Research Article

Journal of Genetic Engineering and Biotechnology Research

Positive Association of Mutations in VKORC1 and CYP2C9 Genes with Venous Thrombo-Embolism (VTE) in Indian Population: A Case Control Study

Babita Kumari, Iti Garg, Chhavi Rai, Usha Panjawani, Bhuvnesh Kumar and Swati Srivastava*

Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organization (DRDO), Lucknow Road, Timarpur, Delhi-110054

***Corresponding author**
Dr Swati Srivastava, Genomics Division, Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organization (DRDO), Lucknow Road, Timarpur, Delhi-110054; Email: sri_swati@rediffmail.com

Submitted: 18 Oct 2019; **Accepted**: 29 Oct 2019; **Published**: 04 Nov 2019

Abstract

Background: *Venous thrombo-embolism (VTE) refers to a blood clot that starts in vein. It is the third most common vascular disease in the world, after myocardial infarction (MI) and stroke, affecting millions of individuals every year. Two main clinical manifestations of VTE include deep vein thrombosis (DVT) and pulmonary embolism (PE), the later one being potentially fatal.*

Objective: *The aim of this study is to investigate whether mutations in two genes VKORC1 and CYP2C9 and subsequent changes in their plasma levels can be used to predict the risk for venous thromboembolism (VTE).*

Method: *A total of five polymorphisms in two genes VKORC1 and CYP2C9 were genotyped in approximately 145 VTE patients and 229 control subjects. The genotyping was done through PCR-RFLP method. Plasma concentrations of VKORC1 and CYP2C9 were estimated using ELISA technique.*

Results: *Genotypic analysis of common polymorphisms in VKORC1 gene showed significantly higher percentage of GG genotype of -1639G>A, CC genotype of 1173 C>T and TT genotype of -497T>G polymorphism in control subjects compared to VTE patients. Also, significantly higher percentage of CC genotype in 416C>T and AA genotype in 1061 A>C polymorphism of CYP2C9*2 and CYP2C9*3 genes respectively was observed in VTE patients in comparison to healthy controls. In addition to genotypic significance, plasma concentrations of both VKORC1 and CYP2C9 were significantly higher in patients group.*

Conclusion: *All the three polymorphisms in VKORC1 gene; -1639G>A, 1173 C>T and -497T>G and two polymorphism 416C>T and 1061A>C of CYP2C9 gene showed significant association with VTE pathophysiology.*

Keywords

Venous thrombo-embolism, Deep vein thrombosis, Pulmonary embolism, Genotyping, CYP4V2, VKORC1

Abbreviations

VTE: Venous thrombo-embolism
DVT: Deep vein thrombosis
PE: Pulmonary embolism
HA: High altitude

Introduction

Venous thrombo-embolism (VTE) is a common pathological condition with an annual incidence of 6 to 29 persons per 100 000 in distinct populations [1]. VTE affects over 10 million people globally every year and it is the third leading vascular disease in the world [2]. It is a widespread multifactorial disease condition associated with multiple inherited and acquired risk factors [3-5]. Two main clinical implications of VTE comprise of deep vein thrombosis (DVT), wherein blood clot formation occurs in deep vein, mostly leg and pulmonary embolism (PE), a potentially life threatening condition where the blood clot lodges in an artery in the lung thereby blocking the blood flow in the lungs. Both these conditions have serious outcomes in both men and women [6]. However most of the complications caused by VTE are preventable after treatment with effective anti-thrombotic drugs and appropriate non-pharmacological interventions, it has strong tendency to recur after stopping anticoagulation therapy [7-9].

The normal hemostasis of body is dependent upon delicate balance between the anticoagulation and the coagulation system. Genetic risk factors of VTE comprise of loss of function mutations such as those in natural anticoagulants like antithrombin, protein C and protein S or gain of function mutations in coagulation genes such as prothrombin mutation G20210A, factor V Leiden [10]. Also,

mutations in other genes related to coagulation pathway, fibrinolytic pathway and endothelial functions play an important role in VTE pathophysiology [11,12]. Family and twin studies have indicated that genetic factors accounts for 60% of risk for DVT [13,14]. However, commonly known genetic risk factors for VTE pathophysiology like F5 rs6025 (Factor V Leiden, FVL) mutation or coagulation inhibitor deficiencies are present only in about 30% of VTE cases [15]. Also, deficiency of natural anticoagulants explains only 1% of DVT cases, [15]. Thus it becomes essential to identify other genetic variants strongly associated with VTE for improved understanding of its pathophysiology. The present study investigates a total of five common mutations in two genes, Vitamin K epoxide reductase complex 1 (VKORC1) and cytochrome P450 family 2 subfamily C member 9 (CYP2C9) in patients of VTE compared to control subjects.

