Homocysteinemia as well as methylenetetrahydrofolate reductase polymorphism are associated with affective psychoses

Andreas Reif*, Bruno Pfuhlmann, Klaus-Peter Lesch

Clinical and Molecular Psychobiology, Department of Psychiatry and Psychotherapy, Julius-Maximilians-University of Würzburg, Füchsinistr. 15, 97080 Würzburg, Germany

Accepted 17 June 2005
Available online 1 August 2005

Abstract

In the recent years, elevated homocysteine plasma levels have been reported to represent a risk factor not only for atherosclerosis, but also to be associated with dementia, depression and—in a gender-specific manner—schizophrenia. Here, we explored a possible association between homocysteinemia and psychiatric disorders. Fasting homocysteine, vitamin B12 and folate were determined in an ethnically homogeneous female population with different psychiatric disorders. Homocysteine was not elevated in females suffering from schizophrenia (mean, 11.6 ± 5.8 µmol/l). As shown previously, increased homocysteine concentrations were associated not only with dementia of different aetiology (mean, 17.2 ± 6.7 µmol/l; \( \chi^2 = 23.39, p < 0.001 \), compared to the schizophrenia group), but also with depressive disorders (mean, 12.9 ± 3.8 µmol/l; \( \chi^2 = 6.88, p = 0.009 \)). B12 and folate levels did not differ between different diagnostic groups. To further explore the connection between homocysteinemia and affective psychoses, a case–control study examining the C677T and the A1298C variants of methylenetetrahydrofolate reductase was conducted. The latter polymorphism not only was associated with affective psychoses in general, but also when divided in unipolar depression and bipolar affective disorder. In conclusion, we suggest that in females homocysteinemia is an unspecific risk factor for organic brain disorders like dementia, and possibly depression, but not for schizophrenia.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Bipolar disorder; Dementia; Depression; Homocysteine; Methylenetetrahydrofolate reductase; Polymorphism; Psychosis; Schizophrenia

1. Introduction

The non-protein amino acid homocysteine (HCY) occurs in humans by the demethylation of nutritional methionine, catalyzed by methyltransferases. Its major catabolic pathways involve the enzymes cystathionine B synthase and methionine synthase; the latter depending on tetrahydrofolate and vitamin B12, so that homocysteine degradation is closely linked to the nutritional status regarding those vitamins. In severe cases of homocysteinemia, which are relatively rare, mutations in key enzymes of homocysteine metabolism can be found. In contrast, most cases of mild homocysteinemia are due to nutritional folate and vitamin B deficiency, and/or reduced glomerular filtration rate. Furthermore, common genetic polymorphisms in the methylenetetrahydrofolate reductase gene explain some of the variance in HCY levels, with C677T being the most extensively investigated SNP. Another variant, acting synergistically with 677T, is the A1298C transition, which was also shown to be associated with higher HCY levels (Weisberg et al., 1998, 2001).

Furthermore, considerable gene × environment interactions do exist: for example, 677T seems to impact with greater effect on vegetarians, compared to controls (Geisel et al., 2003).

HCY is a potent cytotoxin and shows an association with atherosclerosis (Christen et al., 2000; Mangoni and Jackson,
of-convenience”-strategy. The women were recruited consecutively from an admission ward for female inpatients with acute psychiatric disorders. The age ranged from 18 to 92 years. Patients who abused substances or who were substituted with vitamins were excluded from subsequent data analysis (13 subjects). None of the subjects received phenytoine, primidone and phenobarbital, as well as fibrates/cholestermyrine and methotrexate-like immunosuppressants, which influence HCY and folate levels. Four patients were treated with carbamazepine and 12 with valproic acid, however, neither HCY nor folate levels were significantly different ($p > 0.50$, Student’s $t$-test).

For MTHFR genotyping, a total of 136 patients (of both sexes) from Lower Franconia (Germany) were recruited, which were ascertained as inpatients of the Department of Psychiatry. Ninety-one patients suffered from bipolar affective (Bip) disorder; only patients with a minimum of one manic plus one depressive episode were classified as Bip (i.e., strict bipolar-I criteria). A further 45 patients with unipolar depression (MD) were also included in the study, however only when absolutely no signs of bipolarity were present throughout the whole disease course. Those patients fulfilled the DSM-IV criteria of recurring depressive disorder (American Psychiatric Association, 1994), and had at least two depressive episodes. The average age was 50 years for the Bip and 59 years for the MD group, respectively.

