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CASE REPORT

A Rare Prothrombin Gene Mutation C20209T in a South African Patient with Pulmonary Embolism in Pregnancy: a Case Study and Systematic Review

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SUMMARY

Background: **The G202010A prothrombin gene mutation is a documented prothrombotic risk factor in Caucasian patients. Several other mutations have been described within the prothrombin gene, predominantly in non-Caucasians, including the C20209T mutation. The clinical significance of this mutation is uncertain, but it has been associated with thrombotic events and pregnancy complications.**
Methods: **We describe a 28-year-old black South African woman who presented with pulmonary embolism during pregnancy. She was investigated for underlying prothrombotic biomarkers.**
Results: **Genetic screening for the prothrombin G202010A mutation by real-time polymerase chain reaction and melting curve analysis demonstrated an atypical mutant peak. Sequencing confirmed a variant C20209T prothrombin mutation.**
Conclusions: **This is the first report of the C20209T mutation in the Southern African population. It remains uncertain whether genetic testing should be offered routinely to non-Caucasian patients in a resource-limited setting.**
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KEY WORDS

prothrombin gene, prothrombotic genetic risk factors, venous thromboembolic disease

INTRODUCTION

Both acquired and genetic conditions predispose to venous thromboembolism (VTE). Well-described genetic risk factors include the Factor V Leiden and the G202010A prothrombin gene mutation, which have been documented predominantly in Caucasian patients. Although VTE is as common in black patients, the genetic risk factors in this population are poorly elucidated [1]. Several mutational variants within the prothrombin gene occur predominantly in non-Caucasian people [2-5], including the C20209T prothrombin gene mutation. The clinical significance of this mutation, seen with in-

creased frequency in patients of African ethnic origin, is uncertain although it has been associated with thrombosis and obstetric complications [2-4,6]. Only limited reports of this mutation have been described and none in the Southern African population. It is important to assess the clinical significance and prevalence of this mutation to determine whether it has diagnostic and prognostic utility [3,4,7]. In this paper, we report an index presentation of a patient with this mutation, systematically review the available literature, and discuss some of the potential clinical implications and diagnostic dilemmas. The patient provided written informed consent, and ethics approval was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (clearance certificate: M180786).

CASE STUDY

A 28-year-old black South African woman was referred from a primary healthcare facility in Johannesburg at 36 weeks of gestation with shortness of breath and chest pain. She previously had two normal vaginal deliveries at term and did not have a personal or family history of VTE. On examination, although tachycardic and tachypneic, she was hemodynamically stable. A pulmonary embolus (PE) was confirmed on ventilation-perfusion scan, affecting the inferior lingual segment of the left lung. No deep vein thrombosis (DVT) was identified on Doppler investigation of the lower limbs. Echocardiography and chest X-ray were unremarkable.

Laboratory investigations for an underlying hypercoagulable state revealed reduced protein C and S with elevated D-dimer levels, consistent with pregnancy. Genetic screening conducted by real-time polymerase chain reaction (PCR) with subsequent melting curve analysis on the LightCycler®480 (Roche Diagnostics®, Mannheim, Germany) for Factor V Leiden was normal, but the G20210A prothrombin gene analysis demonstrated a mutant peak at a melting temperature of ~51°C and a normal wild type peak at ~60°C (the G20210A prothrombin mutation shows a melting peak at ~54°C) (see Figure 1a). DNA sequence analysis of the prothrombin gene product was performed on the ABI3730 DNA Analyzer (Applied Biosystems®, CA, USA), demonstrating a 20209C/T mutation (see Figure 1b). None of the other previously described mutations (including 20207, 20214, 20218, 20219 and 20221) was noted.

The patient was managed in an obstetric high-care unit on therapeutic-dose enoxaparin (100 mg twice a day subcutaneously). This was continued after discharge at a prophylactic dose of 40 mg daily. The patient had a planned induction of labor at 38 weeks resulting in an emergency Caesarean section for fetal distress and delivery of a healthy 3.5 kg neonate. The postnatal course was uneventful, and the patient was discharged on warfarin therapy to the hematology outpatient clinic.

DISCUSSION AND LITERATURE REVIEW

This is the first report of the C20209T mutation in the Southern African population and was associated with VTE in pregnancy in a young black South African woman.

A full review of the literature (Table 1) revealed that the association of this mutation with venous and arterial thrombotic events and/or obstetric complications (including recurrent pregnancy loss, intra-uterine growth retardation, and unexplained infertility) is controversial. Findings of case studies or series have not been conclusively confirmed by further in-depth studies. Of 81 individuals described with the C20209T mutation, 57 (70%) presented with clinical complications including VTE (34 patients), cerebrovascular accident (CVA) or arterial thrombosis (14 patients), and obstetric complications (12 patients). Ethnic information was available in 55/57 (96%) and gender in 50/57 (88%) of patients. Of these, 39 were of black African descent (predominantly African American) and 34 were female [2-18].

