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VAMP8 and serotonin transporter levels are associated with venous thrombosis risk in a Spanish female population. Results from the RETROVE Project

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ABSTRACT

Introduction: Platelet hyper-reactivity has been associated with thrombosis and high levels of human vesicle-associated membrane protein 8 (VAMP8) and serotonin transporter (SERT). Two polymorphisms (*rs1010* of *VAMP8* gene and in *SERT* gene (*SLC6A4*)) are associated with arterial thrombosis.

Aim: To determine if levels of serotonin, SERT and/or VAMP8 and these polymorphisms are associated with the risk of venous thrombosis.

Material and methods: A total of 324 individuals were included in the RETROVE Study (Riesgo de Enfermedad TROmboembólica VEnosa).

VAMP8, SERT and serotonin were determined by ELISA; polymorphisms of *SLC6A4* and VAMP8 by polymerase chain reaction (PCR) and real time PCR. The venous thrombotic risk was calculated by a logistic regression method to estimate the crude and adjusted OR (adjusted for sex, age, body mass index and venous thrombosis risk co-factors).

Results: Statistically significant high levels of VAMP8 and SERT were found in patients, but not in controls. In contrast, serotonin showed lower levels in patients than in controls. When individuals were studied by gender, only women exhibited a statistically significant difference: the OR for VAMP8 was 3.25 (1.61–6.56 95% CI). The adjusted OR did not change. The OR for SERT was 2.76 (1.36–5.60 95% CI), the adjusted OR was maintained also. For serotonin with OR of 2.62 (1.40–4.92 95% CI), the adjusted OR was not significant. In contrast males did not show significant differences.

No statistically differences between patients and controls were found for both polymorphisms.

Conclusions: VAMP8 and SERT levels are associated with venous thrombosis in a female Spanish population.

1. Introduction

Platelet hyper-reactivity has been associated with venous [1] and arterial [2–5] thrombosis. The hyper-reactive platelet phenotype is characterized by an increase in vitro platelet aggregation after activation with low concentrations of adenosine diphosphate (ADP) and/or epinephrine (EPI). There is a bimodal distribution with hypo-reactive individuals (aggregation < 40%) and hyper-reactive individuals (aggregation > 60%) [6,7].

When platelet aggregation is induced by epinephrine, there is a release of cargo that is contained in platelet granules. The interactions of membrane proteins in platelet plasma membrane (t-SNARE) and in the granules (v-SNARE) form a heteromeric complex. This extends to the two bilayers and mediates membrane fusion and granule cargo release [8].

Human vesicle-associated membrane protein 8 (VAMP-8) is a v-SNARE. It is the first SNARE implicated in the secretion of platelet granules as it was demonstrated by analysis of platelets from knockout mice in VAMP8 [9]. High levels of VAMP8 are found in the hyper-reactive platelet phenotype [10].

Also, the Cardiovascular Health Study [11] reported that there is a strong association between the VAMP8 *rs 1010* single nucleotide

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Table 1
Clinical characteristics of patients and controls in whom VAMP8, SERT and serotonin values were determined.

Baseline characteristics[a]	Patients		Controls		p[†]
	Males	Females	Males	Females	
Gender (n)	66	79	78	101	
Age (year) (mean ± SD)	61.4 ± 17.3	64.6 ± 19.5	44.6 ± 17.3	48.0 ± 19.4	0,0001
BMI ($kg{\cdot}m^{-2}$) (mean ± SD)	27.5 ± 3.9	27.6 ± 4.9	25.5 ± 3.6	25.0 ± 4.2	0,0001
Smoking (n, %)	9 (13.6)	5 (6.3)	17 (21.8)	15 (14.8)	NS
Alcohol consumption (n, %)	35 (53.0)	33 (41.7)	55 (70.5)	45 (44.5)	NS
Hypertension (n, %)	30 (45.4)	36 (45.5)	9 (11.5)	19 (18.8)	0,0001
Dyslipidaemia (n, %)	22 (33.3)	27 (34.2)	12 (15.4)	22 (21.7)	NS
Statins (n, %)	17 (25.7)	20 (25.3)	7 (8.9)	16 (15.8)	NS
Diabetes mellitus (n, %)	8 (12.1)	7 (8.8)	3 (3.8)	4 (3.9)	NS
Autoimmune disease (n, %)	6 (9,09)	10 (12.6)	5 (6.4)	9 (8.9)	NS
Arterial thrombosis background (n, %)	5 (7.6)	6 (7.6)	–	1 (1.0)	0.002
Oral Contraceptives	–	32 (41)	–	55 (54.5)	NS
Non–steroidal anti–inflammatory drugs (n, %)	9 (13.6)	21 (26.6)	14 (17.9)	19 (18.8)	NS
Anti–platelet drugs (n, %)	8 (12.1)	8 (10.1)	1 (1.3)	2 (1.9)	0,023

