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B-vitamins intake, DNA-methylation of One Carbon Metabolism and homocysteine pathway genes and myocardial infarction risk: The EPICOR study

G. Fiorito [a,b,1], S. Guarrera [a,1], C. Valle [a], F. Ricceri [a,b], A. Russo [a,b], S. Grioni [c], A. Mattiello [d], C. Di Gaetano [a,b], F. Rosa [a], F. Modica [a], L. Iacoviello [e], G. Frasca [f], R. Tumino [f], V. Krogh [c], S. Panico [d], P. Vineis [a,g], C. Sacerdote [a,h], G. Matullo [a,b,*]

[a] *Human Genetics Foundation, Torino, Italy*
[b] *Department of Medical Sciences, University of Torino, Italy*
[c] *Department of Preventive and Predictive Medicine, Epidemiology and Prevention Unit, Fondazione IRCSS Istituto Nazionale dei Tumori, Milano, Italy*
[d] *Department of Clinical and Experimental Medicine, Federico II University, Napoli, Italy*
[e] *Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Italy*
[f] *Cancer Registry and Histopathology Unit, "Civile-M.P. Arezzo" Hospital, Ragusa, Italy*
[g] *Epidemiology and Public Health, Imperial College London, UK*
[h] *Cancer Epidemiology, CPO-Piemonte, Torino, Italy*

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Abstract *Background and aims:* Several epidemiological studies highlighted the association between folate and B-vitamins low intake and cardiovascular diseases (CVD) risk. Contrasting results were reported on the relationship between folate intake and DNA-methylation. Folate and B-vitamins may modulate DNA-methylation of specific enzymes which are included in the One-Carbon Metabolism (OCM) and in the homocysteine (Hcy) pathways. The aim of the study was to evaluate whether DNA-methylation profiles of OCM and Hcy genes could modulate the myocardial infarction (MI) risk conferred by a low B-vitamins intake.
Methods and results: Study sample (206 MI cases and 206 matched controls) is a case-control study nested in the prospective EPIC cohort. Methylation levels of 33 candidate genes where extracted by the whole epigenome analysis (Illumina-HumanMethylation450K-BeadChip). We identified three differentially methylated regions in males (TCN2 promoter, CBS 5′UTR, AMT gene-body) and two in females (PON1 gene-body, CBS 5′UTR), each of them characterized by an increased methylation in cases. Functional in silico analysis suggested a decreased expression in cases. A Recursively Partitioned Mixture Model cluster algorithm identified distinct methylation profiles associated to different MI risk: high-risk vs. low-risk methylation profile groups, OR = 3.49, $p = 1.87 \times 10^{-4}$ and OR = 3.94, $p = 0.0317$ in males and females respectively (multivariate logistic regression adjusted for classical CVD risk factors). Moreover, a general inverse relationship between B-vitamins intake and DNA-methylation of the candidate genes was observed.
Conclusions: Our findings support the hypothesis that DNA-methylation patterns in specific regions of OCM and Hcy pathways genes may modulate the CVD risk conferred by folate and B-vitamins low intake.

Abbreviations: CVD, cardiovascular disease; Hcy, homocysteine; OCM, One Carbon Metabolism; DMR, differentially methylated region; RPMM, recursively partitioned mixture model; BMI, body mass index (BMI); WHR, waist-hip ratio; CHD, coronary heart disease.

* Corresponding author. Human Genetics Foundation (HuGeF), Via Nizza 52, Torino, Italy. Tel.: +39 011 6709542/5601; fax: +39 011 2365601.
E-mail address: giuseppe.matullo@unito.it (G. Matullo).

[1] Equally contributed to this work.

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Introduction

Cardiovascular diseases (CVD) are the leading cause of mortality, morbidity and hospitalization in both genders in Europe and North America [1]. Diabetes mellitus, hypercholesterolemia, smoking, hypertension, obesity and physical inactivity are the primary risk factors for these diseases [2,3]. Other risk factors concur to the etiology such as low socioeconomic status, unhealthy dietary habits, alcohol or drugs abuse, lipoproteins, left ventricular hypertrophy. Non-modifiable risk factors include age, male gender, ethnicity and family history [4].

