Genotyping of A1298C polymorphism of the *MTHFR* gene in patients with iron deficiency anemia at the Institute of Hematology and Hemotherapy of Amapá

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ABSTRACT. Iron deficiency anemia mostly affects children and adolescents, women of childbearing age and pregnant women due to their high iron requirements. The *MTHFR* enzyme helps maintain the pool of folate and methionine, avoiding the accumulation of homocysteine. Patients with elevated levels of homocysteine have a wide range of clinical features, including iron anemia, because cysteine is a protein derived from the metabolism of homocysteine and is related to iron homeostasis. The objective of our research was to determine whether the *MTHFR* gene A1298C polymorphism is related to the development of anemia in patients with iron deficiency anemia. We analyzed genetic polymorphisms using blood samples from blood donors who were part of the control group as well as patients with iron deficiency anemia through PCR and electrophoresis techniques. Among 42 patients, 21 had the AC genotype, 5 the CC genotype and 16 the normal AA genotype. In the control group, 58 blood donor samples were analyzed, of which 44 showed the genotype (AA) and 14 were identified as having the heterozygous (AC) genotype; no samples with the CC genotype were identified. Thus, we found a frequency of 0.62 and 0.24 of the

A1298C polymorphism of the *MTHFR* gene for the patient group and the control group, respectively, which is a significant difference. We conclude that the A1298C polymorphism of the *MTHFR* gene may contribute to the development of iron deficiency anemia.

Key words: *MTHFR* Gene Polymorphism; Homocysteine; Iron Deficient Anemia

INTRODUCTION

About 2 billion people worldwide suffer from micronutrient deficiency (Christian and Stewart, 2010). Iron deficiency is the most common deficiency, mainly affecting children and adolescents, women of childbearing age and pregnant women due to their high blood iron requirements (An et al., 2012). As with iron deficiency, catecholamine (vitamin B12) and folate (folic acid) deficiency also cause anemia (Santana et al., 2016). Folic acid functions as a co-enzyme in methylation reactions involved in purine and pyrimidine synthesis and amino acid metabolism. The homocysteine remethylation cycle uses dietary tetrahydrofolate (THF) as the central molecule of this pathway.

The methylation of THF depends on serine (which converts to glycine); from this, methylene tetrahydrofolate reductase enzyme (MTHFR) prepares the folate compound (5, 10-methylene tetrahydrofolate) to provide the methyl (5-methyl-THF) group to the homocysteine generating methionine (Baluz et al., 2002). Hyperhomocysteinemia is caused not only by nutritional factors, but also by polymorphisms in the *MTHFR* gene. Because of the transsulfuration pathway, homocysteine is converted into cysteine, which is related to iron homeostasis (Selhub, 1999). *MTHFR* polymorphisms have been demonstrated in cases of hyperhomocysteinemia, which have been found in many hematologic and tumoral diseases.

An important genetic determinant of hyperhomocysteinemia is a common 677C→T polymorphism in *MTHFR*; a second 1298A→C polymorphism has recently been reported in families with spina bifida and with lymphocyte activity (Weisberg et al., 2001). Friedman et al. (1999) concluded that the A1298C polymorphism results in a decrease in MTHFR activity that is more pronounced in the homozygous polymorphism CC than in the heterozygous AC or homozygous AA.

Therefore, we sought to determine if the *MTHFR* gene A1298C polymorphism is associated with alterations in hematological indices in patients with iron deficiency anemia.

MATERIAL AND METHODS

This work was submitted to the Research Ethics Committee of the Universidade Federal do Amapá through Plataforma Brasil, and required evaluation of the referred scientific research, according to regulations established by the Comitê Nacional de Ética e Pesquisa - CONEP, reference number 466/2012. We obtained approval from the Certificado de Apresentação para Apreciação Ética – CAAE under number 61936616.6.0000.0003.

Fifty-eight blood samples from voluntary blood donors and 42 samples from patients with iron deficiency anemia were collected at the Institute of Hematology and

Hemotherapy of Amapá (HEMOAP). We used the Blood / Tissue DNA Mini Kit for DNA extraction. Subsequently, the blood samples were submitted to A1298C amplification of the *MTHFR* gene by the allele-specific PCR technique according to Ranjith et al. (2003), using: 11 µL of water, 12 µL of MixPCR reagent, 1 µL of each *primer* and 5 µL of DNA extracted from the samples, with a final volume of 30 µL. Then, the samples underwent the following conditions: 94°C for 5 min; 94°C for 30 s; 58°C for 30 s; 72°C for 50 s; 30 cycles and final extension a 72°C for 5 min.