Non-significance is listed as NS.
[a] Number of individuals in each group. The percentages are given in parentheses.
[†] The statistical differences, fixed in a $p < 0.05$, are reported only for descriptive purposes.

polymorphism and the risk of myocardial infarction. This polymorphism is associated also with hyper-reactive platelet phenotypes [10].

It seems to be a “global phenomenon”, so subjects with increased platelet aggregation in response to one inductor (as EPI), show an increase response also to other platelet inductors. Aggregation was inducted in a high percentage of individuals when serotonin (5-TH) was added to sub-maximal concentration of EPI, than when EPI alone was added. Also, the bimodal distribution was retained [7]. The combination of serotonin and ADP caused irreversible aggregation of platelets.

Serotonin has been considered a weak agonist for human platelets, but it enhances platelet activation and pro-coagulant responses. Also, it augments thrombogenesis of injured vascular surfaces [12]. Then, serotonin seems to have a significant agonistic effect on platelets.

There is a receptor for serotonin ($5\text{-}HT_{2A}$) on the platelet surface and a serotonin transporter (SERT) that re-uptakes serotonin. SERT moves serotonin into platelet-dense bodies [13], and is secreted during platelet activation. Hyper-reactive platelet individuals exhibited increased SERT levels and increased binding affinity of serotonin-SERT [7]. SERT is blocked by selective inhibition of serotonin re-uptake (SSRI). Then, the effects of serotonin on platelet activation are weakened and down-regulated the pro-thrombotic tendencies [12]. SERT is present in platelet and in neurons.

A case-control study [14] reported that SSRI is associated with protection against myocardial infarction. Notably, there is a polymorphism in the promoter region of *SERT* gene (*SLC6A4*). It is located around 1 kb from the transcription initiation site and consists of a 44-bp deletion or insertion. The long variant (LL) has more transcriptional activity than the short variant (SS).

The Cas-Témoins de l'Infarctus du Myocarde (ECTIM) Study [15] reported that there is an association with the *LL* genotype of the *SLC6A4* polymorphism and a higher risk of myocardial infarction.

The aim of our study was to determine if VAMP8, SERT and serotonin levels are associated with the risk of venous thrombosis. In addition, we determined if the *rs 1010* SNP of VAMP8 and a polymorphism in the promoter region of the *SLC6A4* are associated with this risk.

2. Materials and methods

2.1. Study design

Patients and controls were recruited from the RETROVE Study (Riesgo de Enfermedad TROmboembólica VEnosa) between 2012 and 2016 in our Thrombosis and Haemostasis Unit (Hospital de la Santa Creu i Sant Pau, Barcelona (Spain)). RETROVE is a prospective, observational, case-control study that consisted of 400 consecutive patients with venous thrombosis and 400 healthy controls. Detailed population baseline characteristics were described previously [16]. The goal of the RETROVE study was to obtain a mathematical algorithm that defines the individual risk of suffering a venous thrombotic event. Controls were not matched intentionally for sex or for age. Thus, any statistical inference was adjusted for these co-variates.

Patients 18 years old were included in our study. They had at least one venous thrombotic event diagnosed with doppler ultrasonography, tomography, magnetic resonance, arteriography, phlebography and pulmonary gammagraphy. As detailed previously [16], a venous thrombotic event was classified as either spontaneous or unprovoked or non–spontaneous or provoked. The provoking factors (one or more) were within three months previous to an event [17,18]. Provoking factors were: surgery, pregnancy or puerperium, immobilization, oral contraceptives, prothrombotic non–neoplastic diseases and other circumstances.

The control individuals were not blood donors and they were not related to the patients. They were distributed according to age and sex in a Spanish population (2001 census).

All of our procedures were approved by the Institutional Review Board of the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain. Written informed consent (in accordance with the Declaration of Helsinki) was obtained from all participants.