Vitamin K epoxide reductase (VKOR) enzyme reduces vitamin K 2,3-epoxide to the active vitamin K hydroquinone which acts as co-factor in production of several coagulation factors. This enzyme is produced by VKORC1 gene, and is a pharmacological target of most popular anticoagulant drug, warfarin [16]. Warfarin acts by targeting VKORC1, leading to reduced production of vitamin K and subsequently clotting enzymes [17]. The presence of non-coding variants in VKORC1 results in decreased production of the VKOR enzyme. Yuan and co-workers demonstrated that VKORC1 (-1639) gene variant decreases transcription of the VKOR gene by 50% thereby increasing patient's sensitivity to warfarin [18].

On the other hand, CYP2C9 is a member of cytochrome P450 family, found primarily in the liver and largely responsible for the degradative metabolism of xenobiotics. Metabolism of warfarin depends on presence of two most common loss of function variants, CYP2C9 *2 (416C>T) and CYP2C9 *3 (1061A>C) in wild type genotype CYP2C9 *1. These mutations can prolong the half-life of drug and can facilitate its anticoagulant effect [19,20]. Several drugs including warfarin are metabolized by CYP2C9, it becomes an important factor in determining therapeutic response, clearance and toxicity of a drug in an individual.

Polymorphisms in the genes VKORC1 and CYP2C9 have been previously shown to affect the dose requirement of warfarin during anticoagulant therapy [21,22]. A recent study on C(1173)T/G(-1639) A polymorphism of VKORC1 in patients with VTE demonstrated that weekly warfarin dose requirement was lower and time to reach therapeutic INR was shorter in homozygote alleles (TT and AA, respectively) [23]. Genotypic variation due to polymorphisms in CYP2C9 and VKORC1 genes play a major role in pharmacokinetics and pharmacodynamics of warfarin respectively [24]. Recent GWAS studies have also shown association of these gene variants with drug metabolism [25]. To the best of our knowledge, no study has reported association of polymorphisms in VKORC1 and CYP2C9 with susceptibility to VTE. Thus the aim of the present study was to evaluate any potential association between polymorphisms in VKORC1 and CYP2C9 genes and their plasma levels with susceptibility of an individual to VTE.

Methodology

Subjects under study

Patients

Patients of venous thrombosis from Army hospital R & R, Delhi and Western command hospital, Chandimandir, Chandigarh, were approached for participation in the study. A total of 145 male VTE patients participated in the study. The written and informed consent of all patients was obtained before beginning the study procedures and the study was conducted in accordance with ethical standards of Helsinki declaration. The protocols followed in the study were approved by Institutional Ethical Committee. All the patients were under 45 years of age and were diagnosed with DVT/cerebral venous thrombosis, (CVT)/portal venous thrombosis, (PVT)/superior sagittal thrombosis (SST), and/or pulmonary thrombo-embolism (PE). The diagnosis was confirmed by atleast one of the neuroimaging or radiological imaging methods. The baseline demographic data such as age, BMI and family history was also recorded for each patient. History regarding the presence of known risk factor such as hypertension, diabetes mellitus, hyper-lipoproteinemias, family history of bleeding disorders, smoking, past surgeryor trauma etc. was also documented.

Controls

Healthy, age and sex matched individuals were selected as controls for this study. A total of 229 control subjects were taken. Baseline demographic data along with history of other VTE related risk factors was documented. Subjects with any history of risk factors were excluded from the study. The study protocol and consent procedure followed was same as that of patients.

Laboratory investigations

Preliminary hematological tests such as complete haemogram and coagulation screening comprising prothrombin time (PT), activated partial thromboplastin time (APTT) and estimation of D-dimer were carried out by standard methods (data not shown).