A sample of DNA probes has been collected from of 284 control subjects consisting of healthy blood donors from the same region as the patient group; the sample was not screened for a history of psychiatric disorders. All control subjects were free of medication, and the study was explained to them, so that the chance that severe psychiatric disorders were present in the control sample was low. Mean age of controls was 35 years.

Diagnoses in all cases were made by consensus of two experienced psychiatrists (A.R., and B.P. or K.-P.L.) by an extensive, semi-structured interview and followed the ICD-10 criteria (World Health Organisation, 1992). If possible, further information was retrieved from family informants and case records from other hospitals to ensure consistent diagnoses. Chart reviews of every patient were done by A.R. None of the subjects showed significant neurological comorbidity, epilepsy, mental retardation, or a history of substance addiction, nor other organic disorders suggesting organic psychiatric disorder. Patients with an ICD-10 diagnosis of schizophrenia or schizoaffective disorder were also diagnosed according to the classification of Leonhard and Beckmann (1995). In all patients, routine laboratory tests and thorough clinical examination were assessed as were smoking status and psychotropic medication. Only patients and volunteers who gave written informed consent after oral as well as written explanation about the investigation were enrolled. The study was approved by the Ethics Committee of the University of Würzburg.
2.2. Homocysteine, folate and B12 determination

Blood was drawn after an overnight fasting period of >10 h to 3 days after admission. Total HCY serum levels were determined quantitatively by a fluorescence-polarization immunoassay (FPIA) with an AxSYM analyser (Abbott, Wiesbaden, Germany). Quantitative vitamin B12 and folate serum levels were determined with an electrochemo-luminescence immunoassay method (ECLIA) performed on an ELECSYS 2010 analyser (Roche Diagnostics, Mannheim, Germany). The cut-off point for HCY elevation was set at 12 μmol/l, representing the upper normal value for HCY in the Central Laboratory of the University of Würzburg.

2.3. Genotyping

DNA was extracted from venous blood by a routine method. Two exonic MTHFR polymorphisms have been determined using standard protocols (Weisberg et al., 2001; Kara et al., 2003), the functional C677T and the A1298C SNPs. Briefly, PCR reactions were performed in a reaction volume of 25 μl, including approximately 50 ng of template genomic DNA, 7 pmol of each primer, 2.5 mM of each dNTP, 10 mM MgCl2, 50 mM KCl, 10 mM Tris–HCl (pH 8.3 at 25 °C), 0.025 mg ml⁻¹ BSA, 0.025% Tween 20, and 1 U of Taq DNA Polymerase. Annealing temperature was 66 °C for A1298C, and 63 °C for C677T (35 cycles). For the determination of C677T, PCR products were digested with HinfI (overnight at 37 °C; fragment sizes: wildtype, 110 bp; SNP, 87 and 23 bp). A1298C PCR amplicons were digested with Fnu4HI (over night at 37 °C; fragment sizes: wildtype, 110 bp; SNP, 87 and 23 bp). A1298C PCR

2.4. Statistics

Statistical analysis of homocysteine levels was performed using the χ² and Student’s t-test by the Statistica 6 software package (StatSoft Germany, Hamburg, Germany). First, global differences between the three diagnostic groups were examined by applying a chi-square test. Thereafter, pair wise comparisons between the diagnostic groups were carried out by a posteriori χ² tests with Bonferroni correction for multiple testing. For the case–control association study, the deviation of genotype frequencies from Hardy–Weinberg equilibrium was determined separately for the two patient populations and the controls by calculating χ²-statistics with df=1 using an online calculator software. Association tests were performed by means of χ²-tests using SPSS for Windows 9.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. Homocysteine, vitamin B12 and folate levels in psychiatric disorders

A total of 120 subjects were enrolled in the first part study of this study, which was to determine HCY, vitamin B12 and folate in psychiatric disorders. According to ICD-10 criteria, 64 suffered from schizophrenia or schizoaffective disorder, 24 from affective psychoses and 32 from dementia or MCI. Mean HCY was 13.4 ± 6.0 μmol/l, mean folate 7.3 ± 3.8 ng/ml and mean B12 512 ± 340 pg/ml. Seventy-six of the 120 patients were smokers. HCY tended to be higher in smokers, which however was not statistically significant (12.1 vs. 13.8 μmol/l, p = 0.16, Student’s t-test). When determined across the whole data set, HCY did not correlate with B12 or folate, respectively (not shown). A positive correlation of HCY with age could be found (Fig. 1; r=0.47).

