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The First Homozygous Family for Prothrombin G20210A Polymorphism Reported in Latin America

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The 20210A allele of the prothrombin gene is associated with increased risk of venous thromboembolism. In this study, we described manifestations of thrombosis in four generations of a Colombian family, with four 20210A homozygous carriers and six 20210G/A heterozygous carriers for polymorphism as well as unrelated participants from the same population. The levels of prothrombin in the 20210A homozygote patients were higher than in the normal 20210G homozygotes (133 ± 11% and 92.3 ± 12.4%, respectively, $P < .01$) and the 20210G/A heterozygotes (133 ± 11% vs. 114.8 ± 24%, $P < .05$). About 2 out of 4 20210A homozygotes and 5 out of 6 20210G/A heterozygous members of this family did not have venous thromboembolism or any other thrombotic manifestation even though one of them had been exposed to thrombotic risk factors. Thus, we posit the effect of 20210A on the thrombotic phenotype in this family seems to be weak.

Keywords: thrombophilia; prothrombin; homozygote; venous thrombosis

Introduction

G20210A polymorphism of the prothrombin gene is a single point change in the 3′ untranslated region (3′ UTR), which decreases hydrolysis of the messenger ribonucleic acid (mRNA) causing prothrombin mRNA accumulation and leads to increased levels of prothrombin in the plasma, as well as increased risk for venous thromboembolism (VTE).[1] The prevalence of this polymorphism among individuals in southern Europe is 2.3% to 6.5%,[2-5] in northern Europe 0.7% to 4.0%,[1,4] and in Latin America 0% to 3.8%.[6-8] In a previous study, we found only one 20210G/A heterozygous carrier among 320 healthy individuals.[9,10]

Homozygous carriers for the 20210A polymorphism are rare and to our knowledge about 90 individuals have been reported, 5 of these from Latin America.[1,7,10-21] Among the homozygous carriers, 14 cases belonged to 6 European families and none were from Latin America. Although the more severe thrombotic risk may be expected in the 20210A homozygotes, the individuals described in the literature are heterogeneous from asymptomatic older individuals to young patients with severe thrombotic events.

We studied different types of inherited thrombophilia as risk factors for deep venous thrombosis at

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Table 1. Demographic Characteristics, Polymorphisms of the G20210A Prothrombin Gene, and Plasma Prothrombin Levels of Family Members

Patient	Age	G20210A	Prothrombin Level (%)	Thrombosis (DVT, PE)	Risk Factors
Maternal grandmother; I: 1	93	GA	94	No	Pregnancy
Maternal grandfather[a]; I: 2	90	GG	72	No	No
Mother; II: 1	60	GA	115	No	Pregnancy
Father[a]; II: 2	62	GA	85	No	No
Proband; III: 1	32	AA	111[b]	DVT, PE	Sedentary life
Sister 1; III: 2	33	AA	122	No	Contraceptive pills, pregnancy
Sister 2; III: 3	31	AA	66[b]	DVT	No
Sister 3; III: 4	31	AA	144	No	No
Sister 4; III: 5	28	GA	111	DVT	Pregnancy
Brother; III: 6	30	GG	ND	No	No
Son of sister 1; IV: 1	6	GA	113	No	No
Son of sister 4; IV: 2	9	GA	109	No	No

NOTES: DVT = Deep venous thrombosis; ND = Not determined; PE = Pulmonary embolism.
[a] Recent death unrelated to venous thrombosis
[b] Values when patients were taking warfarine.

the Hospital Universitario San Vicente de Paúl in Medellín, Colombia[10] and in the context of this research, we have found the first homozygous family for G20210A prothrombin polymorphism reported in Latin America.

Materials and Methods

Patients

In a case-control study of VTE and inherited thrombophilia, 1 male patient homozygous for the prothrombin polymorphism G20210A was identified.[10] This patient along with 11 first and second degree relatives, 2 unrelated patients with a history of VTE, and 20 unrelated healthy donors were included in the study. Ethical approval was obtained by the Research Ethics Committee of the University of Antioquia and informed consent was obtained for all participants.

Determination of G20210A Prothrombin Polymorphism and Levels

Deoxyribonucleic acid (DNA) was extracted from peripheral blood by standard methods. To determine G20210A polymorphism, a previously described protocol was used.[1] Prothrombin levels in plasma were quantified by the coagulometric assay using prothrombin-deficient plasma following the instructions of the manufacturer (HS Plus, Instrumental Laboratory Company, Lexington, Mass).

Statistical Analysis

The results of prothrombin levels in plasma are expressed as mean $\pm$ standard deviation. Analysis of variance (ANOVA) with post hoc Tukey comparisons was used to determine differences between 20210 genotypes using the Prism 4.0 software package. Significance was defined as $P < .05$.

