Thrombin-Activatable Fibrinolysis Inhibitor Polymorphisms and Cerebral Venous Thrombosis in Mexican Mestizo Patients

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Abstract

Thrombin-activatable fibrinolysis inhibitor (TAFI) gene polymorphisms have been proposed as a predisposing factor for cerebral venous thrombosis (CVT). We analyzed the association between CVT and TAFI single-nucleotide polymorphisms (rs3742264, rs2146881, and rs1926447) compared to healthy controls. Mexico Mestizo confirmed cases with CVT and age- and sex-matched controls with no history of venous thrombotic events were recruited from July 2006 to July 2015. Demographic, clinical, and imaging information was included in the analysis. Genotyping single-nucleotide polymorphisms were performed by allele-specific polymerase chain reaction. Allelic univariate analysis, haplotype association, and Hardy-Weinberg equilibrium were assessed. A total of 113 CVT cases (94 females [83.2%]; median age 35 years [interquartile range 27-43 years]) and 134 age- and sex-matched controls were included. The main risk factors for CVT were pregnancy/puerperium (30.9%), oral contraceptive use (19.5%), and hereditary thrombophilia (7.1%). We found no significant association for heterozygous and homozygous models for rs3742264 ($P = .30$ and $P = .69$, respectively), rs2146881 ($P = .90$ and $P = .17$, respectively), or rs1926447 ($P = .40$ and $P = .52$, respectively) compared to controls; these findings were consistent in subgroup and haplotype analyses. In conclusion, TAFI rs3742264, rs2146881, and rs1926447 polymorphisms do not increase the risk of CVT in comparison to healthy controls.

Keywords

cerebral venous thrombosis, thrombin-activatable fibrinolysis inhibitor, single-nucleotide polymorphisms, genetic association analysis

Introduction

Cerebral venous thrombosis (CVT) is a rare medical condition, with a prevalence of 0.5% to 1% of all cerebrovascular diseases in different series.[1-3] In Mexico, a multicenter study showed a frequency of 3% for CVT among patients with all-type acute stroke, which is higher than in other international registries.[4]

The pathophysiology of CVT is complex, as this is considered a multifactorial condition. On the one hand, acquired risk factors (such as oral contraception and exogenous hormone intake, surgery, brain trauma, and pregnancy/puerperium) account for commonly identified conditions. On the other hand, genetic risks (hereditary thrombophilia) have been studied,[5] on the basis that nearly 34% of all patients with CVT in large multicenter registries had this type of prothrombotic condition.[1]

The genetic basis of CVT is widely unknown and has gained attention as a research question because nearly 10% to 25% of cases have no identifiable risk factors.[1,6,7] Certain gene candidates have been studied as the main genetic risk predisposition for CVT; unfortunately, due to the difficulty in achieving a sufficient sample size, multiple studies have failed to show a causative association. Factor V Leyden (G1691A polymorphism),[8] prothrombin mutation (G20210A polymorphism),[8] methylene tetrahydrofolate reductase (C677T polymorphism),[9] plasminogen-activator inhibitor-1

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(4G/4G polymorphism),[10] protein Z (G79A polymorphism),[11] Janus kinase-2 (V617F polymorphism),[12] and thrombin-activatable fibrinolysis inhibitor (TAFI; A505G, C1040T, and C1542G polymorphisms)[13] are a subset of the genes possibly associated with risk of CVT development.

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a metallocarboxypeptidase that, when activated, cleaves C-terminal lysine or arginine residues from peptide substrates; this cleavage induces partial degradation of fibrin and a reduction in plasmin formation, with a subsequent attenuation of fibrinolysis.[14] Polymorphisms from the *TAFI* gene have been studied in venous thrombotic disease, with a debatable association across different studies. In a recent meta-analysis,[15] 3 single-nucleotide polymorphisms (SNPs) were found to have subtle associations with venous thrombosis risk in white individuals; further replication in other populations should be performed to assess these findings. Other polymorphisms that have been described in peripheral thrombotic conditions are uncertain in CVT. We conducted this case–control study to evaluate whether TAFI polymorphisms (G505A [rs3742264], G438A [rs2146881], and C1040T [rs1926447]) are associated with an increased risk of CVT in confirmed Mexican Mestizo cases compared to healthy controls with no history of thromboembolic events.