Restriction fragment length polymorphism (RFLP) based Genotyping

Genotyping was done using DNA isolated from peripheral blood. Blood was collected in K2 EDTA vacutainers. DNA was extracted from blood using QIAamp DNA isolation kit (Qiagen, Germany), according to manufacturer's instructions. Quantitative analysis of high molecular weight DNA was done using DNA/RNA nanodrop 2000 spectrophotometer (Thermo fischer, USA). DNA was also assessed qualitatively on 0.7% agarose gel containing ethidium bromide. 100ng of DNA was loaded in each well and run on 50V for 30 min and visualized under UV. The specific regions of VKORC1 and CYP2C9 genes were amplified using specific PCR primers as detailed in table 1. The composition of final PCR reaction comprised of 100 ng of DNA, 10 pmol of each specific primer (forward and reverse), 200 μM deoxyribo-nucleotide-triphosphate (dNTPs) and 0.6U taq polymerase in total volume of 25 μL reaction buffer (50mM KCL, 20mM Tris-HCL, pH 8.3). Specific restriction enzymes (table 1) were used to digest the amplified PCR at optimized temperature and time. The digested PCR products were mixed with DNA loading dye loaded on agrose gel of concentration between 1.5% to 3%, depending on the band sizes of digested product for electrophoresis. A sample with known profile (positive control) and blank sample (negative control) was run with each experiment. PCR conditions and genotypes are detailed in Table 1.

Table 1: PCR and genotyping details

Gene	SNP region	Primer Details	PCR product	Annealing temperature	Restriction enzyme	Band size (bps)
VKORC1 -1639 G>A rs 9923231	Upstream Promoter	5'-GCCAGCAGGAGAGGGAAATA-3' 5'-AGTTTGGACTACAGGTGCCT-3'	290 bps	58˚C	Msp I	GG=168, 122 GA=290,168, 122 AA=290
VKORC1 1173 C> T rs 9934438	Intron 1	5' -TAGGACTGTCAACCCAGT-3' 5 '-AGTGACATGGAATCCTGA-3'	150 bps	59˚C	Hinf I	CC=150, CT=150, 100, 50 TT= 100, 50
VKORC1 -497 T>G rs2884737	5′ Flanking	5'-ATGGCAAGGCTGGTATAACG-3' 5'-AGGATGGCGGTAGAGATTGA-3'	259 bps	58˚C	Hph I	TT=212, 47 TG=259, 212, 47 GG=259
CYP2C9*2 416 C>T rs1799853	Exon 3, Arg144Cys	5'-GGA GGATGG AAA ACA GAG ACT TA-3 5'-TGAGCT AAC AAC CAG GAC TCA T-3'	396bps	60˚C	AvaII	CC=223, 173 CT=396, 223, 173 TT=396
CYP2C9* 3 1061 A>C rs1057910	Exon 7, Ile359Leu	5'-GCTGTGGTGCACGAC GTC CA GAGATGC -3' 5'-ACA CAC ACT GCC AGA CAC TAGG -3'	298bps	60˚C	NsiI	AA=274, 24 AC=298, 274, 24 CC=298

PCR-RFLP details showing primer sequences, PCR product size, annealing temperature, restriction enzyme and fragment sizes after digestion.

Protein Estimation in plasma

Plasma concentration of VKORC1 and CYP2C9 were determined in both control and thrombotic groups by commercial enzyme-linked immunoassay kits (USCN, China) according to the manufacturer's protocol. Plasma samples were diluted to maintain the concentration well within the range of the kits used. Absorbance was measured by Synergy H4 hybrid reader (Biotek, VT, USA).

Statistical Analysis

Genotypes for each polymorphism in both control and patient groups were counted from agarose gel. Genotypic and allelic frequencies were determined by gene counting and compared by 3 × 2 and 2 × 2 contingency table, respectively. Distribution of genotype frequencies for both the groups was tested on Hardy-Weinberg equilibrium (HWE; 1 degree of freedom). Graph Pad (Prism 8) software was used to calculate statistical significance of genotypes between controls and patients by χ^2 (degree of freedom=2) and Fisher exact test with odds ratio (OR) (2 tailed p-value) and 95% confidence interval (CI). Plasma levels of VKORC1 and CYP2C9 in controls and patients with VTE were presented as mean ± standard error of the mean (SEM) and were tested using one-tailed unpaired t-test using Graph Pad software. Statistical significance criteria were $P < 0.05$ for all tests.

