.

The genomic DNA extracted from patient was amplified with specific forward and reverse primers to detect FVL mutation (c.1601G>A), *F2* gene variant (c.20210G>A), and *MTFHR* gene variants (c.677C>T and c.1298A>C) by polymerase chain reaction (PCR)-restriction fragment length polymorphism (Table 1). The amplified PCR products for FVL mutation (c.1601G>A), *F2* variant (c.20210G>A), and *MTFHR* variants (c.677C>T and c.1298A>C) were digested with HinfI, MboII, and MnlI 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 *F2* mutation (c.20210G>A) was evaluated by PCR-sequencing. In the *F5* gene, heterozygous genotype c.1601G>A was detected (Fig. 1). The presence of the FVL mutation (c.1601G>A) was confirmed by direct PCR product sequencing (Fig. 2). The genotypes heterozygous wild type CT in position 677 and homozygous wild type AA in position 1298 of the *MTHFR* were determined. The *F2* mutation (c.20210G>A) was not detected. Her karyotype showed normal female.

On 4 months later, she was presented with pregnancy at

Table 2. Restriction enzymes used to digest polymerase chain reaction products, and related product size

Restriction enzyme	Polymorphism	Size (bp)
HinfI	MTHFR	
	Wild type allele 677C	198
	Variant allele 677T	175, 23
MboII	MTHFR	
	Wild type allele 1298A	204
	Variant allele 1298C	241
MnlI	Factor V Leiden	
	Wild type allele 1601G	164, 66, 36
	Variant allele 1601A	200, 66

Table 1. Primer used to amplify variant polymorphisms of factor V Leiden (FVL) mutation, coagulation factor II, and *MTFHR*

Polymorphism	Primer sequences (5' to 3')		Size (bp)
	Forward	Reverse	
MTHFR (c.677C>T)	TGAAGGAGAAGGTGTCTGCGGGA	AGGACGGTGCGGTGAGAGTG	198
MTHFR (c.1298A>C)	ATGTGGGGGGAGGAGCTGAC	GTCTCCCAACTTACCCTTCTCC	241
F2 (c.20210G>A)	CCGCCTGAAGAAGTGGATAC	CTTCCTGAGCCCAGAGAGC	197
FVL (c.1601G>A)	TCCCAGTGCTTAACAAGACCA	TGTTATCACACTGGTGCTAA	266

her obstetrical visit. Estimated gestational age was 5 weeks as measured from the patient's last menstrual period, which was confirmed by a first trimester crown-rump length. The couple was counseled about the autosomal dominant nature of FVL mutation. On the intake interview, the patient had no significant past medical history including thromboembolic disease. However, her father was diagnosed with a cerebral infarction and her grandmother died of myocardial infarction. The patient was advised folic acid supplementation and early antenatal registration with thromboprophylaxis in next pregnancy. In view of FVL mutation positive in pregnancy, the patient was started on low dose aspirin (100 mg) once daily and low-molecular-weight heparin (40 mg, Cnoxane; YOOYOUNG Pharm. Co. Ltd., Jincheon, Korea) subcutaneous injection once daily.

