

Alterations in Homocysteine Metabolism Among Alcohol Dependent Patients – Clinical, Pathobiochemical and Genetic Aspects

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Abstract: Addiction research focusing on homocysteine metabolism and its association with aspects of alcohol dependence has revealed important findings. Recent literature on this topic has been taken into account for the review provided.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the homocysteine metabolism. Plasma homocysteine levels are influenced by the single-nucleotide polymorphism (SNP) MTHFR C677T. Besides genetic factors, environmental factors have an impact on homocysteine plasma levels too. Thus, chronic alcohol intake is associated with elevated homocysteine plasma concentrations. Elevation of plasma homocysteine concentration is considered as a predictor for the occurrence of alcohol withdrawal seizures and – as homocysteine is a cardiovascular risk factor – might contribute to the higher risk for myocardial infarction among alcohol dependent patients.

Homocysteine acts as an N-methyl-D-aspartate (NMDA) receptor agonist and has excitotoxic effects. Furthermore, it has been demonstrated that homocysteine has neurotoxic effects especially on dopaminergic neurons. As the rewarding effects of alcohol are mediated by the dopaminergic system, a homocysteine-dependent impairment of the reward system possibly leads to an altered drinking behaviour according to the deficit hypothesis of addiction. Homocysteine is involved in the metabolism of methyl groups and DNA-methylation plays a role in regulation of gene expression. Therefore it has been suggested that homocysteine is an important epigenetic factor. It remains to be determined whether alcohol dependent patients benefit from homocysteine lowering strategies, e.g., *via* supplementation of folate, vitamin B6 and B12. In this respect it is not clear yet, if a supplementation therapy can reduce the risk for the occurrence of alcohol withdrawal seizures.

Keywords: Homocysteine, folate, alcohol, MTHFR, polymorphism, dopaminergic reward system, epigenetics, gene expression.

HOMOCYSTEINE - BIOCHEMISTRY

Homocysteine is a sulphur-containing amino acid, which is produced as intermediate product in the metabolism of the essential amino acid methionine. It is no component of naturally occurring proteins, it is of importance for the one-carbon-metabolism. Homocysteine, folate and methyl group metabolism are linked processes [1]. S-adenosylmethionine (SAM) is a methyl group donor for a number of methyltransferases, resulting in the methylation of substrates such as nucleic acids, proteins and lipids [2]. SAM-dependent methyltransferase reactions lead to the production of S-adenosylhomocysteine (SAH), which is converted thereafter to homocysteine. Homocysteine can be remethylated to methionine *via* folate-dependent or folate-independent mechanisms. The folate-dependent remethylation is catalyzed by the Vitamin B12-dependent enzyme methionine synthase (MS) utilizing a methyl group from 5-methyltetrahydrofolate (5-CH₃-THF). The folate-independent remethylation is catalyzed by Betaine-homocysteine S-methyltransferase (BHMT) using betaine. Homocysteine can also be catabolised through the transsulfuration to cysteine. This pathway begins with the irreversible conversion to cystathionine by cystathionine beta-synthase (CBS), for the reaction described Vitamin B6 is needed as cofactor [3] (Fig. 1).

DETERMINANTS OF PLASMA HOMOCYSTEINE CONCENTRATIONS

Plasma levels of homocysteine are influenced by several factors. Homocysteine levels are higher in men compared to women and they tend to increase with age. An inverse relation to physical activity has been found. Furthermore a dose-dependent relation between the homocysteine level and the number of cigarettes smoked per day has been observed. Nutritional habits have an impact on homocysteine levels too, thus low intake of folate, Vitamin B6 or B12 as well as high coffee consumption are associated with elevated homocysteine levels [5-7]. Taking together the factors described, it has been suggested that the elevation of homocysteine plasma levels may be an expression of unhealthy lifestyles [8, 9]. Plasma homocysteine values are also dependent of renal function, high serum creatinine levels are associated with high homocysteine levels [7].

