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Methylation diet and methyl group genetics in risk for adenomatous polyp occurrence

Mark Lucock [a,*], Zoë Yates [b], Charlotte Martin [a], Jeong-Hwa Choi [a], Emma Beckett [a], Lyndell Boyd [a], Kathleen LeGras [a], Xiaowei Ng [a], Virginia Skinner [c], Ron Wai [c], Jeremy Kho [a], Paul Roach [a], Martin Veysey [c]

[a] School of Environmental & Life Sciences, University of Newcastle, PO Box 127, Brush Rd., Ourimbah, NSW 2258, Australia
[b] Biomedical Sciences and Pharmacy, University of Newcastle, PO Box 127, Brush Rd., Ourimbah, NSW 2258, Australia
[c] Teaching & Research Unit, Central Coast Local Health District, PO Box 361, Gosford, NSW 2250, Australia

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ABSTRACT

Purpose: The aim of this study is to explore whether a methylation diet influences risk for adenomatous polyps (AP) either independently, or interactively with one-carbon metabolism-dependent gene variants, and whether such a diet modifies blood homocysteine, a biochemical phenotype closely related to the phenomenon of methylation.
Methods: 249 subjects were examined using selective fluorescence, PCR and food frequency questionnaire to determine homocysteine, nine methylation-related gene polymorphisms, dietary methionine, 5-methyltetrahydrofolate, vitamins B6 and B12.
Results: 1). Both dietary methionine and 5-methyltetrahydrofolate intake are significantly associated with plasma homocysteine. 2). Dietary methionine is related to AP risk in 2R3R-TS wildtype subjects, while dietary B12 is similarly related to this phenotype in individuals heterozygous for C1420T-SHMT, A2756G-MS and 844ins68-CBS, and in those recessive for 2R3R-TS. 3). Dietary methionine has a marginal influence on plasma homocysteine level in C1420T-SHMT heterozygotes, while B6 exhibits the same effect on homocysteine in C776G-TCN2 homozygote recessive subjects. Natural 5-methyltetrahydrofolate intake is interesting: Wildtype A1298C-MTHFR, heterozygote C677T-MTHFR, wildtype A2756G-MS and recessive A66G-MSR individuals all show a significant reciprocal association with homocysteine. 4). Stepwise regression of all genotypes to predict risk for AP indicated A2756G-MS and A66G-MSR to be most relevant ($p = 0.0176$ and 0.0408 respectively). Results were corrected for age and gender.
Conclusion: A methylation diet influences methyl group synthesis in the regulation of blood homocysteine level, and is modulated by genetic interactions. Methylation-related nutrients also interact with key genes to modify risk of AP, a precursor of colorectal cancer. Independent of diet, two methylation-related genes (A2756G-MS and A66G-MSR) were directly associated with AP occurrence.

1. Introduction

Epigenomic methylation controls gene expression, while methyl groups are also critical for driving neurotransmitter synthesis and thiol regulation, as well as other important areas of metabolism dependent upon methyl group availability. Clinical phenotypes that may be affected by alterations in methylation include those related to cancer, mood disorders and vascular disease [1].

Colorectal cancer (CRC) is the third most common cancer globally, and the fourth most common cancer-related cause of mortality [2]. Australia has particularly high rates, with CRC being the second most commonly diagnosed cancer and the third leading cause of cancer-related deaths [3]. Clinical and histopathological evidence suggests that most CRC arises from pre-existing benign adenomatous polyps (APs) [3,4]. CRC and its benign adenoma antecedent are often linked to nutrition.

Dietary intake of folic acid (especially 5-methyltetrahydrofolate), vitamins B6, B12 and methionine are particularly important as sources of, or key factors in methyl group availability. This may be relevant in tumourgenesis as virtually all CRCs have aberrantly methylated genes [5]. The dietary supply of the former B-vitamins and methionine is critical in maintaining one-carbon metabolism [6]. Within one-carbon transfers, folate, in the form of 5-methyltetrahydrofolate [7] has a pivotal role in supplying a methyl group to convert homocysteine into methionine, a vitamin B12 dependent process involving methionine synthase (MS) and methionine synthase reductase (MSR) (Fig. 1). The

* Corresponding author. Tel.: +61 43484109; fax: +61 2 4348 4145.
E-mail address: Mark.Lucock@newcastle.edu.au (M. Lucock).