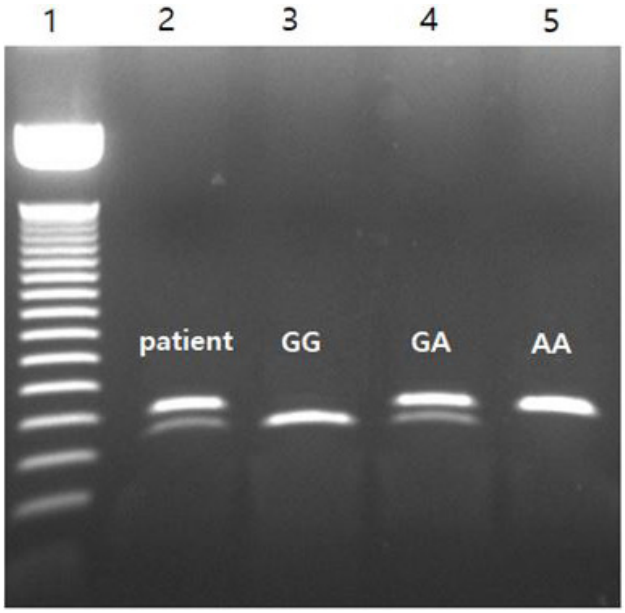

Fig. 1. Polymerase chain reaction (PCR)-restriction fragment length polymorphism for the factor V Leiden mutation was electrophoresed on a 2% agarose gel. The patient was shown to have a heterozygous genotype GA for this mutation. Lane 1, 50 bp DNA marker; Lane 2, PCR product of patient (genotype GA); Lane 3, positive control for genotype GG; Lane 4, positive control for genotype GA; Lane 5, positive control for genotype AA.

Discussion

In this report, we present the case of a Korean woman with a history of RPL that was complicated with the FVL mutation. In several studies, the potential clinical significance of hereditary thrombophilia factors, such as FVL mutation, *F2* gene mutation, plasminogen activator inhibitor-1 gene polymorphism, protein S deficiency, protein C deficiency, and *MTFHR* gene polymorphism has been addressed [11,12]. A thrombophilia may result in impaired maternal fetal circulation by compromising the vascular system associated with significant fetal morbidity and mortality [13]. The FVL mutation has been significantly related to pregnancy complications associated with hypercoagulation, e.g., venous thromboembolism (VTE), hypertensive disorders of pregnancy, late pregnancy loss and stillbirth, placental abruption, and IUGR [14]. Recently, in meta-analysis, FVL mutation was associated with RPL (OR, 1.68; 95% CI, 1.16 to 2.44) [12]. VTE is the leading cause of morbidity and mortality in pregnancy and the postpartum period. VTE occurs in approximately 1 in 1,500 pregnancies, and up to one fourth of untreated deep vein thromboses may lead to pulmonary embolism [15]. Women with a personal history of VTE in a previous pregnancy have a higher prevalence of FVL mutation than those who have never had a VTE [15]. Patients who are heterozygous for this condition are at 3- to 8-fold increased risk for VTE; those who are homozygous are at 10- to 80-fold increased risk [16].

Current expert opinion recommends that management be based on the presence of current VTE, the presence of a past VTE, and risk factors for a VTE during pregnancy. High-risk thrombophilia, including FVL homozygosity, *F2* mutation (c.20210G>A) homozygosity, heterozygosity for FVL mutation and *F2* mutation (c.20210G>A), or antithrombin deficiency, recommend

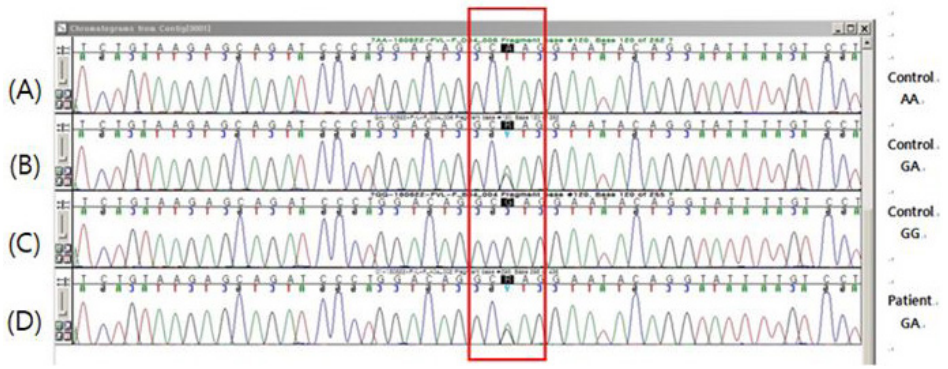

Fig. 2. The chromatogram shows that nucleotide substitution from guanine to adenine at the position of 1601 base in *factor V* gene (c.1601G>A, p.Arg534Gln, NM_000130.4). (A-C) shows the sequences of positive control for genotype AA (A), GA (B), and GG (C), respectively. (D) shows the sequence that patient harbors c.1601G>A (p.Arg534Gln).

prophylactic anticoagulation therapy regardless of VTE history. Low-risk thrombophilia for women who are heterozygous for FVL mutation without previous VTE, such as the present case, the American College of Obstetricians and Gynecologists (2018) [17] recommends surveillance without anticoagulation therapy in pregnancy and postpartum period. However, even though low-risk thrombophilia without previous VTE, if the patient has additional risk factors, such as first-degree relative with a history of a thrombotic episode, or other thrombotic risk factors (e.g., obesity, prolonged immobility, cesarean delivery), postpartum prophylactic anticoagulation therapy is recommended.

