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Case Report

Prothrombin C20209T mutation in deep vein thrombosis: a case report

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Abstract: Thrombophilias is a recognized risk factor for thrombotic events. The prothrombin variant G20210A gene mutation has been commonly examined using polymerase chain reaction (PCR). Currently, in many clinical laboratories, performing the PCR in real-time technique, which, in addition to identifying the G20210A mutation, makes possible the detection of other mutations in the 3'UTR of the prothrombin gene by melting curve analysis, due to the ability of this analysis to be amplicon-dependent (e.g., C20209T, C20221T and A20218G). We report the first case in Chile that describes the atypical prothrombin C20209T mutation, in a 50-year-old male patient diagnosed with deep vein thrombosis in the lower limb and family history of thrombophilia. In the literature, there are few studies of the prevalence and functionality of this mutation; its association with thrombotic events is controversial.

Keywords: C20209T, 3'Untranslated region, thrombophilia, prothrombin, mutation

Introduction

Thrombotic events such as deep vein thrombosis and pulmonary embolism are risk factors that may be acquired and/or inherited. Currently, and as part of the clinical screening tests the Factor V Leiden (FVL) and the prothrombin G20210A mutation are the risk factors most researched in clinical laboratories because are the two most common causes of hereditary thrombophilia.

The prothrombin G20210A mutation located in the region 3'UTR of the gene, is classified as a moderate risk factor for thrombosis. In this site, the pre-mRNA typically receives the cleavage and polyadenylation under endonucleolytic processing. As a result of the mutation, the gene is overexpressed, increasing messenger RNA in the cytoplasm with a consequent increment of prothrombin levels in the plasma, being classified as gain of function mutation [1].

The 3'UTR region of the prothrombin gene is a region prone to mutations. Previous case reports describe other mutations, including C20209T, C20221T, A20218G and T20219A A20207C [2-4]. Among them, C20209T mutation is the most common [5, 6]. Currently, few functional studies have been carried out to determine its clinical significance. Therefore, its association with thrombosis could not be stressed with the same force as the G20210A mutation variant [7, 8].

In this work, we report the first case in Chile that describes the atypical prothrombin C20209T mutation in a patient diagnosed with deep vein thrombosis in the lower limb and family history of thrombophilia.

Case report

A 50-year-old man was admitted to the emergency room following one week of persistent pain of the gastrocnemius musculature in the region of the left popliteal fossa. Deep vein thrombosis (DVT) of the great saphenous and gastrocnemius veins was diagnosed [9, 10]. The patient was initially treated with low-molec-

Table 1. Thrombophilia protocol; Results obtained by performing clinical protocol

Determination	Value	Reference values
Homocysteine	9:09 µmol/L	5-15 µmol/L
Protein S	60%	>58%
Protein C	111.8%	70-140%
Antithrombin III	93.2%	75-125%
Lupus anticoagulant	36 seconds	Less than or equal to 45 seconds
Anticardiolipin antibody	IgG 1.94 GPL IgM 0.64 MPL	Less than 15 GPL or MPL

ular weight heparin and subsequent oral anticoagulant therapy (acenocoumarol) for three months. During his hospitalization the patient's health evolved positively with reduced edema and pain.

The patient had a history of hypertension, dyslipidemia and hyperuricemia treatment (Acerdil D, Zarator and Alopurinol). In his family history it was reported that his father suffered an acute myocardial infarction, and two daughters suffered DVT and pregnancy miscarriages.

According to health center protocol, thrombophilia study was performed with homocysteine levels within normal range and no presence of lupus anticoagulant and anticardiolipin antibodies were detected, C Protein, S protein and antithrombin III levels were within normal range (**Table 1**).

Genetic testing for FVL mutation and G20210A prothrombin gene mutation were performed using DNA isolated from blood samples taken with EDTA and using a High Pure Viral Nucleic Acid Kit (Roche Diagnostics) commercial kit. Real-time PCR was realized for the FVL mutation with the Factor V Leiden Kit and LightCycler instrument (Roche Diagnostics), undetected mutation. For the G20210A prothrombin mutation, Factor II (Prothrombin) G20210A Kit (Roche Diagnostics) was used.