Thirty-two female patients suffered from dementia; 28 of those subjects had HCY levels above the cut-off (Table 1), and 6 had B12 levels below 200 pg/ml. In neither patient, autoimmune gastritis or other disorders were present to explain B12 deficiency, arguing for malnutrition in those subjects. Due to the heterogeneity of diagnoses, we did not perform a correlation of HCY with neuropsychological examinations or neuroimaging measures; however, more severely disabled patients tended to have higher HCY levels. Six of the 24 patients with affective psychoses suffered from bipolar affective disorders, the remaining 18 from recurring depressive disorder. None of these patients had significant renal impairment, but cardiovascular diseases were present in some of the older patients as it was of course the case in the dementia group. Sixteen of the 24 patients had elevated HCY, three patients had decreased B12 in the absence of relevant disorders, and folate was normal in all cases (Table 1). A correlation of elevated HCY with increasing age could be found in this group (r²=0.20).

Fig. 1. Positive correlation of homocysteine with increasing age in female psychiatric inpatients. Open diamonds represent patients with dementia, grey squares schizophrenic patients and closed circles symbolize depressed patients. HCY levels are correlated with age (r=0.47).
Vitamin B12 541

Folate

Table 1
Diagnosis, age, smoking status, HCY, folate and B12 levels of patients suffering from dementia, affective disorders or schizophrenia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Dementia</th>
<th>Affective psychosis</th>
<th>Schizophrenic psychosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>32</td>
<td>24</td>
<td>64</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>55–92</td>
<td>21–84</td>
<td>18–74</td>
</tr>
<tr>
<td>HCY (mean ± SD)</td>
<td>17.2 ± 6.7 μmol/l</td>
<td>12.9 ± 5.8 μmol/l</td>
<td>11.6 ± 5.8 μmol/l</td>
</tr>
<tr>
<td>Folate (mean ± SD)</td>
<td>6.5 ± 4.1 ng/ml</td>
<td>6.8 ± 3.0 ng/ml</td>
<td>8.1 ± 3.9 ng/ml</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>541 ± 438 pg/ml</td>
<td>459 ± 222 pg/ml</td>
<td>507 ± 302 pg/ml</td>
</tr>
</tbody>
</table>

Schizophrenic females had the lowest mean HCY level, normal folate concentrations in all cases and B12 reduction in 4 of 64 patients, 1 of which attributable to autoimmune gastritis also leading to HCY elevation (28.7 mmol/l). Another patient had long-lasting renal insufficiency explaining an HCY of 37.8 μmol/l. Further 19 patients out of 64 had elevated HCY, mostly mild (Fig. 1), in the absence of any obvious underlying pathology. Age did correlate with HCY with a lower $r^2$ (0.15) than in the other groups. Patients with elevated HCY however were significantly older ($p=0.03$, Student’s t-test) than those with normal levels (means, 48 vs. 41 years) and mean HCY concentrations were above the cut-off only in the age groups >50 years (Table 2). When HCY was stratified for folate levels, patients with folate levels at the bottom tertile did not differ in HCY levels from the other patients (mean, 11.4 μmol/l vs. 11.7 μmol/l; n.s.).

In order to test for an association of HCY metabolism with psychiatric disorders, we first screened female inpatients stemming from a small catchment area for HCY, folate and vitamin B12 levels in an observational trial. In this sample, we found increased HCY levels in dementia as well as affective psychoses, so that we chose to examine MTHFR polymorphisms in the second step of the study. Regarding confounding factors, age—but not smoking or medication—was shown to have an effect: older age in the total sample as well as in the different diagnostic categories was associated with increased homocysteine levels.

Table 2
Stratification of homocysteine levels for age (schizophrenic disorders)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Homocysteine (mean, μmol/l)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–29</td>
<td>8</td>
<td>9.9</td>
<td>3.2</td>
</tr>
<tr>
<td>30–39</td>
<td>20</td>
<td>10.4</td>
<td>2.4</td>
</tr>
<tr>
<td>40–49</td>
<td>18</td>
<td>10.1</td>
<td>3.1</td>
</tr>
<tr>
<td>50–59</td>
<td>10</td>
<td>14.6</td>
<td>9.1</td>
</tr>
<tr>
<td>60–70</td>
<td>8</td>
<td>16.1</td>
<td>9.8</td>
</tr>
</tbody>
</table>

Table 3
The MTHFR C677T SNP is not associated with affective psychoses

<table>
<thead>
<tr>
<th>MTHFR</th>
<th>Control group (CTR)</th>
<th>MD</th>
<th>Bip</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>C677T</td>
<td>677</td>
<td>50.0</td>
<td>52.2</td>
</tr>
<tr>
<td>CC</td>
<td>75</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>80</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>21</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>$\chi^2$ (df=2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR vs. MD</td>
<td>$\chi^2=1.09$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR vs. Bip</td>
<td>$\chi^2=2.29$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>0.580</td>
<td></td>
<td>0.319</td>
</tr>
</tbody>
</table>

when increased vs. normal HCY levels were compared, although the power of the test was below the desired value of 0.8 ($\chi^2=2.41$, $p=0.121$, power=0.324 at $\alpha=0.05$).

