

# The clinical impact of MTHFR polymorphism on the vascular complications of sickle cell disease

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## Abstract

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Publication supported by FAPESP.

Received September 23, 2005

Accepted June 20, 2006

Sickle cell disease (SCD) is one of the most common inherited diseases in the world and the patients present notorious clinical heterogeneity. It is known that patients with SCD present activation of the blood coagulation and fibrinolytic systems, especially during vaso-occlusive crises, but also during the steady state of the disease. We determined if the presence of the factor V gene G1691A mutation (factor V Leiden), the prothrombin gene G20210A variant, and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism may be risk factors for vascular complications in individuals with SCD. We studied 53 patients with SCD (60% being women), 29 with SS (sickle cell anemia; 28 years, range: 13-52 years) and 24 with SC (sickle-hemoglobin C disease; 38.5 years, range: 17-72 years) hemoglobinopathy. Factor V Leiden, MTHFR C677T polymorphism, and prothrombin G20210A variant were identified by PCR followed by further digestion of the PCR product with specific endonucleases. The following vascular complications were recorded: stroke, retinopathy, acute thoracic syndrome, and X-ray-documented avascular necrosis. Only one patient was heterozygous for factor V Leiden (1.8%) and there was no prothrombin G20210A variant. MTHFR 677TT polymorphism was detected in 1 patient (1.8%) and the heterozygous form 677TC was observed in 18 patients (34%, 9 with SS and 9 with SC disease), a prevalence similar to that reported by others. No association was detected between the presence of the MTHFR 677T allele and other genetic modulation factors, such as  $\alpha$ -thalassemia,  $\beta$ -globin gene haplotype and fetal hemoglobin. The presence of the MTHFR 677T allele was associated with the occurrence of vascular complications in SCD, although this association was not significant when each complication was considered separately. In conclusion, MTHFR C677T polymorphism might be a risk factor for vascular complications in SCD.

### Key words

- Sickle cell disease
- SC hemoglobinopathy
- Sickle hemoglobinopathies
- Inherited hypercoagulation states
- MTHFR polymorphism

## Introduction

Sickle cell disease (SCD) is one of the most common inherited diseases in the world. In Brazil, 700 to 1000 infants with SCD are estimated to be born each year (1). Even with the same inherited DNA mutation within the  $\beta$ -globin gene, patients present a notorious clinical heterogeneity. Additional genetic factors such as  $\alpha$ -thalassemia, fetal hemoglobin synthesis, and  $\beta$ -globin haplotype have been identified, but none of these can fully explain the differences in clinical expression observed in these patients. Probably, there are other elements that contribute to the many phenotypes of the disease (2-5).

It has been known for a long time that patients with SCD show activation of the blood coagulation and fibrinolytic systems, as well as increased platelet activity and consumption of coagulation inhibitors, especially during vaso-occlusive crises, but also during the steady state of the disease (6-9). Vascular complications are an important and perplexing aspect of the clinical spectrum of sickle cell anemia, although there is controversial evidence surrounding the role of thrombosis in this complication (10,11). Because of the importance of vascular complications in the pathophysiology of SCD, a number of genetic polymorphisms associated with thrombophilia have been studied as potential genetic modifiers of SCD.

In the present study, we investigated the hypothesis that the presence of the factor V gene G1691A mutation (factor V Leiden), the prothrombin gene G20210A variant, and MTHFR (methylenetetrahydrofolate reductase) C677T polymorphism may be a risk factor for vascular complications in individuals with SS and SCD.

## Patients and Methods

We evaluated 53 patients of African descent with SCD, 29 with SS (sickle cell anemia) and 24 with SC (sickle-hemoglobin

C disease) hemoglobinopathy. The study population consisted of 32 women and 21 men. The following vascular complications were identified: stroke by focal neurologic deficit and computed tomography, retinopathy by ophthalmoscopic fundus examination, acute thoracic syndrome by clinical and radiological findings, and avascular necrosis (AVN) of femoral or humeral heads by X-rays. All patients, including those with vascular complications, were on oral folate administration (5 mg/day).

