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Factor V Leiden and prothrombin G20210A mutations and risk of vaso-occlusive complications in sickle cell disease: A meta-analysis through the lens of nephrology

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ABSTRACT

Introduction: Hemolysis is a fundamental feature that contributes to hypercoagulability and thrombotic complications in sickle cell disease (SCD). Factor V Leiden (FVL) and prothrombin G20210A mutations are the most common genetic thrombophilia.

Objectives: The aim of this meta-analysis is to determine the relationship between FVL or prothrombin G20210A and susceptibility of vaso-occlusive complications (VOC) in SCD.

Patients and Methods: For this meta-analysis, eligible studies were retrieved via two systematic searches performed on PubMed, Web of Science and Google Scholar databases. The keywords used in the former search included 'sickle cell anemia', 'SCD', 'Factor V Leiden' and 'G1691A (rs6025) mutation, the latter using the keywords 'sickle cell anemia', 'SCD', 'prothrombin, and 'G20210A (rs1799963).

Results: The final number of studies included was 10 about SCD-VOC and FVL (total patients with VOC 1086 and without VOC 933), and 6 about SCD-VOC and prothrombin G20210A mutation (total patients with VOC 609 and without VOC 674). Meta-analysis in fixed effect model showed that mutant genotypes (GA+AA vs. GG) of the FVL mutation was found to be higher in SCD patients with VOC than in SCD patients without VOC (OR, 3.53; 95% CI, 2.24–5.08; $P < 0.001$). However, the distribution of prothrombin G20210A mutation in SCD patients with or without VOC is similar (OR, 0.900; 95% CI, 0.51–1.59; $P = 0.717$).

Conclusion: Our meta-analysis establishes that the FVL mutation as a high-penetrant risk factor for VOC in SCD patients, whereas the prothrombin G20210A is not associated with the risk of VOC in SCD patients. To further validate the clinical utility of these prothrombotic mutations in SCD patient, large scale and prospective studies in diverse populations are warranted.

Implication for health policy/practice/research/medical education:

This study helps in understanding the role of prothrombotic mutations in sickle cell disease patients. FVL remained as a high-penetrant risk factor for VOC in sickle cell disease patients. The prothrombin G20210A mutation is not associated with VOC in SCD patients.

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Introduction

Sickle cell disease (SCD) is a monogenetic blood disorder that is inherited in an autosomal recessive fashion. This hereditary hemolytic anemia causes chronic vascular manifestations due to intravascular sickling in capillary and vessels (1). The clinical outcome of SCD ranges from mild to moderate to severe with acute to chronic clinical manifestations. Many genetic factors are known to modify the clinical severity of SCD. Amelioration of clinical severity in SCD is directly related to the other genetic determinants such as high HbF levels, Xmn-I polymorphism, Arab Indian haplotype and alpha

thalassemia (2). In addition to these increased levels of acute-phase reactants and inflammatory mediators and nitric oxide scavenging due to hemolysis results in hypercoagulability and thrombotic complications in SCD (3). Coinheritance of protein-C, protein-S or antithrombin deficiency further worsens the hypercoagulable state in SCD patients (4). Further, several studies investigated the association between prothrombotic factors and the risk of venous thromboembolism in SCD patients (5,6).

The most significant risk factors of thromboembolism are the inherited genetic mutations in factor V Leiden (FVL), prothrombin and methylenetetrahydrofolate

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reductase (*MTHFR*) genes (7). FVL refers to the G1691A (Arg506Gln; rs6025) mutation in exon 10 of the factor V (*FV*) gene. The carriers of both heterozygote and homozygote states of FVL mutation showed increased risk of venous thrombosis by exhibiting FV resistance to activated protein C (8). Prothrombin (factor II) is a vitamin K dependent protein that is involved in coagulation. A common mutation in 3'- untranslated region of prothrombin gene (G20210A; rs1799963) associated with elevated levels of prothrombin (9). Several studies have investigated the association between vascular complications and FVL, prothrombin G20210A and *MTHFR* C677T SNPs in SCD patients, but the results are inconclusive (10-19). As regards to the role of *MTHFR* C677T in SCD patients, a meta-analysis including 11 cross sectional studies established that *MTHFR* 677C>T mutation as a high-penetrant risk factor for vaso-occlusive complications (VOC) in SCD patients (20). In the present study we performed a meta-analysis of the available data to determine the relationship between FVL or prothrombin G20210A and susceptibility of VOC in SCD.