In addition genetic factors contribute to the extent of homocysteine plasma concentrations. A mutation within the gene coding for cystathionine beta-synthase (CBS) results in an autosomal recessive inherited hyperhomocysteinemia [10]. Beside CBS the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) plays an important role in one-carbon metabolism. There are known several polymorphisms within the MTHFR-gene [11, 12]. The influence of the single nucleotide polymorphism (SNP) MTHFR C677T on plasma homocysteine levels is well documented [13]. The T-allele causes an exchange of the amino acid alanine for valine in the amino acid sequence which leads to a thermola-

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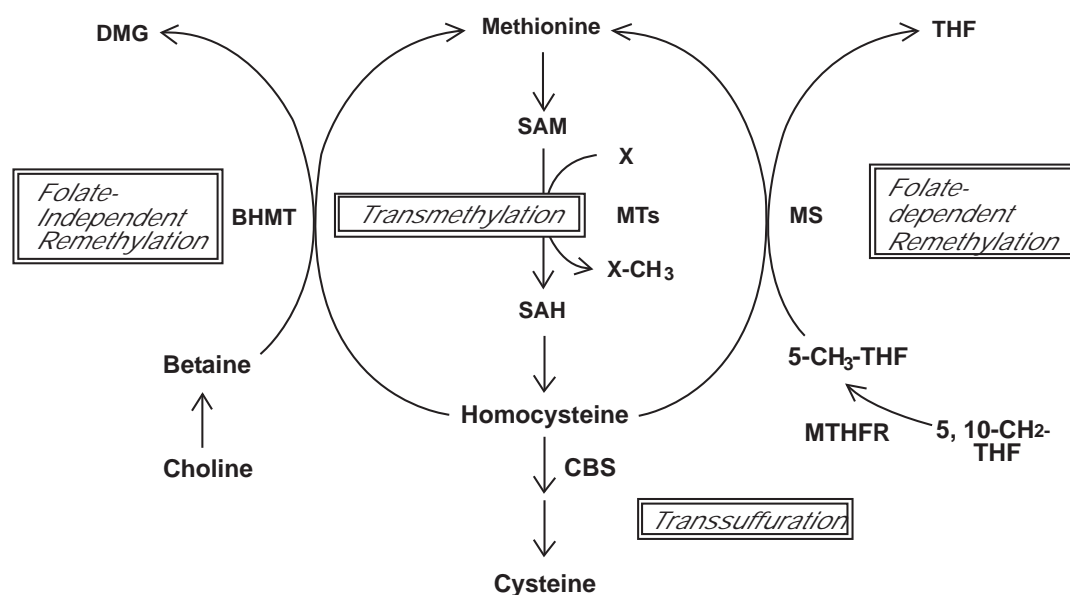


Fig. (1). Homocysteine, folate and methyl-group metabolism. Abbreviations used: BHMT: Betaine-homocysteine S-methyltransferase; CBS: cystathionine beta-synthase; DMG: dimethylglycine; MS: methionine synthase; MTHFR: 5,10-methylenetetrahydrofolate reductase; MTs: methyltransferases; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; THF: tetrahydrofolate (adapted from Williams and Schalinske, 2007 [4]).

bile form of the enzyme. Gudnason *et al.* postulate that this polymorphism explains up to 12.3% of the variance of plasma homocysteine [14]. The homozygous MTHFR 677TT genotype is found in approximately 8-11% of the European Caucasian population and the heterozygous MTHFR 677CT genotype in 39-43% respectively [15, 16].

Investigations of both MTHFR C677T polymorphism and vitamin and folate status revealed a synergistic impact on homocysteine plasma concentrations. This association between both factors is an example for a well studied gene-environmental-interaction [16, 17].

Further investigations dealt with the association between plasma homocysteine and polymorphisms of genes involved in the one-carbon metabolism: e.g. methionine synthase (MS), methionine synthase reductase (MTRR) and catechol-o-methyltransferase (COMT). However the results concerning this topic remain inconsistent, studies finding a positive correlation reveal at best a moderate influence of the SNPs [18-22]. The range for normal plasma homocysteine concentrations is between 5 and 15 $\mu\text{mol/l}$, levels are moderately elevated between 16 and 30 $\mu\text{mol/l}$, intermediately elevated between 31 and 100 $\mu\text{mol/l}$ and markedly elevated above 100 $\mu\text{mol/l}$ [23].

PATHOGENIC CHARACTERISTICS OF HOMOCYSTEINE

Several studies dealt with the relation of homocysteine metabolism and various clinical conditions.

Vasculotoxicity

Elevation of homocysteine plasma levels is considered as independent cardiovascular risk factor [24, 25].

