Gene Variants Associated With Venous Thrombosis: A Replication Study in a Brazilian Multicentre Study

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Abstract

Single nucleotide polymorphisms (SNP) associated with Venous Thromboembolism (VTE) risk have been identified in European and American populations. Replicate SNPs associated with VTE in a Brazilian multicenter case-control study of the Southeast region. Patients with previous VTE assisted at the Outpatient Clinics of 3 centers of the Southeast Brazilian region were compared to normal controls of the same geographic region. We evaluated 29 SNPs associated with VTE risk in other populations, and 90 SNPs for stratification analysis of the population. Due to high admixture of Brazilian population and lack of previous studies, the calculation of the sample power was performed after genotyping. Sample size, allelic frequency and Hardy-Weinberg equilibrium were estimated. The association and odds ratio analyses were estimated by logistic regression and the results were adjusted for multiple tests using Bonferroni correction. The evaluation of the genetic structure similarity in the cases and controls was performed by AMOVA. 436 cases and 430 controls were included. It was demonstrated that this sample has a statistical power to detect a genetic association of 79.4%. AMOVA showed that the genetic variability between groups was 0.0% and 100% within each group. None of the SNPs showed association with VTE in our population. A Brazilian multicenter case-control study with adequate sample power, high genetic variability though no stratification between groups, showed no replication of SNPs associated with VTE. The high admixture of Brazilian population may be responsible for these results, emphasizing the influence of the population genetic structure in association studies.

Keywords

GWAS, genetic association, TEV, SNP, single nucleotide polymorphism, venous thromboembolism, pulmonary embolism, thrombosis

Introduction

Venous Thromboembolism (VTE) is a multicausal disease with an annual incidence of approximately 1 in 1,0001 and is the result of a complex interaction between gene variants and environmental exposures.2 Despite rare and common genetic risk factors having been identified during the last years, these cannot explain the majority of spontaneous VTE. Indeed, the prevalence of these genetic factors can vary according to ancestry.3-6 Furthermore, the genetic or environmental factor responsible for increased thrombosis susceptibility associated to elevation of factor VIII, factor IX and factor FXI has not yet been established.

The HapMap project and the introduction of Genome Wide Association Study (GWAS) strategy enabled the identification of genetic alterations associated to complex diseases.3 This encouraged the search for additional genetic factors that could

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contribute to the knowledge of the widespread clinical heterogeneity of VTE.

Identification of Single Nucleotide Polymorphisms (SNPs) associated with VTE were based on prospective population studies, case-control, and cohorts of patients and families. Many loci have been robustly demonstrated to harbor common and low frequency susceptibility alleles, including ABO, F2, F5, F11, FGG, PROCR and others.7

However, these GWAS studies included only European-ancestry adults from French and Dutch case-control studies, and cohort and case-control studies from the United States, and mostly of Caucasian ethnicity.4-25 The Brazilian population is a highly admixed population and no study has been previously performed in South America.

Therefore, the aim of the present study was to replicate 29 SNPs previously associated with VTE risk, in a multicenter study covering 3 Brazilian centers of the Southeast region. We also evaluated the correlation between SNP genotyping and activity of the corresponding coagulation parameter in samples of participants of one of the centers.

Objective

We sought to replicate 29 SNPs previously associated with VTE risk, in a multicenter study covering 3 Brazilian centers of the Southeast region.

Methods

Selection of Study Participants

All participants provided written informed consent and the study protocol was approved by the institutional Research Ethics Committee (CAAE: 07970212.2.0000.5404).

This is a multicenter case-control study including outpatients and controls from the Southeast region of Brazil. The cases comprised Caucasian and African descendent unrelated patients with a previous diagnosis of deep venous thrombosis (DVT) of the lower limb or pulmonary embolism (PE). They were consecutively recruited from the Outpatient Clinic of Hemoentro Unicamp (Hemocentro / UNICAMP) in Campinas-SP, Thrombophilia Clinic of the Federal University of Rio de Janeiro (UFRJ) in Rio de Janeiro-RJ, and Thrombophilia Clinic of the Hospital Israelita Albert Einstein (HIAE), in São Paulo-SP, from 2009 to 2013. The controls were represented by Brazilian individuals without a history of VTE, from the same geographical area of the cases, matched by age and sex. Control subjects were not-inbred with the participating patients.

