Thrombosis Research 174 (2019) 163–165

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to Editors-in-Chief

Association between *SLC44A2* rs2288904 polymorphism and risk of recurrent venous thromboembolism among Thai patients

ARTICLE INFO

Keywords:
Allele
Genotype
SLC44A2
Thais
Venous thromboembolism

1. Introduction

Venous thromboembolism (VTE) is characterized by clinical manifestations including deep vein thrombosis (DVT), pulmonary embolism (PE) and the less common occurrence of VTE in unusual sites. Generally, the incidence of VTE among Asians is lower than among Caucasians, 13 to 19 *vs.* 100 to 200 of 100,000 annually [1]. Environmental and genetic risk factors are major causes of VTE. The environmental risk factors are divided by unprovoking risk factors including sex, age, ethnic, obesity or high body mass index (BMI) and provoking risk factors including cancer, post operation, hospitalization and immobilization. Genetic risk factors are categorized by mutation functions: loss of function mutations; deficiencies of antithrombin III (ATIII), protein C (PC), protein S (PS) and gain of function mutations; factor V Leiden (FVL) and prothrombin G20210A gene mutations [2]. Deficiencies of ATIII, PC and PS are mainly caused by VTE; while, FVL and prothrombin G20210A gene mutations are rarely found in Asian populations [3,4]. Most patients with VTE are at risk of recurrence during follow-up, especially in the first 6 to 12 months and the risk is significantly higher among patients with unprovoked VTE than among patients with provoked VTE [5].

A related meta-analysis of genome-wide association studies (GWASs) demonstrated the association of a novel locus of *SLC44A2* with VTE susceptibility [6]. The *SLC44A2* gene encodes for HNA-3 antigens, which are associated with a bi-allelic polymorphism caused by a single nucleotide polymorphism rs2288904, SNP (c.461G > A; p.Arg154Gln) in the choline transporter like protein-2. A related study of HNA-3 genotyped by PCR with sequence-specific primer (PCR-SSP) was reported in two Thai blood donor populations. The *SLC44A2*01* allele frequency among central Thais was significantly lower than among northern Thais and Caucasians [7]. However, the study of *SLC44A2* locus regarding Thai patients with VTE is still unknown. This study aimed to determine the association between *SLC44A2* rs2288904 polymorphism and risk of recurrent venous thromboembolism among Thai patients.

2. Patients and methods

Altogether, 109 Thai patients with symptomatic VTE from the Department of Hematology at Phramongkutklao Hospital were included from April to October 2017. Of these, 83, 13 and 13 were DVT, PE and VTE cases in unusual sites, respectively. VTE and PE were documented using Doppler ultrasound examination or venography and ventilation-perfusion lung scan, computed tomography or angiography, respectively. Moreover, the control group with known *SLC44A2* genotypes of 500 unrelated healthy blood donors from a related study was included [7]. The patient demographic data including age, sex, weight, height and BMI was retrieved from medical history. The data of thrombotic events in all patients was observed by retrospective record reviews. Follow-up period was counted in months after stopping the anticoagulant treatment until the secondary diagnosis of VTE. This study was approved by the Committee of Institutional Review Board, Royal Thai Army Medical Department, Bangkok, Thailand and the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathumthani, Thailand.

The genomic DNA of 109 patients was extracted from EDTA blood samples and stored at −20 °C until used. The *SLC44A2* genotyping was performed using a PCR-SSP technique identical to a related study [7].

The association between *SLC44A2* alleles and VTE was calculated using the odds ratio (OR) and 95% confidence intervals (CI). Allele frequencies among patients and controls were compared by Chi-Square test (X^2) and Fisher's exact test. The Kaplan-Meier curves (hazard function) for time to recurrent VTE in different genotypes were plotted and compared by Log-rank test. Hazard ratios (HRs) with 95%CI were calculated using univariate and multivariate Cox regression analysis.

3. Results

In this study, 109 patients with VTE consisting of DVT, PE and VTE in unusual sites were included. In all, 62 men and 47 women had a mean age of 48.18 years and BMI of 24.52 kg m^{-2}. Concerning those three groups, no significant difference was observed in terms of sex, age, BMI and risk group (provoked and unprovoked VTE).

https://doi.org/10.1016/j.thromres.2019.01.001
Received 30 October 2018; Received in revised form 7 December 2018; Accepted 2 January 2019
Available online 03 January 2019
0049-3848/

Table 1
Association of *SLC44A2* with selected Thai patients with VTE.