The role of MTHFR C677T polymorphism for the occurrence of occlusive vascular disease and coronary heart disease (CHD) respectively has also been intensively studied.

Meta-analysis revealed inconsistent findings. An elevated risk for CHD could be shown for individuals with MTHFR 677TT genotype (odds ratio 1.16, 95% confidence interval 1.05-1.28) comparing carriers of MTHFR 677TT genotype with MTHFR 677CC genotype [26] whereas in another meta-analysis, Lewis *et al.* found the association of MTHFR 677TT genotype with CHD dependent on the geographical region where the study has been carried out: in Asia and in the middle east but not in Europe, Australia and north America an effect of MTHFR C677T polymorphism was detected [27].

Vasculotoxic effects of homocysteine are explained by different mechanisms. A homocysteine-mediated endothelial dysfunction, reduction of anticoagulant processes, augmentation of thrombin production, increase in platelet reactivity and an alteration of production of endothelium-derived nitric oxide (eNO) might contribute to the vasculotoxic, thrombogenic and atherogenic qualities of homocysteine [28-30].

Cancerogenesis

There is growing evidence that homocysteine and folate metabolism is involved in cancerogenesis.

Especially for epithelial cancers, such as squamous cell carcinoma of the head and neck, cervical neoplasia or breast cancer an association between cancer risk and high homocysteine and low folate status has been described [31-34].

Regarding the potential role of polymorphisms of genes involved in homocysteine and folate metabolism for cancerogenesis remain inconclusive, the results mainly depend on the type of cancer studied and in which population the study was carried out. A protective effect of MTHFR 677TT, MTHFR 1298CC and MS 2756GG genotype against colorectal cancer is discussed [35-37], whereas for malignant lymphoma, a protective effect was only seen for MTHFR 677TT and MTHFR 1298AA genotype but not for MS

2756GG genotype [38]. However, there are studies supporting no substantial overall association between genetic polymorphism in the one-carbon metabolism and breast cancer risk [39].

It is still discussed, in which way alterations of homocysteine and folate metabolism might lead to an increased cancer risk. Homocysteine may have an impact on DNA damage and genomic DNA methylation [40-42].

Homocysteine and Pregnancy – Embryotoxic Effects

The occurrence of neural tube defects (NTDs) is influenced by folate intake. Homocysteine plasma levels are associated with the risk for NTDs [43]. Folate supplementation during early pregnancy can prevent NTDs [44]. Polymorphisms in MTHFR and MTRR are linked with an increased risk for NTDs [12, 45-47] and recurrent early pregnancy loss [48, 49].

Negative effects in-utero of homocysteine are attributed to embryotoxic characteristics of homocysteine [50], furthermore homocysteine-dependent DNA-methylation possibly alters patterns of gene expression at crucial timepoints during development.

Excitotoxicity and Neurotoxicity

Homocysteine acts as an N-methyl-D-aspartate (NMDA) receptor agonist and thus leads to an increased glutamatergic neurotransmission. It has neurotoxic and excitotoxic effects, this could be demonstrated *in vitro* and *in vivo* studies [51-53].

Homocysteine passes the blood-brain-barrier [54], additional functional disturbance of the blood-brain-barrier leads to an unprotected exposure of the brain to homocysteine. Homocysteine has various consequences for neural cells: oxidative stress, activation of caspases, mitochondrial dysfunction and increase of cytosolic calcium [55, 56]. Taken these factors together, apoptotic processes are initiated. Possibly due to its stimulating effects on neurotransmission, homocysteine can induce epileptic seizures if administered systemically in animal experiments [57, 58]. Another clinical aspect of homocysteine and its action on central nervous system is the development of homocysteine-related cognitive impairment. Nurk *et al.* conducted a six-year follow-up study of 2189 elderly subjects showing a concentration-dependent inverse association of plasma homocysteine with episodic memory assessed with the Kendrick Object Learning test [59]. Homocysteine is considered as a predictor for dementia and Alzheimer's disease [60, 61]. Brain atrophy in healthy elderly subjects, in elderly at risk for Alzheimer's disease and patients diagnosed with Alzheimer's disease is significantly linked with the homocysteine plasma concentration [62-64].

ALCOHOL CONSUMPTION AND HOMOCYSTEINE METABOLISM

Plasma homocysteine concentrations do not increase significantly due to a unique alcohol intoxication among healthy controls [65].