Two investigators from each center interviewed all participants and collected information using a non-validated structured questionnaire. For patients, information regarding gender, age at VTE event, risk factors for VTE (prolonged immobilization, local and general trauma, hospitalization, air travel lasting more than 6 hours or road travel without mobilization per more than 10 hours, 30 days before the VTE episode, surgery, cancer, use of oral contraceptive or hormonal replacement therapy, pregnancy, puerperium, central venous catheter, nephrotic syndrome, personal or familial history of thrombosis, antiphospholipid syndrome) and thrombosis site were collected.

The inclusion criteria included age between 18 and 60 years and confirmed diagnosis of DVT by Doppler ultrasonography and PE by angiotomography, angioresonance or ventilation/perfusion lung scan.

The exclusion criteria were VTE related to cancer, antiphospholipid syndrome, renal, liver or autoimmune disease, and hereditary thrombophilia (deficiency of protein C—PC, protein S—PS, antithrombin—AT, homozygosity or double heterozygosity for FV Leiden and F2 20210A).

For controls, information regarding gender, age at data collection, clinical comorbidities, use of oral contraceptive or hormonal replacement therapy, pregnancy and surgical history, medicine intake including acetylsalicylic acid were collected.

Exclusion criteria for controls included personal or familial history of cardiovascular or VTE episode, non-thrombotic antiphospholipid syndrome, renal, liver or autoimmune disease, hereditary thrombophilia and hormonal therapy or acetylsalicylic acid use.

SNP Selection

Twenty-nine SNPs were selected due to previous association with VTE irrespective of ethnicity or country of origin5-25 (Table 1). In order to represent variability across candidate genes, we performed a systematic SNPtag selection using SNPBROWSERTM v.4.0 software (Applied Biosystems, Foster City, California, USA), based on the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/), a minimum allele frequency (MAF) > 0.05, and a pairwise linkage disequilibrium (LD) of r2 >0.8 (Table 1). SNPs were previously tested by the manufacturer and demonstrated good quality performance.

Furthermore, we used an additional 90 SNPs, randomly selected, based on our previous study and not ancestry informative26 for population stratification analysis. A previous study performed in European individuals showed that any number of SNPs greater than 65 was adequate to evaluate population stratification.27 Based in our previous study26 we developed a bioinformatics pipeline to select randomly SNPs for the population stratification analysis. Briefly, using the International HapMap Project, a SNP frequency vector was built based on the following parameters: MAF > 0.05 and minimum distance between 2 SNPs = 300 kb, to avoid possible LD. In the next step, the pipeline used the sample function in R software v.3.2.3 (http://www.r-project.org) to randomly select the 90 SNPs from the SNP frequency vector. We concentrated SNP selection on chromosomes 2, 7, 10, 15 and 22.28

Table 2 contains information of previous studies showing the odds ratios and their respective confidence intervals as well as the sample size of each study.
**Table 1. Selected SNPs.**

| Locus | SNP      | Alleles* | Global MAF |
|-------|----------|----------|------------|
| NME7  | rs16861990 | A/C      | 0.062      |
| F5    | rs2420371  | A/G      | 0.031      |
| SERPINC1 | rs2227589 | A/G      | 0.129      |
| C4BPB | rs3813948  | A/G      | 0.123      |
| STAB2 | rs4981021  | A/G      | 0.322      |
| SCARA5 | rs9644133 | C/T      | 0.241      |
| BAI3  | rs9363864  | A/G      | 0.334      |
| STAB2 | rs4981022  | C/T      | 0.249      |
| STXB8P5 | rs1039087 | A/G      | 0.457      |
| SCARA5 | rs2726953 | C/T      | 0.263      |
| ST2X   | rs7978987  | A/G      | 0.286      |
| vWF   | rs1063857  | C/T      | 0.336      |
| TC2N  | rs1884841  | C/T      | 0.479      |
| CLEC4M | rs868875   | A/G      | 0.208      |
| SERPINF2 | rs8074026 | C/T      | 0.279      |
| SLC4A2 | rs2288904  | C/T      | 0.183      |
| GP6   | rs1613662  | A/G      | 0.157      |
| GCKR  | rs1260326  | C/T      | 0.293      |
| EDEM2 | rs6120849  | C/T      | 0.157      |
| PROCRI | rs6088735 | C/T      | 0.221      |
| PROCRI | rs867186  | A/G      | 0.096      |
| DNXA6C | rs1413885  | C/T      | 0.218      |
| BAZ1B | rs17145713 | C/T      | 0.172      |
| KNG1  | rs710446   | A/G      | 0.415      |
| CYP4V2 | rs13146272 | A/C      | 0.441      |
| F11   | rs2036914  | C/T      | 0.394      |
| F11   | rs2289252  | C/T      | 0.319      |
| HIVEP1 | rs169713  | C/T      | 0.337      |
| ABO   | rs687621   | C/T      | 0.381      |

*: reference/risk allele; MAF: Minor Allele Frequency.