Table 2: Genotypic and allelic distribution

S. No.	Gene Polymorphism	Study Groups	Genotype (frequency %)			Allele (frequency)		HWE (p-value)
			AA	**AG**	**GG**	**A**	**G**	
1	**VKORC1–1639 G>A**	Patients	18 (12.41)	66 (45.51)	61 (42.06)	102 (35.17)	188 (64.8)	0.98
		Controls	50 (21.83)	64 (27.94)	115 (50.21)	164 (35.80)	294 (64.19)	**0**
			CC	**CT**	**TT**	**C**	**T**	
2	**VKORC1 1173 C> T**	Patients	88 (60.06)	46 (31.72)	11 (7.58)	222 (76.55)	68 (23.44)	0.16
		Controls	161 (70.30)	60 (26.20)	8 (3.49)	382 ((83.40)	76 (16.59)	0.41
			TT	**TG**	**GG**	**T**	**G**	
3	**VKORC1 -497 T>G**	Patients	122 (83.56)	21 (14.38)	3 (2.05)	222 (81.02)	52 (18.97)	0.08
		Controls	178 (77.72)	37 (16.15)	14 (6.11)	393 (85.80)	65 (14.19)	**0**
			CC	**CT**	**TT**	**C**	**T**	
4	**CYP2C9*2 416 C>T**	Patients	44 (30.13)	4 (2.73)	98 (6.71)	92 (31.50)	200 (68.49)	**0**
		Controls	18 (7.86)	43 (18.77)	168 (73.36)	79 (17.24)	379 (82.75)	**0**
			AA	**AC**	**CC**	**A**	**C**	
5	**CYP2C9* 3 1061 A>C**	Patients	44 (30.13)	69 (47.26)	33 (22.60)	157 (53.76)	135 (42.23)	0.55
		Controls	44 (19.21)	80 (34.93)	105 (45.85)	168 (36.68)	290 (63.31)	**0.0001**

Results

Clinical characteristics of subjects under study

The mean age of patients was 32 years (SD 3.96). No significant difference existed between mean age of patients and controls. All the subjects (both VTE patients and controls) were Indian Army personnel, they were physically active and had BMI in normal range (<29.9 Kg/m^2). While ~70 patients were diagnosed with DVT, others had CVT, PVT, SST or PE. Haemogram of patients was also normal barring a few who were hyperlipidimic (data not shown). Ethnic diversity of the subjects under study was not considered.

Genotypic variation in VKORC1

Out of the three SNP regions genotyped for VKORC1 gene, –1639G>A, 1173C> T and -497 T>G, two showed statistically significant genotypic difference between VTE patients and control group, –1639G>A (χ2=13.47, p=0.001) and -497T>G (χ2=16.46, p-value=0.0003) (Table 3). In both these SNPs, no significant difference was observed at the allelic level (p=0.87 and p=0.09 for -1639G>A and -497T>G respectively). For -1639G>A SNP, the prevalence of AA genotype (21.83%) was predominant in control group compared to patient group AA=12.41) whereas the striking difference was observed in case of heterozygous AG genotype frequency in patients (45.51%) and control group (27.94%). For -497T>G SNP, the control group had lower percentage of TT genotype (77.72%) compared to patients (83.56%).

The other SNP of VKORC1 gene, 1137C>T showed significant difference between patients and controls only at the allelic level (p-value for Fischer's exact test =0.02), but not at genotypic level (χ2=5.11, p=0.07). CC genotype was predominant in control group (70.30%) compared to patients group (60.06%). Prevalence of C allele (83.40%) in controls was higher than in patients (76.55%).

Table 3: Statistical analysis for polymorphic SNPs

S. No.	Gene Polymorphism	χ2 (df=2)	p-value	Fischer's exact	OR	CI
1	VKORC1–1639 A>G	13.47	0.001	0.87	0.99	0.88-1.10
2	VKORC1 1173 C>T	5.11	0.077	0.022	1.54	0.53-2.22
3	VKORC1 -497 T>G	16.46	0.0003	0.09	1.41	0.94-2.09
4	CYP2C9*2 416 C>T	45.55	<0.0001	<0.0001	0.54	0.42-0.71
5	CYP2C9* 3 1061 A>C	21.04	<0.0001	<0.0001	0.49	0.37-0.67

Genotypic variation of CYP2C9

Present study targeted two common SNPs of CYP2C9 gene, CYP2C9*2 416C>T and CYP2C9*3 1061A>C. Both the SNPs showed statistically significant difference between the two study groups, both at genotypic and allelic level between the patients and control group (Table 3).

CYP2C9*2 416C>T polymorphism was significantly associated with VTE and showed higher percentage of CC genotype in patients (30.13%) compared to controls (7.86%) (χ2=45.55, p<0.0001) as shown in table 3. At the allelic level also, the percentage occurrence of C allele was significantly higher (31.50%) in patients compared to controls (17.24%, p<0.0001).

Another polymorphism studied CYP2C9*3 1061A>C, showed significantly higher percentage of AA genotype (30.13%) and A allele (53.76%) in VTE patients in comparison to controls (AA=19.21%, A=36.58%) with χ2 of 21.04 and p value < 0.0001 (Table 3).