There is a controversial debate concerning the impact of moderate alcohol consumption on homocysteine plasma concentrations. As moderate alcohol consumption is linked

with a decreased risk for CHD [66], it has been tried to explain the so-called "French paradox" by changes in homocysteine and folate metabolism. However, epidemiological studies revealed contradictory results: the findings range from a negative [67, 68] to a positive [69, 70] until no association [14] between the two variables "alcohol consumption" and "plasma homocysteine". A J-curved relation has been suggested by several studies [71-73]. The different type of beverage used in the populations studied seems to account for the inconsistent results. Plasma homocysteine levels were not affected by moderate beer drinking, even though a positive association between alcohol consumption and plasma homocysteine could be shown for drinking spirits or wine [69,70]. In studies applying a controlled design, these observations could only be partly verified: Van der Gaag *et al.* were able to show that wine and spirit consumption leads to increased homocysteine plasma levels whereas consumption of beer did not [74]. The authors argued that this might be due the high vitamin B content of beer. In contrast to this result, Beulens *et al.* found out that beer consumption did not affect plasma homocysteine but lead to increased plasma vitamin B6 and decreased vitamin B12 levels [75]. In another controlled study, an increase of homocysteine plasma concentrations was independent of which alcoholic beverage was consumed. The elevation of homocysteine was accompanied by a decrease of plasma folate among the red wine and spirits group, whereas the folate status remained unchanged among the participants consuming beer [76].

It was shown that chronic alcohol intake among alcohol dependent patients redounds to markedly elevated homocysteine plasma concentrations [77-79], the data indicated that actively drinking alcoholics had twice the level of homocysteine in their plasma than did the healthy controls [78]. This is consistent with the finding of Hultberg *et al.* median values of homocysteine plasma concentration were 10.0 $\mu\text{mol/l}$ in the control group compared to 20.5 $\mu\text{mol/l}$ in the group of actively drinking alcoholics. It is noteworthy to mention that this effect seems to be reversible, abstinent alcoholics had a plasma homocysteine concentration of 9.5 $\mu\text{mol/l}$ [77].

In another study, plasma homocysteine levels were found to be significantly correlated with the extent of alcoholisation assessed with the blood-alcohol concentration among alcoholic subjects at admission [79]. The mean value of plasma homocysteine levels fell after cessation of drinking from 33.6 $\mu\text{mol/l}$ to 13.9 $\mu\text{mol/l}$ on day 3 after admission.

Several mechanisms contributing to the hyperhomocysteinemia have been discussed. A direct inhibition of MS by acetaldehyde, which is an alcohol breakdown product might cause an elevation of homocysteine levels [80]. Homocysteine is metabolized *via* remethylation and transsulphuration, for both reactions, folate, vitamin B6 as well as vitamin B12 are essential co-factors [5]. Reduced availability of folate, vitamin B6 and vitamin B12 cause an impairment of homocysteine metabolism. Low folate intake, poor absorption, decreased hepatic uptake and retention, increased urinary excretion of folate account for the folate deficiency observed among alcohol dependent patients [81]. Genetic factors involved in homocysteine and folate metabolism also may contribute to the association between elevated homocysteine plasma concentrations and alcohol dependence. An excess of MTHFR 677T-allele found among alcohol dependent pa-

tients compared to healthy control subjects potentially affects homocysteine plasma levels [82].

CLINICAL RELEVANCE OF ALTERATIONS OF HOMOCYSTEINE METABOLISM AMONG ALCOHOL DEPENDENT PATIENTS

As chronic alcohol intake is associated with both elevated plasma homocysteine levels [78] and an increased risk of myocardial infarction and stroke [83-85], a causal chain between these factors can be assumed.

Similarly, an important role for cancerogenesis has been ascribed to alterations in homocysteine metabolism and chronic alcohol consumption [86]. Thus, findings revealing a modified risk for esophageal cancer and colorectal cancer due to an interaction between alcohol consumption and MTHFR C677T polymorphism and MS A2756G polymorphism respectively are in accordance with this suggestion [87, 88].

