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ORIGINAL STUDY

Association Between *TSPAN15* (rs78707713) and *SLC44A2* (rs2288904) Genetic Polymorphisms and Venous Thromboembolism in Cancer Patients

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Abstract

Objectives: **To assess the relationship between *TSPAN15* and *SLC44A2* polymorphisms and venous thromboembolism (VTE) in cancer patients was evaluated with correlation to PROTECHT scoring system and survival data.**

Background: **Although VTE is common in cancer patients, the underlying mechanisms are complex and poorly understood.**

Patients and methods: **This study enrolled two groups: 50 VTE-cancer patients in group I and 50 cancer patients with no VTE in group II. Full history, general examination, histopathological diagnosis, and clinical stage were recorded. Complete blood count, coagulation profile, and analysis of *TSPAN15* (rs78707713) and *SLC44A2* (rs2288904) single-nucleotide polymorphisms by PCR technique were done. Radiological examinations were done according to the clinical scenario. PROTECHT score and survival analysis were performed.**

Results: **VTE-cancer patients had high-grade cancer, increased incidence of metastasis with shorter progression-free survival versus those without VTE. Partial immobilization, hospitalization, and gemcitabine-based chemotherapy showed a significant relationship with VTE in cancer patients. *SLC44A2* and *TSPAN15* mutant alleles were more prevalent in group I. *SLC44A2* (AG + GG) and *TSPAN15* (CC) genotypes were identified as VTE independent risk factors.**

Conclusion: **VTE is associated with higher tumor grades and metastatic disease, and it is linked to shorter progression-free survival in cancer patients. Screening of *SLC44A2* (rs2288904) and *TSPAN15* (rs78707713) genetic variants may enhance the ability to predict VTE risk in cancer patients.**

Keywords: Cancer, PROTECHT score, Solute carrier family 44 member 2, tetraspanin 15, Venous thromboembolism

1. Introduction

The significance of cancer-associated thrombosis has been widely recognized for physicians who deal with venous thromboembolism (VTE) as well as for oncologists. VTE is currently estimated to occur annually in 0.5 % of cancer patients as opposed to 0.1 % of the population in general [1].

VTE is a prevalent cardiovascular illness caused by a complex interaction of inherited and acquired elements that influence disease risk [2].

It is well known that genetic single-nucleotide polymorphisms (SNPs) are linked to disease risk.

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Two novel VTE-associated SNPs, tetraspanin 15 (*TSPAN15*) rs78707713 and solute carrier family 44 member 2 (*SLC44A2*) rs2288904, have been identified by genome-wide association studies [3].

The tetraspanin 15 (*TSPAN15*) gene is found on chromosome 10q22 and is of the tetraspanin family. Tetraspanins are proteins of small size composed of one small extracellular loop (EC1) and one large extracellular loop (EC2) as well as four typical and hydrophobic transmembrane domains (TM1−TM4) [4]. *TSPAN15* plays a role in the cellular trafficking and activity of ADAM metallopeptidase domain 10 (ADAM10), that produces cleavage of many proteins such as tumor necrosis factor-alpha and E-cadherin [5].

SLC44A2 regulates the transport of choline into mitochondria. Choline is essential for ATP production, which is required for the maximum activation of platelets. *SLC44A2* was found to initiate thrombosis in a mouse model of venous stenosis; however, the exact mechanism was not discovered [6].

The aim of this work is to evaluate the association between *TSPAN15* and *SLC44A2* polymorphisms and VTE in cancer patients was evaluated with correlation to PROTECHT scoring system and survival data.

2. Patients and Methods

2.1. Patients

This case−control study was conducted through cooperation between Medical Biochemistry and Molecular Biology, Clinical Oncology and Nuclear Medicine, Internal Medicine, Pathology, Clinical Pathology, and Radio-diagnosis Departments, Faculty of Medicine, Menoufia University, in the period from August 2021 to April 2023. One hundred cancer patients were recruited, treated, and followed up. Group I included 50 consecutive cancer patients with VTE and group II selected 50 cancer patients without VTE as a control with matched (age group, sex, and primary cancer diagnosis) to group I.

Ethical approval

An approval from the ethical committee (IRB 7/2021BIO3-2) was obtained and all participants provided written informed consent.

2.2. Methods

All patients underwent full history taking and family history as well as full clinical examination, histopathological diagnosis including histopathological type and subtype together with grading by interpretation of hematoxylin and eosin-stained slides by expert pathologist. Patients were assessed for the presence of VTE symptoms based on clinical suspicion depending on clinical examination and history taking, symptoms and signs of limb swelling, cardiopulmonary symptoms, or both, as well as VTE risk factors including immobilization, hospitalization, chemotherapy, and surgery. Group I patients were included based on the presence of VTE on imaging, whereas group II patients were selected cancer patients with a matched age group, sex, and primary cancer diagnosis but without VTE.

For all patients, all clinical data and treatment received were collected. Performance status was assessed according to the Eastern Cooperative Oncology Group (ECOG) [7] and BMI was calculated [8]. Patient's staging was done according to American Joint Committee of Cancer (AJCC), 8th edition [9]. Furthermore, patients were categorized according to the stages into nonmetastatic stages including stage I, II, III versus metastatic stage IV.

PROTECHT score was calculated for each patient to assess the risk to develop VTE. The PROTECHT score is a modified version of the Khorana score and is based on its five predictive factors, which include the following: when clinical risk factors are present, extremely high risk cancer sites (such as pancreatic, stomach, or primary brain cancer) are given two points, whereas high risk cancer sites are given one point (lung, ovarian, or bladder cancer). The following prechemotherapy values are also given one point each: platelet count more than 350×10^9/l, hemoglobin more than 6.2 mmol/l, leukocyte count more than 11×10^9/l, and BMI more than 35 kg/m^2. Treatment with chemotherapy that contains carboplatin, cisplatin, or gemcitabine each contributes one point to the PROTECHT prediction score. Patients with a score of 3 or above were regarded to be at high risk for VTE, whereas those with a score of 0−2 were thought to be at moderate or intermediate risk [10].