Heterozygosity analysis

The percentage of heterozygosity was higher in patients group compared to controls in three SNPs under study, i.e, CYP2C9*2, CYP2C9*3 and VKORC1 1173C>T. While in VKORC1 -1639 A>G, the percentage of heterozygosity was equal in both the study groups, it was lower in patients compared to controls in VKORC1 -497T>G (Figure 1).

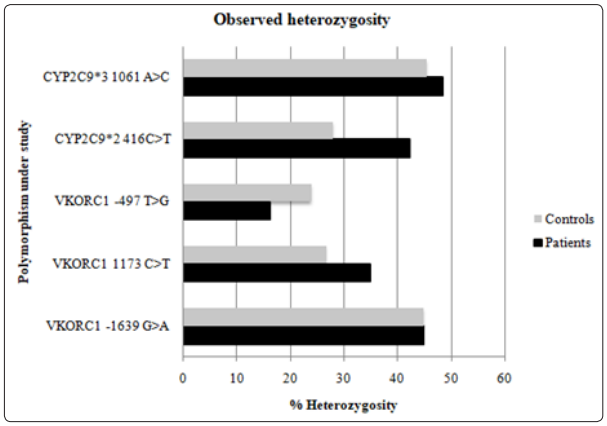

Figure 1: Percentage heterozygosity in both Patients and Controls group for 5 SNPs under study: VTE patients group showed higher heterozygosity in comparison to controls in case of CYP2C9*3 1061A>C, CYP2C9*2 416C>T and VKORC1 1173C>T

Association of plasma concentration of VKORC1 and CYP2C9 with VTE

The plasma concentration of both VKORC1 and CYP2C9 was measured in patients and control groups (figure 2). It was found that plasma levels of VKORC1 and CYP2C9 both were significantly higher in patient's group. The mean concentration of VKORC1 in patients was found to be 1210±123.7 pg/mL compared to control subjects with mean concentration of 599.6±54.60 pg/mL. The difference between the means of the study groups was 610.8±135.2 pg/mL with confidence interval (CI) of 338.5 to 883.2 (p<0.0001) (Figure 2a).

In case of CYP2C9, the mean concentration in patients group was 5958 ± 2032 pg/mL compared to controls with mean levels 2318±274.9 pg/mL. The difference between the means of the study groups was 3639±1701 pg/mL with CI of 165.2 to 7113 (p=0.04) (Figure 2b).

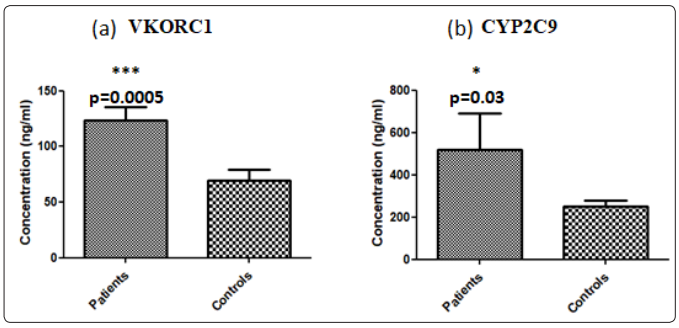

Figure 2: Plasma levels of VKORC1 and CYP2C9 in both VTE patient and control groups. Statistical significance was at P < 0.05: VTE patients group showed significantly higher concentrations of both VKORC1 and CYP2C9 compared to control group

Discussion

Till date, the impact of genetic polymorphisms in VKORC1 and CYP2C9 genes on susceptibility of an individual towards VTE remains unclear. This study attempts to evaluate the role of common mutations in these two genes with events of venous thrombosis.

Genetic factors are important determinants for individual's susceptibility towards disease and drug response. Personalized medicine approach is based on information about genetic risk factors of a person. It is a promising strategy to improve patient's health care, particularly in regard to anticoagulation treatment [26-28]. Despite availability of several new generation direct oral anticoagulants, vitamin K antagonists (VKA) are widely used for anticoagulation treatment. Thus it is extremely important to ensure its effective delivery and safety [29]. Major problems related to the use of VKAs are excessive bleeding or even thrombo-embolic events, depending upon the coagulation and anticoagulation status of an individual [30,31]. The instability of anticoagulation treatment is largely due to variation in VKAs response in individuals, resulting from polymorphisms in VKORC1 and CYP2C9 gene, both of which play an important role in VKA metabolism [32]. Polymorphisms in these two genes have been previously linked to pharmacogenetics of warfarin in order to understand inter-individual variability for deciding the accurate warfarin dosing [33-36].