Although a maternal periconceptional ingestion of alcohol does not seem to affect the risk for the occurrence of NTDs [89] which has been linked to folate metabolism, the use of alcohol during pregnancy can cause fetal alcohol syndrome (FAS). Pre- and postnatal growth retardation, craniofacial anomalies, heart defects, CNS malformation are main symptoms of FAS [90]. In addition to a direct toxic effect of alcohol on the embryo's CNS causing neuroapoptosis [91] and an impaired development of mitochondria [90], an indirect homocysteine-mediated effect contributing to neurotoxicity can be discussed.

Brain atrophy can be regarded as clinical sign of neurotoxicity which is detectable *via* neuroimaging. Among healthy elderly people [64] and patients with Alzheimer's disease [62] the extent of brain atrophy is significantly correlated with homocysteine plasma levels. This finding could be confirmed for alcohol dependent patients [92]. Due to the neurotoxic effects of homocysteine and reversibility of both alcohol-induced hyperhomocysteinemia and brain atrophy, a causal relationship can be assumed.

The glutamatergic system is involved in mediating craving as a crucial aspect of alcohol dependence [93]. Acamprostate is used as anti-craving drug acting as an NMDA receptor antagonist resulting in a decrease of glutamatergic neurotransmission [94, 95]. Homocysteine stimulates NMDA receptors and thus leads to an increased glutamatergic neurotransmission [52]. Therefore it has been assumed that the extent of craving during withdrawal assessed with the Obsessive Compulsive Drinking Scale (OCDS) is influenced by plasma homocysteine levels. However, in a study carried out by Hillemecher *et al.* no evidence was provided for a connection between homocysteine metabolism and craving [96].

A typology established by Lesch distinguishes alcohol dependent patients in four subtypes: type 1 is characterized by marked withdrawal symptoms including withdrawal seizures, type 2 shows pre-morbid conflicts and anxiety, type 3 evolves from a permissive milieu and suffers from mood-changes before becoming alcohol dependent and type 4 exhibits pre-alcoholic cerebral injuries and serious social problems [97]. Significant higher plasma homocysteine concentrations were found in patients classified to Lesch type 1 with a history of withdrawal seizure compared to the corre-

sponding patients classified to Lesch types 2-4 [98]. Taken together alcohol dependent patients having a seizure during alcohol withdrawal regardless of their Lesch-classification had significantly higher plasma homocysteine levels on admission than patients without a withdrawal seizure (84,7 +/- 29,8 $\mu\text{mol/l}$ vs 30,2 +/- 23,2 $\mu\text{mol/l}$; $p=0,0007$). The difference between both groups persists during detoxification of alcohol. Seizure patients also had significantly lower folate levels and higher blood alcohol concentrations. Values of plasma homocysteine level have been therefore suggested as a predictor of alcohol withdrawal seizures [99, 100]. If homocysteine plasma concentrations on admission for detoxification treatment are higher than 40 $\mu\text{mol/l}$, therapeutic consequences in terms of an additional administration of anti-convulsant medication can be considered. Furthermore, patients with a previous alcohol withdrawal seizure had significantly higher homocysteine levels compared to patients without a history of withdrawal seizure (mean 42.0 $\mu\text{mol/l}$ vs 22.5 $\mu\text{mol/l}$; $p<0.0001$) [101] pointing out that risk for the occurrence of withdrawal seizures may be a trait marker.

HOMOCYSTEINE AND ALTERATIONS OF THE DOPAMINERGIC SYSTEM

Dopamine is metabolized into its final metabolite homovanillic acid (HVA) by the action of two enzymes: catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) [102]. Plasma HVA is a marker estimating the central dopaminergic activity [103]. It has been demonstrated *in vivo* and *in vitro* studies that homocysteine has toxic effects especially on dopaminergic neurons [104, 105]. After intraperitoneal injections of homocysteine homocysteine levels in the striatal regions of mouse brains increased while locomotor activities and levels of HVA significantly decreased [54].

One study investigated the association of homocysteine and HVA levels in humans: among patients suffering from a depression increased plasma homocysteine concentrations were associated with lower HVA cerebrospinal fluid (CSF) levels as well as with the extent of depressivity assessed with the HAMILTON depression scale [106]. In depressed patients with a history of alcoholism HVA levels measured in CSF were found to be lower compared to healthy controls and to patients with a major depressive episode without a history of alcoholism [107]. Until now, it remains unclear whether the association between homocysteine plasma levels and depression or HVA levels respectively is more pronounced among alcohol dependent patients compared to patients with a major depression. A positive effect of folate and vitamin B12 treatment on depressive symptoms has been postulated [108, 109], in further studies it should be tried to elucidate a potential benefit of folate and vitamin B supplementation in regard of depressive symptoms especially among alcohol dependent patients.