Materials and Methods

Literature search criteria

For this meta-analysis, eligible studies were retrieved via two systematic searches performed on PubMed, Web of Science and Google Scholar databases. The keywords used in the former search included 'sickle cell anemia', 'SCD', 'Factor V Leiden' and 'G1691A (rs6025) mutation, the latter using the keywords 'sickle cell anemia', 'SCD', 'prothrombin, and 'G20210A (rs1799963). No data or language restrictions were applied to search the relevant papers and the last search was updated on January 17, 2019. The inclusion criteria adopted to select the studies included in the present meta-analysis is as follows: (1)

case-control study; (2) evaluating the association between FVL/Prothrombin G20210A mutation and vascular complication of SCD; and (3) containing the count for FVL/Prothrombin G20210A genotypes to calculate the odds ratio (OR) and confidence interval (CI). Author, publication date, ethnicity, genotyping data for the FVL/Prothrombin G20210A mutations in SCD patients with or without vascular complications were extracted from each paper. As none of the selected papers having all three genotypes and the presence of selection in comparison group, Hardy-Weinberg equilibrium was not tested for independent papers. The inter study heterogeneity was assessed by Cochran's Q test and inconsistency value I^2 . To assess the strength of the associations between the FVL/Prothrombin G20210A mutations with susceptibility to vascular complication, crude OR and corresponding 95% CI limits were calculated and two-sided p-values were reported. Fixed-effect method was used to pool results in the dominant model. High-resolution forest plots were prepared to depict both OR and 95% CI limits. To assess publication bias we examined funnel plots. As there is low inter study heterogeneity, a sensitivity analysis was not performed. The analysis was conducted using a web tool MetaGenyo (21).

Results

Characteristics of studies

The process adopted for retrieval and selection of papers in this meta-analysis is shown in Figure 1. The baseline characteristics of the included studies are summarized in Table 1. All included studies provided data on the prevalence of both FVL and prothrombin G20210A or any one of the mutations in SCD patients with or without VOC. Data on the prevalence of FVL in SCD patients were provided in 10 studies (10-19), and data on the prevalence

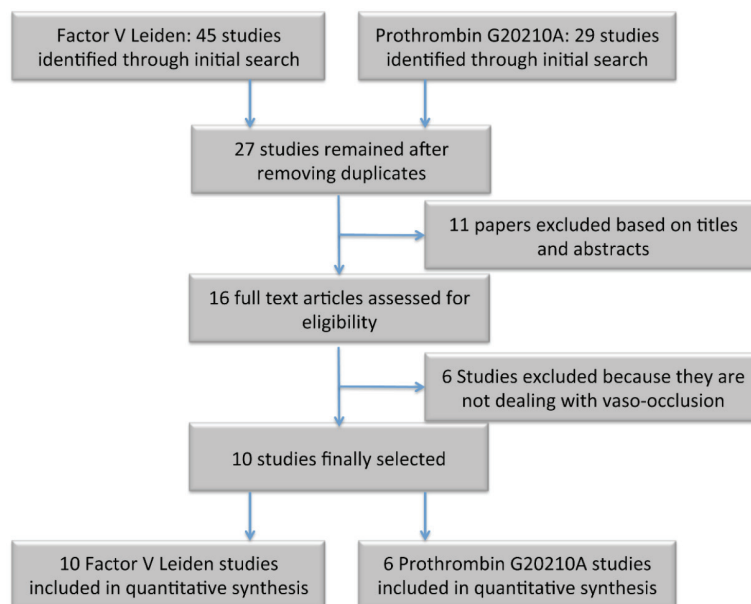


Figure 1. Schema describing the steps taken during study selection.