Warfarin is the most popular anticoagulant used for treatment of venous thrombosis. Although the clinical significance of warfarin is established since decades, its use possess difficulties in managing the risk of complications [37-38]. Inappropriate dosing of warfarin has substantial risk of both major and minor haemorrhage [41,42]. The dosage requirement for achieving target anticoagulation is highly variable due to inter-individual variability and largely genetically determined [43-45].

VKORC1 gene encodes for Vitamin K epoxide reductase protein which is the key enzyme in vitamin K cycle [16,17]. This enzyme production is the rate limiting step in physiological process of vitamin K recycling [46]. Variants of VKORC1 may affect the availability of reduced vitamin K, which is particularly essential for production of several coagulation factors such as factor VII, factor IX and factor X [47]. We investigated three genetic polymorphisms in VKORC1 gene. All three of them were found to be significantly associated with VTE either at genotypic or at allelic level. -1639G>A polymorphism in the promoter region of VKORC1 is believed to be the main cause for low dose phenotype [48]. Luciferase assay has demonstrated that activity of G allele is increased over activity of A allele by 44%. Thus the carriers of A allele respond to lower initial dose of warfarin compared to carriers of G allele, whereas heterozygous respond to intermediate dose of warfarin [49]. In concurrence with this, our finding demonstrated that AA genotype in -1639A/G polymorphism prevailed predominantly in control group. This implied that control group has overall lower production of VKORC1 compared to VTE patients group. The higher VKORC1 producing genotypes (GG+AG) were 87.57% in patients compared to 78.15% in controls. This is further confirmed by our ELISA results wherein VTE patients showed significantly increased plasma concentrations of VKORC1.

1173C>T polymorphism in VKORC1 is near perfect linkage disequilibrium with -1639 G>A and is also associated lower dose warfarin phenotype [48]. Present study finds no significant genotypic variation in patient and control group for this SNP. Percentage of higher protein producing genotypes (CC+CT) was equivalent for the patients (91.78%) and control groups (96.5%). However, statistically significant allelic difference was observed (OR=1.54, 0.53-2.22).

For -497T>G polymorphism, TT genotype was predominant in patients (83.56%) in comparison to controls (77.72%). The difference was statistically significant both at genotypic and allelic level seem to be pre-disposing a person to VTE. We infer that presence of T allele seem to be linked to production of higher VKORC1 and could be contributing to VTE pre-disposition.

In the present study we found that the patients group predominantly possessed C allele in case of CYP2C9*2 416C>T polymorphism (OR=0.54, CI=0.42-0.71) and A allele in case of CYP2C9*3 1061A>C polymorphism (OR=0.49, CI=0.37-0.67). Earlier reports have demonstrated mutation frequencies for different CYP2C9 variants *1, *2, and *3 to be 81%, 11%, and 8%, respectively [50,51]. We also found that the plasma concentration of CYP2C9 was significantly higher in patients group compared to control. We infer that presence of the C allele and A allele in CYP2C9*2 416C>T and CYP2C9*3 1061A>C polymorphism could be associated with increased plasma concentration of CYP2C9 and may present as the risk factors for VTE development.

Results of present study support the hypothesis that the genotypic variations in VKORC1 and CYP2C9 are associated with individual's susceptibility towards VTE.

Conclusion

In summary, polymorphisms in VKORC1 and CYP2C9 can significantly alter their plasma concentration. We have demonstrated the association of SNPs in the VKORC1 and CYP2C9 genes and their increased plasma level with VTE susceptibility in Indian population. The findings of present study could be used to predict the role of these emerging biomarkers that may be useful to increase the predictability of a VTE event in male Indian population.

Declarations

Ethical Approval and Consent to Participate

This study was approved by Institutional ethical committee. A written and informed consent was obtained from all participants before recruiting them for study.

Conflict of Interest

The author(s) declare(s) that they have no conflict of interests

Funding

The present work was funded by Defence Research and Development Organization (DRDO)

Acknowledgements

Authors are extremely thankful to all the volunteers of Indian Army who participated in the study. We are also thankful to Prof. (Dr) MA Ashraf, Jamia Millia Islamia (Former Scientist 'E' at DIPAS) for his efforts in initial collection of blood samples.