**Genotyping**

The SNPs study was carried out using a high-performance genotyping platform (TaqMan® OpenArray Genotype System—Applied Biosystems), based on real-time PCR, and the plate used was120 SNPs for 24 samples.

Genomic DNA was extracted and purified using standard procedure.29 The concentration of the extracted DNA samples was determined in a spectrophotometer (Thermo Scientific NanoDrop™ 1000 Spectrophotometer). The DNA samples had A260/A230 and A260/A280 ratios from 1.8 to 2.0; the concentrations of the samples were adjusted to 50 ng/μl.

DNA analysis for both, FV Leiden (G1691A) and F2 G20210A mutation was performed using a polymerase chain reaction (PCR) method using a TaqMan® SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA).

The OpenArray TaqMan assays for genotyping were performed using the QuantStudio™ 12 K Flex Real-Time PCR System (Applied Biosystems, CA, USA) according to the manufacturer’s recommended operating guideline. DNase/RNase-free distilled water was used in each assay as a non-template control (NTC).

The genotype data were analyzed using the OpenArray® SNP Genotyping Analysis software (version 1.0.5) (Applied Biosystems, CA, USA).

**Statistical Analysis**

We evaluated the allele frequency of genotyped SNPs in terms of Hardy-Weinberg equilibrium (p-value > 0.05), MAF > 0.05 and pairwise LD (r2 > 0.8) by HAPLOVIEW v.4.2 software.30

In order to evaluate whether population substructure is similar, we performed the Analysis of MOlecular VAriance (AMOVA) by ARLEQUIN v3.5.1.2 software,31 using the 90 additional SNPs as analysis factors. AMOVA partitions the source of genetic variance into 2 components: within-groups, between-groups, and measures the population differentiation due to genetic structure by the fixation index (Fst). We considered Fst <0.05 as the absence of population stratification,26 as well as the between-groups variance component < 0.7, as this was the smallest value found when comparing populations within regions worldwide.32

The analysis of genetic associations was performed by logistic regression test. The power to detect genetic associations was determined from the post hoc statistical power of the sample in the software GPOWER33 with the following parameters: logistic regression test; 2-tailed; OR = 1.4; α = 0.002; n = 875.

The correction of the multiple testing was performed using the Bonferroni correction.

**Results**

**Study Population**

In this case-control study, patients and controls of both genders, aged between 18 and 60 years were included.

In the period from 2009 to 2013, 1258 samples of participants were collected, of which 149 were from HIAE, 638 were from UFRJ and were 471 from Unicamp. Of these, 392 samples were discarded for not meeting purity and/or DNA quantification standards.

**Clinical and Demographic Characteristics**

Of the 436 cases and 430 controls, 206 cases and 230 controls came from Hemocentro /Campinas, 134 cases and 148 controls came from UFRJ/Rio de Janeiro, and 96 cases and 52 controls came from HIAE/São Paulo. Of those patients 4.8% were heterozygous for FV Leiden and 5.1% heterozygous for F2 G20210A mutation. This sample has a statistical power to detect a genetic association of 79.4%. The age and gender of patients and controls separated by each study center are shown in Table 3.

**Population Stratification Analysis**

AMOVA results showed Fst<0.05 overall loci, and a between-group variance component < 0.7; a st h i s was the smallest value found when comparing populations within regions worldwide.32
Association Analysis of SNPs With VTE Risk

The SNP average genotypes call rate was 99.7% (standard deviation = 4.7%) in the candidate genes and 99.4% (standard deviation = 12.2%) for SNPs used in the stratification analysis.

Among the 29 SNPs genotyped in the candidate genes, 4 SNPs (rs2227589, rs169713, rs687621, rs2288904) in the cases and 4 SNPs in the controls (rs2726953, rs687621, rs1884841, rs868875) did not follow the Hardy-Weinberg equilibrium (HWE) and were excluded. None of the SNPs failed frequency test (MAF < 0.05) (Table 6). With these results we excluded SNPs without Hardy-Weinberg equilibrium in the controls.