Materials and Methods

Study Population

Consecutive patients with first-ever confirmed CVT treated at the Instituto Nacional de Neurologia y Neurocirugia (a third-level neurological referral center in Mexico City) were recruited from July 2006 to July 2015. Data from these patients were extracted from a retrospective data set collected prospectively from our institutional stroke registry. Inclusion criteria consisted of an acute (less than 30 days) CVT confirmed by magnetic resonance imaging (MRI) and 3-dimensional (3D) gadolinium-enhanced magnetic resonance venography (MRV), computed tomography (CT), and CT venography (CTV) or conventional angiography; some cases had more than 1 imaging modality for confirmation of the diagnosis. Cases were required to have complete outpatient follow-up for at least 90 days. Patients were required to be older than 18 years; both parents and their 4 grandparents were required to be of Mexican Mestizo origin.

All patients had a standardized diagnostic follow-up, laboratory, and treatment protocol according to institutional guidelines for CVT. The data set included demographics, medical history, risk factors, CVT onset time, arrival time at the hospital, complications, treatment, anatomical characteristics of the venous vessels, procedures, and ambulatory status at discharge for each patient. Risk factors and predisposing conditions for CVT, such as smoking, anemia, recent use of contraceptives, history of abortions or miscarriage, pregnancy or puerperium, and thrombophilia or antiphospholipid syndrome, were investigated in both the groups. Clinical outcomes were defined using a modified Rankin score (mRs) as follows: a good clinical outcome consisted of mRs 0 to 2, and a bad clinical outcome consisted of mRs >2. Cases were labeled as provoked (with the presence of 1 or more known risk factor for thrombosis) or unprovoked (in the absence of any known risk factor) CVT in the analysis.

The control group included unrelated healthy Mexican Mestizo volunteers recruited from the Institutional Blood Bank. These participants were sex- and age-matched, without a history of venous thrombotic events; additionally, both parents and their 4 grandparents were of Mexican Mestizo origin. All participants (cases and controls) gave their informed consent for this study, which was approved by the Local Ethics Committee.

Blood Sampling and Genotype Analysis

Whole blood samples were extracted from patients and controls and stored at −25°C. Genomic DNA was extracted from peripheral leukocytes that were isolated from the acid–citrate–dextrose–anticoagulated blood of all patients and controls using standard methods reported by Miller and collaborators.[16]

Genotyping of TAFI polymorphisms (G438A [rs2146881], G505A [rs3742264], and C1040T [rs1926447]) was performed using TaqMan SNP genotyping assays (C__16136443__10, C__1872266__20, and C__11697322__10, respectively) in a Step One Plus real-time polymerase chain reaction system (Applied Biosystems, California) according to the supplier's methodology. Hardy-Weinberg equilibrium was assessed for the different genotypes of the 3 SNP loci.

Statistical Analysis

Categorical variables were expressed as frequencies and percentages; continuous variables were expressed as the means (standard deviation) or median and interquartile range (IQR) when appropriate, according to a normality distribution test (Kolmogorov-Smirnov analysis). Common risk factors for thrombosis (smoking, anemia, recent use of contraceptives, history of abortions or miscarriage, pregnancy or puerperium, and thrombophilia or antiphospholipid syndrome) were compared between cases with CVT and controls using a χ^2 test for categorical variables. Allelic frequencies were calculated using a gene-counting method. An odds ratio (OR) and 95% confidence interval (CI) were calculated to evaluate the association magnitude; Hardy-Weinberg equilibrium was assessed using Pearson χ^2 test. *P* values <.05 were considered statistically significant. All statistical analyses were performed using the statistical package SPSS (version 20.0, IBM Inc, Armonk, New York).

Sample size for cases and controls was calculated to achieve a power of 80%, assuming an α level of 95%, an exposure rate of 10%, and an OR of 2.0. These values led to a sample size of 100 participants for each arm. To evaluate the combined effect from risk alleles, genotype association was assessed taking into account the number of risk alleles in a logistic model adjusted

Table 1. General Characteristics of Cases Versus Controls.

	Cases, n = 113 (%)	Controls, n = 131 (%)	Total, N = 244 (%)	P
Median age, years (IQR)	35 (27-43)	37 (27-45)	36 (27-45)	.66[a]
Female	94 (83.1)	106 (80.9)	200 (81.9)	.64
Family history of venous thrombosis				
CVT	4 (3.5)	5 (3.8)	9 (3.7)	.87
Any other site	16 (14.1)	4 (3.1)	20 (8.2)	.005
Risk factors				
Oral contraceptive	22 (19.5)	10 (7.6)	32 (13.1)	<.001
Pregnancy	15 (13.3)	1 (0.8)	16 (6.5)	<.001
Puerperium	20 (17.7)	0 (0)	20 (8.2)	<.001
Hereditary thrombophilia	8 (7.1)	0 (0)	8 (3.3)	<.001
Antiphospholipid syndrome	3 (2.7)	0 (0)	3 (1.2)	<.001
Cancer	1 (0.9)	1 (0.8)	2 (0.8)	.91

Abbreviations: IQR, interquartile range; CVT, cerebral venous thrombosis.
[a]Mann-Whitney U test.

by age and gender, with covariates for CVT risk (including hereditary and acquired thrombophilia). Haplotype analysis was performed using SNPstats software also adjusting for sex and risk factors.

