

## C677T polymorphism of the MTHFR gene and variant hemoglobins: a study in newborns from Salvador, Bahia, Brazil

Polimorfismo C677T no gene da MTHFR e hemoglobinas variantes: um estudo em recém-nascidos de Salvador, Bahia, Brasil

Fábio David Couto <sup>1</sup>  
 Elisângela Vitória Adorno <sup>1,2</sup>  
 Joelma Figueiredo Menezes <sup>1</sup>  
 José Pereira Moura Neto <sup>1</sup>  
 Marco Antônio Vasconcelos Rêgo <sup>3</sup>  
 Mitermayer Galvão dos Reis <sup>1</sup>  
 Marilda Souza Gonçalves <sup>1,2</sup>

### Abstract

*The C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR) is associated with an increase in total homocysteine serum levels (tHcy), described as a risk factor for cardiovascular disease. Eight hundred forty-three neonates from two different maternity hospitals, one public and another private, in Salvador, Bahia, Brazil were screened for this polymorphism by PCR and RFLP. The T-allele frequency in the total sample was 0.23, and the prevalence rates of heterozygous and homozygous carriers were 36.2% and 5.3%, respectively. The T-allele frequency differed and the T/T genotype was more prevalent at the private maternity hospital. The hemoglobin (Hb) profile was investigated by HPLC in 763 newborns. The frequency of variant Hb was higher at the public than at the private maternity hospital. The association of the C677T polymorphism and the Hb profile was investigated in 683 newborns, showing a relatively high frequency of variant Hbs and the T allele. These data could provide an important basis for further studies focusing on potential risks of vaso-occlusive events in these individuals.*

*Newborns Infant; Polymorphism; Hemoglobinopathies*

### Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine (Hcy) metabolism. A single point mutation causing a C → T substitution at nucleotide 677 of the MTHFR gene has been associated with a thermolabile enzyme form of low biological activity <sup>1</sup>. High total homocysteine (tHcy) serum or plasma levels have been attributed to the presence of the C677T MTHFR polymorphism, mainly in association with low folate and vitamin B12 levels <sup>2,3,4</sup>. The role of high tHcy serum or plasma levels received considerable interest after these factors were implicated as an important risk factor for cardiovascular disease in a study examining children's aorta fragments <sup>5</sup>; the study proposed that high homocysteine levels play a role in arteriosclerosis genesis.

Sickle-cell anemia (SS) is a monogenic disorder with worldwide distribution and high prevalence in Brazil <sup>6</sup>. The mutant sickle hemoglobin results from a single nucleotide substitution: (GAG → GTG) at the sixth codon of the beta-globin gene. Sickle cell anemia displays a heterogeneous clinical presentation, unpredictable clinical development, and is characterized by chronic hemolytic anemia and vaso-occlusive events that result in acute pain crises, with chronic and progressive tissue damage <sup>7</sup>. Vascular alterations are commonly observed among these patients <sup>8</sup>, and SS and SC disease

<sup>1</sup> Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brasil.

<sup>2</sup> Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, Brasil.

<sup>3</sup> Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brasil.

#### Correspondence

Marilda Souza Gonçalves  
 Laboratório de Patologia e Biologia Molecular, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão 121, Salvador, BA  
 40295-001, Brasil.  
 mari@cpqgm.fiocruz.br

carriers have an increased risk of arterial vascular disease and venous thrombosis <sup>9</sup>.

The high turnover of red blood cells (RBC) observed in sickle-cell patients is associated with lower serum levels of folate and vitamin B12 <sup>10</sup>, leading to the increased tHcy serum or plasma levels that have been implicated in vascular endothelium injury <sup>11</sup>. Even in the absence of genetic predisposition or traditional risk factors such as C-reactive protein and MTHFR, the increased tHcy levels have been considered an important predictor of mortality in patients with angiographically defined coronary artery disease <sup>12</sup>.

Bahia, a State in Northeast Brazil, has a high interethnic admixture. The strong African gene pool <sup>13</sup> has resulted in a high frequency of hemoglobinopathies, with a prevalence of S heterozygotes (AS) reaching 14% in groups of African descendants <sup>14</sup>. In this article we report on a molecular characterization of the C677T MTHFR gene polymorphism in a newborn population from two different maternity hospitals in Salvador, investigating its association with the presence of variant hemoglobins.

## Material and methods

### Subjects

A total of 843 newborns were studied. After obtaining approval from the Research Ethics Committee of the Oswaldo Cruz Foundation, we enrolled newborns from the Tsylla Balbino public maternity hospital and the Santo Amaro private maternity hospital. Although both are located in Salvador, the socioeconomic characteristics of women giving birth at these two hospitals are quite different. Sample collection occurred from May to December 2000. Eight hundred forty-three newborn babies were screened for the C677T MTHFR gene polymorphism and 763 for the hemoglobin profile.

### Blood collection

Pediatricians and nurse staff collected a cord-blood sample from each infant, using EDTA anticoagulant. Samples were refrigerated at 4°C for a maximum of eight hours before the hemoglobin profile investigation.