Progression-free survival (PFS) among our patients was defined as the time interval from the initiation of therapy until the disease progressed.

Duplex ultrasound examination was performed for patients with limb swelling, using General Electric (LOGIQ E10) equipment, linear array transducers (2−9 and 6−15 MHz) were utilized for the femoral, popliteal, calf veins, and tibial venous segments. The iliac veins and IVC were assessed using a curvilinear transducer (1−6 MHz).

The veins were assessed for absent or diminished vein compressibility, visualization of thrombus, flow absence on spectral color Doppler, absence or impairment of flow augmentation, and loss of spontaneous plasticity with respiration.

Computed tomography (CT) pulmonary angiography was performed for patients with chest pain and elevated D-dimer levels or clinical suspicion, using GE (REVOLUTION) 128 multidetector CT scanner. Dual-energy CT technique with single breath hold. The protocol includes intravenous administration of 300 mg/ml of iodinated contrast material at 3–5 ml/s, with the time for the pulmonary artery being optimized using bolus tracking and automated triggering. Axial soft tissue sections of 1 and 2 mm as well as coronal and sagittal reformats were included in the reconstruction.

2.3. Laboratory investigations

Complete blood count (CBC), coagulation profile; prothrombin concentration, and activated partial thromboplastin time (APTT) and analysis of *TSPAN15* and *SLC44A2* SNPs by PCR technique were done.

2.4. Blood sampling

Sample collection: under complete aseptic conditions, 6 ml of blood were collected; 4 ml were divided into two EDTA tubes to do CBC and SNP analysis and 1.8 ml were added to 0.2 ml of sodium citrate (3.2 %) to perform prothrombin concentration and APTT.

2.5. Assay procedure

CBC was performed on the automated hematology analyzer, Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan). Prothrombin concentration and APTT were done using Stago STA compact automated coagulation analyzer (Diagnostica Stago, France).

Analysis of *TSPAN15* (rs78707713) and *SLC44A2* (rs2288904) using an assay called allelic discrimination, that detects sequence variations in a single nucleic acid. In each reaction, two primer/probe pairs are included in order to genotype two putative variations located at the SNP site in a template sequence. Initially, DNA extraction was extracted from whole blood followed by SNP detection by real-time PCR.

DNA extraction: whole blood DNA extraction was done using the Quick-g DNA TM MiniPrep kit from Zymo Research (USA). DNA amplification, establishing the plate document, the start of the PCR run, and analyzing the results, were done with the use of Applied Biosystems 7500 (software version 2.0.1; Foster City, California, USA).

Preparation of the reaction mixture for genotyping: TaqMan Master Mix II (2 ×), primers, and probes were provided by Applied Biosystems. The manufacturer described the probes sequences as follows: *SNP1 (rs78707713): [VIC/FAM]:AGCTTAGCATGTGAGCAAAACAGCG [T/C]GGAGGCCACGTGGCTGAGGGTAGGT.For*SNP2(rs2288904): [VIC/FAM] TGGCAGGGAGTGGCTGAGGTGCTTC [A/G]AGATGGTGACTGCCCTGCTGTCCTC. All solutions were gently centrifuged and vortexed. In order to prepare a reaction mixture of 25 μl, the following elements were added: 6.25 μl of nuclease-free water, 12.5 μl of master mix, and 1.25 μl of SNP assay. Proper mixing of the master mix was done before dispensing into the PCR tubes. The addition of 5 μl (0.1 μg/μl) of DNA template to each unknown reaction, and 5 μl of DNAse-free water to the negative control reaction was done as well as gentle mixing and brief centrifugation of mixtures.

The cycler was loaded with the tubes, using real-time PCR equipment, Applied Biosystems 7500: a denaturation step for 4 min at 94 °C, 50 cycles of annealing for 25 s at 50 °C, extension for 40 s at 72 °C, and final extension for 3 mi at 72 °C.

2.6. Statistical analysis

Data were analyzed through IBM SPSS statistics, version 20.0 (IBM Corp., Armonk, New York, USA). To verify the normality of data distribution, Shapiro test was used. To compare categorical variables between groups, χ^2 test (Fisher or Monte Carlo) was applied. Comparing two groups with quantitative variables that were normally distributed was done using the Student *t* test. Whereas, when comparing two groups with quantitative variables that were not normally distributed, the Mann–Whitney test was applied. Analysis of variance was used in the comparison between different studied categories. Different groups with quantitative variables that were abnormally distributed were compared using the Kruskal–Wallis test. With the use of the Hardy–Weinberg equation, the population of the examined sample was explored to identify its equilibrium. Odd ratio (OR) was calculated to determine the ratio of the odds and 95 % confidence interval (CI) of an event that occurs in one risk group to the odds of it occurring in the nonrisk group. Kaplan–Meier was used to detect the significant relation with the PFS. At the 5 % level, significance of the results was determined.

3. Results

The current study recruited 100 patients classified into two groups: VTE and non-VTE-cancer patients. Both groups were age, sex, and cancer diagnosis

matched. The most frequent histopathological type was carcinoma (Fig. 1a).

In group I: cancer patients with VTE; deep venous thrombosis was more common in right lower limb in 29 of patients. Out of the 50 patients in group I, only two patients had bilateral deep venous thrombosis, and six patients developed pulmonary embolism (Fig. 1b).