Several studies focused on the inverse relationship between B-vitamins intake and CVD risk [5]. As an example, it is well established that folates and some B-vitamins (B2, B6 and B12, folic acid) introduced with diet can reduce serum homocysteine (Hcy) levels promoting its re-methylation to methionine [6], and that an elevated plasma Hcy level is an independent risk factor for CVD [7].

In humans and in animal models, global decreased DNA-methylation was observed in atherosclerotic lesions, a condition linked to low intake of folates, methionine-rich diet, and elevated plasma Hcy levels [8], although human supplementation studies reported contrasting results [9,10]. DNA-methylation of CpG sites in the gene promoter region is an important determinant of gene expression, having an inverse relationship [11]. The mechanism by which folate and B-vitamins intake may modulate DNA-methylation depends on the activity of specific enzymes, many of which are included in the One Carbon Metabolism (OCM) [12]. The OCM is a complex network of biochemical reactions, involving the transfer of one-carbon groups

Table 1 List of candidate genes.

Gene ID	Gene name	Gene selection criteria
AHCY	adenosylhomocysteinase	[a]hsa00270:Cysteine and methionine metabolism
ALDH1L1	aldehyde dehydrogenase 1 family, member L1	[a]hsa00670:One carbon pool by folate
AMT	aminomethyltransferase	[a]hsa00670:One carbon pool by folate
APOE	apolipoliterature and/or gene function/interaction E	gene function and/or protein−protein interaction
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/ IMP cyclohydrolase	[a]hsa00670:One carbon pool by folate
BHMT	betaine-homocysteine methyltransferase	[a]hsa00270:Cysteine and methionine metabolism
CBL	Cas-Br-M (murine) ecotropic retroviral transforming sequence	gene function and/or protein−protein interaction
CBS	cystathionine-beta-synthase	[a]hsa00270:Cysteine and methionine metabolism
CTH	cystathionase (cystathionine gamma-lyase)	[a]hsa00270:Cysteine and methionine metabolism
DHFR	dihydrofolate reductase	[a]hsa00670:One carbon pool by folate
DNMT1	DNA (cytosine-5-)-methyltransferase 1	[a]hsa00270:Cysteine and methionine metabolism
FOLH1	folate hydrolase (prostate-specific membrane antigen) 1	[a]hsa00670:One carbon pool by folate
FOLR1	folate receptor 1	gene function and/or protein−protein interaction
FOLR2	folate receptor 2	gene function and/or protein−protein interaction
FTCD	formiminotransferase cyclodeaminase	gene function and/or protein−protein interaction
GART	phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase	[a]hsa00670:One carbon pool by folate
MAT1A	methionine adenosyltransferase I, alpha	[a]hsa00270:Cysteine and methionine metabolism
MAT2B	methionine adenosyltransferase II, beta	[a]hsa00270:Cysteine and methionine metabolism
MTHFD1	methylenetetrahydrofolate dehydrogenase (NADP + dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase	[a]hsa00670:One carbon pool by folate
MTHFD1L	methylenetetrahydrofolate dehydrogenase (NADP + dependent) 1-like	[a]hsa00670:One carbon pool by folate
MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP + dependent) 2, methenyltetrahydrofolate cyclohydrolase	[a]hsa00670:One carbon pool by folate
MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP + dependent) 2-like	gene function and/or protein−protein interaction
MTHFR	5,10-methylenetetrahydrofolate reductase (NADPH)	[a]hsa00670:One carbon pool by folate
MTHFS	5,10-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)	[a]hsa00670:One carbon pool by folate
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	[a]hsa00270:Cysteine and methionine metabolism,[a]hsa00670:One carbon pool by folate
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase	gene function and/or protein−protein interaction
NNMT	nicotinamide N-methyltransferase	gene function and/or protein−protein interaction
PON1	paraoxonase 1	gene function and/or protein−protein interaction
RFC1	replication factor C (activator 1) 1, 145 kDa	gene function and/or protein−protein interaction
SHMT1	serine hydroxymethyltransferase 1 (soluble)	[a]hsa00670:One carbon pool by folate
SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	[a]hsa00670:One carbon pool by folate
TCN2	transcobalamin II; macrocytic anemia	gene function and/or protein−protein interaction
TYMS	thymidylate synthetase	[a]hsa00670:One carbon pool by folate

[a] KEGG, Kyoto Encyclopedia of Genes and Genomes, Nucleic Acids Res. 2000; 28:27−30.