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Sponsorship: Supported by a grant (B.L., M.L., S.B., T.H.) from the German Research Foundation (DFG: BL 577/5-1).

Abstract
The objective of this study was to investigate whether polymorphisms of genes that are involved in one-carbon metabolism (dihydrofolate reductase, methionine synthase reductase, methylenetetrahydrofolate reductase, reduced folate carrier 1 and transcobalamin II) influence DNA methylation in 106 patients with alcoholism. In the multivariate model no genotype showed significant effects on DNA methylation.

Keywords: polymorphisms, one-carbon metabolism, alcoholism, addiction
There is growing evidence that alcoholism leads to alterations in homocysteine and folate plasma levels which is followed by alterations of global as well as promoter-specific DNA methylation (Bleich et al., 2006, Bönsch et al., 2004). This study was performed to investigate whether functionally relevant polymorphisms of genes involved in one-carbon metabolism modify global DNA methylation in patients with alcoholism.

We investigated 106 patients (86 men, 20 women, age: mean = 43.7 years, SD = 8.6 years) who were admitted for alcohol detoxification treatment. All of them had an established diagnosis of alcohol dependency according to DSM-IV. Analysis of global genomic DNA methylation and of polymorphisms were performed as described previously (Bönsch et al., 2004; Linnebank et al., 2006). In detail we analyzed the polymorphisms dihydrofolate reductase (DHFR) c.594+59del19bp (change of transcript level), methionine synthase reductase (MTRR) c.66A>G (p.I49M), methylenetetrahydrofolate reductase (MTHFR) c.677C>T (A222V), reduced folate carrier 1 (RFC 1) c.80G>A (p.R27H) and transcobalamin II (TCNII) c.776C>G (p.P259R). We applied a significance level of \( \alpha \leq 0.05 \) (two-sided) using a multivariate general linear model computing the genotypes as fixed factors and DNA methylation as well as fasting total plasma homocysteine as dependent variables. The study was approved by the local Ethics Committee of the University of Erlangen-Nuremberg. All participants gave written informed consent.

The genotype frequencies were as follows:

- **DHFR c.594+59del19bp**: absent/absent/absent/present/present/present: 0.30/0.57/0.13,
- **MTRR c.66A>G**: AA/AG/GG: 0.29/0.60/0.10,
- **MTHFR c.677C>T**: CC/CT/TT: 0.45/0.42/0.12,
- **RFC 1 c.80G>A**: GG/AG/AA: 0.17/0.52/0.31,
- **TCNII c.776C>G**: CC/CG/GG: 0.26/0.47/0.26.

In the multivariate model no genotype showed significant effects on homocysteine plasma levels or on DNA methylation: (I) DNA methylation (R2=.095, adjusted R2=-.001; F=.993, p=.455; TCNII: F=.097, p=.908; RFC 1: F=1.412, p=.249; MTHFR: F=.836, p=.436; DHFR: F=1.714, p=.186; MTRR: F=.538, p=.586) (II) homocysteine (R2=.056, adjusted R2=-.043; F=.565, p=.838; TCNII: F=.063, p=.939; RFC 1: F=.404, p=.669; MTHFR: F=.651, p=.524; DHFR: F=.558, p=.574; MTRR: F=1.235, p=.295). Our study was sufficiently powered to detect an overall effect of the genotypes explaining at least 10% of the variance of global methylation (R2>0.1, 1-ß=0.8).

Contrary to our expectations and previously reported data in colorectal cancer (Mokarram et al., 2008) the investigated polymorphisms did not have any significant effect on global DNA methylation. This might be up to two reasons. (I) It might be a tissue specific effect. We analysed DNA from peripheral leukocytes and did not investigate brain tissue. (II) The missing impact might be due to the investigation of alcohol dependent patients. Global DNA methylation was quantified in peripheral blood of patients that were admitted for detoxification. Therefore the missing impact of the investigated genetic polymorphisms on global DNA methylation probably depends on the underlying disease. Also heterogeneity within the group of alcohol dependent patients may account to the insignificant results. It cannot be excluded that investigation of patients suffering from other diseases or investigation of healthy control persons respectively may result in more significant results.

The investigated polymorphisms were chosen because of effects on global DNA methylation reported before. Nevertheless there are other polymorphisms that influence one-carbon metabolism that will be interesting subjects of future studies.
In summary, the presented data does not support the hypothesis that functionally relevant polymorphisms of one-carbon metabolism have a major modifying effect on global DNA methylation or on homocysteine plasma levels in patients with alcoholism. This study focused on an impact of the investigated genetic polymorphisms on global DNA methylation. Further studies should explore whether there are significant effects of the investigated polymorphisms on site specific DNA methylation.


