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Study of the ethnic origin of the prothrombin gene *(F2)* in the admixed Venezuelan population.

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Key words: prothrombin gene (*F2*); *F2* polymorphism 20210 G>A; ethnic origin; rs2070850; rs2070852; rs2282686; rs1799963.

Abstract. The prothrombin (coagulation factor II), codified by the *F2* gene, is the precursor of thrombin that cleaves fibrinogen, leading to a blood clot formation. The *F2* mutation 20210 G>A (c.*97G>A) is associated with prothrombin thrombophilia, and carriers have a higher than average risk for developing deep venous thrombosis. The 20210A variant is almost absent in populations other than Caucasoid European, and was not found in a Venezuelan population sample of 160 healthy individuals. To assess the possible ethnic origin of the *F2* gene in our admixed population, four intragenic SNPs (rs2070850, rs2070852, rs2282686 and rs1799963), with different allelic frequencies according to ethnic groups, were studied and compared with the main 1000 Genomes Project super-populations. The results showed intermediate allelic frequencies in all the SNPs, without differentiating a single specific population, confirming the joint ancestral genetic contribution of the parental populations in Latin America: Spaniards, Africans and Amerindians. Our allelic frequency distribution of the *F2* polymorphisms was closer to the AMR (American admixed) subset population of the 1000 Genomes Project. According to this ethnic composition, there is a low probability of detecting carriers of the risk allele 20201A in the Venezuelan healthy general population.

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Origen étnico del gen de la protrombina (*F2*) en la población venezolana.

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Palabras clave: Gen protrombina (*F2*); polimorfismo *F2* 20210 G>A; origen étnico; rs2070850; rs2070852; rs2282686; rs1799963.

Resumen. La protrombina (factor de coagulación II), codificada por el gen *F2*, es un precursor de la trombina, la cual escinde al fibrinógeno, conducente a la formación de un coágulo de sangre. La mutación *F2* 20210 G>A (c. * 97G> A) se asocia con trombofilia y los portadores tienen un riesgo superior al promedio de desarrollar trombosis venosa profunda. La variante 20210A está casi ausente en poblaciones distintas a la europea caucasoidea, y no se encontró en una muestra de 160 individuos sanos de la población venezolana. Para evaluar el posible origen étnico del gen *F2* en nuestra población mezclada, se estudiaron cuatro SNP intragénicos (rs2070850, rs2070852, rs2282686 y rs1799963) con diferentes frecuencias alélicas, según los grupos étnicos y se compararon con las principales superpoblaciones del Proyecto 1000 Genomas. Los resultados mostraron frecuencias alélicas intermedias en todos los SNP, sin diferenciar una única población específica, lo que confirma la contribución genética ancestral conjunta de las poblaciones parentales en América Latina: españoles, africanos y amerindios. Nuestra distribución de frecuencias alélicas de los polimorfismos de *F2* fue más parecida al subconjunto AMR (American admixed population) del Proyecto 1000 Genomas. De acuerdo con esta composición étnica, existe una baja probabilidad de detectar portadores del alelo de riesgo 20201A en la población general sana venezolana.

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INTRODUCTION

Prothrombin is a proenzyme that is converted to thrombin by the prothrombinase complex (Factor Xa and Factor Va, assembled on a phospholipidic surface in the presence of Ca^{+2}). Thrombin cleaves fibrinogen, which triggers a self-aggregation process that end with clot formation. Approximately, 49% of prothrombin levels seem to be genetically regulated (1). The prothrombin polymorphisms 20210 G>A (c.*97G>A, rs1799963) (2) and 19911 A>G (c.1726-59 A>G, rs3136516) (3) have been associated to increased prothrombin plasma levels, and both represent independent risk factors for venous thrombosis (4-6). The 20210A genetic variant has different prevalence according to the ethnic group, being more frequent in Caucasians, especially Spaniards and Southern Europeans (French, Italians, Greeks) (7-12). We have assessed the prothrombin polymorphism G20210A in a Venezuelan population sample, recruiting 160 healthy subjects from the metropolitan area of Caracas, as described elsewhere (13). All of them were homozygous for the ancestral allele G.

The allele A is a worldwide infrequent variant, which is absent in Asian and African populations, and is at very low frequencies in some European populations: Azorian (0.018), Basque (0.020), Spaniards (0.015), Cypriot (0.039), French (0.021) (ALFRED database, https://alfred.med.yale.edu), with a global Minor Allele Frequency (MAF) of 0.0036 (1000 Genomes Project, http://www.internationalgenome.org). In the American subset populations (AMR) included in the 1000 Genomes Project (Mexican, Colombian, Peruvian and Dominicans), the allele A frequency is 0.0144, similar to those found in the above mentioned populations. The absence/low frequency of the allele A in non-Caucasian populations have been attributed to an ancient founder effect, occurred after the divergence of Africans from non-Africans (14). On the other hand, its frequency was found between two to ten-fold increased in patients with idiopathic vein thrombosis compared to healthy control subjects (2). Thus, the absence of the allele A in this Venezuelan cohort prompted us to assess the probable *F2* gene ethnic origin in the admixed Venezuelan populations.

MATERIALS AND METHODS

Population

A cohort of 160 healthy subjects (aged between 25 to 60 years old) from the metropolitan area of Caracas was selected. A 5 mL blood sample was withdrawn in 15% EDTA-Na_4, and DNA was extracted as described elsewhere (13). The project was approved by the Bioethical Committee of the Venezuelan Institute for Scientific Research (IVIC). A signed informed consent was obtained from all participants.

Prothrombin polymorphism G20210A.

Genotyping of the G20210A polymorphism (rs1799963) was performed by DNA PCR amplification followed by *Hind* III restriction endonuclease digestion, as previously described (13).