	Patients $N = 92$	Controls [7] $N = 500$	OR (95%CI)	X^2 (DF)	p value
Genotype model					
*SLC44A2*01/*01*	59 (64.1%)	264 (52.8%)	NA	5.74 (2)	0.0565
*SLC44A2*01/*02*	30 (32.6%)	190 (38.0%)			
*SLC44A2*02/*02*	3 (3.3%)	46 (9.2%)			
Allele model					
*SLC44A2*01*	148 (80.4%)	718 (71.8%)	1.603	NA	0.0163[a]
*SLC44A2*02*	36 (19.6%)	282 (28.2%)	(1.091–2.356)		

Abbreviations: CI, confidence interval; DF, degrees of freedom; NA, not applicable; OR, odds ratio.
[a] p value from Fisher's exact test.

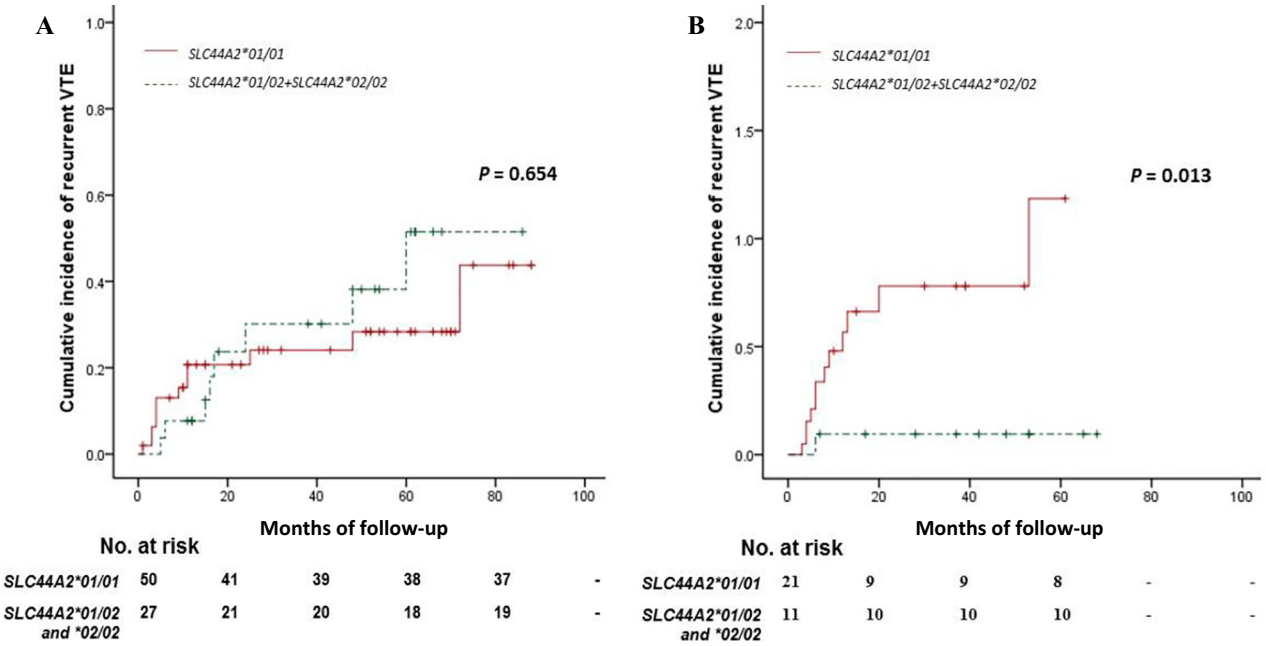

Fig. 1. Cumulative incidence of recurrent VTE compared by different genotypes of *SLC44A2* in patients with provoked (A) and unprovoked VTE (B) (p-value from Log-rank test).