Results

G20210A Prothrombin Polymorphism

The proband, a 32-year-old man who developed VTE of the right iliac, femoral, and popliteal veins complicated with pulmonary embolism, was 20210A homozygous for prothrombin G20210A polymorphism. Two sisters of the proband developed VTE of iliofemoral veins; one was a 20210A homozygote and the other a 20210G/A heterozygote. The demographic characteristics and the genetic determination of G20210A polymorphism of the family are shown in Table 1 and Figure 1A.

Prothrombin Levels

To correlate prothrombin G20210A genotypes with plasma prothrombin levels, we tested 11 members of the family, 2 unrelated patients with a history of VTE carrying the 20210G/A allele, and 20 unrelated healthy individuals, 19 of which carry the 20210G allele. We observed that the higher levels of prothrombin correlated with the number of 20210A

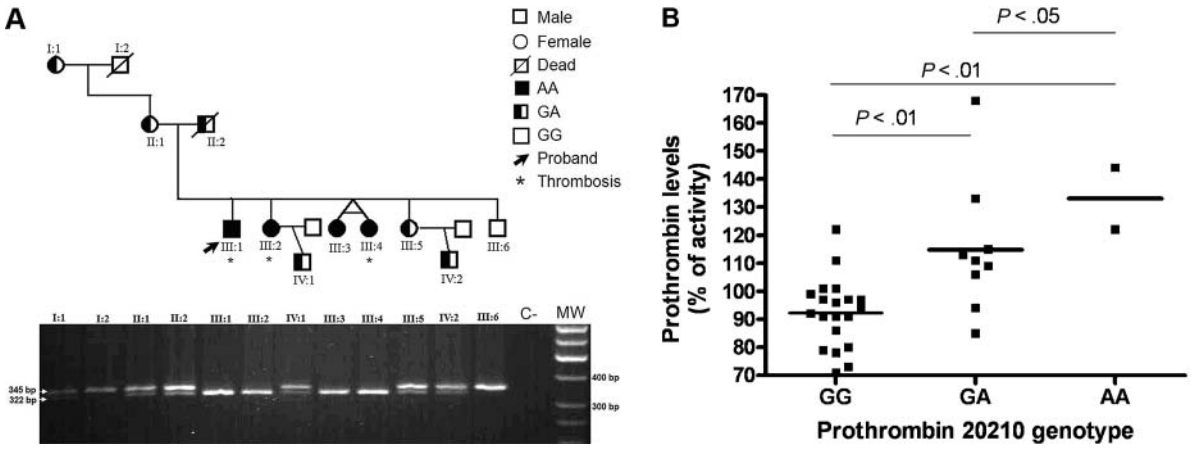

Figure 1. Genetic and biochemical characterization in a family with homozygous carriers for the prothrombin 20210A gene polymorphism. A, Family genealogy and agarose gel of polymerase chain reaction (PCR)-amplified exon 14 and 3′ UTR of the prothrombin gene from 12 family members. The 20210G sequence of the prothrombin gene displays a band of 345 bp after *Hind*III digestion; the 20210G/A heterozygous genotype is characterized by two fragments of 345 and 322 bp; the absence of the 345 bp fragment and the presence of only the 322 bp fragment is distinctive of the 20210A homozygous genotype. C–: negative control without deoxyribonucleic acid (DNA) and molecular weight (MW) of 100 bp. B, Plasma levels of prothrombin from 1 member of the family and 19 unrelated healthy individuals carrying the 20210G allele; 6 members of the family, 1 healthy donor, and 2 unrelated patients with a history of venous thromboembolism (VTE) carrying 20210G/A and 2 members of the family homozygous for the 20210A allele.

alleles in the prothrombin gene (A/A: 133% ± 11%, G/A: 114.8% ± 24%, and G/G: 92.4% ± 12.1%; Figure 1B). Two 20210A homozygous carriers were excluded from prothrombin level analyses because they were taking warfarin, which inhibits prothrombin synthesis.

Discussion

This appears to be the first study documenting a family in Latin America with carriers of G20210A polymorphism of the prothrombin gene, including four 20210A homozygotes and six 20210G/A heterozygous individuals. Two out of four 20210A homozygotes did not have VTE or any other thrombotic manifestation, even though one of them had been exposed to thrombotic risk factors such as oral contraceptives and pregnancy. Moreover, five out of six 20210G/A heterozygous members of this family did not have VTE and even some of them had pregnancies and or had reached an advanced age without any thrombotic symptoms.

The expression of the phenotype of venous thrombosis, a complex genetic disorder, is dependent on the interaction of the gene products from several loci as well as on environmental and/or acquired influences. It seems that heterozygous G20210A prothrombin polymorphism is not an important risk factor for thrombosis in this family; these findings are in agreement with the results proposed by others authors in different regions of the world[1,7,10-21] showing that the G20210A polymorphism is not a risk factor for thrombosis as high as it should be expected.

In conclusion, based on the literature and on our results, we consider the possibility that the prothrombin G20210A polymorphism acts as a gene with minor influence on the prothrombin plasma levels and VTE. Consequently, the prothrombin G20210A polymorphism does not seem to play a critical role in the phenotype of thrombosis, even in 20210A homozygous carriers.

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