We began our study after the RETROVE Project had started, so we were able to obtain Platelet-rich plasma (PRP) for measurement of VAMP8, SERT and serotonin in 324 individuals: 145 patients with venous thrombosis (66 men and 79 women) and 179 healthy controls (78 men and 101 women) selected chronologically in the same previous conditions of RETROVE project but in a shorter period of time (between November 2013 to May 2015). Table 1 lists the clinical characteristics of this population. We found that age, body mass index and hypertension had statistical differences. These parameters also had statistical differences in the total RETROVE population [16] consisting of 400 consecutive patients with venous thrombosis and 400 healthy controls.

Table 2 shows the venous thrombosis events that were classified as either unprovoked or spontaneous or provoked or non–spontaneous.

2.2. Blood collection

Blood was collected 6 months after the most recent thrombotic event. Anti-vitamin K was withdrawn and the blood samples were taken

Table 2
Characteristics of 145 consecutives thrombotic events where VAMP8, SERT and serotonin levels were determined.

	Spontaneous			Non-spontaneous		
	M[a]	F[a]	Total[b]	M[a]	F[a]	Total[b]
Isolated deep vein thrombosis	25 (52.0)	21 (44.7)	46 (48.4)	10 (55.5)	11 (34.3)	22 (44.0)
No isolated deep vein thrombosis	8 (16.6)	5 (10.6)	13 (13.7)	3 (16.6)	6 (18.7)	9 (18.0)
Isolated pulmonary embolism	13 (27.0)	19 (40.4)	32(33.6)	5 (27.7)	14 (43.7)	19 (38.0)
Visceral thrombosis	1 (2.0)	–	1 (1,0)	-	–	-
Venous sinus thrombosis	1 (2.0)	2 (4.2)	3 (3.1)	–	1 (3.1)	1 (2.0)
Total[c]	48 (33.1)	47 (32.4)	95(65.5)	18 (12.4)	32 (22.1)	50 (34.5)

[a] The percentages between parentheses were obtained from the number of thrombosis types divided by the total number of males (M) or females (F).
[b] The percentages between parentheses were obtained from the number of thrombosis types divided by the total number of spontaneous or non–spontaneous events.
[c] The percentages between parentheses were obtained from the number of male (M), female (F) or total divided by the total number of patients.

after a washout period of at least 21 days or 36 h for heparin. Also, we required a week for anti-platelet therapy (aspirin or clopidogrel) or for other drugs that affect platelet function such as non-steroidal anti-inflammatory (NSAID). For SERT and serotonin analysis, 4 patients and 2 controls were excluded because they were being treated with serotonin reuptake inhibitor (SSRI). Blood samples were collected from the antecubital vein and immediately anticoagulated with 1/10 volume of 0.129 M sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation at 160*g* for 10 min. Platelet-poor plasma (PPP) was obtained by centrifugation at 2000*g* for 20 min. It was frozen and stored at −40 °C until analyzed.

2.3. Laboratory determinations

VAMP 8 was determined with human vesicle-associated membrane protein 8 (VAMP8) ELISA kit by CUSABIO (Houston, USA). Platelets were isolated: PRP was washed with an equal volume using a phosphate buffer solution (Na_2HPO_4-KH_2PO_4) and centrifugation at 4500*g* for 10 min. The platelet pellet was frozen at −40 °C. Before analysis, the platelet pellet was resuspended with Triton X-100 and cooled on ice for 1 h. The final concentration was expressed as the number of platelets (pg/10^9 PLT).

SERT was determined by a commercial kit: ELISA for serotonin transporter (SERT) by USCN Life Science Inc. (Wuhan, Hubei) according to the manufacturer's instructions. Platelets were isolated as described above. The final concentration was expressed as the number of platelets (pg/10^9 PLT).

Serotonin was determined by a serotonin ELISA kit (IBL international GMBH, Hamburg, Germany) according to the manufacturer's instructions. Platelets were isolated as described above. The platelet pellet was resuspended with 200 μL of distilled water. The final concentration was expressed as the number of platelets (ng/10^9 PLT).

2.4. Genetic analysis

DNA was isolated from peripheral blood leukocytes by a standard protocol [19].

The **VAMP8 *rs 1010* single nucleotide polymorphism**: Genotyping for *rs1010* was performed using the *TaqMan* SNP Genotyping by allelic assay (ID C_2091644_20; Applied Biosystems, Foster City, CA, USA). Fluorescence data were obtained using an ABI 7500 Real Time PCR System (Applied Biosystem).