Our results showed that 64 % of patients with VTE had metastatic disease (stage IV) versus 30 % of non-VTE-cancer patients. Sixty-four percent of cancer patients with VTE had a high-grade tumor versus 44 % of cancer patients without VTE. The laboratory parameters such as hemoglobin, platelet count, total leukocyte count, prothrombin concentration, and APTT did not statistically differ between groups. As regards risk factors for VTE, only partial immobilization and hospitalization had a significant relationship with the incidence of VTE in cancer patients. Among various lines of treatment used, gemcitabine-based chemotherapy was related to an increased VTE risk. Thirty percent of group I patients had high risk PROTECHT score of more than 3 points versus 20 % in group II. Grade III had the highest percentage in the two groups, representing 61.4 and 45.5 % in groups I and II, respectively, with no significant *P* value. The most frequent *SLC44A2* (rs2288904) genotype was GG (56 %) followed by AG (42 %) in group I. Whereas, GG and AG genotypes were equally distributed (40 %) in group II with significant *P* value of 0.013. The most frequent *TSPAN15* (rs78707713) genotypes were TC (58 %) and CC (34 %) in group I versus TC (62 %) and TT (22 %) in group II with significant *P* value of 0.037. Both of mutant G allele of *SLC44A2* (rs2288904) and the mutant C allele of *TSPAN15* (rs78707713) were more significantly frequent in group I in comparison with group II. Furthermore, the *SLC44A2* (rs2288904) polymorphism showed significance with the dominant and additive models. Whereas *TSPAN15* (rs78707713) polymorphism was significant with the recessive and additive models. The haplotype model

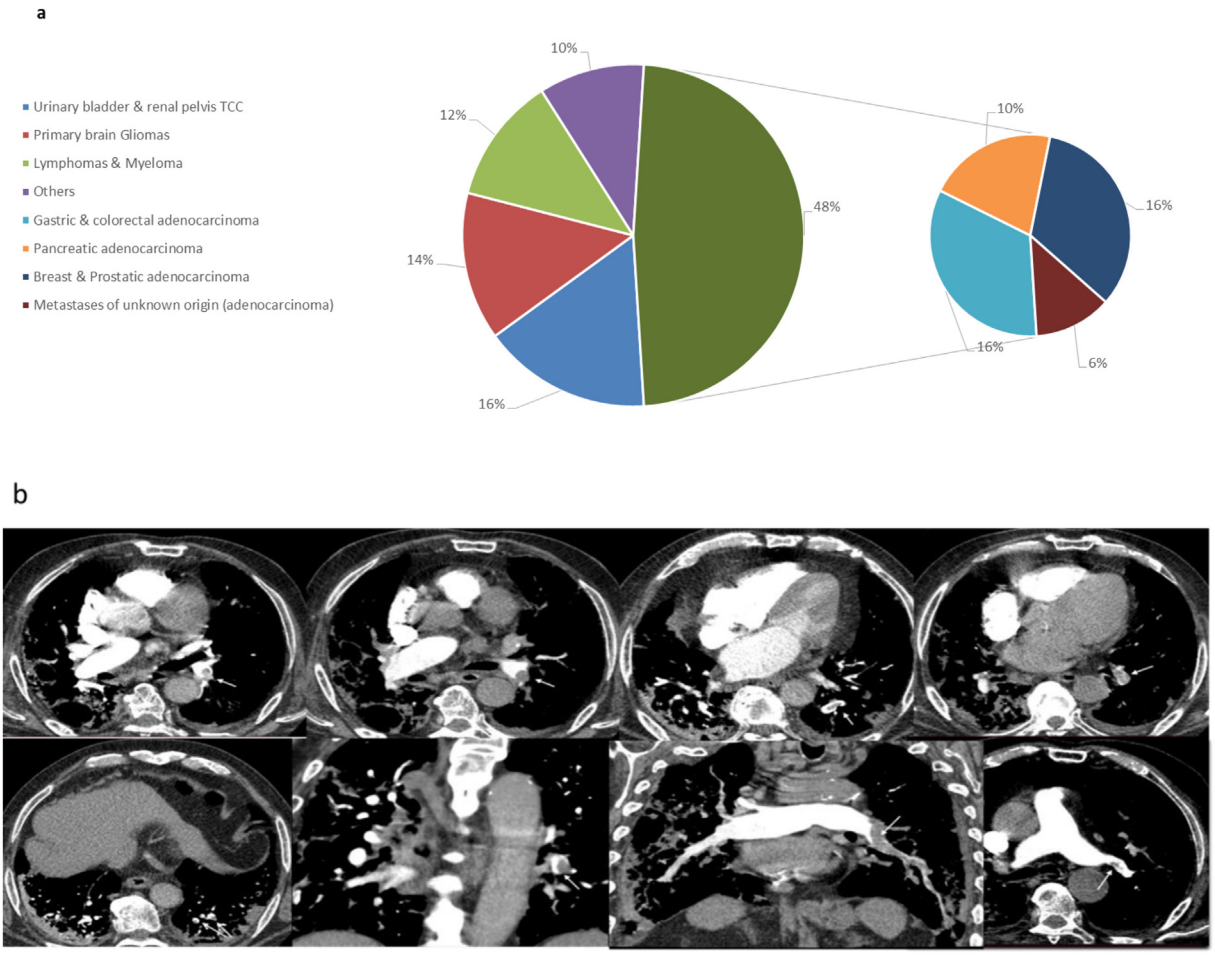

Fig. 1. (a) For both groups patients' primary cancer diagnosis and histopathological subtypes. (b) CT pulmonary angiography. CT, computed tomography. Axial, coronal and coronal oblique images show pulmonary embolism of left main pulmonary and left lower lobe segmental and subsegmental branches (arrows).