Results

A total of 125 patients with confirmed CVT were recruited during the study period. In all, 12 patients were excluded (5 patients with incomplete medical records, 4 patients with incomplete follow-up information, and 3 patients who did not give informed consent). A total of 113 cases with CVT were finally selected according to inclusion criteria (94 females [83.1%]; median age 35 years (IQR 27-43 years]), while 131 age- and sex-matched participants (106 females [80.9%]; median age 37 years (IQR 27-45 years]) were recruited as controls. Table 1 summarizes demographic data and risk factors for cases and controls.

Clinical and Imaging Analysis of CVT Cases

Family history of CVT, deep venous thrombosis, or pulmonary thromboembolism was present in 20 (17.7%) cases. The main clinical characteristics of patients with CVT included focal seizures in 50 (44.2%) cases, mono/hemiparesis in 39 (34.5%) cases, aphasia in 14 (12.4%) cases, and mental status disturbance in 25 (22.1%) cases. At the end of the follow-up period, 105 (92.9%) patients had a good prognosis (mRs 0-2); no deaths were recorded during the follow-up period.

Confirmatory imaging studies included 80 patients with MRI/MRV, 45 with CT/CTV, and 8 with digital subtraction angiography; certain cases required more than 1 study modality for confirmatory diagnosis and optimal anatomical evaluation of the venous vessels. The superior sagittal sinus was the most commonly affected vessel (81 [71.7%] cases), followed by

Table 2. Genotype Distribution of the 3 *TAFI* Gene SNPs in Patients With CVT Versus Control Participants.

	Cases, n = 113 (%)	Controls, n = 131 (%)	Total, N = 244 (%)	P
rs3742264				
GG	74 (56.5)	55 (48.7)	129 (52.9)	.22
GA	48 (36.6)	48 (42.5)	96 (39.3)	.35
AA	9 (6.9)	10 (8.8)	19 (7.8)	.56
rs2142264				
GG	88 (77.9)	91 (69.5)	179 (73.4)	.14
GA	24 (21.2)	39 (29.8)	63 (25.8)	.13
AA	1 (0.9)	1 (0.8)	2 (0.8)	.91
rs1926447				
CC	78 (69.0)	84 (64.1)	162 (66.4)	.42
CT	31 (27.4)	45 (34.4)	76 (31.1)	.24
TT	3 (2.7)	2 (1.5)	5 (2.0)	.53

Abbreviations: TAFI, thrombin-activatable fibrinolysis inhibitor; SNPs, single-nucleotide polymorphisms; CVT, cerebral venous thrombosis.

Table 3. Association of 3 *TAFI* Gene SNPs in Patients With CVT Versus Control Participants.

	Cases, n = 113 (%)	Controls, n = 131 (%)	Total, N = 244 (%)	OR (95% CI)	P
rs3742264					
GG	74 (56.5)	55 (48.7)	129 (52.9)	0.7 (0.4-1.2)	.22
GA+AA	57 (50.4)	58 (44.2)	115 (47.1)	1.3 (0.8-2.2)	.23
rs2142264					
GG	88 (77.9)	91 (69.5)	179 (73.4)	1.5 (0.8-2.7)	.14
GA+AA	25 (22.1)	40 (30.5)	65 (26.6)	0.6 (0.3-1.1)	.13
rs1926447					
CC	78 (69.0)	84 (64.1)	162 (66.4)	1.2 (0.7-2.1)	.42
CT+TT	34 (30.1)	47 (35.9)	81 (33.2)	0.8 (0.4-1.3)	.34

Abbreviations: TAFI, thrombin-activatable fibrinolysis inhibitor; SNPs, single-nucleotide polymorphisms; CVT, cerebral venous thrombosis; OR, odds ratio; CI, confidence interval.

36 (31.9%) cases involving left transverse sinus and 31 (27.4%) involving right transverse sinus thrombosis. Parenchymal lesions included 50 (44.2%) cases with focal edema/venous infarction, and 38 (33.6%) cases had hemorrhagic lesions at the index admission brain CT or MRI.