The **polymorphism of the *SLC6A4* promoter** was analyzed by direct PCR as previously described [20] with minor modifications. The final amplicon product consisted of 406/450-bp fragments (*S* and *L* alleles, respectively). PCR was performed with Master Mix Promega (Madison, USA).

2.5. Statistics analyses

The controls and patients were compared by the Mann-Whitney test. Interquartiles (25th and 75th percentiles) and median were calculated. 75th percentile was selected as the cut off (≥1930 pg/10^9 PLT) of VAMP8 levels and (≥784 pg/10^9 PLT) of SERT levels. A 25th percentile was selected as cut off (≤115 ng/10^9 PLT) of serotonin levels. The interaction between these biomolecules was analyzed for age and sex by an ANOVA. The $\chi 2$ test was used for group comparisons of frequencies. A logistic regression method was used to estimate both the crude and adjusted odds ratio (OR) of venous thrombosis risk with 95% confidence intervals. A report of previous studies [21,22] stated that potential confounders can add partiality in the determination of the risk factors for thrombosis. With venous thrombosis, age, sex, body mass index (BMI), Factor V Leiden (FVL) and high levels of Factor VIII (FVIII) ((≥217%) and Factor von Willebrand (FvW) (≥183%) were potential confounders. To avoid confusion, we calculated the venous thrombosis risk applying three statistical models. The venous thrombosis risk models were for VAMP8: 1) unadjusted or crude (model 1); 2) adjusted for age (model 2); 3) adjusted for age, BMI, FVL, FVIII and FvW; for SERT and serotonin: 1) unadjusted or crude (model 1); 2) adjusted for BMI (model 2); 3) adjusted for age and BMI (model 3). Finally, p values < 0.05 were considered statistically significant.

3. Results

We found differences for categorical variables as shown in Table 1. Also, we observed 145 consecutive events of thrombosis. There was more spontaneous (n = 95, 65.5%) than non-spontaneous venous thrombosis (n = 50, 34.5%) (Table 2).

It is notable that VAMP8 and SERT showed statistically significant higher levels in patients than in controls. In contrast, serotonin showed lower levels in patients than in controls as is shown in Table 3.

We found that the function biomolecules were affected by sex.

Our analysis of VAMP8 showed a clear interaction with sex (p = 0.028). The control values of women were lower than the control values of men and patients (Fig. 1a).

Table 3
Levels of VAMP8, SERT and serotonin in patients and in controls.

	Median		25th percentile		75th percentile		p
	Patient	Control	Patient	Control	Patient	Control	
VAMP8 (pg/10^9 PLT)	1498	1138	903	536	2372	1930	0,001
SERT (pg/10^9 PLT)	636	543	454	366	989	784	0,001
Serotonin (ng/10^9 PLT)	144	172	69	115	206	263	0,013