Our replication results showed that none of the 25 candidate SNPs was associated with VTE risk in this Brazilian population. Results of the association analyses are reported in Table 7.

Discussion

A central goal of human genetics is to identify genetic risk factors for common, complex diseases. GWAS, though powerful to identify SNPs associated to VTE risk, present limitations. Other studies included:
Recently GWAS of VTE linked SNPs located in genes that encode proteins associated with coagulation and haemostasis, such as receptor glycoprotein VI (GP6), AT (SERPINC1), PC receptor (PROCR), F11 and F5. There are SNPs in genes associated to protein involved in pathways associated to DVT, such as HIVEP1 and GCR. Otherwise, some SNPs are functional variants and others may only be markers in linkage

Table 4. Clinical Characteristics of Patients.

| Source of variation          | HIAE      | Unicamp   | UFRJ      | Total     |
|------------------------------|-----------|-----------|-----------|-----------|
| Lower limbs                  | 58 (60.4%)| 174 (84.5%)| 115 (85.8%)| 347 (79.6%)|
| Pulmonary Embolism—PE        | 16 (16.7%)| 21 (10.2%)| 18 (13.4%)| 55 (12.6%)|
| Lower limbs associated with PE| 22 (22.9%)| 11 (5.3%)  | 1 (0.7%)   | 34 (7.8%)  |
| Spontaneous                  | 43 (44.8%)| 52 (25.2%)| 89 (66.4%)| 184 (42.2%)|
| Provoked VTE                 | 53 (55.2%)| 154 (74.8%)| 45 (33.6%)| 252 (57.8%)|
| Hormonal anticonceptive hormone replacement therapy | 10 (18.9%) | 62 (40.3%) | 2 (4.4%) | 74 (29.4%) |
| Surgery                      | 4 (7.5%)  | 34 (22.1%)| 13 (28.9%)| 51 (20.2%)|
| Pregnancy/Puerperium         | 4 (7.5%)  | 36 (23.4%)| 6 (13.3%) | 46 (18.3%)|
| Immobilization               | 12 (22.6%)| 7 (4.5%)  | 9 (20%)   | 28 (11.1%)|
| Travel                       | 9 (17%)   | 7 (4.5%)  | 3 (6.7%)  | 19 (7.5%)  |

Table 5. Analysis of Molecular Variance (AMOVA).

| Source of variation | Sum of squares | Variance components | Percentage variation |
|---------------------|----------------|---------------------|----------------------|
| Among Populations   | 14.191         | 0.00089             | 0.000                |
| Within populations  | 24.020.005     | 1.491.077           | 100.00               |
| Total               | 24.034.196     | 1.490.988           |                      |

Table 6. Presented HWE in Case and Controls and MAF for Each SNPs.\(^a\)