Genetic Analysis

Distribution of alleles among cases with CVT and controls for rs3742264 (G = 72.5%, A = 27.4%; P = .84), rs2142264 (G = 86.2%, A = 13.7%; P = .16), and rs1926447 (C = 82.4%, T = 17.6%, P = .17) did not deviate from Hardy-Weinberg equilibrium. Allelic and genotypic frequencies for the 3 TAFI polymorphisms (rs3742264, rs2146881, and rs1926447) were compared between cases with CVT and controls (Table 2).

As seen in Table 3, no model was associated with significantly increased CVT risk in cases relative to controls. Common dominant models for rs3742264 (GA+AA, OR = 1.3

Table 4. Haplotype Association With Response in the Entire Population (unadjusted).[a]

Variant	rs3742264	rs2146881	rs1926447	Frequency[b]	OR (95% CI)	P[c]
H1	G	G	C	0.53	1.00	–
H2	A	G	C	0.27	0.87 (0.57-1.32)	0.50
H3	G	A	T	0.11	1.42 (0.77-2.63)	0.26
H4	G	G	T	0.05	0.69 (0.30-1.59)	0.38
H5	G	A	C	0.02	0.41 (0.10-1.73)	0.23
Rare	*	*	*	0.006	0.00 (inf-inf)	1

Abbreviations: OR, odds ratio; CI, confidence interval.
[a]N = 244.
[b]Summary frequencies for cases and controls.
[c]Global haplotype association P value: .21.

[95% CI, 0.8-2.2]; $P = .23$), rs2142264 (GA+AA, OR = 0.6 [95% CI, 0.3-1.1]; $P = .13$), and rs1926447 (CT+TT, OR = 0.8 [95% CI, 0.4-1.3]; $P = .34$) polymorphisms showed no significant association with CVT risk. Due to the lack of significant association between genotypes and CVT risk, no further analysis was performed adjusting for covariates (age, gender, pregnancy, puerperium, hereditary thrombophilia, or oral contraceptive use).

When performing haplotype analysis, linkage disequilibrium was found between rs2146881 ($D' = 0.998$) and rs1926447 ($D' = 0.8109$); comparing rs2146881 with rs1926447 ($D' = 0.8262$) similarly demonstrated linkage disequilibrium. Seven haplotypes were detected (GGC, AGC, GAT, GGT, GAC, AGT, and AAC), with the highest frequency for GGC in cases and controls (53.2%) and the lowest frequency for AGT and AAC (<1%). Association analysis (crude) for haplotypes indicated no association with risk of CVT (Table 4); a sex-adjusted analysis produced the same result.

Discussion

In the present study, rs3742264, rs2146881, and rs1926447 genotypes and haplotype analysis indicated no increased risk of CVT compared to healthy controls in a Mexican Mestizo sample; this is the first exploratory analysis in our population based on genetic risk association.

The TAFI antigens attenuate fibrinolysis secondary to the activation (through the effect of tissue plasminogen activator) and conversion of plasminogen into plasmin on the surface of a fibrin clot.[17] The relationship of TAFI functional deficits with venous thrombotic states has been studied,[18-20] and these deficits are less prominent in arterial thrombotic conditions (acute myocardial infarction and stroke),[21,22] with inconclusive findings regarding the association of SNPs from this gene with risk of a prothrombotic state.

Two recent meta-analyses of the 3 SNPs from the present study[15,24] have analyzed associations between these variants and risk of venous thrombosis. Both studies showed that the rs2146881 polymorphism is not correlated with venous thrombosis risk. In subgroup analyses, one of these studies found that the G allele and GG genotype of the rs3742264 polymorphism in non-Asian populations may increase the risk of venous

thrombosis diseases,[23] while the AA genotype of rs3742264 and the TT genotype of the rs1926447 polymorphism were significantly associated with decreased risk of venous thrombosis.[15] These findings were consistent for patients with CVT, with no significant association for thrombosis risk.[23]

The null finding in the present study is consistent with previous published studies of TAFI SNPs and CVT risk: 2 studies (one in a German cohort with 77 consecutive CVT cases that focused on the rs2146881 polymorphism[24] and another in 59 consecutive Turkish CVT cases examining the rs3742264, rs2146881, and rs1926447 polymorphisms[25]) showed no association. However, haplotype analysis of TAFI SNPs was performed in a Brazilian cohort with 72 consecutive CVT cases, and final analyses indicated that the GTC haplotype for the 3 SNPs increased the risk of CVT in comparison to controls and venous thromboembolism (encompassing deep vein thrombosis and pulmonary embolism) cases, with an OR = 2.67 (95% CI, 1.13-6.63).[13] We did not detect this risk association in our sample, where the GGC haplotype was more frequent.