The melting curve analysis of the prothrombin G20210A mutation showed a different pattern in the patient sample compared with the heterozygous mutation-positive control and homozygous normal allele. In heterozygous carriers, G20210A mutation showed two peaks at 49°C and 59°C for the normal allele and mutated allele, respectively, whereas in the patient sample, peaks at 54°C and 59°C were observed; the latter corresponds to a normal allele, but the peak at 54°C is atypical (**Figure 1**).

The sample was sequenced using the Big Dye Terminator Kit v1.1 on an ABI Prism 310 Genetic Analyzer, showing nucleotide change from cytosine to thymine at position 20209 in the 3'-untranslated region of the prothrombin gene, determining the C20209T mutation (**Figure 2**).

Discussion

This case is the first report of the prothrombin G20210A mutation in Chile. It is possible that similar cases were not previously reported because few laboratories perform real-time PCR by melting curve analysis, which can detect this mutation. Commonly, laboratories perform the traditional restriction enzyme digestion and PCR method because of lower cost, where the action mechanism of the restriction enzymes is targeted nucleotide so that this procedure is only shown as an enzyme that interacts with nucleotide 20210 and does not allow identification of other mutations in the region.

Several cases have been reported around the world with this new mutation, but no prevalence values have been established yet, although some authors suggest a higher prevalence in black populations as most reports include African-Americans or subjects of African descent [3, 5]. Itakura et al. [11] conducted a three-part study on U.S. African-American population. The first part was a prevalence study of 1078 individuals for C20209T mutation, obtaining a rate of 0.37%. The second and third parts of the study researched the association between mutation C20209T mutation and infarction in patients with sickle cell anemia and deep vein thrombosis, respectively, and found no significant association. The authors state that although no association was observed in this study, the role of this mutation as a risk factor for thrombotic events should not be dismissed. With a low prevalence it is difficult to determine whether a significant association exists.

It has been suggested that the prothrombin variant C20209T mutation being adjacent to the G20210A prothrombin gene, and as part of the dinucleotide signal to the cleavage site in polyadenylation, could have the same or similar mechanism of action increasing the risk of

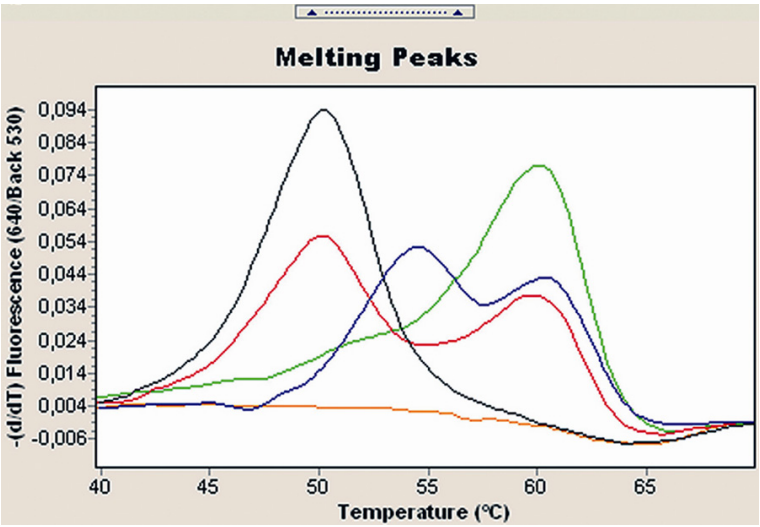

Figure 1. Graphic melting curves. Green curve shows a peak at 59°C, which corresponds to a normal homozygote. Red curve with two peaks, one at 49°C and other at 59°C corresponding to a heterozygous mutant. In black, a peak at 49°C that corresponds to a homozygous mutant. Blue curves with two peaks, one at 59°C, which corresponds to a normal allele and another peak at 54°C corresponding to an atypical mutant allele. In the orange ran a negative control.

