The MTHFR C677T polymorphism and global DNA methylation in oral epithelial cells

Isabela Tatiana Sales de Arruda, Darlene Camati Persuhn and Naila Francis Paulo de Oliveira
Departamento de Biologia Molecular, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, João Pessoa, PB, Brazil.

Abstract
DNA methylation is mediated by DNA methyltransferases (DNMTs) that add a methyl group to the 5'-carbon of cytosine. The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10- methylenetetrahydrofolate to 5-methyltetrahydrofolate in the rate-limiting step of the cycle involving the methyl donor S-adenosyl-L-methionine (SAM). The MTHFR C677T polymorphism results in a thermolabile enzyme with reduced activity that is predicted to influence the DNA methylation status. In this study, we investigated the impact of the MTHFR C677T polymorphism on the global DNA methylation of oral epithelial cells obtained from 54 healthy subjects. There were no significant differences in global DNA methylation among the MTHFR CC, CT and TT genotypes (p = 0.75; Kruskal-Wallis test).

Keywords: DNA methylation, epigenetic, MTHFR C677T, oral epithelial cells, polymorphism.
Received: April 2, 2013; Accepted: June 16, 2013.

DNA methylation is dependent on a methyl donor and S-adenosyl-L-methionine (SAM) is the primary methyl group donor for most biological methylation reactions (Chiang et al., 1996). SAM is generated by the methionine cycle in which 5-methyltetrahydrofolate transfers single methyl groups to homocysteine in a reaction catalyzed by methionine synthase to produce methionine. After donating the methyl group, 5-methyltetrahydrofolate is converted to tetrahydrofolate and then to 5,10-methylenetetrahydrofolate by serine hydroxymethyltransferase. 5,10-methylenetetrahydrofolate is a key substrate that can be directed towards nucleotide biosynthesis or methionine regeneration. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that can be used in the methionine cycle to generate SAM (Schwann and Rozen, 2001). The MTHFR C677T polymorphism leads to the amino acid alanine being replaced by valine (p.Ala222-Val) and the production of a thermolabile variant of MTHFR with 30% less enzyme activity (Sharp and Little, 2004). The MTHFR C677T polymorphism has been suspected to induce hypomethylation and then activate proto-oncogenes, which could explain the association between this polymorphism and some types of cancer (Liu et al., 2012; Saberi et al., 2012; Izmirli, 2013), including oral cancer (Sailasree et al., 2011). This hypomethylation has been suggested to involve the entire genome or specific CpG sites (Matsubayashi et al., 2005; Graziano et al., 2006; Zhu et al., 2011).

Variations in the DNA methylation profile are common among healthy individuals and may be gender-, age- and tissue-specific (El-Maarri et al., 2007; Thompson et al., 2010; Fernandez et al., 2012). Even within the same age group, gender and cell types, inter-individual variations are still observed and are influenced by environmental and genetic factors (Valenza-Schaerly et al., 2001; Weaver et al., 2004; Fraga et al., 2005; Sinclair et al., 2007; Bjornsson et al., 2008).

The aim of this study was to assess the influence of the MTHFR C677T polymorphism on global DNA methylation in oral epithelial cells in young subjects with no systemic disorders or visible alterations in the oral mucosa.

This study was approved by the Institutional Review Board of the Federal University of Paraíba (protocol number 0427/2012). All volunteers were informed about the nature of the proposed research and provided written informed consent. A convenience sample of unrelated male and female subjects > 19 years old was recruited for the study. All subjects were in good general health and had at least 20 healthy teeth. The exclusion criteria included any systemic disorder, pregnancy or lactation, smoking habit and the systemic use of antibiotics or anti-inflammatory medicines within six months prior to baseline. Individuals

Send correspondence to Naila Francis Paulo de Oliveira. Depar- tamento de Biologia Molecular, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Cidade Universitária, Campus I, 58059-900 João Pessoa, PB, Brazil. E-mail: naila_fran- cис@yahoo.com.br.
with a history of oral disease or with clinical signs of damage to the oral mucosa were excluded. The subjects were classified into one of three categories based on their MTHFR genotype, viz. CC, CT and TT.

