

SHORT COMMUNICATION

**A C677T Methylenetetrahydrofolate Reductase (MTHFR)
Polymorphism and G20210A Mutation in the Prothrombin
Gene of Sickle Cell Anemia Patients from Northeast Brazil**

**Fábio David Couto,¹ Wendell Vilas Boas,¹ Isa Lyra,^{2,4} Ângela Zanette,⁴
Marie France Dupuit,² Mari Ney Tavares Almeida,²
Mitermayer Galvão Reis,¹ and Marilda Souza Gonçalves^{1,2,*}**

¹Laboratório de Patologia e Biologia Molecular, Centro de Pesquisas Gonçalo Moniz,
Fundação Oswaldo Cruz (FIOCRUZ), Salvador-Bahia, Brasil

²Universidade Federal da Bahia, Salvador-Bahia, Brasil

³Complexo Pediátrico Professor Hosanah de Oliveira, Ambulatório de Hematologia
Pediátrica, Hospital Universitário Professor Edgar Santos da Universidade
Federal da Bahia, Canela, Salvador-Bahia, Brasil

⁴Ambulatório de Hematologia, Fundação de Hematologia e Hemoterapia da Bahia
(HEMOBA), Salvador-Bahia, Brasil

ABSTRACT

The C677T methylenetetrahydrofolate reductase (MTHFR) gene polymorphism and the G20210A mutation at the 3' untranslated region (3'UTR) of the prothrombin gene may be considered to be genetic risk factors that contribute to the clinical heterogeneity in sickle cell disease. The current study investigated a group of sickle cell (SS) patients from Salvador-Bahia, Northeast Brazil in order to determine the prevalence of these polymorphisms, using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. Out of 69 SS patients

*Correspondence: Dr. Marilda Souza Gonçalves, Laboratório de Patologia e Biologia Molecular, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Rua Waldemar Falcão 121, Brotas, Salvador-Bahia, CEP: 40.295-001, Brasil; Fax: +55-71-356-2155; E-mail: mari@cpqgm.fiocruz.br.

diagnosed with the C677T MTHFR gene polymorphism, 13 (18.6%) were heterozygous and four (5.7%) homozygous. The G20210A mutation was not found in 50 SS patients investigated. These results became important once the C677T MTHFR gene polymorphism was found to be an independent risk factor for vascular disease, a common clinical event in sickle cell disease.

Key Words: Methylenetetrahydrofolate reductase (MTHFR); Prothrombin gene; Sickle cell anemia; Salvador-Bahia, Brazil.

Sickle cell disease is one of the most common and complex genetic disorders found worldwide, mainly among people of African origin. The clinical heterogeneity of sickle cell disease is well documented and many genetic and environmental factors have been considered to influence its pathophysiology. α -Thalassemia (thal), different β^S -globin gene haplotypes, fetal hemoglobin (Hb) levels, G6PD deficiency, cell hydration, nutritional and other environmental conditions, are intrinsic and extrinsic factors described as potential modifiers of the sickle cell clinical phenotype (1).

Prothrombin and methylenetetrahydrofolate reductase (MTHFR) are some of the target genes investigated in thromboembolism events in general and in sickle cell anemia patients (2,3). Both these genetic polymorphisms are supposed to be involved in vascular alterations. They have been investigated in groups of individuals carrying other genetic risk factors for vascular disease, such as sickle cell anemia (4).

Bahia, a state located in northeast Brazil, shows an elevated racial admixture resulting in a pool of inherited genetic diseases (5). The heterozygous state of sickle cell disease (Hb AS) reaches 14%, and sickle cell anemia up to 1.6% in a group of Bahians of African descent (6). In this context, it is useful to investigate the prevalence of two genetic markers, the C677T MTHFR gene polymorphism and the G20210A mutation at the 3'untranslated region (3'UTR) of the prothrombin gene, in a group of sickle cell anemia patients from Salvador-Bahia, Brazil, in order to identify individuals at risk of developing early coagulopathies.

The study was developed in a group of 69 sickle cell patients attending the out patient clinic at the Blood Center of Bahia (HEMOBA), Salvador, Bahia, Brazil. There were 35 (50.7%) males and 34 (49.3%) females, with a mean age of 18.7 years (from 1 to 73 years). Approval was obtained from the Oswaldo Cruz Foundation Institutional Ethical Committee, Salvador-Bahia, Brazil. Peripheral blood samples were collected from each patient by physicians and nursing staff, into EDTA vacutainer tubes. Samples were refrigerated at 4°C for a maximum of 8 hours before the Hb profile investigation. Hemoglobin analyses were performed by cation exchange high-performance liquid chromatography (HPLC; VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. DNA was isolated from peripheral blood leukocytes using the GFX™ Genomic Blood DNA Purification KIT (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), following the manufacturer's instructions. The polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were used to investigate the G20210A mutation of the prothrombin gene and the C677T MTHFR gene polymorphism (7,8). The statistical analysis was performed using EPI Info Software version 6.04 (16). A *p* value of less than 0.05 was considered statistically significant.

Table 1. Prevalence of the C677T MTHFR gene polymorphism among SS patients from Salvador-Bahia, Brazil.

Mutations	Frequency among SS patients			Total
	Wild type (%) (n)	Heterozygotes (%) (n)	Homozygotes (%) (n)	
C677T MTHFR	75.7 (52)	18.6 (13)	5.7 (4)	100.0 (69)
G20210A Prothrombin	100.0 (50)	–	–	100.0 (50)

Of the 69 sickle cell patients diagnosed with the C677T MTHFR gene polymorphism, 13 (18.6%) were heterozygous and four (5.7%) homozygous. No statistically significant difference was found among the T allele distribution and gender, $\chi^2 = 1.81$, $p = 0.405$. The G20210A prothrombin gene mutation at the 3'UTR was not found in the 50 sickle cell patients investigated (Table 1).

Identifying genetic risk factors for preventive or therapeutic measures have become a common practice in clinical follow-up of sickle cell anemia patients (9). The frequency of the C677T single nucleotide polymorphism in the MTHFR gene is different among different ethnic groups (10). In Salvador, the T allele frequency among 843 newborns was 0.23, with 36.2% heterozygous and 5.3% homozygous carriers (11). In previous studies, the homozygous state for the C677T MTHFR gene polymorphism was not found among Brazilian Blacks (12) or was only found with a prevalence of 1.5% (13). Considering that the majority of the Bahian population is of African descent, the prevalence of the C677T MTHFR gene polymorphism found in the present study was considered to be high.

The G20210A mutation at the 3'UTR of the prothrombin gene shows a heterogeneous worldwide geographic distribution. Our results in relation to the prothrombin gene mutation is in accordance with the low prevalence of prothrombin gene alterations in Black Africans (14,15). However, we need to increase the number of patients in order to confirm the real prevalence of this gene alteration among sickle cell anemia patients from Salvador-Bahia, Brazil. Further studies about the association between this single nucleotide polymorphism and phenotype need to be done among the Bahian sickle cell anemia patients. In conclusion, the prevalence of the heterozygous and homozygous genotypes for the C677T MTHFR gene polymorphism was considered high when one takes into consideration the African origin of this population group.

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