Table 1. Comparison between the two studied groups according to clinical data, laboratory investigations, risk factors, and current treatment

	Group I ($N = 50$) [n (%)]	Group II ($N = 50$) [n (%)]	Test of significance	P
Age (years)				
<60	8 (16)	8 (16)	$\chi^2 = 0.0$	1.000
≥60	42 (84)	42 (84)		
Mean ± SD	65.72 ± 9.38	67 ± 8.3	$t = 0.699$	0.486
Sex				
Male	23 (46)	27 (54)	$\chi^2 = 0.640$	0.424
Female	27 (54)	23 (46)		
Smoking	18 (36)	19 (38)	$\chi^2 = 0.043$	0.836
BMI (kg/m^2)				
Underweight	3 (6)	2 (4)	$\chi^2 = 0.337$	$^{MC}P = 1.000$
Normal	16 (32)	16 (32)		
Overweight	15 (30)	15 (30)		
Obesity	16 (32)	17 (34)		
Mean ± SD	27.64 ± 7.54	27.30 ± 7.13	$t = 0.232$	0.817
Performance status				
PS 0	6 (12)	19 (38)	$\chi^2 = 25.175$[a]	<0.001[a]
PS 1	22 (44)	29 (58)		
PS 2	13 (26)	0		
PS 3	9 (18)	2 (4)		
Presence of metastasis (stage IV)	32 (64)	15 (30)	$\chi2 = 11.602$[a]	0.001[a]
Type of metastasis	$N = 32$	$N = 15$		
Bone	7 (21.9)	3 (20)	$\chi^2 = 0.021$	$^{FE}P = 1.000$
Visceral	18 (56.3)	11 (73.3)	$\chi^2 = 1.261$	0.261
Soft tissue	1 (3.1)	0	$\chi^2 = 0.479$	$^{FE}P = 1.000$
Multiple	6 (18.8)	1 (6.7)	$\chi^2 = 1.176$	$^{FE}P = 0.404$
Tumor grade				
Low grade	18 (36.0)	28 (56.0)	$\chi^2 = 4.026$[a]	0.045[a]
High grade	32 (64.0)	22 (44.0)		
Laboratory investigations				
Hemoglobin (g/dl)				
Mean ± SD	11.69 ± 1.93	11.91 ± 1.69	$t = 0.618$	0.538
Platelet count (× 10^3/μl)				
Mean ± SD	251.2 ± 110.2	267.2 ± 104.6	$t = 0.742$	0.460
Total leukocyte count (× 10^3/μl)				
Median (minimum–maximum)	8.35 (0.30–71)	8.75 (3–66.50)	$U = 1180.0$	0.629
Prothrombin concentration (%)				
Mean ± SD	87.91 ± 8.48	86.99 ± 9.75	$t = 0.503$	0.616
Partial thromboplastin time (s)				
Mean ± SD	26.15 ± 0.81	26.07 ± 0.98	$t = 0.434$	0.666
Risk factors for VTE				
Past history of VTE	16 (32)	15 (30)	$\chi^2 = 0.047$	0.829
Immobilization (partial)	20 (40)	2 (4)	$\chi^2 = 18.88$[a]	<0.001[a]
Hospitalization	42 (84)	31 (62)	$\chi^2 = 6.139$[a]	0.013[a]
DM	6 (12)	6 (12)	$\chi^2 = 0.0$	1.000
Family history of DVT	11 (22)	8 (16)	$\chi^2 = 0.585$	0.444
Current treatment				
Chemotherapy	27 (54.0)	26 (52.0)	$\chi^2 = 0.040$	0.841
Hormonal	2 (4.0)	0	$\chi^2 = 2.041$	$^{FE}P = 0.495$
Radiotherapy	16 (32.0)	20 (40.0)	$\chi^2 = 0.694$	0.405
Best supportive care	2 (4.0)	2 (4.0)	$\chi^2 = 0.0$	$^{FE}P = 1.000$
Targeted therapy	3 (6.0)	2 (4.0)	$\chi^2 = 0.211$	$^{FE}P = 1.000$
Gemcitabine-based chemotherapy	8 (16)	0	$\chi^2 = 8.696$	$^{MC}P = 0.006$[a]
Platinum based chemotherapy	17 (34)	16 (32)	$\chi^2 = 0.045$	0.832
PROTECHT score				
0	9 (18.0)	8 (16.0)	$\chi^2 = 2.259$	0.520
1	12 (24.0)	18 (36.0)		
2	14 (28.0)	14 (28.0)		
≥3 points	15 (30.0)	10 (20.0)		
Low risk <3 points	35 (70.0)	40 (80.0)	$\chi^2 = 1.333$	0.248

(*continued on next page*)

Table 1. (continued)

