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Research Articles

The methylenetetrahydrofolate reductase 677TT genotype and folate intake interact to lower global leukocyte DNA methylation in young Mexican American women☆

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Abstract

DNA methylation is an epigenetic feature associated with X chromosome inactivation, genomic imprinting, transcriptional silencing of genes, and genomic stability. Folate provides a labile source of methyl groups that may be used for cellular methylation reactions, including DNA methylation. The methylenetetrahydrofolate reductase (MTHFR) 677C→T variant is an important determinant of folate nutriture and may influence DNA methylation. This study sought to assess the influence of the MTHFR C677T genotype on global leukocyte DNA methylation in young (age range = 18-45 years) Mexican American women (N = 43: CC, n = 14; CT, n = 12; TT, n = 17). Subjects consumed a folate-restricted diet (135 μg of dietary folate equivalents per day) for 7 weeks followed by folate treatment with 400 or 800 μg of dietary folate equivalents per day for another 7 weeks. Global leukocyte DNA methylation was assessed via the cytosine extension assay at weeks 0, 7 (after folate restriction), and 14 (after folate treatment). No main effect of MTHFR C677T genotype or folate intake was detected at any time point during the study. However, at the end of folate treatment (week 14), DNA methylation was lower ($P < .05$) among women with the MTHFR 677TT genotype than among those with the CT or CC genotype. Because it is unlikely that folate treatment would result in methyl group loss, we suggest that there was a delay in the DNA methylation response to folate intake. Overall, these data suggest that the MTHFR 677TT genotype and folate interact to lower global leukocyte DNA methylation patterns in young Mexican American women.

Keywords: MTHFR; Folate; Folic acid; Women; DNA methylation; Human

1. Introduction

DNA methylation is an epigenetic feature associated with X chromosome inactivation, genomic imprinting, transcriptional silencing of genes, and genomic stability [1]. Aberrations in DNA methylation are associated with numerous pathologies, including cancer. Cytosine DNA methylation occurs primarily on cytosine-phosphate-guanine (CpG) dinucleotides and is catalyzed by a family of DNA methyltransferases that transfer methyl groups from S-adenosylmethionine to cytosine residues. S-adenosylmethionine is formed in the methionine cycle, where folate serves as an important source of methyl groups that are used by S-adenosylmethionine.

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Folate, in a variety of forms, functions to donate and accept one-carbon units in a metabolic system referred to as one-carbon metabolism. The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is a key player in one-carbon metabolism because it diverts one-carbon units toward methylation reactions (5,10-methylenetetrahydrofolate → 5-methyltetrahydrofolate) at the expense of nucleotide synthesis. A common single-nucleotide polymorphism in the MTHFR gene involving a cytosine (C) → thymine (T) transition at nucleotide 677 is associated with reduced enzyme activity [2], higher plasma total homocysteine [3], and altered risk for chronic diseases and congenital anomalies [2,4].

To date, few studies have assessed the influence of folate intake and/or the MTHFR 677C→T polymorphism on DNA methylation under controlled conditions [5-7]. Because of the relationship between aberrations in DNA methylation and disease risk, more studies are needed to more fully delineate the effect of folate intake and relevant common genetic variants on DNA methylation. This study sought to investigate the influence of the MTHFR 677C→T variant on global leukocyte DNA methylation in young Mexican American women consuming controlled folate intakes. This study is the first to report that the MTHFR 677TT genotype interacts with folate intake to lower global leukocyte DNA methylation in young women consuming controlled folate intakes.

2. Methods and materials

2.1. Subjects

From 1999 through 2002, self-reported Mexican American women, defined as having 2 parents possessing the same race/ethnicity, were recruited among staff and students of the Cal Poly Pomona University and from the surrounding Southern California communities. Additional inclusion criteria were having the appropriate MTHFR C677T genotype, being a nonsmoker, being nonanemic, being a non-supplement user (within the past 3 months), having no chronic drug use problem, having no antifolate medication regimen, having no history of chronic disease, not being pregnant, not planning a pregnancy, not lactating, and having a normal blood chemistry profile. The screening and experimental procedures were reviewed and approved by the institutional review board of the Cal Poly Pomona University for human subject use, and informed consent was obtained from each participant.

2.2. Experimental design

This was a 14-week controlled feeding study with 7 weeks of folate restriction followed by 7 weeks of folate treatment with 400 or 800 μg of dietary folate equivalents (DFEs) per day and has been described in detail in another article [3]. During folate restriction (weeks 0-7), the subjects consumed a low-folate diet providing 135 μg of DFEs per day. During folate treatment (weeks 8-14), the subjects continued to consume the folate-restricted diet providing 135 μg of DFEs per day in addition to intakes of 156 and 391 μg of supplemental folic acid per day (Sigma, St Louis, Mo) for total folate intakes of 400 and 800 μg of DFEs per day, respectively. The folic acid supplement was prepared as a solution [3] and consumed with the meals. The recommended daily allowance or adequate intake for all other essential nutrients was provided as a combination of the diet and supplements in the form of pills [3].

2.3. Analytic methods

2.3.1. Global DNA methylation

The cytosine extension assay [8] with minor modifications [9] was used to assess global DNA methylation. DNA was extracted from mononuclear cells [10], and genomic DNA (0.75 μg) was digested with an excess of methylation-sensitive *Hpa*II restriction endonuclease according to the manufacturer's protocol (New England Biolabs, Beverly, Mass). A second DNA aliquot (0.75 μg) was digested simultaneously with methylation-insensitive isoschizomer *Msp*I (New England Biolabs), and a third DNA aliquot (0.75 μg) was incubated without a restriction enzyme and served as a background control. The single-nucleotide extension reaction was performed in a 40-μL polymerase chain reaction mixture containing 0.75 μg of genomic DNA, 1× NEBuffer 2 (New England Biolabs), 10 U of Klenow Fragment/exo- (New England Biolabs), and 4 μmol/L of [^{3}H]dCTP (2′-deoxycytidine 5′-triphosphate; American Radiolabeled Chemicals, Inc, St Louis, Mo). After incubation, the sample (35 μL) was applied to a Whatman DE-81 ion-exchange filter paper (Whatman, Florham Park, NJ) that was washed, air dried, and processed for scintillation counting. Each sample was run in duplicate. The [^{3}H]dCTP incorporation into DNA was expressed as mean disintegrations per minute (dpm) for every 0.75 μg of DNA. The absolute percentage of double-stranded unmethylated CCGG sites was calculated as follows: (*Hpa*II-induced dpm/*Msp*I-induced dpm) (100). In addition to running the undigested control, we included an aliquot of lambda DNA as a positive control suitable for high levels of [^{3}H]dCTP incorporation, an aliquot of lambda DNA that had been methylated completely in vitro by the action of M•*Sss*I as a negative control suitable for low levels of [^{3}H]dCTP incorporation, and an aliquot of human pooled DNA as an intermediate control. Because of the large sample number, the samples were run in batches. All batches, to which the investigator performing biochemical tests was blinded, contained the controls described. In addition, each batch (n = 14) contained 1 subject and occasionally 2 subjects with the CC, CT, and TT genotypes. For each subject within a batch, weeks 0, 7, and 14 were analyzed simultaneously in duplicate. The intraassay coefficient of variation was 4% based on the sample duplicates. The interassay coefficients of variation were 11.6%, 14.8%, and 8.3% based on the negative control, pooled plasma, and positive control, respectively.