Concerning the pathogenesis of Parkinson's disease a key aspect is an impairment of the dopaminergic system. During long-term treatment of Parkinson's disease with levodopa, an elevation of plasma homocysteine levels has been observed [110], whereas the extent of homocysteine elevation seems to depend on the genotype of MTHFR C677T-polymorphism [111]. Due to the neurotoxic impact of homocysteine on dopaminergic neurons, it has been suggested that long-term

levodopa therapy for Parkinson's disease may accelerate the course of disease by elevating homocysteine [17].

Similar conditions can be assumed for the dopaminergic system and addictive behaviour. The mesolimbic dopamine system appears to play a critical role in reinforcing alcoholism and in the rewarding effects of alcohol leading to the so called reward deficit hypothesis of addiction in order to explain addictive behaviour [112, 113]. As chronic alcohol consumption is associated with an elevation of homocysteine plasma levels, a homocysteine-mediated impairment of the dopaminergic reward system possibly leads – in terms of a vicious circle – again to increased alcohol intake.

According to this hypothesis there is growing evidence that HVA – as an indicator for central dopaminergic neuronal activity – is significantly lowered among alcohol dependent patients [114, 115]. Furthermore, it was shown that HVA levels are significantly influenced by MTHFR C677T-polymorphism in a group of alcohol dependent patients but not in a group of healthy controls. Carriers of MTHFR C677T T-allele with the diagnosis alcohol dependence had significantly lower HVA plasma levels compared to homozygote carriers of MTHFR C677T C-allele with the diagnosis alcohol dependence giving a further indicator for a link between homocysteine and monoamine metabolism [116]. In order to explain this finding, beside neurotoxic, direct metabolic mechanisms are also likely: homocysteine and monoamine metabolism are probably linked through tetrahydrobiopterin (BH4), the rate-limiting pteridine cofactor required in the synthesis of monoamine neurotransmitters [117]. Other genetic factors with a potential impact on HVA levels have been studied revealing no consistent results [115, 118, 119].

GENETICS

The role of MTHFR C677T-polymorphism among alcohol dependent patients is of special interest. Because of the alcohol-induced impairment of homocysteine metabolism in alcohol dependent patients, plasma homocysteine levels are strongly influenced by MTHFR C677T-polymorphism [120]. A hyperhomocysteinemia (more than $15\mu\text{mol/l}$) was observed in 84.2% of MTHFR C677T TT-genotype carriers, in 54.3% of heterozygote MTHFR C677T CT-genotype and in 31.6% patients with MTHFR C677T CC-genotype.

Elevated homocysteine plasma levels are considered as a trait marker for the risk for withdrawal seizures. Thus, it has been suggested that genetic factors contribute to the risk for withdrawal seizures. A trend for the MTHFR C677T T-allele frequency could be demonstrated applying a case-control design study: T-allele frequency increased from 0.28 in healthy control subjects to 0.33 in alcohol dependent patients suffering from mild withdrawal symptoms up to 0.40 in alcohol dependent men with a history of withdrawal seizure. In addition, differences between healthy male controls and male alcohol dependent patients regarding the MTHFR C677T T-allele frequency turned out to be significant (0.27 vs 0.37; $p=0.03$) [82].

Taking into consideration that plasma homocysteine levels were associated with certain subtypes of Lesch's classification [98], a study was undertaken in order to elucidate a potential relationship between subtypes of Lesch's classification and different MTHFR genotypes. In addition to MTHFR

C677T-polymorphism, MTHFR G1793A and MTHFR C393A-polymorphism have been investigated. Comparing patients classified to Lesch subtype 4 with patients classified to Lesch subtype 2+3, a significant higher frequency of MTHFR C677T TT-genotype was detected. Concerning the non-synonymous MTHFR C393A-polymorphism [121], significantly more MTHFR C393A A-carriers were found in patients classified to Lesch subtype 4. No differences between the Lesch subtypes were shown for MTHFR G1793A-polymorphism [122].