	Group I ($N = 50$) [n (%)]	Group II ($N = 50$) [n (%)]	Test of significance		P
High risk ≥3 points	15 (30.0)	10 (20.0)			
Median (minimum–maximum)	2 (0–5)	1 (0–5)			
	Group I ($N = 50$)	Group II ($N = 50$)	χ^2 (P)	P_0	OR (95 % CI)
SLC44A2 rs2288904					
AA	1 (2)	10 (20)	8.721[a] (0.013[a])		1.000
AG	21 (42)	20 (40)		0.032[a]	10.50 (1.230–89.686)
GG	28 (56)	20 (40)		0.015[a]	14.0 (1.657–118.31)
HWE (χ^2 P)	1.723 (0.189)	1.389 (0.239)			
Dominant (AG + GG vs. AA)	49/1	40/10	8.274 (0.004[a])	0.019[a]	12.250 (1.504–99.798)
Recessive (GG vs. AA + AG)	28/22	20/30	2.564 (0.109)	0.111	1.909 (0.862–4.227)
Additive (GG/AG/AA)	28/21/1	20/20/10	8.721 (0.013[a])	0.014[a]	2.202 (1.175–4.127)
Allele					
A	23 (23)	40 (40)	6.697[a] (0.010[a])		1.000
G	77 (77)	60 (60)		0.010[a]	2.232 (1.208–4.124)
TSPAN15 rs78707713					
TT	4 (8)	11 (22)	6.573[a] (0.037[a])		1.000
TC	29 (58)	31 (62)		0.139	2.573 (0.736–8.992)
CC	17 (34)	8 (16)		0.015[a]	5.844 (1.413–24.17)
HWE (χ^2 P)	2.979 (0.084)	2.989 (0.084)			
Dominant (TC + CC vs. TT)	36/4	39/11	3.843 (0.050)	0.059	3.244 (0.956–11.001)
Recessive (CC vs. TT + TC)	17/33	8/42	4.320 (0.038[a])	0.041[a]	2.705 (1.040–7.036)
Additive (CC/TC/TT)	17/29/4	8/31/11	6.573 (0.037[a])	0.013[a]	2.389 (1.202–4.747)
Allele					
T	37 (37)	53 (53)	5.172[a] (0.023[a])		1.000
C	63 (63)	47 (47)		0.024[a]	1.920 (1.092–3.378)
Haplotype					
AT	13	26	9.828[a] (0.020[a])		1.000
AC	10	14		0.505	1.429 (0.500–4.081)
GT	24	27		0.192	1.778 (0.750–4.216)
GC	53	33		0.004[a]	3.212 (1.450–7.114)

CI, confidence interval; DVT, deep venous thrombosis; FE, Fisher Exact; HWE, χ^2 for goodness (if $P < 0.05$ – not consistent with HWE.); LL, lower limit; MC, Monte Carlo; OR, Odd's ratio; *t*, Student *t* test; *U*, Mann–Whitney test; UL, Upper Limit; VTE, venous thromboembolism; χ^2, χ^2 test.
Low grade: includes grade I, II for nonprostatic carcinoma and low Gleason grade for prostatic carcinoma.
High grade: includes III, IV for nonprostatic carcinoma and high Gleason for prostatic carcinoma.
Group I: cancer patients with venous thromboembolism.
Group II: cancer patients without venous thromboembolism.
P: *P* value for comparing between the two studied groups.
P_0: *P* value for OR.
[a] Statistically significant at *P* value less than or equal to 0.05.

revealed that combined G allele of *SLC44A2* (rs2288904) and C allele of *TSPAN15* (rs78707713) increased the risk for developing VTE by 3.212 times ($P0 = 0.004$) (Table 1).

In each group, the association between *SLC44A2* (rs2288904) genotypes and different variables was investigated. Only PROTECHT score showed statistical significance in group I. Approximately, 46 % of GG genotype had high risk score compared with 0 % of AA genotype and 9.5 % of AG genotype. The PROTECHT score was also significant with *TSPAN15* (rs78707713) genotypes in group I. Approximately, 53 % of CC patients had high PROTECHT score points versus 20.7 % of TC and 0 % of TT patients. Additionally, TC and CC in VTE-cancer patients showed a significant higher cancer grade (Table 2).

Regarding survival data, VTE incidence in cancer patients was associated with shorter PFS, significant *P* value (0.001) (Table 3 and Fig. 2a). In cancer patients with VTE (group I), the median for the duration between cancer diagnosis and VTE diagnosis was 3 months for a range of 1–12 months. Comparing *TSPAN15* (rs78707713) genotype CC versus TT + TC, survival data showed shorter PFS for CC genotype patients in both groups (Table 3 and Fig. 2b). Comparing *SLC44A2* (rs2288904) GG genotype versus AA + AG, survival data showed shorter PFS for GG genotype patients in group I with 12 versus 15 months for AA + AG genotypes.

Table 2. Relation between SLC44A2 (rs2288904), TSPAN15 (rs78707713) and different risk factors for VTE in each group.