In 12 of the females, obstetric complications were the only presenting finding, and this was more common in Caucasian patients ($p = 0.0592$) [2-4,8,14,15]. Arterial thrombosis was more common in male than in female patients ($p = 0.0143$) [4,6-8,12,16].

Epidemiological studies suggest that patients of black African descent have a similar or higher incidence of VTE and present at a younger age (47.5 - 56.2 years) than Caucasian patients [1,5,7,9]. Our analysis of published studies suggests that black patients with the C20209T prothrombin gene mutation present with VTE at least 10 years earlier (34.5 years, $n = 18$) than black patients without it [2-18]. Female patients with the mutation also presented with thrombosis at an earlier age (34.5 years, $n = 22$) compared with males (50 years, $n = 15$) ($p = 0.0009$). This earlier presentation may be partially attributed to the presence of additional risk factors including pregnancy and oral contraceptive use which were seen in 53% of all female patients and 33% of females with VTE [2-4,8,14,15]. Overall 51% of patients with a C20209T mutation had an associated risk factor [3,4,6-11,14,16]. Recurrent thrombotic episodes were reported in 12 patients [3,4,6,10,13].

CONCLUSION

In this case presentation, we describe VTE in a pregnant black South African patient. Studies suggest that black individuals that carry the C20209T mutation may be at increased risk of thrombotic events which present at a younger age, particularly in females with additional prothrombotic risk factors such as pregnancy or oral contraceptive use [2-4,8,14,15].

The possible prothrombotic pathophysiological mechanism of the C20209T prothrombin gene mutation [9,13, 14] and the potential added risk associated for either thrombosis or pregnancy complications is uncertain. For

Table 1. Systematic review of the literature documenting cases of C20209T mutation in association with thrombotic events, stroke or obstetric complications [2-18].

	Female (n = 34)	Male (n = 16)	Gender not specified (n = 7)	p-value
Ethnicity				
African descent	24 (71%)	10 (63%)	5	
Caucasian descent	10 (29%)	4 (25%)	-	
Hispanic descent	-	2 (13%)	-	
Unknown	-	-	2	
Median age at presentation of thrombotic event (years) [IQR]				
All patients	34.5 [23.3 - 43.8] (n = 22)	50 [41 - 59] (n = 15)		0.0009
African descent	34 [21 - 43] (n = 18)	44 [41 - 57] (n = 9)		0.0040
Other descent	40.5 [28.3 - 72.3] (n = 4)	55 [44.8 -84.5] (n = 6)		0.2571
Clinical presentation				
Venous thrombotic event	18 (53%) [1]	10 (63%) [1, 2]	6 (86%)	> 0.9999
Superficial thrombosis	1	1		
DVT	10	4		
PE	2	3		
DVT and PE	4	1		
Other	1	1		
Arterial thrombotic event	5 (15%) [1]	8 (50%) [1, 2]	1 (14%)	0.0143
Cerebrovascular accident	4	6		
Peripheral vascular disease	-	1		
Myocardial infarction	1	1		
Documented obstetric complication (recurrent pregnancy loss, intra-uterine growth retardation or infertility)	12 (35%)	-		
Associated risk factors	23 (68%)[3]	6 (38%)[3]		0.0661
Use of combined oral contraceptive pill or pregnancy	18	-		
Trauma or surgery	2	1		
Other (additional prothrombotic mutations, malignancy, infectious diseases, sickle cell disease)	7	6	1	

[1] Two patients had DVT and cerebrovascular accident.
[2] One patient had PE and myocardial infarction.
[3] Five patients had more than one risk factor.
Abbreviations: DVT - deep vein thrombosis, IQR - Interquartile range, n - number, PE - pulmonary embolism.

this reason, it is unclear whether genetic testing should be offered routinely to non-Caucasian patients in a resource-limited setting, particularly when the therapeutic implications of the testing are uncertain. [4,8] Heterogeneity of the reported clinical presentation (arterial or venous thrombosis and obstetric complications) also complicates the definition of a target population for testing. The mutation appears to act synergistically with other prothrombotic risk factors. This raises concerns about risk factors prevalent in our population including Human Immunodeficiency Virus (HIV) and *Mycobacterium tuberculosis* infection.