Oral epithelial cell samples were collected by mouthwash with 3% dextrose and centrifuged at 3000 rpm for 10 min (Treviatto and Line, 2000). DNA was purified using DNazol® (Invitrogen) based on the manufacturer’s recommendations. The MTHFR C677T genetic polymorphism was detected by PCR-RFLP, as previously described (Arruda et al., 1997). Global DNA methylation levels were assessed with an ELISA-based commercial kit (MDQ1, Imprint® methylated DNA quantification kit; Sigma Aldrich, St. Louis, MO, USA) according to the manufacturer’s recommendations. The global DNA methylation levels among groups were compared using the Kruskal-Wallis test while the relationship between DNA methylation and age or gender was assessed using analysis of variance (ANOVA) and Students unpaired t-test, respectively. A value of p < 0.05 indicated significance. All data analyses were done using the software package Prism 5.0 (GraphPad Inc., La Jolla, CA, USA).

Fifty-four subjects 19-25 years old were enrolled in the study. Of these, 17 were CC (four males and 13 females; mean age: 21.4 ± 2.9 years; mean ± SD), 19 were CT (five males and 14 females; mean age: 21.8 ± 2.1 years) and 18 were TT (11 males and seven females; mean age: 22.7 ± 2.8 years) based on their MTHFR genotype. There were no significant differences in the global DNA methylation levels (expressed as a percentage) among the CC, CT and TT genotypes (p = 0.75; Kruskal-Wallis test) (Figure 1). In addition, the percentage of global DNA methylation was unrelated to age (p = 0.07; ANOVA) or gender (p = 0.30; Students unpaired t-test).

Studies of the effect of the MTHFR C677T polymorphism on DNA methylation have focused primarily on the pathological implications of this phenomenon. Few studies have examined the influence of this polymorphism on DNA methylation in healthy subjects. This is the first study to evaluate this association in oral epithelial cells and no significant differences were detected among the genotypes. A previous study of normal colonic mucosa found no influence of the MTHFR C677T polymorphism on global DNA methylation in this tissue, although a tendency towards a decrease in global methylation was observed in smokers with low folate levels who carried the C677T substitution (Pufulete et al., 2005). In addition, studies using lymphocytes found no correlation between the MTHFR C677T polymorphism and decreased DNA methylation in the genome overall (Narayanan et al., 2004; Ono et al., 2012) or at specific CpG sites (Hirsch et al., 2008; Wernimont et al., 2011), regardless of the folate concentration.

However, the existence of this association remains controversial because some studies detected a positive correlation when folate levels were low (Stern et al., 2000; Friso and Choi, 2002; Friso et al., 2002). Folate consumption stimulates the metabolic pathway leading to the biosynthesis of homocysteine that is subsequently converted to its active form, tetrahydrofolate, which is a substrate for the enzyme MTHFR. MTHFR plays a central role in folate metabolism by irreversibly converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate. This product provides methyl groups for methionine synthesis, which in turn is required for the synthesis of SAM, the primary methyl group donor (Fox and Stover, 2008).

In addition to aging (Bjornsson et al., 2008; Grönninger et al., 2010), the influence of environmental effects on DNA methylation is a confounding factor in establishing a correlation between polymorphisms and modifications of the DNA methylation profile. Indeed, environmental factors such as diet (Heijmans et al., 2008; McKay et al., 2012), alcohol consumption (Philibert et al., 2012; Zhang et al., 2013), tobacco use (Flom et al., 2011; Wangsri et al., 2012), radiation exposure (Grönninger et al., 2010; Chaudhry and Omaruddin, 2012) and air pollution (Tarantini et al., 2009; Salam et al., 2012) have been shown to modulate DNA methylation.

**Acknowledgments**

We thank the volunteers who donated oral mucosa samples. ITSA was supported by a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

**References**


conceptional B vitamin and methionine status. Proc Natl Acad Sci USA 104:19351-19356.

Associate Editor: Angela M. Vianna-Morgante

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.