3.2. Methylene tetrahydrofolate polymorphisms and affective psychoses

As we found an increase of HCY in affective disorders, we subsequently investigated whether MTHFR polymorphisms, which lead to increased HCY levels, are associated with MD or Bip, respectively. To do so, the C677T as well as the A1298C SNPs of MTHFR were determined in a second sample consisting of 136 patients and 284 controls. While C677T, being the most frequently examined MTHFR polymorphism, was not associated with affective psychoses (Table 3), the 1298C variant was significantly associated with both Bip and MD (Table 4) arguing that variation in the MTHFR gene contributes to the genetic risk for affective psychoses.

4. Discussion

Regarding confounding factors, age—but not smoking or medication—was shown to have an effect: older age in the total sample as well as in the different diagnostic categories was associated with increased homocysteine levels.

Table 4
MTHFR A1298C is associated with affective psychoses

<table>
<thead>
<tr>
<th>MTHFR</th>
<th>Control group (CTR)</th>
<th>MD</th>
<th>Bip</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>A1298C</td>
<td>1298</td>
<td>47</td>
<td>51.1</td>
</tr>
<tr>
<td>AA</td>
<td>75</td>
<td>40.8</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>96</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>13</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>$\chi^2$ (df=2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR vs. MD</td>
<td>$\chi^2=6.65$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR vs. Bip</td>
<td>$\chi^2=6.25$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>0.036</td>
<td></td>
<td>0.044</td>
</tr>
</tbody>
</table>
4.1. Homocysteinemia and psychiatric disease

Evidence for HCY being a cardiovascular and cerebrovascular risk factor mainly stems from cross-sectional and case–control studies, while prospective studies (which are less in number) do not present convincing evidence for this (Christen et al., 2000). Furthermore, there is increasing evidence that homocysteinemia is associated with dementia of various aetiology (VD, AD; Reutens and Sachdev, 2002), but similar to cardiovascular disease a causal role therein has not been proven yet. In accordance with previous studies, we detected elevated HCY in almost all of our patients with neurodegenerative disorders. While a pathophysiological rationale for homocysteinemia in AD is obvious, considering a potential role of HCY in atherosclerosis, its occurrence in AD is less clear. Besides increasing awareness of vascular impairment being a risk factor in AD as well (de la Torre, 2002), HCY may contribute to AD pathology by oxidative stress (Starkebaum and Harlan, 1986), increasing β-amyloid mediated cell death (White et al., 2001), gliotoxic effects (Maler et al., 2003) and glutamate neurotoxicity through stimulation of the NMDA receptor (Lipton et al., 1997).

Whether homocysteinemia correlates with cerebral atrophy in dementia as well is not fully understood yet. Nevertheless, preliminary studies demonstrated that homocysteinemia is associated with hippocampal and global brain atrophy in elderly, non-demented subjects (den Heijer et al., 2003), and also with neuroradiologic disease progression in AD (Clarke et al., 1998). These results are in line with biochemical and molecular findings and further argue for HCY being a potential neurotoxic agent promoting neurodegeneration, although more studies are clearly needed to resolve this issue. Furthermore, a correlation with disease severity has to be established. A pilot study argued for positive correlation of HCY with Mini Mental Status Exam test results (Leblhuber et al., 2000). The unspecific neurotoxic and pro-oxidative actions of HCY could explain its diagnostic unspecificity and HCY may thus be considered as an aggravating factor in dementing disorders of various etiology.

Comparatively few studies aimed to investigate a possible involvement of HCY in depression (Fava et al., 1997; Bottiglieri et al., 2000; Penninx et al., 2000; Naismith et al., 2002; Tiemeier et al., 2002). Data from the Rotterdam study (Tiemeier et al., 2002) argued for a relationship of an impaired homocysteine pathway in depression and the Hordaland Homocysteine Study (Bjelland et al., 2003) showed that homocysteinemia as well as the MTHFR C677T variant are related to depression in a large non-clinical population. Most importantly, those studies (with the exception of one large study arm of the Hordaland study) were mainly conducted in elderly subjects, which is reasonable when pertinent concepts of vascular dysfunction in late-onset depression are taken into account (“vascular depression”; Alexopoulos et al., 1997). This disease entity is thought to delineate a subgroup of depressive disorders, in which vascular dysfunction is proposed to underlie psychiatric symptoms especially in elderly suffering from cerebrovascular disease.