### Hemoglobin analysis

Hemoglobin analyses were performed by cation-exchange high performance liquid chromatography (HPLC) (BioRad Variant®, California,

USA), according to the manufacturer's instructions.

### DNA extraction

DNA was isolated from cord-blood leukocytes using the GFX® Genomic Blood DNA Purification KIT (Amersham Pharmacia Biotech Inc., Piscataway, USA), following the manufacturer's instructions.

### C677T MTHFR gene polymorphism

C677T MTHFR gene polymorphism was investigated by PCR, as previously described by Frosst et al. <sup>1</sup>. The generated 198 bp PCR product was digested by the *Hinf* I restriction enzyme (New England BioLabs Inc., Tozer Road, Beverly, MA, USA), resulting in 175 and 23 bp fragments in the homozygous T state, and 198, 175, and 23 bp fragments in heterozygotes. The wild type remains undigested, preserving the original 198 bp fragment.

## Results

The total T-allele frequency was 0.23, with a prevalence of 36.2 and 5.3% for the C/T and T/T genotypes, respectively. However, the allele frequency was higher among newborns of the Santo Amaro maternity hospital than from the newborns of the Tsylla Balbino maternity hospital, with a statistically significant difference from the T/T genotype distribution (Table 1).

As shown in Table 2, out of 763 newborns, 60 were FAS heterozygotes (53 from Tsylla Balbino and seven from Santo Amaro); 30 were FAC heterozygotes (27 from Tsylla Balbino and three from Santo Amaro); one was FC, six were FSC, and one was FS (all from Tsylla Balbino). The frequency of variant hemoglobins was higher at the Tsylla Balbino than the Santo Amaro maternity hospital, with a statistically significant difference for FAS,  $\chi^2 = 14.14$  and  $p$  value  $< 0.001$ , and for FAC genotype distributions,  $\chi^2 = 7.80$  and  $p$  value = 0.005. The observed relationship between the C677T MTHFR gene polymorphism and different hemoglobin patterns was established in a total of 683 samples (Table 3).

## Discussion

The T-allele frequency of the C677T MTHFR polymorphism has been found to vary among different populations and ethnic groups, with a

lower frequency among Africans<sup>15</sup>. Krieger et al.<sup>13</sup> estimated in a sample from Northeast Brazil that it was some 97% of the way to interethnic panmixia. An intensive African slave trade occurred from the 16<sup>th</sup> to the 19<sup>th</sup> centuries<sup>16</sup>, and more than five million Africans of several nationalities entered Brazil between 1850 and 1950<sup>17</sup>. In Brazil, Arruda et al.<sup>18</sup> found a T-allele frequency of 10% among people of European descent; 1.45% among Blacks; and 1.2% among Indians. Perez et al.<sup>19</sup>, studying children with spina bifida and controls, found prevalence rates for the T/T genotype of 7.64 and 10.32%, respectively. The T-allele frequency of 0.23 and the prevalence of C/T and T/T genotypes found in this report were higher than those observed in other studies of African and African-descent populations worldwide<sup>20,21,22</sup> and lower than those found in studies of Europeans<sup>23</sup>. This result was probably influenced by the high rate of racial admixture in the Bahian population. The T/T genotype displayed a statistically significant difference between the groups of newborns from the two maternity hospitals, with a lower frequency at the Tsylla Balbino maternity Hospital. The mothers who gave birth at the Santo Amaro maternity hospital are primarily representative of the European-descendent portion of the Bahian population, confirming the higher T-allele frequencies previously described in this continental group<sup>24</sup>.

In contrast to the C677T MTHFR gene polymorphism distribution, the variant hemoglobin frequencies were higher at the Tsylla Balbino than at the Santo Amaro maternity hospital. These differences are probably due to the ethnic composition of the newborns from the two hospitals. Azêvedo et al.<sup>14</sup> showed an increase in variant hemoglobin allele frequencies in European-descendent Bahians, which were not restricted to African-descendent groups, but with intermediate numbers among mulattos.

The frequencies of C/T or T/T genotypes among FAS newborns were 35.7% and 5.4%, respectively. The T-allele presence has been associated with increased tHcy serum levels. Khajuria & Houston<sup>25</sup> show *in vitro* evidence of a direct mechanism by which homocysteine, at physiologically and pathophysiologically relevant concentrations, may induce thrombosis.

Even though sickle cell patients routinely using folic acid to improve erythropoiesis, folate status can be found at sub-clinical levels when compared to Hb AA normal control serum folate levels<sup>10</sup>. Lowenthal et al.<sup>26</sup> hypothesized that the concentration of folate required to normalize plasma homocysteine lev-

Table 1

Prevalence of the C677T MTHFR gene polymorphism among 843 newborns from the Tsylla Balbino and Santo Amaro maternity hospitals. Salvador, Bahia, Brazil, 2000.

