“20209C-T” a Variant Mutation of Prothrombin Gene Mutation in a Patient with Recurrent Pregnancy Loss

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Abstract. Recurrent pregnancy loss is considered when a female undergoes at least two consecutive spontaneous abortions or more than two alternately. This condition affects approximately 5% of women in reproductive age. Several causes of recurrent abortion have been established, but nevertheless, approximately half of all cases remain unexplained. Thrombophilic disorders have been suggested as a possible cause of recurrent miscarriage. A single 20210 G-A mutation of the 3'-untranslated region of (F2) has been reported as a cause of inherited thrombophilia. The F2 G-A mutation affects 1% to 4% of the US population, and its prevalence is higher among Caucasian women of Southern European descendants. Studies of G20210A polymorphism have also shown conflicting associations with recurrent abortions. In addition to G20210A polymorphism, other mutations affecting the F2 gene have been associated with thrombosis and/or pregnancy complications.

Introduction

Recurrent pregnancy loss is considered when a female undergoes at least two consecutive spontaneous abortions or more than two alternately [1]. This condition affects approximately 5% of women in reproductive age. Several causes of recurrent abortion have been established, but nevertheless, approximately half of all cases remain unexplained. Thrombophilic disorders have been suggested as a possible cause of recurrent miscarriage [2]. Women with thrombophilic disorders can suffer from a hypercoagulable state with abnormal placentation and pregnancy complications, such as preeclampsia, intrauterine growth restriction, placental abruption, and stillbirth [3-5]. Both heterozygosity and homozygosity for a common single 20210 G-A mutation of the 3'-untranslated region of (F2) have been reported as a cause of inherited thrombophilia. The F2 G-A mutation affects 1% to 4% of the US population and its prevalence is higher among Caucasian women of Southern European descendants. This mutation is associated with a 20% to 50% increase in prothrombin plasma levels, and affected women have a three-fold increased risk of venous thrombosis [6]. Studies of G20210A polymorphism have also shown a strong association between the polymorphism and a recurrent abortion; the mutation is associated with early recurrent miscarriage (OR 2.56, 95% CI 1.04-6.29) and late non-recurrent abortion (OR 2.30, 95% CI 1.09-4.87) [7]. In addition to G20210A polymorphism, other mutations affecting the F2 gene have been associated with thrombosis and/or pregnancy complications [8].

Case Report

The patient was a 34-year-old Caucasian woman without a family history of thrombosis. She was referred to our hospital with a history of two late spontaneous abortions that had occurred at 15 and 16 weeks of gestation, respectively. The fertility study was negative. The study of thrombophilia was negative for protein C, free-protein S, antithrombin, factor VIII:C, anti-beta-2-Glycoprotein I antibodies (IgM/IgG), anticardiolipin antibodies (IgM/IgG), presence of lupus anticoagulant, MTHFR mutation (C677T), and Factor V Leiden mutation (G1691A), except for the molecular
study of prothrombin mutation (G20210A). To assess prothrombin gene mutations in our laboratory, DNA was isolated from peripheral blood using a MagNA Pure LC DNA Isolation Kit (Roche Diagnostics). The sample was tested for the presence of the G20210A mutation by PCR, using a Factor II (Prothrombin) G20210A Kit (Roche Diagnostics) with a Roche LightCycler instrument.

A melting-curve analysis revealed a wild type allele at 60°C and an atypical melting point at 54°C (Figure 1A), which corresponds to an uncommon prothrombin allele. The DNA sequence of the prothrombin gene (ABI3100xl capillary sequencer, Applied Biosystems) in this patient showed a C→T transition at nucleotide 20209 (Figure 1B).

The patient was diagnosed as a heterozygous carrier of the mutation C20209T prothrombin gene. The family study showed the presence of this mutation in the patient’s father; the patient received 40 mg of subcutaneous enoxaparin on a daily basis once the pregnancy was clinically established. Induction of labor was scheduled during the 39th week of gestation; a healthy baby was then delivered.

**Discussion**

The treatment of pregnant women with a prothrombin mutation is controversial, but several authors have reported that the use of thromboprophylaxis with LMWH has been shown to be useful in preventing recurrent thrombosis in this setting. Furthermore, preliminary evidence from case series suggests that this strategy may also be helpful in preventing miscarriage and in improving neonatal and maternal outcomes [10, 11].

The clinical features and biochemical activity of the 20209C-T variant are not fully understood in this context. The functional activity has not been systematically investigated. It has been widely demonstrated that the 20210G-A mutation is related to an increase in plasma prothrombin levels [12] and is associated with an increased risk of the development of thrombotic events and/or obstetrical complications. Our patient showed normal prothrombin levels. This could be explained by the absence of the 19911G allele, which has been shown to increase prothrombin levels in combination with 20209 C>T. It seems rational that the close proximity of the two mutations could produce the same type of clinical symptoms. The 20209C-T variant has mainly been found in individuals of African-American offspring who have had thrombotic events and/or obstetrical complications in the past. To date, no case of a Caucasian patient carrying this mutation has been reported. The present findings indicate that the mutation is not confined to a small ethnic group of African-American patients but could also affect individuals of European ancestry.

An important technical point to take into account in order to identify polymorphisms close the G20210A mutation is to use of adequate genotyping methods. In cases of homozygosity, direct sequencing should be performed to exclude double heterozygotes. In this report we describe a case of
C20209T prothrombin mutation with history of recurrent pregnancy loss in a Caucasian woman. This case can provide further evidence that variant C20209T might have an important role as a pathogenic factor for thrombophilic events and/or obstetrical complications. Further study is needed in order to confirm these findings.

References