F2 intragenic polymorphisms

Three intragenic prothrombin (*F2*) polymorphisms: c.240+83 C>T (IVS 2; rs2070850), c.423-7 G>C (IVS 5; rs2070852) and c.1472+251 T>C (IVS 11; rs2282686), which have clearly different allelic frequencies in distinct ethnic groups were selected. Primers were designed with Primer-BLAST software (at https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and each SNP was detected by restriction analysis, as follow:

rs2070850 (C>T): (f) 5'-GAGAGTGCGTGGAGGAGAC and (r) 5'-CATGTCATGGAGCTGCACA, product size 286 bp. Allele T produces a recognition site for *Bsm*I restriction enzyme.

rs2070852 (G>C): (f) 5'-CCACCATGGGCTGAGAAC and (r) 5'-CATTCCTGCCTCCTCACG, product size 195 bp. Allele C is recognized by the restriction endonuclease *Mnl*I.

rs2282686 (T>C): (f) 5'- AAGAGCCCC TTTCCCTTTTC and (r) 5'- GGTGAAACCCA CCAGTCTCT, product size 266 bp. Allele T abolishes the restriction site for *Sma*I enzyme.

Statistical analysis

Allelic frequencies were calculated by direct counting. Hardy-Weinberg equilibrium was assessed for each SNP, using the χ^2 test.

RESULTS

All the ancestors (up to two generations back) of the individuals recruited in the present study were born in Venezuela. The 160 individuals studied were homozygous for the wild type allele G of the prothrombin polymorphism G20210A.

The allele frequencies for the three SNPs are summarized in Table I. All of them were in Hardy-Weinberg equilibrium.

The allelic frequencies obtained for each polymorphism was compared to the five super-populations reported in the 1000 Genomes Project: Admixed American (AMR), European (EUR), African (AFR), South Asian (SAS) and East Asian (EAS) (Table I).

TABLE I
ALLELIC FREQUENCIES OF THE THREE INTRAGENIC PROTHROMBIN (*F2*) POLYMORPHISMS IN DIFFERENT POPULATIONS.

Populations	rs2070850 C>T Ancestral allele: C	rs2070852 G>C Ancestral allele: G	rs2282686 T>C Ancestral allele: C
VZL	C=0.780 T=0.220	G=0.439 C=0.561	T=0.406 C=0.594
AMR	C= 0.718 T=0.282	G=0.416 C=0.584	T=0.590 C=0.409
EUR	C=0.879 T=0.121	G=0.292 C=0.708	T=0.709 C=0.290
AFR	C=0.970 T=0.030	G=0.873 C=0.126	T=0.210 C=0.790
SAS	C=0.830 T=0.170	G=0.293 C=0.706	T=0.707 C=0.292
EAS	C=0.438 T=0.562	G=0.697 C=0.303	T=0.301 C=0.698

VZL: Venezuelan (present study); AMR: American; EUR: European; AFR: African; SAS: South Asian; EAS: East Asian. Number of chromosomes in the Venezuelan sample: rs2070850 (n= 304); rs2070852 (n= 312); rs2282686 (n=320).

Two of the three studied polymorphisms had intermediate frequencies for each allele, different from that of the main parental populations (European, African and Asian).

DISCUSSION

The prothrombin 20210G>A transition (c.*97G>A, rs1799963), despite being located in the 3' untranslated region of the gene, has been associated with elevated plasma prothrombin levels that raises the risk of thrombotic events. The pathophysiologic mechanism of this genetic variation seems to be a *gain-of-function* mutation that produces an enhanced 3' end processing and increased mRNA accumulation, with the consequent increase in protein synthesis (15).

First reports of allelic frequencies showed that 18% of probands from thrombophilic families and 6% of nonrelated patients with deep vein thrombosis carried the allele A, contrasting with the lower frequencies in healthy controls (between 1 to 2%) (2, 16). Subsequent additional studies found that the allele A is almost absent in non Caucasoids populations. In the Venezuelan sample of 320 chromosomes of healthy individuals its frequency was null, even though at least one carrier is expected. This motivated us to assess the probable ethnic origin of the *F2* gene, using population-specific intragenic polymorphic markers.

Venezuelan populations are the result of the genetic admixture of African, European and Amerindian ethnic groups (17). We chose three intragenic prothrombin (*F2*) polymorphisms: c.240+83 C>T (IVS 2; rs2070850), 423-7 G>C (IVS 5; rs2070852) and c.1472+251 T>C (IVS 11; rs2282686), which have clearly different allelic frequencies between distinct ethnic groups.

It was not observed an allelic disequilibrium in the *F2* gene polymorphisms studied of the *F2* gene that allows to propose a greater similarity with a particular ethnic group, as have been reported for other populations (Table I), where one of the alleles

have a higher frequency compared to other super-population samples.

The AMR population subgroup of 1000 Genomes Project was the exception, which showed greater similarity with the Venezuelan population. The individuals included in the AMR group belong to American admixed populations with the same parental origins as Venezuelan population (Spanish, Amerindian and African). Thus, the intermediate allelic frequencies observed in Venezuelans confirmed the mixed ethnic origin of our population, and the similar genetic background between the Ibero-American populations. The absence of 20210A allele in the 320 chromosomes examined suggests that in this cohort, the Caucasian/Spanish contributions must be low, besides being a sample of healthy individuals, in whom the A allele should be less frequent.

In conclusion, a unique, discernible and/or probable ethnic origin of the *F2* gene in the very heterogeneous healthy population of the metropolitan area of Caracas (Capital District) cannot be established with the intragenic polymorphisms used, although a clearly admixed pattern of the population was supported.

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