| SNP            | Gene        | Chr. | HWE, Cases | HWE, Controls | HWE, Total | MAF% |
|----------------|-------------|------|------------|---------------|------------|------|
| rs1413885      | DNAJC6      | 1    | 0.3598     | 0.2784        | 0.8875     | 0.4568|
| rs16861990     | NME7        | 1    | 0.5585     | 0.2212        | 0.1969     | 0.3218|
| rs2420371      | F5          | 1    | 0.7807     | 1             | 1          | 0.1005|
| rs2227589*     | SERPINC1    | 1    | 0.01876    | 0.4754        | 0.2597     | 0.2208|
| rs3813948      | C4BPB       | 1    | 0.1947     | 0.4376        | 0.7832     | 0.2583|
| rs1260326      | GCKR        | 2    | 0.3104     | 0.1946        | 0.102      | 0.4439|
| rs710446       | KNG1        | 3    | 0.7599     | 0.7697        | 0.6221     | 0.4833|
| rs13146272     | CYP4V2      | 4    | 0.2066     | 0.5986        | 0.6413     | 0.3446|
| rs2036914      | F11         | 4    | 0.7199     | 0.9096        | 0.869      | 0.3073|
| rs2289252      | F11         | 4    | 0.1199     | 0.2444        | 0.3802     | 0.404 |
| rs169713*      | HIVEP1      | 6    | 0.01995    | 0.2999        | 0.4549     | 0.2081|
| rs9363864      | BA13        | 6    | 0.6818     | 0.6087        | 0.8853     | 0.4173|
| rs1039084      | STX8P5      | 6    | 0.08157    | 0.2322        | 0.8301     | 0.4369|
| rs17145713     | BAZ1B       | 7    | 0.3928     | 0.4771        | 0.2389     | 0.3911|
| rs2726953**    | SCARA5      | 8    | 0.2058     | 0.01769       | 0.0142     | 0.08067|
| rs9644133      | SCARA5      | 8    | 0.5652     | 0.1492        | 0.1455     | 0.237 |
| rs687621**     | ABO         | 9    | 0.01406    | 0.0313        | 0.0008949  | 0.4727|
| rs1063857      | VWF         | 12   | 0.622      | 1             | 0.6404     | 0.1846|
| rs4981022      | STAB2       | 12   | 0.4788     | 0.6255        | 0.9439     | 0.4821|
| rs4981021      | STAB2       | 12   | 0.8199     | 0.7467        | 1          | 0.3389|
| rs7978987      | STX2        | 12   | 0.7266     | 0.5844        | 0.9365     | 0.3294|
| rs1884841***   | TC2N        | 14   | 0.8147     | 0.004088      | 0.0266     | 0.3113|
| rs8074026      | SERPINF2    | 17   | 0.8206     | 0.2692        | 0.4766     | 0.3303|
| rs868875***    | CLEC4M      | 19   | 0.5892     | 0.04017       | 0.06269    | 0.3846|
| rs2288904*     | SLCC4A2     | 19   | 0.04182    | 0.1167        | 0.8097     | 0.1783|
| rs1613662      | GP6         | 19   | 0.6439     | 0.3756        | 0.8106     | 0.3286|
| rs6120849      | EDEM2       | 20   | 0.6115     | 1             | 0.6722     | 0.4623|
| rs6088735      | PROCR       | 20   | 0.4411     | 0.1251        | 0.6509     | 0.2639|
| rs867186       | PROCR       | 20   | 0.4672     | 0.72          | 0.395      | 0.2901|

\(^a\)Seven SNPs Were Not in HWE.
SNPs not following Hardy-Weinberg equilibrium in cases (*) and in controls (**).
We recently performed an analysis of an admixed population of the same geographical area of the individuals included in this study and demonstrated that some non-inverted haplotypes were more similar to Native-American haplotypes than to European haplotypes, in contrast to what was found in other American admixed populations. We showed that individuals who display the same global ancestry could exhibit remarkable differences in the distribution of local ancestry blocks.43

A SNP identified in a study with a sufficient sample size and replicated in an independent dataset from the same population, confirmed the effect in the GWAS target population.44 However, reviews of literature based on associations between SNPs and VTE have not always been robustly confirmed in studies aimed for replication, even in the same population.15,17,19,36 Failure to replicate in a different population does not necessarily invalidate the original study and suggests that the SNP has an ethnic-specific effect.

Austin et al., (2011) evaluated 10 SNPs identified in 3 European case-control studies as risk factors for VTE in Caucasian and Afro descendants Americans. Some SNPs were replicated in Caucasians; however, the association with F11 SNPs after adjustment for multiple comparisons among Afro descendants was not confirmed. In this setting, we correctly performed Bonferroni correction in our analysis.43

An important point is the relation of one SNP with another established genetic factor. In a case-control study,45 the authors described an association of VTE with 4 SNPs (ATP1B1, NME7, BLZF1, and SLC19A2). However, after a replication study and control for FV Leiden (F5 rs6025), no additional information over that provided by patients characteristics and F5 rs6025T alone could be added. They found that FV Leiden is inherited as a haplotype block that spans 7 genes on chromosome 1q24.2, including those that have been associated with VTE risk, such as ATP1B1, NME7, BLZF1, C1orf114, SLC19A2, F5 and SELP.

Phenotype comparable to those reported in the initial study should be used in all replication studies. Our patients presented phenotype very similar to those described in the original studies, including VTE of the lower limbs and PE, which is a strength of our study.