Ethnic variation among TAFI haplotypes and SNPs should be analyzed based on genetic replication in the Mexican Mestizo population. Zwingerman et al[26] showed that the Thr325Ile polymorphism demonstrated allele frequency variation by geographical region among European, Asian, and African populations, a finding that has also been reported in other studies.[27,28] When analysis was limited to cases of European origin, a significant difference was observed associated with the risk of venous thrombosis (OR = 0.83; $P < .021$).[26] A previous meta-analysis examined the association between the Thr325Ile SNP and coronary artery disease, with an increased global risk association (OR = 1.25, 95% CI, 1.02-1.54) in mixed ethnic groups; however, in a subgroup analysis of European cases, this association did not reach statistical significance (OR = 1.13, 95% CI, 0.90-1.40).[29]

The Mexican Mestizo genetic pool (Amerindian, Hispanic Caucasian, and African) could play a role in elucidating the SNP behavior of the *TAFI* gene and associated risk association for CVT.[30] In a previous report from Zorio et al,[31] Thr325Ile SNP frequencies were similar among cases with myocardial infarction and controls in a young Hispanic sample, with an increase in TAFI activity in cases not related to the SNP distribution. Moderate changes in TAFI plasma levels do not alter

its function due to a threshold-dependent mechanism[26]; therefore, TAFI activity analysis in Mexican Mestizo patients could be useful for explanatory evaluation of this association.

Other functional SNPs could influence TAFI expression: −2345 2G/1G and −1690 A/G polymorphisms increased the risk of atherosclerotic ischemic stroke in Chinese population.[32] Combined promoter and coding TAFI SNP analysis could be key to understanding occurrence, severity, and functional outcomes in CVT.

Sex subgroup analyses have found differences in SNP associations in CVT. In a meta-analysis, the minor allele of the rs3742264 SNP in a dominant model (AA+GA vs GG) was more frequent in female controls, and the Thr325Ile polymorphism was protective in Europeans independently of sex[26]; despite this finding, 15% of SNPs have a sex-dependent susceptibility that could explain sexual dimorphism for thrombotic events.[33] In our sample, no differences in genotype, allele, or haplotype were observed between sexes in cases versus controls, which is similar the Ala147Thr and Thr325Ile SNPs and the risk of ischemic stroke in the Japanese population.[28]

Certain limitations of the current study should be acknowledged. First, SNP association analysis is not intended to establish causality, due to the difficulty in replicating clear causal relationships in clinical practice, especially in multifactorial conditions such as CVT.[34,35] Exploratory analysis of the behavior of candidate genes increases the necessity of performing studies such as genomic-wide association analysis, which can lead to prioritization of these genes, fine mapping of functional variants, and potentially identification of SNPs associated with disease risk in admixed populations.[36] Second, CVT is a rare medical condition, which leads to a difficulty in sample recruitment for adequate power to detect results (despite the fact that our study included the largest consecutive CVT case sample to date). Third, TAFI antigen levels were not measured in the present study; therefore, associations between different SNP and these levels were not evaluated; from previous studies, the association from 505A, −438G, and 1040C allele genotypes predicts an increase in TAFI levels, with a subsequent risk of venous thrombosis, meanwhile 505G carriers associated with lower TAFI antigen levels, and lower risk of thrombosis[37]; therefore, this should be evaluated in future analysis in our sample to determine risk association. And finally, small sample size is a limitation to exclude or confirm association between the analyzed SNPs, despite the fact that CVT is a rare medical condition and the association risk from previous studies is mild.[23-25]

In conclusion, our study indicates that in Mexican Mestizo CVT cases relative to healthy controls, no risk associations were found for the rs3742264, rs2146881, and rs1926447 polymorphisms or in haplotype analysis of the *TAFI* gene.

Authors' Note

All authors contributed equally to this work, discussed the results and implications, commented on the manuscript at all stages, and finally approved the final version to be published.

Antonio Arauz conceived and supervised the study and drafted the manuscript; Nayelli Argüelles conceived the study proposal, collected samples and data; Aurelio Jara performed genetic analysis, and was involved in data analysis and interpretation; Jorge Guerrero performed genetic analysis, and was involved in data analysis and interpretation; and Miguel A. Barboza performed the statistic analysis and drafted the manuscript.

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