The study was approved by the local Ethics Committee on human research. After written informed consent was obtained, approximately 5 mL whole blood was collected in EDTA and stored at  $-4^{\circ}\text{C}$  until DNA extraction. Factor V Leiden mutation, MTHFR C677T polymorphism, and prothrombin G20210A variant were identified by PCR followed by further digestion of the PCR product with specific endonucleases, as described in Refs. 12-14.

The significance of the difference between medians was determined by the Mann-Whitney U-test. Fisher's exact test was used to compare the frequency of observations in contingency tables. Odds ratios (OR), as an estimate of relative risk, and 95% confidence intervals (95% CI) were calculated using standard methods. A two-tailed  $P < 0.05$  value was considered to be statistically significant. Statistical tests were performed using the Instat2 software (GraphPad, San Diego, CA, USA).

## Results

Median age was lower for SS patients (28 years, range: 13 to 52 years) than for SC patients (38.5 years, range: 17 to 72 years), with the difference being almost statistically significant ( $P = 0.059$ ). Median hemoglobin level was lower for SS patients (7.95 g/dL) than for SC patients (11.53 g/dL), whereas fetal hemoglobin level was higher for SS patients (6.2%) than for SC patients (2.1%). These differences were both

significant ( $P < 0.001$ ).

The vascular complications investigated in the present study were more frequent in SC patients (83.3%) than in SS patients (34.5%), as shown in Table 1. Retinopathy was significantly more frequent in SC patients, as expected, whereas acute thoracic syndrome was only recorded among SS patients (Table 1).

Only one patient was heterozygous for the factor V Leiden mutation (1.8%) and there was no prothrombin G20210A mutation. Because of the low frequency observed, the relationship between these mutations and other epistatic factor was not considered further.

MTHFR 677TT polymorphism was detected in 1 patient (1.8%) and the heterozygous form 677TC was observed in 18 patients (34%), 9 with SS and 9 with SC disease. No association was detected when we compared the presence of the MTHFR 677T allele and other genetic modulation factors such as  $\alpha$ -thalassemia,  $\beta$ -globin gene haplotype and fetal hemoglobin.

The presence of the MTHFR 677T allele was associated with the occurrence of vascular complications in SCD, although this association was not significant if each complication is considered separately (Table 2). Taking all 4 complications together, the OR was 4.8 (95% CI: 1.3-17.3,  $P = 0.020$ ). The association was still significant when we considered stroke, retinopathy and AVN, with an OR of 3.5 (95% CI: 1.1-11.5,  $P = 0.047$ ).

## Discussion

Although HbS cells are indispensable for the generation of the disease, several other phenomena, affected by a multitude of genes other than the one directly mutated (epistatic effect), play an important role (15). The importance of thrombosis in the pathophysiology of SCD has been recently recognized. Considerable evidence suggests that endo-

Table 1. Prevalence of stroke, avascular necrosis (AVN), retinopathy, and acute thoracic syndrome (ATS) in patients with sickle cell disease (SCD).

	SCD patients (N = 53)	SS patients (N = 29)	SC patients (N = 24)
Cases with vascular complication	30 (56.6%)	10 (34.5%)	20 (83.3%)*
Stroke	1 (1.8%)	1 (3.4%)	0 (0.0%)
AVN	2 (3.8%)	1 (3.4%)	1 (4.2%)
Retinopathy	16 (30.1%)	1 (3.4%)	15 (62.5)
ATS	4 (7.5%)	4 (13.8%)	0 (0.0%)
Combined complications <sup>#</sup>	7 (13.2%)	3 (10.3%)	4 (16.7%)
Episodes of vascular complication			
Stroke	3 (5.6%)	3 (10.3%)	0 (0%)
AVN	8 (15.1%)	3 (10.3%)	5 (20.8%)
Retinopathy	21 (39.6%)	2 (6.9%)	19 (79.2%)*
ATS	6 (11.3%)	6 (20.7%)	0 (0%)*

SS = sickle cell anemia; SC = sickle-hemoglobin C disease.

<sup>#</sup>4 patients had AVN + retinopathy; 1 patient had stroke + AVN; 1 patient had stroke + ATS; 1 patient had AVN + retinopathy + ATS.