The possibility of differences in selection criteria when compared to other European and American studies would have influenced the confirmation of the association of some of the SNPs with VTE risk. MARTHA cases are patients referred to thrombophilia centers generally younger than FARIVE cases, recruited from the general population.11,13 In addition, MARTHA population was enriched with Factor V Leiden or F2 G20210A mutations, 50% in cases and 41% in controls, compared to 18% and 8% in FARIVE, respectively. All 3 centers that selected patients for our study were thrombophilia reference centers. We excluded patients with known hereditary thrombophilia associated with a high risk of VTE that could explain per se the symptomatic thrombosis, like anticoagulant deficiency, and homozygosity or double heterozygosity FV Leiden and F2 20210A.

### Table 7. Results of Logistic Regression.

| SNP     | Gene    | Chr | p Value | OR (95%) | Beta   |
|---------|---------|-----|---------|----------|--------|
| rs1413885 | DNAJC6  | 1   | 0.8326  | 0.97 (0.81-1.19) | -0.2114 |
| rs16861990 | NME7   | 1   | 0.7783  | 0.97 (0.79-1.19) | -0.2815 |
| rs2420371 | F5      | 1   | 0.8701  | 0.97 (0.70-1.35) | -0.1635 |
| rs2227589 | SERPINC1| 1   | 0.7179  | 1.04 (0.83-1.31) | 0.3612  |
| rs3813948 | C4BPB  | 1   | 0.6074  | 1.06 (0.85-1.33) | 0.5138  |
| rs1260326 | GCKR   | 1   | 0.1021  | 1.18 (0.97-1.45) | 1.635   |
| rs710446  | KNG1   | 1   | 0.2365  | 0.88 (0.73-1.08) | -1.184  |
| rs1314627 | CYP4V2 | 1   | 0.1545  | 0.86 (0.70-1.06) | -1.424  |
| rs2036914 | F11    | 1   | 0.8942  | 1.01 (0.83-1.23) | 0.133   |
| rs2289252 | F11    | 1   | 0.7541  | 0.96 (0.78-1.20) | -0.3133 |
| rs169713  | HIVEP1 | 1   | 0.8959  | 0.98 (0.77-1.26) | -0.1309 |
| rs9363864 | BA13   | 1   | 0.6209  | 1.25 (1.03-1.53) | 2.244   |
| rs1039084 | STXB5  | 1   | 0.7562  | 1.03 (0.85-1.26) | 0.3104  |
| rs1714571 | BAZ1B  | 1   | 0.5033  | 0.93 (0.76-1.14) | -0.6693 |
| rs9644133 | SCARA5 | 1   | 0.3685  | 0.90 (0.72-1.13) | -0.8993 |
| rs1063857 | VWF    | 1   | 0.5455  | 1.08 (0.84-1.39) | 0.6046  |
| rs4981022 | STAB2  | 1   | 0.6194  | 0.95 (0.78-1.16) | 0.4967  |
| rs9363864 | BA13   | 1   | 0.6209  | 1.25 (1.03-1.53) | 2.244   |
| rs1039084 | STXB5  | 1   | 0.7562  | 1.03 (0.85-1.26) | 0.3104  |
| rs1714571 | BAZ1B  | 1   | 0.5033  | 0.93 (0.76-1.14) | -0.6693 |
| rs9644133 | SCARA5 | 1   | 0.3685  | 0.90 (0.72-1.13) | -0.8993 |
| rs1063857 | VWF    | 1   | 0.5455  | 1.08 (0.84-1.39) | 0.6046  |
| rs4981022 | STAB2  | 1   | 0.6194  | 0.95 (0.78-1.16) | 0.4967  |
| rs4981022 | STAB2  | 1   | 0.7009  | 0.96 (0.78-1.16) | -0.3841 |
| rs7978987 | STX2   | 2   | 0.5746  | 0.94 (0.77-1.16) | -0.5613 |
| rs8074026 | SERPINC2| 2   | 0.1908  | 1.01 (0.86-1.23) | 0.1152  |
| rs2288904 | SLC4A2 | 2   | 0.4278  | 1.11 (0.86-1.44) | 0.793   |
| rs1613662 | GP6    | 2   | 0.9988  | 1.00 (0.81-1.23) | 0.001454 |
| rs120849   | EDEM2  | 2   | 0.5842  | 1.05 (0.87-1.29) | 0.5473  |
| rs608735  | PROC   | 2   | 0.5641  | 1.06 (0.86-1.33) | 0.5768  |
| rs861186   | PROC   | 2   | 0.5256  | 1.07 (0.86-1.34) | 0.6348  |

Chr: chromosome.

disequilibrium (LD) with functional variants that remain to be identified, like MYH7B, EDEM2, BAZ1B.14,18

It is important to note that although SNPs are the most frequent genetic variation in the human genome, the majority has a minimal biological impact and confer a modest increase of disease risk,35 and this is not different for VTE.