References

1. White RH, Zhou H, Romano PS (1998) Incidence of idiopathic deep vein thrombosis and secondary thromboembolism among ethnic groups in California. Ann Inter Med 128: 737-740.
2. Di Nisio M, van Es N, Büller HR (2016) Deep vein thrombosis and pulmonary embolism. Lancet 388: 3060-3073.
3. Homans J (1954) Thrombosis of the deep leg veins due to prolonged sitting. N Engl J Med 250: 148-149.
4. Hull RD, Raskob GE (1986) Prophylaxis of venous thromboembolic disease following hip and knee surgery. J Bone Joint Surg Am 68: 146-150.
5. Lippi G, Franchini M, Favaloro EJ (2009) Unsuspected triggers of venous thromboembolism-trivial or not so trivial? Semin Thromb Hemost 35: 597-604.
6. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, et al. (2007) Incidence and mortality of venous thrombosis: a population based study. Journal of Thrombosis and Haemostasis 5: 692-699.
7. National Health and Medical Research Council (2011) Stop the Clot: Integrating VTE Prevention Guideline Recommendations into Routine Hospital Care. 3rd edition. National Health and Medical Research Council.
8. Palareti G (2012) Recurrent venous thromboembolism: what is the risk and how to prevent it? Scientifica (Cairo) 2012: 391734.
9. Hansson PO, Sörbo J, Eriksson H (2000) Recurrent venous thromboembolism after deep vein thrombosis: incidence and risk factors. Arch Intern Med 160: 769-774.
10. Hotoleanu C (2017) Genetic Risk Factors in Venous Thromboembolism. Review. Adv Exp Med Biol 906: 253-272.
11. Kumari B, Srivastava S, Chatterjee T, Vardhan R, Tyagi T, et al. (2014) Study of associated genetic variants in Indian subjects reveals the basis of ethnicity related differences in susceptibility to venous thromboembolism. Thrombosis 182762.
12. Kumari B, Prabhakar A, Sahu A, Chatterjee T, Tyagi T, et al. (2017) Endothelin-1 Gene Polymorphism and Its Level Predict the Risk of Venous Thromboembolism in Male Indian Population. Clinical and Applied Thrombosis/Hemostasis 23: 429-437.
13. Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, et al. (2000) Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic Analysis of Idiopathic Thrombophilia. Am J Hum Genet 67: 1452-1459.
14. Larsen TB, Sorensen HT, Skytthe A, Johnsen SP, Vaupel JW, et al. (2003) Major genetic susceptibility for venous thromboembolism in men: a study of Danish twins. Epidemiology 14: 328-332.
15. Rosendaal FR (1999) Venous thrombosis: a multicausal disease. Lancet 353: 1167-1173.
16. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, et al. (2004) Identification of the gene for vitamin K epoxide reductase. Nature 427: 541-544.
17. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, et al. (2004) Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 427: 537-541.
18. Yuan HY, Chen JJ, Lee MT, Wung JC, Chen YF, et al. (2005) A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. Human Molecular Genetics 14: 1745-1751,
19. Ross S, Pare G (2013) Pharmacogenetics of antiplatelets and anticoagulants: a report on clopidogrel, warfarin and dabigatran. Pharmacogenomics 14: 1565-1572.
20. McMillin GA, Melis R, Wilson A (2010) Gene-based warfarin dosing compared with standard of care practices in an orthopaedic surgery population: a prospective, parallel cohort study. Ther Drug Monit 32: 338-345.
21. Shalia KK, Doshi SM, Parikh S, Pawar PP, Divekar SS, et al. (2012) Prevalence of VKORC1 and CYP2C9 gene polymorphisms in Indian population and its effect on warfarin response. J Assoc Physicians India 60: 34-38.
22. Eriksson N, Wadelius M (2012) Prediction of warfarin dose: why, when and how? Pharmacogenomics 13: 429-440.
23. Kabalak PA, Savaş İ, Akar N, Demir N, Eğin Y (2018) Frequency of vitamin K oxidoreductase complex subunit-1 (VKORC1) polymorphisms and warfarin dose management in patients with venous thromboembolism. Pharmacogenomics J 18: 646-651.
24. Biswas M, Bendkhale SR, Deshpande SP, Thaker SJ , Kulkarni DV, et al. (2018) Association between genetic polymorphisms of CYP2C9 and VKORC1 and safety and efficacy of warfarin: Results of a 5 years audit. Indian Heart Journal 70: S13-S19.
25. Takeuchi F, McGinnis R, Bourgeois S, Barnes C, Eriksson N, et al. (2009) A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 5:e1000433.
26. Hatz MH, Schremser K, Rogowski WH, et al. (2014) Is individualized medicine more cost effective? A systematic review. Pharmacoeconomics 32: 443-55.
27. Zineh I, Pacanowski M, Woodcock J (2013) Pharmacogenetics and coumarin dosing–recalibrating expectations. N Engl J Med 369: 2273-2275.
28. Abohelaika S, Wynne H, Avery P, Kamali F (2015) Influence of CYP2C9 polymorphism on the fall in International Normalized Ratio in patients interrupting warfarin therapy before elective surgery. J Thromb Haemost 13: 1436-1440.
29. Furie B (2013) Do pharmacogenetics have a role in the dosing of vitamin K antagonists? N Engl J Med 369: 2345-2346.
30. Connolly SJ, Pogue J, Eikelboom J, Flaker G, Commerford P, et al. (2008) Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. Circulation 118: 2029-2037.
31. Gallagher AM, de Vries F, Plumb JM, Hass B, Clemens A, et al. (2012) Quality of INR control and outcomes following venous