\* $P < 0.05$  compared to SS (Fisher's exact test).

Table 2. Odds ratio for the association between the 677T allele and vascular complications in sickle cell disease.

MTHFR	Vascular complication		Odds ratio	95% CI
	AVN +	AVN -		
CC	3	31	1 <sup>#</sup>	
CT/TT	5	14	3.7	0.8-17.7
	R +	R -		
CC	11	23	1 <sup>#</sup>	
CT/TT	10	9	2.3	0.7-7.4
	ATS +	ATS -		
CC	3	31	1 <sup>#</sup>	
CT/TT	3	16	1.9	0.4-10.7
	Stroke +	Stroke -		
CC	2	32	1 <sup>#</sup>	
CT/TT	1	18	0.9	0.08-10.5
	AVN + R + stroke	No AVN, R or stroke		
CC	13	21	1 <sup>#</sup>	
CT/TT	13	6*	3.5	1.1-11.5
	AVN + R + stroke + ATS	No vascular complication		
CC	15	19	1 <sup>#</sup>	
CT/TT	15	4*	4.8	1.3-17.3

AVN = avascular necrosis; R = retinopathy; ATS = acute thoracic syndrome.

<sup>#</sup>Reference risk factor. \* $P < 0.05$  for comparison between patients with and without vascular complication with regard to the distribution of the allele 677T (Fisher's exact test).

thelial damage and thrombosis play important contributory roles in the vaso-occlusive complications of SCD (10,16).

We found only 1 patient in 53 SS + SC subjects heterozygous for factor V Leiden and no patient with prothrombin mutation, as expected, since these polymorphisms are more frequent in Caucasians.

The MTHFR 677TT genotype was detected in 1.8% of SCD patients, which is similar to the prevalence reported by others, ranging from 1 to 2.8% (17-20). Balasa et al. (18) found a prevalence of 1% for this genotype among 110 normal American Blacks, whereas its prevalence among Caucasians ranges from 10 to 20%, depending on the geographic distribution (21-26). In Brazil, despite the great miscegenation, there seems to be a high proportion of Caucasians within normal individuals, since Morelli et al. (27) reported a 9% prevalence of 677TT and Franco et al. (28) found a 16.6% prevalence.

We found that the 677T allele was associated with vaso-occlusive complications in patients with SCD. Some investigators did not find an association between MTHFR C677T and clinical manifestations in SCD: Cumming et al. (17) evaluated stroke in SS patients and Zimmerman et al. (19) evaluated the occurrence of stroke and AVN in SS, SC and S $\beta^0$  thalassemia patients. In a Brazilian study conducted on 73 patients with sickle cell anemia, SC hemoglobinopathy and S $\beta^0$  thalassemia, none presented homozygosity for the MTHFR 677T allele, including those with vascular complications such as stroke or deep vein thrombosis (29).

On the other hand, Kutlar et al. (30) found a significant association between the 677T allele and AVN in SS patients, which has also been reported in individuals without SCD (31).

Hyperhomocystinemia is a significant risk factor for venous thrombosis in patients without known acquired thrombophilia or other genetic risk factors for venous thrombosis, underscoring the importance of homocysteine evaluation in thrombotic disease (27). The role of moderate hyperhomocystinemia as a cause of stroke in adults has been well documented, and it has been suggested that high homocysteine levels may be a risk factor for the development of stroke in patients with SCD as well (32). Morelli et al. (27) found that homocystinemia levels are significantly higher in homozygotes for the MTHFR C677T mutation both in patients with venous thromboembolism and controls.

Chronic ongoing hemolysis in sickle cell patients causes a high demand for folate, necessary for normal erythropoiesis. The presence of the 677T allele, which is associated with high homocysteine levels, may aggravate the effect of this amino acid on the vascular system. Actually, Houston et al. (33) showed a clear influence of hyperhomocystinemia on the risk of stroke in SS patients, although their folate status was normal.

In conclusion, our data suggest that MTHFR C677T polymorphism might be a risk factor for vascular complications in SCD, probably contributing to the deleterious effect of hyperhomocystinemia caused by folate imbalance.

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