Genotype	Tsylla Balbino (%)	Santo Amaro (%)	Total (%)
C/C	379 (61.0)	114 (51.4)	493 (58.5)
C/T	216 (34.8)	89 (40.1)	305 (36.2)
* T/T	26 (4.2)	19 (8.6)	45 (5.3)
Total	n = 621	n = 222	n = 843
Allele	Allele frequency	Allele frequency	Allele frequency
C	0.78	0.71	0.77
T	0.22	0.29	0.23

n = number of samples; \* Differences in T/T genotype,  $\chi^2 = 8.08$ ; p = 0.004.

Table 2

Hemoglobin profile distribution among newborns from the Tsylla Balbino and Santo Amaro maternity hospitals. Salvador, Bahia, Brazil, 2000.

Hemoglobin pattern	Tsylla Balbino (%)	Santo Amaro (%)	Total (%)
FA	421 (82.7)	244 (96)	665 (87.2)
FAS*	53 (10.4)	7 (2.8)	60 (7.9)
FAC**	27 (5.3)	3 (1.2)	30 (3.9)
FC	1 (0.2)	-	1 (0.1)
FSC	6 (1.2)	-	6 (0.8)
FS	1 (0.2)	-	1 (0.1)
Total	n = 509	n = 254	n = 763

n = number of samples; \*  $\chi^2 = 14.14$  and p value < 0.001;

\*\*  $\chi^2 = 7.80$  and p value = 0.005.

Table 3

The C677T MTHFR polymorphism and hemoglobin patterns among 683 newborns from the Tsylla Balbino and Santo Amaro maternity hospitals. Salvador, Bahia, Brazil, 2000.

Hemoglobin profile	C677T MTHFR gene Polymorphism			Total
	C/C (%)	C/T (%)	T/T (%)	
FA	349 (59)	211 (35.6)	32 (5.4)	592
FAS	33 (58.9)	20 (35.7)	3 (5.4)	56
FAC	18 (66.7)	8 (29.6)	1 (3.7)	27
FC	-	1 (100.0)	-	1
FSC	4 (66.6)	1 (16.7)	1 (16.7)	6
FS	-	1 (100.0)	-	1
Total	n = 404	n = 242	n = 37	n = 683

n = number of samples.

els in patients with sickle cell disease may be higher than that of normal controls, and that patients with sickle cell disease have a higher nutritional requirement for folic acid than the general population. These facts can contribute to raising tHcy serum levels, described as a risk factor for coronary artery disease<sup>27</sup>. The T-allele presence found in the newborns in this study could provide an important basis for further studies focusing on potential risks of vaso-occlusive events in these individuals.

The Bahia State Foundation for Hematology and Transfusion Therapy (HEMOBA) has regis-

tered 942 SS patients, and data from the neonatal screening program for variant hemoglobins from APAE (the Association of Parents and Friends of Children with Disabilities) in Salvador, Bahia reports one SS for every 423 newborns. Blood samples from these SS patients and newborns can be referred to specialized laboratories to perform molecular investigation of the C677T polymorphism in the MTHFR gene. In our opinion, polymorphisms in genes responsible for folate and homocysteine metabolic pathways should be investigated in Bahia and other SS populations worldwide.

## Resumo

*O polimorfismo C677T no gene da MTHFR tem sido associado ao aumento dos níveis séricos de homocisteína total (tHcy), descrito como fator de risco para o desenvolvimento de doenças cardiovasculares. Oitocentos e quarenta e três recém-nascidos (RNs), de duas maternidades diferentes, uma pública e a outra privada, em Salvador, Bahia, Brasil foram triados para o polimorfismo C677T por PCR e RFLP. A frequência do alelo T foi de 0,23 e as prevalências dos genótipos C/T e T/T foram de 36,2% e 5,3%, respectivamente. A frequência do alelo T diferiu e a prevalência do genótipo T/T foi mais elevada entre os RNs da maternidade privada. O perfil de hemoglobinas (Hb) foi determinado por HPLC em 763 RNs. A frequência de Hbs variantes foi mais elevada entre os RNs da maternidade pública Tsylla Balbino do que na maternidade privada do Hospital Santo Amaro. A associação do polimorfismo C677T e o perfil de Hbs foram estudados em 683 RNs, apresentando frequência elevada da coexistência do alelo T e Hb variantes. Estes resultados podem ser utilizados como base para estudos futuros sobre riscos potenciais de eventos vaso-oclusivos nestes indivíduos.*

*Recém-nascidos; Polimorfismo; Hemoglobinopatias*

## Contributors

F. D. Couto participated in the blood sample collection in the hospitals, hemoglobin analysis, DNA extraction, molecular analyses, and drafting of the manuscript. E. V. Adorno, J. F. Menezes, and J. P. Moura Neto participated in the blood sample collection in the hospitals, hemoglobin analysis, and DNA extraction. M. Rego participated in the statistical analysis. M. G. Reis participated in the interpretation of the results and drafting and revision of the manuscript. M. S. Gonçalves supervised the laboratory analyses, interpretation of the results, and drafting and revision of the manuscript.

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