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Alcohol abuse and cigarette smoking are associated with global DNA hypermethylation: Results from the German Investigation on Neurobiology in Alcoholism (GINA)

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ABSTRACT

Recent studies have shown that smoking and alcoholism may be associated with altered DNA methylation and that alcohol consumption might induce changes in DNA methylation by altering homocysteine metabolism. In this monocenter study, we included 363 consecutive patients referred for hospitalization for alcohol detoxification treatment. Blood samples were obtained on treatment days 1, 3, and 7 for measurement of global DNA methylation in leukocytes by liquid chromatography tandem mass spectrometry. Genomic DNA was used for genotyping the following seven genetic variants of homocysteine metabolism: cystathionine beta-synthase (CBS) c.844_855ins68, dihydrofolate-reductase (DHFR) c.594 + 59del19bp, methylenetetrahydrofolate-reductase (MTHFR) c.677C > T and c.1298A > C, methyltetrahydrofolate-transferase (MTR) c.2756A > G, reduced folate carrier 1 (RFC1) c.80G > A, and transcobalamin 2 c.776C > G. Multivariate linear regression showed a positive correlation of global DNA methylation with alcohol consumption and smoking on day 1 of hospitalization. DNA methylation was not correlated with homocysteine or vitamin plasma levels, nor with the tested genetic variants of homocysteine metabolism. This suggests a direct effect of alcohol consumption and smoking on DNA methylation, which is not mediated by effects of alcohol on homocysteine metabolism.

Introduction

Tobacco and alcohol are the most commonly used noxious compounds worldwide. Cigarette smoking and alcoholism significantly increase the risk for a variety of medical and psychiatric conditions and different forms of cancer (Thun et al., 1997; Zaridze et al., 2009). Because of the high prevalence of alcoholism and tobacco abuse and their negative health consequences, it is important to understand the mechanisms involved in alcohol and tobacco dependence and toxicity. A growing number of studies have shown that alcoholism and chronic alcohol intake in non-addicted subjects may be associated with altered DNA methylation (Bönsch, Lenz, Reulbach, Kornhuber, & Bleich, 2004; Harlaar & Hutchison, 2013; Starkman, Sakharkar, & Pandey, 2012). Some studies have demonstrated an additive effect of alcohol consumption and smoking on changes in DNA methylation (Thapar, Covault, Hesselbrock, & Bonkovsky, 2012). Alcohol might induce changes in DNA methylation by altering homocysteine metabolism (Bleich et al., 2000; Bleich & Hillemacher, 2009; Bönsch et al., 2004; Varela-Rey, Woodhoo, Martinez-Chantar, Mato, & Lu, 2013).

DNA methylation depends on S-adenosylmethionine (SAM) as a methyl group donor. The demethylated residue of SAM is S-adenosylhomocysteine (SAH), which is reversibly hydrolyzed to homocysteine. In a vitamin B6-dependent pathway, homocysteine can be trans-sulfurated to cystathionine and cysteine (Fig. 1). Alternatively, homocysteine can be remethylated, depending on the essential co-factors folate, vitamin B2, and vitamin B12. Because

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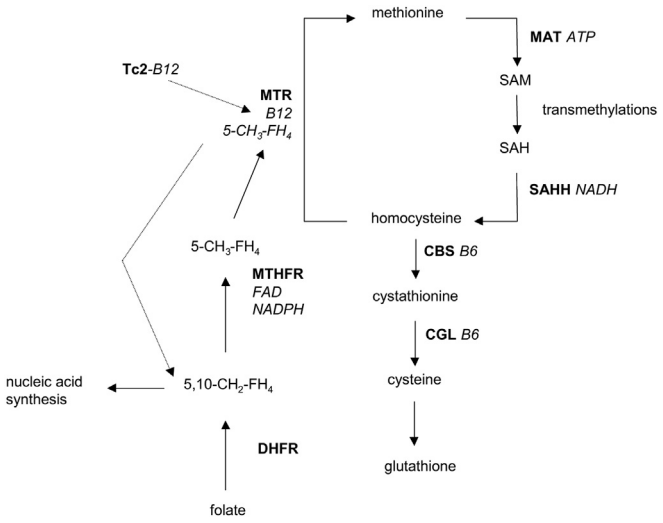

Fig. 1. Homocysteine metabolism. The sulfur-containing amino acid methionine is activated to S-adenosylmethionine (SAM), which is a ubiquitous methyl group donor. The degradation product of SAM is S-adenosylhomocysteine (SAH), which is hydrolyzed to homocysteine. Homocysteine can be remethylated to methionine and SAM via methionine synthase (MTR), which depends on derivatives of folate and vitamin B12 as cofactors. Lack of these vitamins is a common cause of hyperhomocysteinemia (Mudd et al., 2001). The folate derivative is synthesized by methylenetetrahydrofolate reductase (MTHFR) and dihydrofolate reductase (DHFR), and the derivative of vitamin B12 is transported by transcobalamin 2 (Tc2). Alternatively, homocysteine can be transsulfurated by vitamin B6-dependent cystathionine β-synthase (CBS) and cystathionine gamma-lyase (CGL) to cysteine as a component of glutathione. Due to the existence of several functional variants in the genes involved in homocysteine metabolism, and to differences in dietary vitamin and amino acid uptake, disorders of homocysteine metabolism exhibit marked inter-individual differences.

vitamin deficiency commonly occurs in alcohol-dependent patients, alcohol-induced changes in DNA methylation and homocysteine might be explained by vitamin deficiencies (Cravo & Camilo, 2000; Heese et al., 2012). Not only the vitamin status, but also genetic variants, may modify folate, vitamin B12, and homocysteine metabolism (Stover, 2011). Alcohol-induced changes of DNA methylation are possibly influenced by these genetic variants, which are common in the general population.