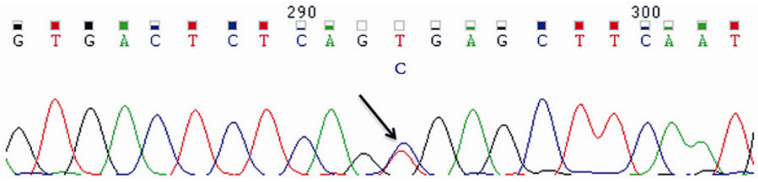

Figure 2. Fragment sequencing of the amplicon obtained by PCR 3'UTR end of the prothrombin gene. Arrow indicates nucleotide 20209 where two peaks were observed, one representing a thymine base and the other a cytosine base.

thrombotic events [4]. Danckwardt et al. [7] performed genetic constructs with normal and mutated 20209 alleles, analyzed the level of transcription and observed similar results of the functional studies of the G20210A prothrombin mutation [1]. That is, an increase in the expression of messenger RNA and consequent protein increased, albeit more moderate. In contrast, van der Putten et al. [8] performed a functional assay for C20209T prothrombin mutation, finding opposite results to Danckwardt et al. [7], who argue that there is a relation between the presence of mutation and clinical presentation of thrombosis, and given the special characteristics in the 3'-untranslated region of the prothrombin gene where the absence of uracil residues, essential for the recruitment of the transcription machinery, is compensated by mutations that add to thymine in this region and results in functional gain of the gene [12, 13]. Despite their differences, both authors agree that larger studies are required to establish a definitive association between prothrombin C20209T mutation and risk of thrombosis.

Studies by Schrijver et al. [14] reported three cases of African-American women with pregnancy complications carrying atypical mutations of the prothrombin, where one was a heterozygous carrier of the mutation C20221T and two were heterozygous carriers of the mutation C20209T. In addition, Danckwardt et al. [7] described three cases of Moroccan-Jewish women with obstetric complications who were carriers of the mutation C20209T.

The relation between the prothrombin G20210A mutation and obstetric complications such as preeclampsia, abortions, placental abruption, and intrauterine growth restriction, is considered controversial. Despite several studies that found an association, there is no consensus on the magnitude of this risk factor and specific association with obstetric complications, namely the result of differences in study design [15-17]. In reference to mutation C20209T there is even less information available and its association with obstetric complications can only be inferred from the reported cases in the literature.

Interestingly, in our case report, the patient had two daughters who suffered miscarriages with no apparent cause or diagnosis. This would strongly suggest the need for a G20210A mutation study by melting curve analysis for the daughters, in view of the probability of being mutation C20209T carriers like their father, would provide clinicians with important infor-

mation for future pregnancies. It might be considered that the real-time PCR test with melting curve analysis is not very specific because, in addition to detecting the prothrombin gene G20210A mutation, other mutations can be determined [2-5, 18], and amplicon sequencing is necessary to know which mutation is present when outliers occur. However, this remains a problem, given the low prevalence of this mutation, especially in our population. Furthermore, the association of PCR with melting curve analysis gives us more information than traditional PCR, and is of high value in patients where other causes of risk of thrombotic events have been ruled out [19], and yet they suffer them repeatedly.

The value of the melting curve analysis (hybridization probes) in PCR studies is highlighted as it gives us the assurance that the amplified product is sought because the melting temperature is specific for each amplicon and gives us the possibility of identifying other amplification products that were obtained with the same primers.

This report demonstrates the importance of performing adequate tests to find outliers in prothrombin variant G20210A mutation in our population, although particular mutations are associated with a particular race or region and because of globalization and immigration, they can reach our environment; therefore, we must know and possess the necessary technology for diagnosis.

Disclosure of conflict of interest

None.

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