Regarding the results of *SLC44A2* (rs22889904) allele detections by PCR-SSP among 109 patients with VTE and healthy controls, homozygous *SLC44A2*01/*01* was the most common in both groups (71 of 109 *vs.* 264 of 500), followed by heterozygous *SLC44A2*01/*02* (35 of 109 *vs.* 190 of 500) and homozygous *SLC44A2*02/*02* (3 of 109 *vs.* 46 of 500). When allele frequencies of the patients and the controls were compared, the frequency of *SLC44A2*01* was found to significantly increase among patients (81.2% *vs.* 71.8%, $p = 0.005$, OR: 1.70, 95%CI: 1.18–2.45). However, common underlying genetic risk factors may be involved in VTE; 17 of 109 patients comprising 8 and 7 inherited PC and PS deficiencies and another 2 FVL mutation were excluded. The association between omitted patients with VTE and *SLC44A2* was analyzed (Table 1). Even though the genotype model of *SLC44A2*01* and *SLC44A2*02* showed no significant difference, we found convincing evidence the *SLC44A2*01* allele that was significantly associated with VTE compared with controls ($p = 0.016$, OR: 1.60, 95% CI: 1.09–2.36). Additionally, we did not observe any difference between *SLC44A2*01* and *SLC44A2*02* allele frequencies and three different clinical manifestations of Thai patients with VTE.

Among 109 patients, 35 (32.1%) presented recurrent VTE. All eight patients with PC deficiency had recurrent thrombotic event ($p < 0.001$). No significant difference was observed among recurrent and non-recurrent patients with VTE when stratified by sex, diagnosis, risk of thrombosis, patients with antiphospholipid syndrome, FVL mutation and PS deficiencies ($p > 0.05$).

In addition, the risk association of recurrent VTE was analyzed by comparing homozygous risk allele *SLC44A2*01/*01* with combined non-risk allele including *SLC44A2*01/*02* and *SLC44A2*02/*02*. Univariate and multivariate Cox regression analyses were performed with individual genotypes to determine their association with the risk of recurrent VTE. In all patients, no significant association was found among three *SLC44A2* genotypes and regarding the recurrence risk on both univariate (HR: 0.63, 95%CI: 0.29–1.37, $p = 0.229$) and multivariate analyses after adjusting for inherited and acquired risk factors of VTE (HR: 1.78, 95%CI: 0.82–3.89, $p = 0.142$). Interestingly, a significant association between *SLC44A2*01/*01* genotype and risk of VTE recurrence was observed among patients with unprovoked VTE on both univariate (HR: 2.68, 95%CI: 1.30–5.52, $p = 0.008$) and multivariate Cox regression analyses (HR: 4.18, 95%CI: 1.88–9.30, $p < 0.001$).

The cumulative incidence of recurrent VTE during follow-up time among patients with provoked and unprovoked VTE was calculated using Kaplan-Meier analysis (Fig. 1). The median ± SD of follow-up time was 32.0 ± 26.6 months. Only patients with unprovoked VTE having *SLC44A2*01/*01* genotype showed a significant difference in the cumulative incidence of recurrent VTE compared with *SLC44A2*01/*02* and *SLC44A2*02/*02* genotypes (Fig. 1B, Log-rank test, $p = 0.013$). No significant difference was observed among patients with provoked VTE (Fig. 1A, Log-rank test, $p = 0.654$).

4. Discussion

The well-known causes of VTE include genetic, acquired, behavior and disease combinations. Moreover, deficiencies of PC, PS and ATIII are common genetic risk factors for VTE in Caucasian and Asian populations [3,4]. Single nucleotide variants in coagulation factors and several unknown functions including *HIVEP1*, *TSPAN15*, *SLC44A2* and *ORM1* are associated with increased risk of thrombosis [6]. Additionally, the *TLR9* rs5743839 polymorphism is associated with the risk of VTE recurrence especially among patients with unprovoked VTE [8].

Baseline characteristics results among 109 Thai patients with VTE showed no significant difference, the male to female ratio of 1.32 was consistent with related studies, but one study among Thai patients revealed a ratio of 0.48. However, patients' ages were similarly represented in the same early middle age [1,2]. Obesity is also a risk factor for thrombosis, our patient groups were considered as non-obese.