Table 1. Characteristics of the studies included in the meta-analysis of FVL and prothrombin G20210A

Author	Population	Age (y)	Vaso-occlusive Complications	Total No. of samples	FVL		Prothrombin G20210A					
					VOC		No VOC		VOC		No VOC	
					GG	GA+AA	GG	GA+AA	GG	GA+AA	GG	GA+AA
Andrade et al (10)	Brazilians	14 - 62	Stroke	73	4	1	4	1	8	1	63	1
Zimmerman et al (11)	African Americans	>10	AVNF	101	16	0	16	0	16	0	85	0
			Stroke	101	27	0	27	0	27	0	74	0
Kutlar et al (12)	African Americans	<10	Thrombosis	20	1	9	1	9	-	-	-	-
Farra et al (13)	Lebanese	NA	Severe VOC	163	83	10	83	10	89	4	64	6
Hatzlhofer et al (14)	Brazilians	5-72	Severe VOC	277	174	3	174	3	174	3	98	2
Hamdy et al (15)	Egyptians	3-18	VOC	50/100*	10	9	10	9	47	3	49	1
Nishank et al (16)	Indian	16 ± 6.5	Chest pain	150	60	17	60	17	-	-	-	-
			Bone joint pain	150	103	22	103	22	-	-	-	-
			Abdominal Pain	150	58	16	58	16	-	-	-	-
			Fatigue	150	75	19	75	19	-	-	-	-
Sedky et al (17)	Egyptians	NA	Stroke	40	1	5	1	5	-	-	-	
Kangne et al (18)	Indian	1-47	VOC	126	100	3	100	3	-	-	-	
Samarah et al (19)	Palestinians	16 ± 9.9	Joint pain	117	41	16	41	16	55	4	54	4
			Chest pain	117	52	18	52	18	57	4	52	4
			Abdominal Pain	117	48	14	48	14	61	4	48	4
			Splenomegaly	117	53	18	53	18	49	3	60	5

*Total Number of samples for FVL is 50 for prothrombin 100.

AVNF: Avascular necrosis of the femoral head; VOC: vaso-occlusive complications; CVD: Cerebrovascular disease; NA: Not available.

of prothrombin G20210A were provided in 6 studies (10,11,13-15,19).

Association of the FVL mutation With VOC in SCD

Ten studies compared the prevalence of total FVL mutation in SCD patients with (n = 1086) or without VOC (n = 933). The heterogeneity among studies was not significant ($I^2 = 19.8\%$; $P = 0.233$). By using a fixed effects model, the prevalence of total FVL mutations was found to be higher in SCD patients with VOC than in SCD patients without VOC (OR, 3.53; 95% CI, 2.24–5.08; $P < 0.001$) (Figure 2A). The funnel plot of studies evaluating the role of FVL appeared symmetric (Figure 3A), suggesting the absence of publication bias (Egger's test P value = 0.1017).

Association of the prothrombin G20210A mutation with VOC in SCD

Six studies compared the prevalence of total prothrombin G20210A mutation in SCD patients with (n=609) or without VOC (n=674). The heterogeneity among studies was not significant ($I^2=0\%$; $P=0.739$). Using a fixed-effects model, the prevalence of total prothrombin G20210A mutation was similar in SCD patients with VOC and without VOC (OR, 0.900; 95% CI, 0.51–1.59; $P = 0.717$) (Figure 2B). The funnel plot of studies evaluating the role of prothrombin G20210A suggests evidence of asymmetry or publication bias (Figure 3B).

Discussion

SCD patients are at risk of variety of VOC as a result of hemolysis and superimposed thrombosis (22). A recent study using a population-based data set from the state of California, described the prevalence of venous thromboembolism (VTE) and its consequences in SCD patients (23). FVL and prothrombin G20210A mutations are the most common genetic thrombophilias known to increase the risk of initial VTE (9, 24). The findings of our meta-analysis suggest that the FVL mutation in SCD patients increases the risk of VOC. There is no evidence of publication bias or statistical heterogeneity between FVL studies of this meta-analysis. As regards to prothrombin G20210A, our meta-analysis showed that the prevalence of 20210A allele carriers in SCD patients with or without VOC is similar.