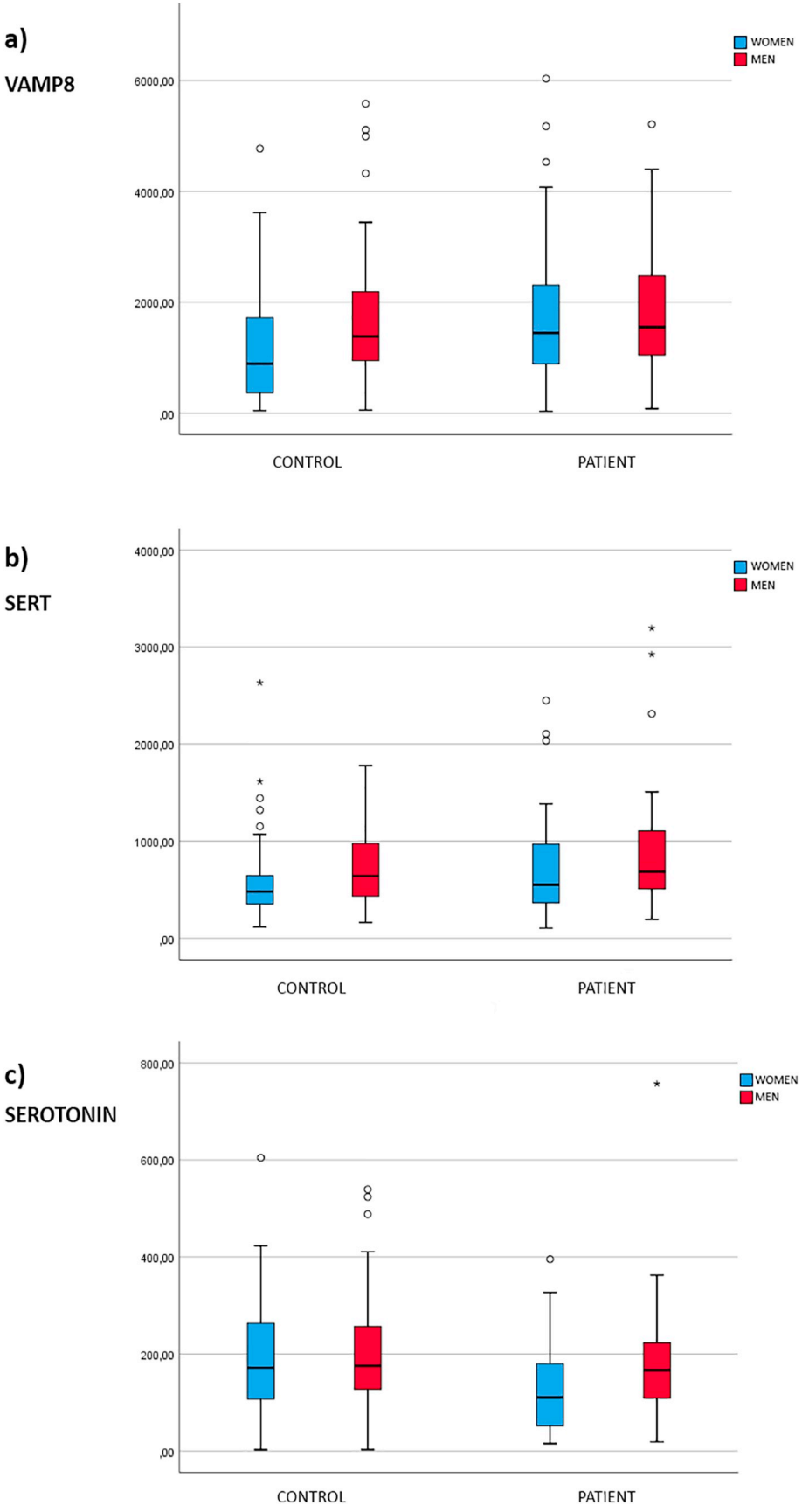

Fig. 1. Levels of VAMP 8 (a), SERT (b), and serotonin (c) by sex.

Table 4
75th percentile of VAMP8 (≥1930 pg/10^9 PLT) and SERT (≥784 pg/10^9 PLT) and 25th percentile of serotonin (≤115 ng/10^9 PLT) in women and men.

		Women		Men	
		Patient	Control	Patient	Control
VAMP8	< 1930 (pg/10^9 PLT)	49 (62%)	85 (84.2%)	45 (68.2%)	50 (64.1%)
	≥1930 (pg/10^9 PLT)	30 (38%)[a]	16 (15.8%)[a]	21 (31.8%)	28 (35.9%)
	Model 1 OR (95% CI)	3,25 (1.61–6,56) p = 0,001		0,83 (0,41–1,67) NS	
	Model 2 OR (95% CI)	3,07 (1,43–6,59)p = 0,004		0,84 (0,39–1,83) NS	
	Model 3 OR (95% CI)	3,43 (1,41–8,35) p = 0,006		0,59 (0,23–1,49) NS	
SERT	< 784 (pg/10^9 PLT)	51 (66.2%)	84 (84.8%)	37 (57.8%)	49 (62.8%)
	≥784 (pg/10^9 PLT)	26 (33.8%)[a]	15 (15.2%)[a]	27 (42.2%)	29 (37.2%)
	Model 1 OR (95% CI)	2.76 (1.36–5.6) p = 0,004		1.24 (0.63–2.42) NS	
	Model 2 OR (95% CI)	2.39 (1.14–4.99) p = 0,021		1.35 (0.67–2.73) NS	
	Model 3 OR (95% CI)	2.25 (1.08–4.70) p = 0,030		0.98 (0.46–2.12) NS	
Serotonin	≤115 (ng/10^9 PLT)	40 (51,9%)[a]	28 (29.2%)[a]	18 (29.0%)	15 (19.7%)
	> 115 (ng/10^9 PLT)	37 (48.1%)	68 (70.1%)	44 (71.0%)	62 (80.3%)
	Model 1 OR (95% CI)	2.62 (1.40–4.92) p = 0,003		1.69 (0.77–3.71) NS	
	Model 2 OR (95% CI)	2.17 (1.13–4.17) p = 0,020		1,48 (0.65–3.36) NS	
	Model 3 OR (95% CI)	1.67 (0.85–3.29) NS		1.62 (0.72–3.66) NS	