This study was the first to specifically identify the association of *SLC44A2*01* and Thai patients with VTE, similar to a related study in other populations [6]. Moreover, patients with unprovoked VTE, having homozygous *SLC44A2*01/*01* genotype, were significantly associated with a higher risk of VTE recurrence than those of the other two genotypes. The *SLC44A2* gene is well-known to encode for HNA-3 antigens, implicated in severe transfusion-related acute lung injury (TRALI) by neutrophil extracellular traps formation, which related to VTE development in a mouse model [9]. Even though the mechanism of *SLC44A2* alleles involved in thrombus formation remains ambiguous because this gene does not relate to coagulation pathways and thrombotic biomarkers. The association of *SLC44A2* alleles with VTE might involve in von Willebrand factor (vWF). Lacking of *SLC44A2* in transgenic mice demonstrated a reduction of plasma vWF levels and an altered response upon vascular damage indicated that *SLC44A2* contributed to hemostasis upon injury [10]. Our results provide not only a clear link between *SLC44A2*01* allele and a risk of VTE but also support the abovementioned study. Other separated or combined genetic factors such as *TSPAN15*, *PROCR, KNG1 GP6* and *TLR9* associated among patients with VTE cannot be excluded [6,8]. Additional studies with a larger sample size are suggested to confirm the risk association of VTE.

In conclusion, the *SLC44A2*01* allele is associated with VTE and constitutes a potential marker for VTE recurrence among Thai patients. Screening for this allele among patients at risk may be helpful in predicting and preventing recurrent VTE development.

Funding

This study was supported by the Thammasat University Research Fund.

Conflict of interests

The authors state that they have no conflicts of interest to declare.

References

[1] J.A. Heit, F.A. Spencer, R.H. White, The epidemiology of venous thromboembolism, J. Thromb. Thrombolysis 41 (2016) 3–14.
[2] M. Crous-Bou, L.B. Harrington, C. Kabrhel, Environmental and genetic risk factors associated with venous thromboembolism, Semin. Thromb. Hemost. 42 (2016) 808–820.
[3] P. Angchaisuksiri, Venous thromboembolism in Asia–an unrecognised and undertreated problem? Thromb. Haemost. 106 (2011) 585–590.
[4] W. Prayoonwiwat, P. Arnutti, M. Hiyoshi, O. Nathalang, C. Suwannasophon, R. Kokaseam, et al., Detection of factor V Leiden in Thai patients with venous thrombosis, Asian Pac. J. Allergy Immunol. 18 (2000) 105–108.
[5] P. Prandoni, F. Noventa, A. Ghirarduzzi, V. Pengo, E. Bernardi, R. Pesavento, et al., The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients, Haematologica 92 (2007) 199–205.
[6] M. Germain, D.I. Chasman, H. de Haan, W. Tang, S. Lindström, L.-C. Weng, et al., Meta-analysis of 65,734 individuals identifies TSPAN15 and SLC44A2 as two susceptibility loci for venous thromboembolism, Am. J. Hum. Genet. 96 (2015) 532–542.
[7] O. Nathalang, K. Intharanut, K. Siriphanthong, S. Nathalang, N. Leetrakool, Risk estimation of HNA-3 incompatibility and alloimmunization in Thai populations, PLoS One 10 (2015) e0116905.
[8] A. Ahmad, K. Sundquist, B. Zöller, P.J. Svensson, J. Sundquist, A.A. Memon, Association between TRL9 rs56743836 polymorphism and risk of recurrent venous thromboembolism, J. Thromb. Thrombolysis 44 (2017) 130–138.
[9] E.K. Storch, C.D. Hillyer, B.H. Shaz, Spotlight on pathogenesis of TRALI: HNA-3a (CTL2) antibodies, Blood 124 (2014) 1868–1872.
[10] J. Tilburg, R. Adili, T.S. Nair, M.E. Hawley, D.C. Tuk, M. Jackson, et al., Characterization of hemostasis in mice lacking the novel thrombosis susceptibility gene SLC44A2, Thromb. Res. 171 (2018) 155–159.

Dollapak Apipongrat[a], Tontanai Numbenjapon[b], Wichai Prayoonwiwat[b], Pasra Arnutti[c], Oytip Nathalang[a,*]
[a] *Graduate Program in Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumtani, Thailand*
[b] *Division of Hematology, Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand*
[c] *Department of Pathology, Phramongkutklao College of Medicine, Bangkok, Thailand*
E-mail address: oytipntl@hotmail.com (O. Nathalang).

[*] Corresponding author at: Graduate Program in Medical Technology, Faculty of Allied Health Sciences, Thammasat University, 99 Moo 18 Paholyothin Road, Klongnung, Klongluang, Pathumtani 12120, Thailand.