Although this mutation is not specific to a geographical region or ethnic group, it appears more prevalent in people of black African descent. Most studies have been conducted in African American populations and local African studies are absent. Further studies are indicated to raise awareness and improve understanding of its

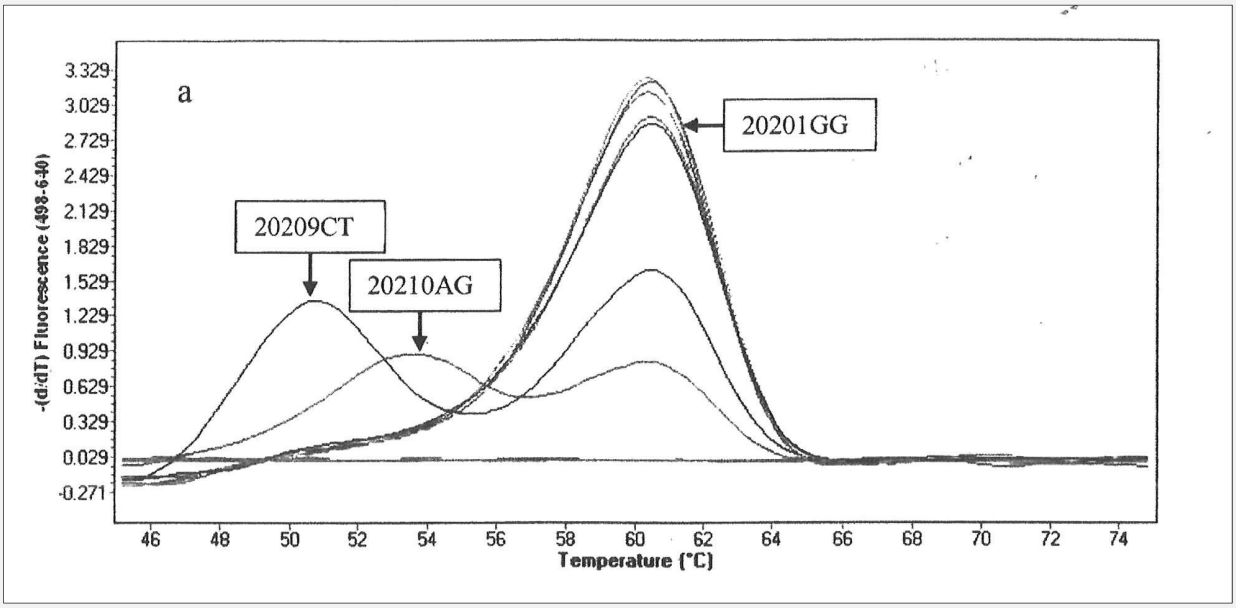

Figure 1a. Melting curve analysis: prothrombin 20209CT, 202010GA, and wild type 20210G melting peaks.

The melting temperature of the C20209T mutant in this patient was ~51°C. The typical prothrombin gene mutation at 202010AG (melting temperature ~54°C) and the wild-type (melting temperature ~60°C) are included for reference.

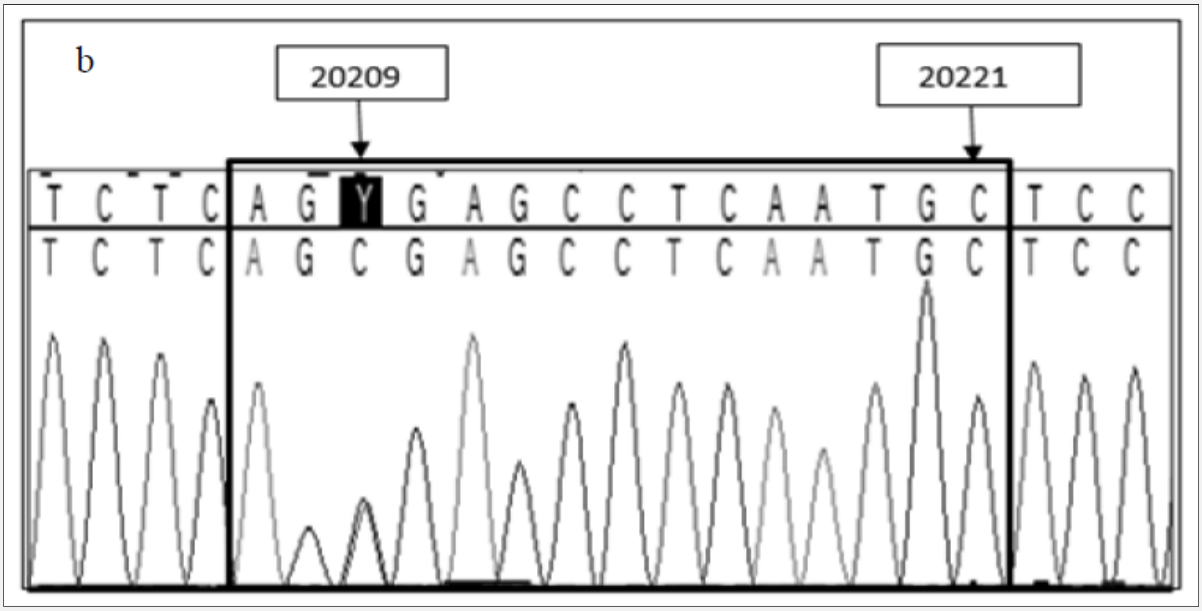

Figure 1b. The prothrombin gene sequence.

The patient was found to be heterozygous for the C20209T mutation.

contribution to the development of pathological thrombosis.

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Declaration of Interest:
The authors declare no conflict of interest.

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