Non-significance is listed as NS.
The *odds ratios* (OR) within the 95% confidence intervals (CI) in parentheses for all of the models. By VAMP8: Model 1: non-adjusted model. Model 2: adjusted for age. Model 3: adjusted for age, BMI, FVL, FVIII and FvW.
By SERT and serotonin: Model 1: non-adjusted model. Model 2: adjusted for BMI. Model 3: adjusted for age and BMI.
[a] Significant different results.

Table 5
Non-spontaneous and spontaneous venous thrombosis in patients and controls in the 75th percentile of VAMP8 (≥1930 pg/10^9 PLT). Non-significance is listed as NS.

		VAMP8 percentile 75th			
		< 1930 (pg/10^9 PLT)	≥1930 (pg/10^9 PLT)	Total	
Women	Controls	85	16	101	
		84,20%	15,80%	100,00%	
	Patients with **non-spontaneous** thrombotic event	22	10	32	
		68,80%	31,3%*	100,00%	
	Patients with **spontaneous** thrombotic event	27	20	47	**p = 0,002**
		57,40%	42,6%*	100,00%	
	Total	134	46	180	
		74,40%	25,60%	100,00%	
Men	Controls	50	28	78	
		64,10%	35,90%	100,00%	
	Patients with **non-spontaneous** thrombotic event	13	5	18	
		72,20%	27,80%	100,00%	
	Patients with **spontaneous** thrombotic event	32	16	48	**NS**
		66,70%	33,30%	100,00%	
	Total	95	49	144	
		66,00%	34,00%	100,00%	

*Significant different results.
Relevant.

We found that SERT levels were sex-depended (p = 0.0001). It is clear that women have lower levels of SERT than men both in patients and in controls (Fig. 1b).

Sex affects serotonin levels also (p = 0.011). The values for women were lower than for men in patients and in controls (Fig. 1c).

The levels of VAMP8 and SERT were studied in individuals who were in the 75th percentile (≥1930 pg/10^9 PLT by VAMP8 levels and ≥784 pg/10^9 PLT by SERT levels): A higher percentage of patients had levels in the upper percentile 75th as by VAMP8 as SERT levels (35.2% vs 24.6%; difference 10.6%, p = 0.049 and 40.2% vs 24.9%; difference 15.3%, p = 0.002 respectively).

3.1. VAMP8

When gender was included in our study of VAMP8 levels, we noted a significant difference but only in women: 38.0% vs 15.8%; difference of 22.2%; p = 0.001. The associated thrombotic OR was 3.25 (1.61–6.56 95% CI). The OR did not change when co-variates were included (OR = 3.43 (1.41–8.35 95% CI) p = 0.006) (Table 4).

3.2. SERT

As noted previously, when gender was included, women exhibited significant differences: 33.8% vs 15.2%; difference of 18.6%; p = 0.004. The associated thrombotic OR was 2.76 (1.36–5.60 95% CI). When the co-variates were included, the OR was maintained (OR = 2.25 (1.08–4.70) (p = 0.030)) (Table 4).

Importantly, males did not show significant differences between patients and controls with VAMP8 or with SERT as shown in Table 4.

We studied the effect of serotonin in individuals in the 25th percentile (≤115 ng/10^{9}PLT):

3.3. Serotonin

Serotonin behaved opposite to SERT and VAMP8. It showed a higher percentage of patients with levels under the 25th percentile (40.8% vs 26.3%; difference 14.5% p = 0.006). When individuals were studied by sex, a significant difference was obtained in women (51.9% vs 29.2%; difference 22.7%; p = 0.004). The associated thrombotic OR was 2.62 (1.40–4.92 95% CI). When age was included, the significant difference was lost (OR = 1.67 (0.85–3.29 95% CI). Males did not show differences between patients and controls (Table 4).

We found an association in the 75th percentile of VAMP8 levels and spontaneous venous thrombosis also in women (42.6% vs 31.3% difference 11.3% p = 0.002) as shown in Table 5.

***The VAMP8 rs 1010* single nucleotide polymorphism and *SLC6A4* polymorphism**: No statistical differences between patients and controls were found as is show in Table 6.