In a cohort of 363 patients with alcohol dependency, we analyzed changes of DNA methylation and attempted to identify parameters related to homocysteine metabolism, which may mediate or modify the association of alcohol and DNA methylation, i.e., plasma levels of homocysteine and vitamins involved in homocysteine metabolism as well as genetic variants of homocysteine metabolism.

Materials and methods

Patients

The present study is part of the German Investigation on Neurobiology in Alcoholism (GINA) (Heese et al., 2012). Consecutive patients were recruited from the Department of Addiction and Psychotherapy of the LVR-Clinic in Bonn, Germany (Heese et al., 2012). All participants were diagnosed with alcohol dependency according to ICD-10 and were included in the study on admission for alcohol detoxification. Patients were mainly detoxified with clomethiazole following a symptom-triggered regime using the Banger Score (Banger, Philipp, Herth, Hebenstreit, & Aldenhoff, 1992). If, for clinical reasons, clomethiazole could not be used, benzodiazepines were administered. Patients diagnosed with dependence from other substances were excluded. Daily alcohol consumption was calculated per day, according to patients' self-reported alcohol consumption during the last week before admission to the hospital. Fasting blood samples were obtained on days 1 (admission), 3, and 7 of the detoxification treatment. Blood samples were centrifuged and consecutive serum and lithium heparin plasma samples were stored at −80 °C immediately after collection. Homocysteine and global DNA methylation were assessed at all three time points, while vitamin serum levels were obtained at admission.

This study was approved by the local ethics committee. All patients gave their informed written consent.

Biochemical measurements

Serum alcohol concentrations were measured by an enzymatic test (alcohol dehydrogenase method) with a Dimension Vista™ system (Siemens Healthcare Diagnostics, Eschborn, Germany).

Serum alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, and gamma glutamyl transferase (GGT) activity were measured by means of an enzymatic test (ALTI method, AST method, GGT method) with a Dimension Vista™ system (Siemens Healthcare Diagnostics). Reference intervals for ALT ranged up to 45 IU/L for men and 34 IU/L for women, reference intervals for AST ranged up to 35 IU/L for men and 31 IU/L for women, and reference intervals for GGT ranged up to 55 IU/L for men and 38 IU/L for women.

Serum carbohydrate-deficient transferrin (CDT) and serum transferrin were measured by means of particle-enhanced immunonephelometry using a BN Prospec™ System (Siemens Healthcare Diagnostics). Reference intervals given by Siemens Healthcare Diagnostics ranged from 28.1 to 76.0 mg/L (1st to 99th percentile). CDT values with reference to the results obtained with the N antiserum to human transferrin ranged from 1.19 to 2.47%.

Blood cell count was measured using a Sysmex XE 5000™ System (Sysmex Corporation, Kobe, Japan). Erythrocyte count was performed in a separate measuring channel according to the principle of impedance measurement with hydrodynamic focusing. Measurement of hematocrit was based on the precise erythrocyte count. The impulse of each cell is proportional to its cell volume. Cumulative impulse height summation adds the analyzed impulses and corresponding cell volumes in a defined sample volume to reach the hematocrit value. Mean corpuscular volume (MCV, [femtoliter]) was calculated from hematocrit [%] × 10 divided by erythrocyte count [million/μL].

Homocysteine was determined by fully automated particle-enhanced immunonephelometry with a BN II System (Siemens Healthcare Diagnostics, Eschborn, Germany) by enzymatic conversion to S-adenosyl-homocysteine (SAH). The reference range for homocysteine is 5.8–11.9 μmol/L. The intra-assay coefficient of variation of the homocysteine assay was 3.4% (mean: 11 μmol/L, $n = 20$); the inter-assay coefficient was 5.6% (mean: 11 μmol/L, $n = 20$).

Plasma concentrations of vitamin B1, vitamin B2, and vitamin B6 were analyzed using commercially available HPLC assays (Chromsystems Instruments & Chemicals GmbH, Munich, Germany) on an HPLC system with a fluorescence detector (Agilent Series 1200, Agilent, Waldbronn, Germany). Reference intervals given by Chromsystems were 66.5–200 nmol/L for vitamin B1, 174–471 nmol/L for vitamin B2, and 35.2–110.5 nmol/L for vitamin B6. The intra-assay coefficient of variation of the vitamin B6 assay was 5.3% (mean: 10.4 μg/L; $n = 20$), while the inter-assay coefficient of variation was 6.5% (mean: 10.5 μg/L; $n = 20$).

Plasma concentrations of vitamin B12 and folate were measured by means of a competitive chemiluminescence immunoassay with an Access™ Immunoassay System (Beckman Coulter, Krefeld, Germany), according to the manufacturer's instructions. The reference