Most psychiatric disorders can not be attributed to mutations in a single gene. Recent research has demonstrated that epigenetic mechanisms have long-lasting effects within neurons regulating gene activity without impairing the DNA code [123]. DNA methylation is an important mechanism of gene regulation leading to gene repression. DNA methylation occurs by transferring a methyl group from SAM to cytosine residues at the dinucleotide sequence CpG, this reaction is catalyzed by DNA methyltransferases [124, 125]. Although CpG sequences within the genome are usually strongly methylated, those at the promoter regions of genes are methylated to a lesser extent, and thus, the amount of DNA methylation at the promoter correlates with the extent of gene inactivation. The donation of carbon atoms for maintenance of methylation patterns is mainly realized through the folate cycle. In healthy subjects, it was shown that global DNA methylation status in peripheral leukocytes depends on plasma homocysteine levels [126]. Because alterations of homocysteine and folate metabolism in alcoholics are well documented, homocysteine-dependent changes of global DNA methylation caused by chronic alcohol intake have been postulated. A significant increase of genomic DNA methylation among alcohol dependent patients has been observed. Additionally, DNA methylation significantly correlated with plasma homocysteine concentrations [127].

Further research has focused on the regulation of alpha synuclein promoter. Alpha synuclein plays a critical role in dopaminergic neurotransmission, it regulates synaptic dopamine homeostasis and influences the expression of dopamine synthesis genes [128, 129]. An elevated expression of alpha synuclein mRNA in alcohol dependent patients has been demonstrated, which is associated with the extent of craving [130]. Bönsch *et al.* observed a significant increase of the alpha synuclein promoter DNA methylation which was significantly dependent on homocysteine plasma levels in alcohol dependent patients [131].

Another study dealt with the expression regulation of homocysteine-induced endoplasmic reticulum protein (HERP), a protein involved in the intracellular defense system. Analyzing alcohol dependent patients and healthy controls, a significant elevation of HERP promoter DNA methylation was found in alcohol dependent patients, which again was associated with plasma homocysteine levels. Furthermore, HERP mRNA expression was significantly lower in patients with alcohol dependence compared to healthy controls [132].

CONCLUSIONS AND FUTURE PROSPECTS

Homocysteine metabolism contributes to several important aspects of alcohol dependence. Further controlled studies are needed trying to elucidate whether alcohol dependent

patients benefit from homocysteine lowering strategies, e.g., *via* supplementation of folate, vitamin B6 or vitamin B12 respectively. In this respect it is not clear, if a supplementation therapy can reduce the risk for the occurrence of alcohol withdrawal seizures or has an influence on the extent of depression or craving.

Further evidence for the suggested linkage between homocysteine and dopamine metabolism and its relevance for addiction is needed. In view of the findings for alcohol dependence the question arises whether the results are specific for alcohol dependence or if they are transferable to other types of addiction.

Concerning the important field of research on gene expression and the involvement of homocysteine metabolism herein, further studies are needed focusing on clinical relevance for alcohol dependency.

Learning Objectives – Summary:

- Homocysteine metabolism is involved in important aspects of alcohol dependence.
- Elevated homocysteine concentrations on admission for alcohol detoxification treatment can be considered as predictor for withdrawal seizures.
- The risk for the occurrence of alcohol withdrawal seizures is modified by MTHFR C677T-polymorphism.
- Homocysteine has neurotoxic effects on the dopaminergic system.
- Homovanillic acid levels are significantly influenced by MTHFR C677T-polymorphism among alcohol dependent patients but not among healthy controls.
- Homocysteine and folate metabolism are involved in the regulation of gene expression.

Future Research Questions:

- Do alcohol dependent patients profit from homocysteine lowering strategies e.g. *via* supplementation of folate, vitamin B6 or vitamin B12?
- Is there further evidence for a linkage between homocysteine and monoamine metabolism?
- In which extent do neurotoxic effects of homocysteine contribute to an impairment of dopaminergic reward system?
- Are the findings regarding the association between alcohol dependence and homocysteine metabolism specific for alcoholism or are they transferable to other types of addictions?
- Can homocysteine-dependent changes in gene expression explain clinical aspects of alcohol dependence?

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Received: August 6, 2007

Revised: August 12, 2007

Accepted: September 10, 2007