thromboembolism. Clin Appl Thromb Hemost 18: 370-378.
32. Voora D, Eby C, Linder MW, Milligan PE, Bukaveckas BL, et al. (2005) Prospective dosing of warfarin based on cytochrome P-450 2C9 genotype. Thromb Haemost 93: 700-705.
33. Aithal GP, Day CP, Kesteven PJ, Daly AK (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 353: 717-719.
34. Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, et al. (2005) The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood 106: 2329-2333.
35. Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, et al. (2002) Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. JAMA 287: 1690-1698.
36. D'Andrea G, D'Ambrosio RL, Di Perna P, Chetta M, Santacroce R, et al. (2005) A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 105: 645-649.
37. Hirsh J, Dalen J, Anderson DR, Poller L, Bussey H, et al. (2001) Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. Chest 119: 8S–21S.
38. Hylek EM, D'Antonio J, Evans-Molina C, Shea C, Henault LE, et al. (2006) Translating the results of randomized trials into clinical practice: the challenge of warfarin candidacy among hospitalized elderly patients with atrial fibrillation. Stroke 37:1075-1080.
39. Tapson VF, Hyers TM, Waldo AL, Ballard DJ, Becker RC, et al. (2005) Antithrombotic therapy practices in US hospitals in an era of practice guidelines. Arch Intern Med 165: 1458-1464.
40. Wittkowsky AK (2004) Effective anticoagulation therapy: defining the gap between clinical studies and clinical practice. Am J Manag Care 10: S297-306.
41. Rettie AE, Tai G (2006) The pharmocogenomics of warfarin: closing in on personalized medicine. Mol Interv 6: 223-227.
42. Kamali F (2006) Genetic influences on the response to warfarin. Curr Opin Hematol 13: 357-361.
43. Momary KM, Shapiro NL, Viana MA, Nutescu EA, Helgason CM, et al. (2007) Factors influencing warfarin dose requirements in African-Americans. Pharmacogenomics 8: 1535-1544.
44. Kealey C, Chen Z, Christie J, Thorn CF, Whitehead AS, et al. (2007) Warfarin and cytochrome P450 2C9 genotype: possible ethnic variation in warfarin sensitivity. Pharmacogenomics 8: 217-225.
45. Schelleman H, Chen Z, Kealey C, Whitehead AS, Christie J, et al. (2007) Warfarin Response and Vitamin K Epoxide Reductase Complex 1 in African Americans and Caucasians. Clin Pharmacol Ther 81: 742-747.
46. Wajih N, Sane DC, Hutson SM, Wallin R (2005) Engineering of a Recombinant Vitamin K-dependent γ-Carboxylation System with Enhanced γ-Carboxyglutamic Acid Forming Capacity. The Journal of Biological Chemistry 280: 10540-10547.
47. Stafford DW (2005) The vitamin K cycle. J Thromb Haemost 3: 1873-1878.
48. Owen RP, Gong L, Sagreiya H, Klein TE, Altman RB (2010) VKORC1 pharmacogenomics summary. Pharmacogenet Genomics 20: 642-644.
49. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, et al. (2005) Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 352: 2285-2293.
50. Moridani M, Fu L, Selby R (2006) Frequency of CYP2C9 polymorphisms affecting warfarin metabolism in a large anticoagulant clinic cohort. Clin Biochem 39: 606-612.
51. Schwarz UI, Ritchie MD, Bradford Y (2008) Genetic determinants of response to warfarin during initial anticoagulation. N Engl J Med 358: 999-1008.