Inherited thrombophilia has a different prevalence between various ethnic groups [6,7]. According to previous study of RPL in Korean women, FVL mutation was not found in any women and protein S deficiency was found 13.5% [11]. Another study for thrombophilia in Korean patients with VTE suggests that FVL mutation testing is unnecessary for Korean patients with VTE [10]. To our acknowledgment, this is the first case of FVL mutation in Korean. It is important to have a guideline for evaluation and management for Korean RPL women based on the evidence and our circumstances.

In conclusion, during pregnancy, patients with FVL mutation are at increased risk for VTE, intrauterine fetal death, IUGR, placental abruption, and preeclampsia. It is important to have a good knowledge of FVL mutation and its potential impact on pregnancy. Inherited thrombophilia has shown the ethnic difference signify the need for consideration in screening and interpretation. Appropriate counselling and clinical intervention should be considered during pregnancy to prevent adverse pregnancy outcomes.

References

1. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. Fertil Steril 2010;93:1234-43.
2. Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368:601-11.
3. Brenner B. Inherited thrombophilia and pregnancy loss. Thromb Haemost 1999;82:634-40.
4. Younis JS, Ohel G, Brenner B, Ben-Ami M. Familial thrombophilia--the scientific rationale for thrombophylaxis in recurrent pregnancy loss? Hum Reprod 1997;12:1389-90.
5. Dahlbäck B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. Haemostasis 1994;24:139-51.
6. Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995;346:1133-4.
7. De Stefano V, Chiusolo P, Paciaroni K, Leone G. Epidemiology of factor V Leiden: clinical implications. Semin Thromb Hemost 1998;24:367-79.
8. Kim TW, Kim WK, Lee JH, Kim SB, Kim SW, Suh C, et al. Low prevalence of activated protein C resistance and coagulation factor V Arg506 to Gln mutation among Korean patients with deep vein thrombosis. J Korean Med Sci 1998;13:587-90.
9. Chang JD, Hur M, Lee SS, Yoo JH, Lee KM. Genetic background of nontraumatic osteonecrosis of the femoral head in the Korean population. Clin Orthop Relat Res 2008;466:1041-6.
10. Kim S, Song I, Kim HK, Huh S. Thrombophilia in Korean patients with arterial or venous thromboembolisms. Ann Surg Treat Res 2016;90:340-5.
11. Lee GS, Park JC, Rhee JH, Kim JI. Etiologic characteristics and index pregnancy outcomes of recurrent pregnancy losses in Korean women. Obstet Gynecol Sci 2016;59:379-87.
12. Sergi C, Al Jishi T, Walker M. Factor V Leiden mutation in women with early recurrent pregnancy loss: a meta-analysis and systematic review of the causal association. Arch Gynecol Obstet 2015;291:671-9.
13. Laurini R, Laurin J, Marsál K. Placental histology and fetal blood flow in intrauterine growth retardation. Acta Obstet Gynecol Scand 1994;73:529-34.
14. Bloomenthal D, von Dadelszen P, Liston R, Magee L, Tsang P. The effect of factor V Leiden carriage on maternal and fetal health. CMAJ 2002;167:48-54.
15. Dizon-Townson DS, Nelson LM, Jang H, Varner MW, Ward K. The incidence of the factor V Leiden mutation in an obstetric population and its relationship to deep vein thrombosis. Am J Obstet Gynecol 1997;176:883-6.
16. Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism. A meta-analysis involving approximately 120,000 cases and 180,000 controls. Thromb Haemost 2009;102:360-70.
17. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins–Obstetrics. ACOG practice bulletin No. 196 summary: thromboembolism in pregnancy. Obstet Gynecol 2018;132:243-8.