		Genotype of SLC44A2 rs2288904					
		With venous thromboembolism			**Without venous thromboembolism**		
		AA (n = 1)	**AG (n = 21)**	**GG (n = 28)**	**AA (n = 10)**	**AG (n = 20)**	**GG (n = 20)**
Performance status	PS 0, 1, 2	1 (100.0%)	18 (85.7%)	22 (78.6%)	10 (100.0%)	19 (95.0%)	19 (95.0%)
	PS 3	0 (0.0%)	3 (14.3%)	6 (21.4%)	0 (0.0%)	1 (5.0%)	1 (5.0%)
	χ^2 (^{MC}p)	0.905 (0.765)			0.700 (1.000)		
PROTECHT score	Low risk < 3 points	1 (100.0%)	19 (90.5%)	15 (53.6%)	8 (80.0%)	15 (75.0%)	17 (85.0%)
	High risk ≥ 3 points	0 (0.0%)	2 (9.5%)	13 (46.4%)	2 (20.0%)	5 (25.0%)	3 (15.0%)
	χ^2 (^{MC}p)	8.295* **(0.015*)**			0.724 (0.897)		
Grade	Low	1 (100.0%)	9 (42.9%)	8 (28.6%)	5 (50.0%)	11 (55.0%)	12 (60.0%)
	High	0 (0.0%)	12 (57.1%)	20 (71.4%)	5 (50.0%)	9 (45.0%)	8 (40.0%)
	χ^2 (p)	2.763 (^{MC}p = 0.223)			0.284 (0.868)		
Presence of metastasis	No	0 (0.0%)	7 (33.3%)	11 (39.3%)	7 (70.0%)	14 (70.0%)	14 (70.0%)
	Yes	1 (100.0%)	14 (66.7%)	17 (60.7%)	3 (30.0%)	6 (30.0%)	6 (30.0%)
	χ^2 (p)	0.752 (0.857)			0.0 (1.000)		
Gemcitabine based chemotherapy	No	1 (100.0%)	19 (90.5%)	22 (78.6%)	10 (100.0%)	20 (100.0%)	20 (100.0%)
	Yes	0 (0.0%)	2 (9.5%)	6 (21.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	χ^2 (p)	1.703 (^{MC}p = 0.533)			–		
Immobilization	No	0 (0.0%)	14 (66.7%)	16 (57.1%)	10 (100.0%)	19 (95.0%)	19 (95.0%)
	Yes (partial)	1 (100.0%)	7 (33.3%)	12 (42.9%)	0 (0.0%)	1 (5.0%)	1 (5.0%)
	χ^2 (^{MC}p)	1.893 (0.374)			0.700 (1.000)		
Hospitalization	No	0 (0.0%)	4 (19.0%)	4 (14.3%)	4 (40.0%)	5 (25.0%)	10 (50.0%)
	Yes	1 (100.0%)	17 (81.0%)	24 (85.7%)	6 (60.0%)	15 (75.0%)	10 (50.0%)
	χ^2 (p)	0.828 (^{MC}p = 0.764)			2.674 (0.263)		
		Genotype of TSPAN15 rs78707713					
		With venous thromboembolism			**Without venous thromboembolism**		
		TT (n = 4)	**TC (n = 29)**	**CC (n = 17)**	**TT (n = 11)**	**TC (n = 31)**	**CC (n = 8)**
Performance status	PS 0, 1, 2	3 (75.0%)	22 (75.9%)	16 (94.1%)	10 (90.9%)	31 (100.0%)	7 (87.5%)
	PS3	1 (25.0%)	7 (24.1%)	1 (5.9%)	1 (9.1%)	0 (0.0%)	1 (12.5%)
	χ^2 (^{MC}p)	2.799 (0.289)			4.111 (0.137)		
PROTECHT score	Low risk < 3 points	4 (100.0%)	23 (79.3%)	8 (47.1%)	10 (90.9%)	24 (77.4%)	6 (75.0%)
	High risk ≥ 3 points	0 (0.0%)	6 (20.7%)	9 (52.9%)	1 (9.1%)	7 (22.6%)	2 (25.0%)
	χ^2 (p)	6.316* **(^{MC}p = 0.041*)**			1.071 (0.682)		
Grade	Low	2 (50.0%)	14 (48.3%)	2 (11.8%)	5 (45.5%)	19 (61.3%)	4 (50.0%)
	High	2 (50.0%)	15 (51.7%)	15 (88.2%)	6 (54.5%)	12 (38.7%)	4 (50.0%)
	χ^2 (p)	6.892* **(^{MC}p = 0.033*)**			1.062 (0.654)		
Presence of metastasis	No	0 (0.0%)	10 (34.5%)	8 (47.1%)	5 (45.5%)	26 (83.9%)	4 (50.0%)
	Yes	4 (100.0%)	19 (65.5%)	9 (52.9%)	6 (54.5%)	5 (16.1%)	4 (50.0%)
	χ^2 (^{MC}p)	2.811 (0.234)			7.477* (0.019*)		
Gemcitabine based chemotherapy	No	3 (75.0%)	24 (82.8%)	15 (88.2%)	11 (100.0%)	31 (100.0%)	8 (100.0%)
	Yes	1 (25.0%)	5 (17.2%)	2 (11.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	χ^2 (p)	0.898 (0.851)			–		
Immobilization	No	3 (75.0%)	15 (51.7%)	12 (70.6%)	10 (90.9%)	31 (100.0%)	7 (87.5%)
	Partial	1 (25.0%)	14 (48.3%)	5 (29.4%)	1 (9.1%)	0 (0.0%)	1 (12.5%)
	χ^2 (p)	1.905 (0.447)			4.111 (0.137)		
Hospitalization	No	0 (0.0%)	4 (13.8%)	4 (23.5%)	5 (45.5%)	13 (41.9%)	1 (12.5%)
	Yes	4 (100.0%)	25 (86.2%)	13 (76.5%)	6 (54.5%)	18 (58.1%)	7 (85.7%)
	χ^2 (p)	1.165 (0.483)			2.588 (0.274)		

χ^2: Chi square test; MC: Monte Carlo.
p: p value for comparing between genotypes of SLC44A2rs2288904.
p: p value for comparing between genotypes of TSPAN15rs78707713.
*: Statistically significant at p ≤ 0.05.

For group II, PFS was almost equal with 21 months for both genotype groups. In both patients' groups, the difference was statistically insignificant (Table 3 and Fig. 2c).

The univariate analysis risk factors of thrombosis showed that stage IV disease, high-tumor grade, immobilization, hospitalization, *SLC44A2* rs2288904 (AG + GG) and *TSPAN15* rs78707713 (CC) were

Table 3. Progression-free survival (months) for both patients' groups with TSPAN15 (rs78707713) and SLC44A2 (rs2288904) different genotypes

	Mean	% End of study	Log rank	
			χ^{22}	*P*
Patients groups				
Group I	13.91	42.0	16.698*	<0.001*
Group II	21.78	76.0		
TSPAN15 (rs78707713) gene in group I				
TT + TC	14.61	48.5	1.027	0.311
CC	12.55	29.4		
TSPAN15 (rs78707713) gene in group II				
TT + TC	22.33	81.0	4.152*	0.042*
CC	18.88	50.0		
SLC44A2 (rs2288904) gene in group I				
AA + AG	15.61	50.0	0.916	0.338
GG	12.57	35.7		
SLC44A2 (rs2288904) gene in group II				
AA + AG	21.70	73.3	0.238	0.626
GG	21.90	80.0		

Group I: cancer patients with venous thromboembolism.
Group II: cancer patients without venous thromboembolism.

significant risk factors, however, the multivariate analysis showed that only stage IV disease, immobilization, *SLC44A2* rs2288904 (AG + GG) and *TSPAN15* rs78707713 (CC) were the independent risk factors (*P*£0.05) (Table 4).