GWAS that identified SNPs linked to VTE included predominantly Caucasian populations, and only one study included Afro descendants,36 with a poor representation of other regions and admixed populations. We performed the first case-control study in South America, in a Brazilian admixed population, with the aim to replicate some of these SNPs previously demonstrated in other populations. However, our replication results showed that none of the 25 candidates SNPs were associated with VTE risk in this Brazilian population.

Factors that can explain the fact by which GWAS findings in European populations may not be transferable to admixed American cohorts are differences in population substructure, including a specific distribution of ancestry tracts in the genome,37 a positive selection of alleles among populations and even within a population, and genetic variants that could have functional effects.38

Brazil is a genetically trihybrid population, with the genomic inheritance of European, sub-Saharan African, and Native-American populations.39-42 It has been described different proportion of global ancestries among these 3 populations and other admixed American populations,13,22,23 and in Brazilian individuals from diverse geographical regions.19,20
We genotyped FV Leiden and F2 20210A mutation in 1104 VTE patients assisted at Hemocentro Unicamp from 2004 to 2019 and found a low prevalence of these mutations than that described in Europe (4.4% and 4.1%, respectively). These results emphasize the ethnic differences of a Brazilian admixed population when compared to European.

We noted a great variability in sex distribution between the 3 participating centers, with a higher number of women patients from Unicamp. The healthcare of Unicamp include a secondary/tertiary general hospital, a hematology clinic at Hemocentro / Unicamp, and a woman hospital (CAISM). Patients that presented DVT were assisted at outpatient clinic of Hemocentro / Unicamp, after hospital discharge. This could explain the higher number of women in this sample. It is important to point that we performed statistical analysis according to sex, but no difference was found (data not shown).

About 58% of our patients presented provoked VTE, but 55.5% were associated to mild risk factors. This percentage was even higher in those patients assisted at Hemocentro / Unicamp (68.2%), which provided the most patients and controls included in this study. The inclusion of only spontaneous DVT would be desirable to avoid underestimation of genetic influence. Otherwise, VTE is a complex disease and the association between acquired and genetic risk factors can play a role to trigger the thrombotic episode.

Lack of reproducibility of genetic associations can be attributed to lack of comparability between cases and controls, increasing the risk of biases as there can be heterogeneity in exposure to environmental challenges and population stratification, particularly in admixed populations. In our study, as we were aware of these critical points, we included patients and controls of the same geographical area. Indeed, we genotyped 90 SNPs to exclude stratification of the population analyzed and our results confirmed that the 2 groups had similar genetic structures, which allowed us to perform an unbiased genetic association analysis.

Small sample size is a frequent problem and can result in insufficient power to detect minor contributions of one or more alleles. We calculated our sample size according to previous studies in populations of European ancestry, which presented a large variation in OR. Our sample size presented 79.4% of statistical power to detect genetic association, with allele frequencies greater than 5%. However, all of our OR were lower than 1.1, and our study was underpowered to determine whether any association between the SNPs and DVT existed in our population. Despite the attention given to many of the important points in a replication study, we consider this a limiting factor of our study.

Despite having included a large number of SNPs, as those associated with biochemical traits related to VTE and fibrinolytic system, the allele frequency of the SNPs was lower than expected. A recent meta-analysis that included 7,507 VTE and 52,632 controls, only confirmed 9 loci previously associated with VTE.

The genetic heterogeneity of the Brazilian population probably in part explains this lower allele frequencies, highlighting the influence of population genetic structure in association studies.

Further studies designed to investigate new genes, common, rare and regulatory variants will possibly contribute to the knowledge and the use of genetic analysis to determine individual VTE risk in the clinical practice.

Conclusions

In a Brazilian multicenter case-control study, with high genetic variability though no stratification between groups, there was no replication of SNPs associated with VTE in GWAS, previously described for European and United States adults, mostly of Caucasian ethnicity. The high admixture of the Brazilian population may be a critical point for these results, emphasizing the influence of the population genetic structure in association studies.

To our knowledge this was the first study that evaluated SNPs associated with VTE in the South America, in Brazilian population.

Declaration of Conflicting Interests

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