4.2. Homocysteinemia and depression: a vascular link?

In our sample, which has the disadvantage of a small size, homocysteinemia—although to a lesser extent compared to dementia—could be observed in depressive disorders. On closer examination of the depressive patients, we noted that homocysteinemic subjects were older, had more vascular disorders and cognitive impairment and thus were more susceptible to suffer from “vascular depression”. In contrast, younger patients with recurring depressive episodes or bipolar affective disorders mainly had normal HCY levels. We thus speculate that homocysteinemia may play a role in later-onset depression, possibly by being a vascular risk factor which contributes to the pathology of “vascular depression”.

While the connection between cerebrovascular disorder and depression might still be explained by vascular lesions in critical brain regions, it remains an unresolved question why depression occurs also more frequently in cardiovascular disease. Genetic variation of MTHFR with subsequent homocysteine elevation may constitute a possible link between cardiovascular risk and affective disorders on a genetic as well as functional basis. This makes MTHFR an apparent candidate gene in coronary disease with depression.

4.3. Homocysteinemia, MTHFR polymorphism and schizophrenia

The evidence for an involvement of HCY in schizophrenia is poor. Besides sporadic case reports, few studies provided positive results: Regland et al. (1997) reported to find higher HCY levels and MTHFR C677T mutation in psychotic subjects more often. In a further case–control study investigating both MTHFR C677T and A1298C, an association of both SNPs with neuroleptic-responsive schizophrenia was found (Sazci et al., 2003). Those findings could, however, not be replicated by other groups (Virgos et al., 1999; Yu et al., 2004), the latter of which also performed haplotype and TDT analyses. Susser et al. (1998) restricted a possible relationship between schizophrenia and homocysteinemia to low-folate subjects only. Most recently, in a large sample of 193 schizophrenic subjects which were matched to >700 controls, a positive association was found for young, male schizophrenics and HCY elevation (Levine et al., 2002). In a follow-up study to this, HCY was determined on the admission (Applebaum et al., 2004), likewise as in the present study, to exclude poor hospital nutrition as the underlying cause of homocysteinemia. Again, homocysteinemia was found in young, schizophrenic males, but not in females.

In accordance with Levine et al. (2002) and Applebaum et al. (2004), we could not detect homocysteinemia in younger psychotic women. When schizophrenic patients
were stratified for age (Table 2), our results were similar to previously published data (Levine et al., 2002): mean HCY levels were elevated only in the age groups over 50 years; nutrition or lifestyle during disease progression might play a role in this phenomenon. All patients in this group, however, had an onset of psychosis before the age of 50. Together, we thus suggest that homocysteinemia does not play a major role in the pathophysiology of schizophrenia in women. There is still no convincing explanation for the gender-specific effect of homocysteinemia in schizophrenia and one might only speculate whether biological or rather socioeconomic factors like low vitamin intake due to impoverishment play a role.

5. Conclusion

The authors suggest that homocysteinemia and MTHFR genotype is a risk factor for, or associated with, affective disorders. For future studies, it is justified to investigate whether it has a special role in the proposed disease entity “vascular depression” and whether it interacts with other genes shown to play a role both for vascular and affective disorders, like NOS-III (Reif et al., in press). In women, HCY elevation however does not seem to play a role in the pathophysiology of schizophrenic psychoses, although this finding has to be replicated in larger and case-controlled studies.

Acknowledgement

We are indebted to Alex Strobel, Institute of Psychology II, Dresden University of Technology, Dresden, Germany, for performing the statistical tests for the case–control association study. We gratefully acknowledge T. Töpfer for excellent technical assistance and the Central Laboratory of the University of Würzburg (Chair: Prof. Dr. U. Walter) for the determination of homocysteine, folate and vitamin B12. This study was supported by the Deutsche Forschungsgemeinschaft (Grant RE1632/1-1 to A.R., KFO 125/1-1 to A.R. and K.P.L. and SFB581 to K.P.L.), Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (IZKF Würzburg, 01KS9603) and the European Commission (NEWMOOD LSHM-CT-2003-503474).

References