4. Discussion

Patients with cancer are more susceptible to developing VTE. Thrombotic events are linked to interruptions in cancer therapy, a decline in life quality, and an increase in morbidity and death, all of which raise the cost of healthcare [11].

TSPAN15 plays a role in the platelet's amyloid precursor protein cleavage [12], whose regulation is suggested as a novel factor in the VTE pathophysiology [13].

SLC44A2 is one of the genetic loci that regulates mitochondrial energetics, controlling platelet activation and thrombosis [14].

This study is the first to identify the potential relationship between *SLC44A2* (rs2288904) and *TSPAN15* (rs78707713) polymorphisms and the risk of VTE in cancer patients.

Several scores are available to help clinicians in the prediction of VTE-cancer risk and to guide them in selecting candidate patients for thromboprophylaxis. The most well-known is the Khorana score, that categorizes patients as low (0 points), intermediate (1−2 points), and high (≥3 points) risk. To better stratify the VTE risk among cancer patients, other scores including the PROTECHT score (Prophylaxis of Thromboembolism during Chemotherapy), have been developed [15]. Based on the PROTECHT score of this study, 30 % of group I patients had high risk for VTE.

This study showed that 64 % of VTE-cancer patients had a high-tumor grade versus 44 % of cancer patients without VTE. Ahlbrecht et al. [16] reported similar findings.

Our study results showed that 64 % of patients with VTE had metastatic disease (stage IV) versus 30 % of non-VTE-cancer patients. Visceral metastasis was the most common to occur in each group. Walker et al. [17] reported similar findings.

The only significant predisposing factors for VTE among cancer patients in this study, were partial immobilization and hospitalization.

Immobilization and chemotherapy potentiate thromboembolism development in cancer patients versus noncancer patients. Several cancer therapies promote occurrence of VTE [18].

Chemotherapy has contributed to VTE development in patients with cancer over the past few decades [19]. Chemotherapy-related thrombosis is higher in patients with cancer by six to seven times [17].

Regarding *SLC44A2* (rs2288904) SNP, our results showed significantly higher mutant GG and AG genotypes, and significant excessive frequencies of the mutant G allele versus the A allele in cancer patients with VTE than that of non-VTE-cancer patients, considering that the G allele can increase the risk for VTE by OR 2.232 with 95 % CI (1.208−4.124).

Germain et al. [20] confirmed the association of *SLC44A2* (rs2288904) SNP with VTE.

This study illustrated a significant link of *TSPAN15* (rs78707713) polymorphism with VTE in cancer patients when compared with those without VTE, with a significant predominance of the TC and CC genotypes and the C allele. Thus, we assume that the C allele increases the risk for VTE (OR) 1.920-fold with 95 % CI (1.092−3.378). Trégouët and Morange [21] reported similar findings.

The meta-analyzed replication results by Germain and colleagues showed a relation between *TSPAN15* (rs78707713) and VTE. The common T allele of *TSPAN15* (rs78707713) was associated with an OR for VTE of 1.42. The overall OR for VTE with *TSPAN15* (rs78707713) was 1.31 [20].

Contrary to our results, Hernandez et al. [22] reported a lack of association between *TSPAN15* (rs78707713) SNP and VTE in African Americans.

The relations between *TSPAN15* (rs78707713) and *SLC44A2* (rs2288904) genotypes and different parameters were investigated in each group in this study. In *TSPAN15* (rs78707713) SNP, tumor grade and PROTECHT score were the two