Primers used in the amplification with their respective base pair sizes and temperatures for the identification of alleles C and A of the *MTHFR* gene are given in Table 1.

Table 1. Primer fragment sequences of *MTHFR* gene used with respective sizes and temperatures.

Ringed fragment	size	temp
1298 CF: 5'- CTT TGG GGA GCT GAA GGA-3'	120 bp	58°C
1298 CR: 5'- ACA AAG ACT TCAAAG ACA CTT G-3'		
1298 AF: 5'- GGA GCT GAC CAG TGA AGA-3'	77 bp	58°C
1298 AR: 5'- TGT GAC CAT TCC GGT TTG-3'		

The electrophoresis technique was used to identify and visualize the fragments, which could be observed in 1.5% agarose gel, where we obtained the following standards for the A1298C polymorphism. 120bp and 77bp fragments, identified the heterozygote (AC); 120bp fragment, identified homozygous CC; 77 bp fragment, identified homozygous AA.

After the molecular analyses, we made a statistical analysis with the quantitative results obtained in a comparison of the absolute and relative values for each *MTHFR* gene genotype: A1298C, C1298C and A1298A Tables 2 and 3. Using the values found in Table 3, we applied a chi-square test.

RESULTS AND DISCUSSION

The 42 patient samples analyzed for the *MTHFR* gene A1298C polymorphism resulted in 26 patients with polymorphism, 21 patients with heterozygous genotype (AC) and 5 homozygous genotype (CC), while 16 did not show the polymorphism, with homozygous genotype (AA). The control samples analyzed from 58 blood donor samples resulted in 44 with genotype (AA) and 14 samples were identified with heterozygous polymorphism (AC) genotype; no samples with the CC genotype were identified (Tables 2 and 3 and Figure 1).

Table 2. Genotyping result of 42 patient samples with iron deficiency anemia.

A1298C	C1298C	A1298A
n= 21	n= 5	n= 16
50%	12%	38%

Table 3. Comparison of subjects with and without A1298C *MTHFR* gene polymorphism in patients and control group.

Patients (n= 42)					Control Group (n= 58)			
Gene	with	%	without	%	with	%	without	%
<i>MTHFR</i>	26	62	16	38	14	24	44	76

The chi-square test result was equal to 14.5. Therefore, for a P-value = 0.05 and degree of freedom = 1, the H_0 is rejected. Thus, the polymorphism A1298C of the *MTHFR* gene is related to iron deficiency anemia, because it is more frequent in patient groups.

Figure 1. Electrophoresis of seven patients with polymorphism A1298C of the *MTHFR* gene, all of which are heterozygous AC. This result can be seen in the two bands with 120bp and 77bp fragments.

Findings of genes such as *GRX5*, *DMT1* or *TMPRSS6* involved in iron deficiency suggest that this condition may be more frequent than previously assumed (Saad, 2010). These discoveries of new genes show that iron deficiency anemia is not just of nutritional origin as was previously believed. Ueland et al. (2001) concluded that *MTHFR* polymorphisms may be associated with increased risk for various diseases, possibly mediated by altered folate and homocysteine metabolism.

Although we did not find studies on the influence of *MTHFR* gene polymorphism in patients with iron deficiency anemia, we can emphasize that gastric complications are related to low iron absorption from the diet. Thus, among research conducted on the *MTHFR* polymorphism, we highlight that of Miao et al. (2002) in his studies of the Chinese population relating to polymorphism of this gene with the esophageal cancer and gastric adenocarcinoma. Another relevant study was that of Curtin et al. (2004), who observed a relationship of the *MTHFR* polymorphism of the A1298C gene with colon cancer and concluded that this polymorphism may increase the risk of this cancer.

We obtained a frequency of 0.62 and 0.24 of the A1298C polymorphism of the *MTHFR* gene for the patients and control groups, respectively. These data show a higher frequency in the patient group compared to the control group (2.58 times higher). Therefore, it is possible there is a relationship between A1298C polymorphism of the *MTHFR* gene and the development of iron deficiency anemia caused by deficient regulation of iron in the

body from inadequate production of MTHFR, an enzyme responsible for acting in various metabolic pathways related to protein metabolism, including cysteine, which is one of the essential factors in iron regulation.

CONCLUSIONS

This is a genetic polymorphism that has not been previously associated with iron deficiency anemia. Based on molecular and statistical analyses, we conclude that there is a relationship between the development of iron deficiency anemia in patients who have MTHFR A1298C polymorphism due to deficient metabolism of homocysteine.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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