4. Discussion

One important result of our study is that in a Spanish population, levels of VAMP8 and SERT are associated with venous thrombotic risk,

Table 6
Allelic frequencies of VAMP8 *rs1010 SNP* and SERT genotype (polymorphism in the promoter region of the *SLC6A4*). Non-significance is listed as NS.

VAMP (genotype)	AA	AG	GG	p
Patient (%)	34	48	18	NS
Control (%)	36	42	22	NS
SERT (genotype)	LL	LS	SS	p
Patient (%)	33	45	22	NS
Control (%)	27	47	26	NS

but only in women. By controlling the potential confusion, the thrombotic risk factor did not decrease after adjusting for age. In our study the controls were not matched with patients for age and sex due to our study design. Our controls were younger than the patients. Nevertheless, our results were clear-cut. As far as we know, there are no other studies that examined this association.

High levels of VAMP8 and SERT are associated with platelet hyperreactivity. Interestingly this phenotype has been associated with females [6]. Berlin et al. [23] found a higher platelet aggregation in women of fertile age than in post-menopausal women and men. This could be a mechanism to prevent heavy menstrual bleedings. Also, we found higher levels in women of fertile age than in menopausal women (results not shown).

To account for the relationship between stress and thrombotic events, it has been proposed that a synergistic interaction occurs between adrenergic and serotonergic stimuli [24,25]. These thrombotic events include myocardial infarction and sudden death. Different types of receptors control the actions of serotonin that are ended by a single serotonin transporter (SERT). So, SERT is the main mechanism that regulates plasma serotonin levels. It prevents vasoconstriction and thus ensures a stable blood flow [26]. In our study, we found that low levels of Serotonin were associated with high venous thrombotic risk, but only in women. High levels of SERT might explain the decrease in the levels of serotonin that we observed.

Platelet pathology has been associated classically with arterial thrombosis. We analyzed the association of VAMP8 and SERT with venous thrombosis adjusted by arterial risk co-factors as body mass index (BMI) and as well, venous risk cofactors as FVIII and FvW high levels.

Our group has reported recently [16] that short times to occlusion of PFA-100 (as EPI as ADP) are associated with venous thrombosis and suggested that these mechanism could be associated with platelet adhesion. We did not find a correlation with VAMP8, SERT or serotonin levels and short values of PFA (both in ADP and in EPI) (data not shown). These biomolecules are related to platelet aggregation not in platelet adhesion so, our results support the hypothesis that the mechanism that is related to short values of PFA-100 with thrombosis could cause platelet adhesion.

An important finding of our study is that there is an association between VAMP8 levels and spontaneous venous thrombosis in women. We analyzed a SNP of VAMP8 that it is associated with high levels of VAMP8 and myocardial infarction: the *rs 1010* SNP in *VAMP8*. It has been observed in five independent patient populations [11,27,28]. We did not find an association between this SNP and venous thrombosis. We analyzed other polymorphism in the *SERT* gene (polymorphism in the promoter region of the *SLC6A4*). This polymorphism is associated [15] with myocardial infarction risk, but we did not find this association with venous thrombosis. Often, polymorphisms associated with arterial thrombosis have not been found associated with venous thrombosis.

There are some limitations in our study. First, because we are a reference center, it is possible that a sample with random group of patients could have lower thrombotic risk than our sample. Second, all biomolecules were determined at least 6 months after the thrombotic event. Thus, if these phenotypes were studied during the acute phase, our results might have been different. Third, there could be a possible selection bias; patients and controls were selected chronologically between November 2013 and May 2015. Our population was smaller (324 individuals) than the total RETROVE population (800 individuals) but the statistical differences found in some of the baseline characteristics between patients and controls (age, body mass index and hypertension) were also found statistical different in the total RETROVE population [16]. Fourth, as it is a case-control study, it is not possible to demonstrate causality; therefore, we demonstrate the association between these biomolecules and the thrombotic risk. Finally, the number of individuals in our study may not provide enough statistical power. Further studies with a larger population may determine the validity of our findings.

In conclusion, our results showed that VAMP8 and SERT levels are related to venous thrombotic risk in women. Although there are two polymorphisms in the gene of these proteins associate with their levels and myocardial infarction, we did not find an association of their allelic frequencies and venous thrombotic risk. Further studies are necessary to confirm our results.

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Declaration of competing interest

The authors report no conflicts of interest.

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