The carriers of FVL mutant proteins confer a greater risk of developing deep vein thrombosis compared with normal individuals (25). Further, the carriers of prothrombin G20210A mutation are associated with a 3-fold greater risk of venous thrombosis (26). However these mutations show distinctive worldwide distribution. Both FVL and prothrombin G20210A mutations are prevalent among Caucasians and found to be rare among other populations, including Africans (27). Several studies have focused on the prevalence of FVL and prothrombin G20210A mutations in SCD patients to determine a

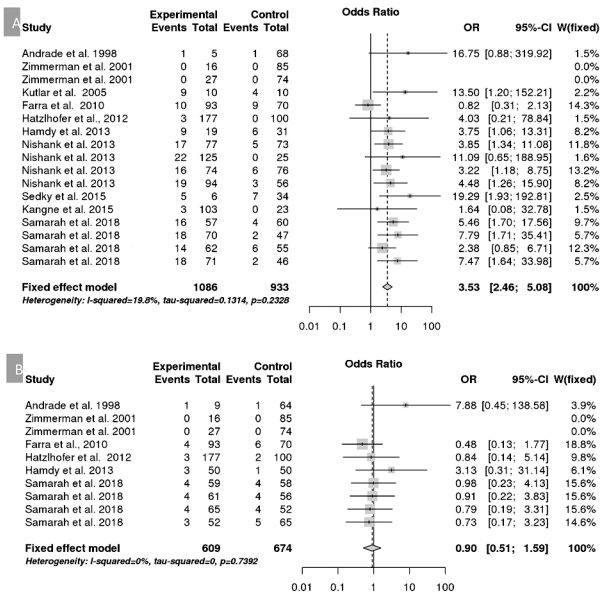


Figure 2. Forest plot of the association of prothrombotic mutations and VOC. A: Factor V Leiden; B: prothrombin G20210A; Experimental: with vascular complications; Control: without vascular complications; Events: GA + AA; OR: odds ratio; CI: confidence interval; W: weight.

possible association with various VOC. High frequency of FVL reported in Indian (16), Iranian (28), and Palestinian SCD patients (19). High frequency of prothrombin G20210A mutation was documented in Tunisian SCD patients (29). In contrast to this a low prevalence of FVL and prothrombin G20210A was reported in African American (30), Saudi Arabian (31) and Afro-Brazilian SCD patients (32).

Conclusion

Although the SCD is a monogenetic disorder, the susceptibility of VOC in SCD is multifactorial and involving both genetic and environmental factors, each with a small effect on VOC risk. Further, the heterogeneity in VOC poses a great challenge to researchers focusing on VOC pathogenesis and therapy in SCD patients. Hence, it is difficult to accurately predict the VOC risk by analyzing a single genetic variation. To further validate the clinical utility of these prothrombotic mutations in SCD patient, large scale and prospective studies in diverse populations are warranted.

Author's contribution

LVKSB is the single author of the paper.

Conflicts of interest

None declared.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

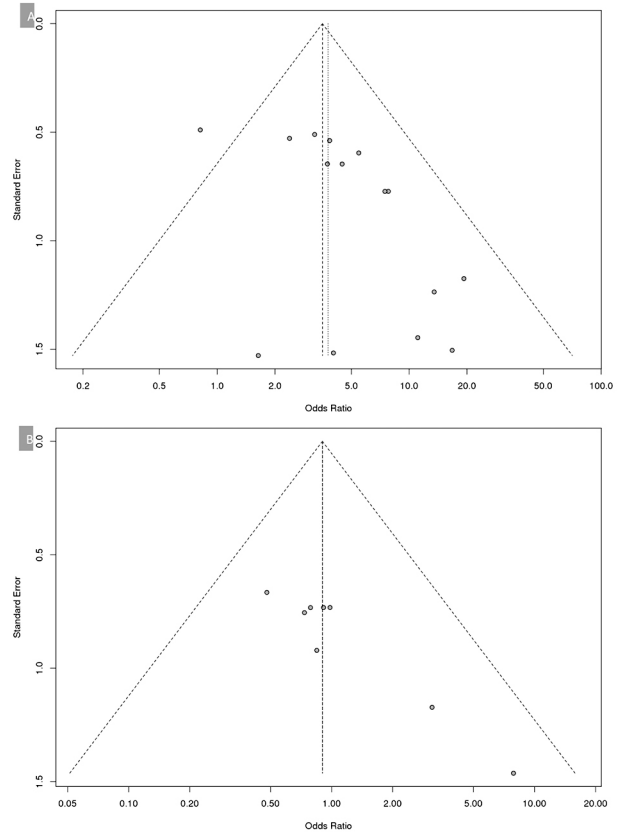


Figure 3. Publication bias for the prothrombotic mutations and VOC in SCD. A: Factor V Leiden; B: Prothrombin G20210A.

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