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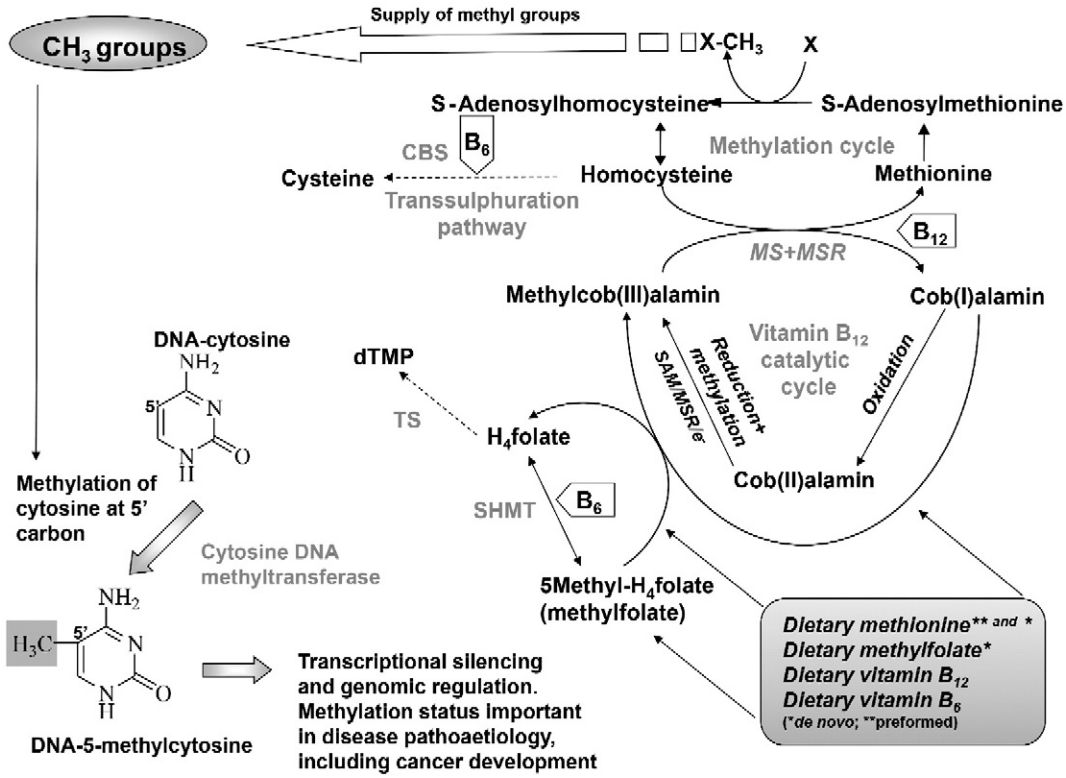

Fig. 1. Dietary sources of preformed methyl groups and associated metabolism, including *de novo* synthesis of methionine. The figure shows where nutrients interact as cofactors and the role of methyl groups in gene expression.

major enzymatic source of one-carbon units in one-carbon metabolism is vitamin B6 dependent serinehydroxymethyl transferase (SHMT), and the key enzyme directing these one-carbon units to methyl group metabolism is methylenetetrahydrofolate reductase (MTHFR) [7].

De novo methionine derived from one-carbon metabolism provides around half our methionine requirement; diet provides the other half. Methionine is converted to S-adenosylmethionine, the universal methyl donor for methylation of a wide variety of biological substrates [8]. Other enzymes are also crucial to the balance between utilising one-carbon units for *de novo* methyl groups or dTMP, which is needed for DNA synthesis. Ultimately, both of these phenomena are critical determinants of tumourgenesis.

A shortage of 5-methyltetrahydrofolate or related components of a healthy 'methylation diet' will decrease the biosynthesis of S-adenosylmethionine, limiting the availability of methyl groups for methylation reactions. DNA hypomethylation in humans consuming a low folate diet has been reported in a number of studies [9–11], and it is reasonable to postulate that an individual's risk of developing a benign adenoma or subsequent CRC is influenced by B-vitamin nutrition. Vegetables, particularly green leafy and cruciferous vegetables, are a major source of folate. Studies suggest that both folate intake and folate status are inversely associated with neoplastic risk [12]. Common polymorphisms of the genes responsible for one-carbon metabolism have also been associated with colorectal neoplasia, providing further evidence for a causal relationship between folate and neoplasia [13,14].

This study examines whether a methylation diet can influence risk for AP either independently or interactively with one-carbon metabolism-dependent gene variants C677T-MTHFR, A1298C-MTHFR, A66G-MSR, A2756G-MS, C1420T-SHMT, 2R3R-TS, 1494del6-TS, C776G-TCN2 and CβS-844ins. The study also examines whether such a diet can modify the blood homocysteine level, a biochemical phenotype closely related to the phenomenon of methylation.

2. Materials and methods

Two hundred and forty nine participants (56.2% female 43.8% male) at Gosford Hospital (NSW) undergoing routine screening for colonic pathology agreed to participate. Subjects were between 40 and 89 years of age at the time of examination (overall mean age 63.3) and were mentally competent to complete a food frequency questionnaire (FFQ) interview. Local Human Research Ethics Committee approval was given and informed consent obtained prior to volunteers participating in the study.

2.1. DNA analysis

Folate gene variants were examined using polymerase chain reaction (PCR) to amplify blood DNA followed by restriction enzyme digestion and gel electrophoresis; 2R3R-TS (rs34743033) and 1494del6-TS (rs16430), C1420T-SHMT (rs1979277), C776G-TCN2 (rs1801198), C677T-MTHFR (rs1801133), A1298C-MTHFR (rs1801131), A2756G-MS (rs1805087), A66G-MSR (rs10380) and CβS-844ins (no rs) were analysed according to the following methods respectively: Horie et al. [15], Ulrich et al. [16], Heil et al. [17], Pietrzyk et al. [18], Van der Put et al. [19–21], Wilson et al. [22] and Tsai et al. [23]. The term wildtype assumes ancestral genotype.

2.2. Food Frequency Questionnaire for intake of native 5-methyltetrahydrofolate, vitamins B6, B12, and methionine

Estimated daily intake of nutrients was assessed by interviewer administered FFQ. The questionnaire was extensive, covering 225 food items and all food groups. Subjects were also asked to provide a list of all supplements they were taking and were asked about these during the FFQ interview.

The FFQs were analysed using Foodworks™ 2.10.146 (Xyris Software, Brisbane, QLD, Australia). This package uses a number of food