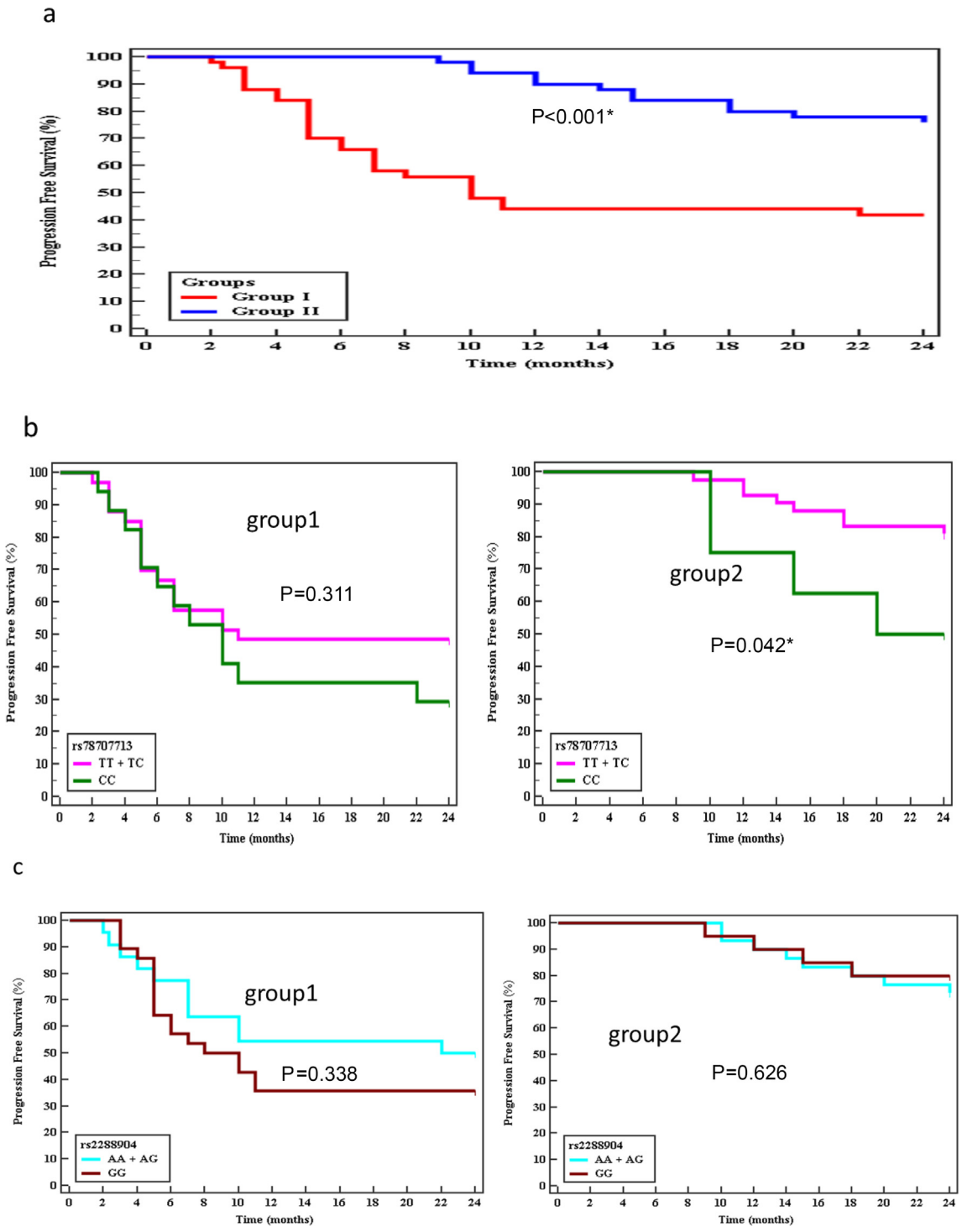

Fig. 2. (a) Kaplan–Meier survival curve for progression-free survival for both patients groups. (b) Kaplan–Meier survival curve for progression-free survival with TSPAN15 (rs78707713) gene for groups I and II. (c) Kaplan–Meier survival curve for progression-free survival with SLC44A2 (rs2288904) gene for cancer with groups I and II.

parameters that showed statistical significance in group I. For *SLC44A2* (rs2288904) genotypes, only PROTECHT score was statistically significant in group I.

Among our studied cancer patients, the PFS in VTE-cancer patients was 13.91 versus 21.78 months in non-VTE-cancer patients. Similarly, several case–control studies showed decreased survival

Table 4. Univariate and multivariate logistic regression analysis for the parameters affecting cancer patients with venous thromboembolism (N = 50 vs. 50)

	Univariate		[a]Multivariate	
	P	OR (LL−UL 95 % CI)	*P*	OR (LL−UL 95 % CI)
SLC44A2 rs2288904 (AG + GG)	0.019[b]	12.250 (1.504−99.798)	0.024[b]	38.560 (1.634−909.790)
TSPAN15 rs78707713 (CC)	0.041[b]	2.705 (1.040−7.036)	0.035[b]	4.002 (1.106−14.486)
Presence of metastasis	0.001[b]	4.148 (1.798−9.573)	0.021[b]	4.098 (1.232−13.637)
Past history of VTE	0.829	1.098 (0.470−2.564)		
High risk PROTECHT score	0.251	1.714 (0.683−4.301)		
High tumor grade	0.046[b]	2.263 (1.013−5.052)	0.925	0.944 (0.286−3.112)
Immobilization	<0.001[b]	16.00 (3.487−73.408)	0.001[b]	16.269 (3.133−84.487)
Hospitalization	0.016[b]	3.218 (1.248−8.299)	0.262	2.011 (0.593−6.818)
Gemcitabine-based chemotherapy	0.999	−		
Age	0.483	0.984 (0.940−1.029)		

CI, confidence interval; LL, lower limit; OR, odd's ratio; UL, upper limit; VTE, venous thromboembolism.
[a] All variables with $P < 0.05$ was included in the multivariate.
[b] Statistically significant at *P* value less than or equal to 0.05.

rates in cancer patient who experienced VTE versus cancer patients without VTE [23].

This study identified six significant risk factors for VTE: *SLC44A2* rs2288904 (AG + GG), *TSPAN15* rs78707713 (CC), metastatic disease, high-tumor grade, immobilization and hospitalization. Four of them, *SLC44A2* rs2288904 (AG + GG), *TSPAN15* rs78707713 (CC), metastatic disease and immobilization were independent risk factors. Yang et al. [24] reported that immobilization was a predisposing factor attributing to VTE occurrence in patients with gynecological surgery. On the other hand, Mandala et al. [25] and Yang and colleagues identified that having a history of VTE was an independent risk factor for developing VTE in cancer patients receiving adjuvant chemotherapy and in patients with gynecological surgery, respectively [23].

4.1. Conclusion

VTE is associated with advanced tumor stage and shorter PFS in cancer patients. Genetic testing for *SLC44A2* (rs2288904) and *TSPAN15* (rs78707713) SNPs may enhance VTE prediction in cancer patients, both SNPs are linked to higher PROTECHT score. Furthermore, *TSPAN15* (rs78707713) SNP may have prognostic value for further study on a larger group of patients.

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Declaration of Competing Interest

There are no conflicts of interest.

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