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Influence of Methylenetetrahydrofolate Reductase Gene Polymorphisms on Homocysteine Concentrations after Nitrous Oxide Anesthesia

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Background: Mutations in the methylenetetrahydrofolate reductase (*MTHFR*) gene (677C>T, 1298A>C) cause elevated plasma homocysteine concentrations and have been linked to fatal outcomes after nitrous oxide anesthesia. This study tested the hypothesis that patients with common *MTHFR* 677C>T or 1298A>C mutations develop higher plasma homocysteine concentrations after nitrous oxide anesthesia than wild-type patients.

Methods: In this prospective, observational cohort study with blinded, mendelian randomization, the authors included 140 healthy patients undergoing elective surgery. All patients received 66% nitrous oxide for at least 2 h. The main outcome variable, plasma total homocysteine, and folate, vitamin B_{12}, and holotranscobalamin II were measured before, during, and after surgery. After completion of the study, all patients were tested for their *MTHFR* 677C>T or 1298A>C genotype.

Results: Patients with a homozygous *MTHFR* 677C>T or 1298A>C mutation (n = 25) developed higher plasma homocysteine concentrations (median [interquartile range], 14.9 [10.0–26.4] μM) than wild-type or heterozygous patients (9.3 [7.5–15.5] μM; n = 115). The change in homocysteine after nitrous oxide anesthesia was tripled in homozygous patients compared with wild-type (5.6 μM [+60%] *vs.* 1.8 μM [+22%]). Only homozygous patients reached average homocysteine levels considered abnormal (> 15 μM). Plasma 5-methyl-tetrahydrofolate concentrations increased uniformly by 20% after nitrous oxide anesthesia, indicating the inactivation of methionine synthase and subsequent folate trapping. Holotranscobalamin II concentrations remained unchanged, indicating no effect of nitrous oxide on vitamin B_{12} plasma concentrations.

Conclusions: This study shows that patients with a homozygous *MTHFR* 677C>T or 1298A>C mutation are at a higher risk of developing abnormal plasma homocysteine concentrations after nitrous oxide anesthesia.

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NITROUS oxide inhibits vitamin B_{12} (cobalamin) by irreversibly oxidizing the cobalt atom of cobalamin.[1,2] This leads to a subsequent inhibition of enzymes requiring cobalamin in its coenzyme form. Inhibition of vitamin B_{12} lasts several days because of the irreversible nature of the chemical reaction.[3,4] Among the enzymes that require active vitamin B_{12} as a cofactor, methionine synthase (gene symbol *MTR*, EC 2.1.1.13) is crucial because it is located at the juncture of two pathways: homocysteine remethylation and the folate cycle (fig. 1). Therefore, inhibition of methionine synthase *via* oxidized cobalamin results in a sustained increase of plasma homocysteine concentrations and lack of biologically active folate ("folate trapping") that can be used for the conversion of homocysteine to methionine.[5,6]

In the methylenetetrahydrofolate reductase (*MTHFR*) gene, which is operant in the folate cycle, two common single nucleotide polymorphisms have been described (*MTHFR* 677C>T, *MTHFR* 1298A>C) that are associated with a reduced enzyme activity.[7,8] Together, both polymorphisms have a combined prevalence of approximately 20% in the Western European population.[9] *MTHFR* 677C>T results in decreased formation of active folate, methyltetrahydrofolate,[10] which in turn leads to an increase in plasma total homocysteine concentrations.[7] The *MTHFR* 677C>T polymorphism is considered the single most important genetic determinant of plasma homocysteine.[11]

Recently, two reports describing children carrying several polymorphisms and mutations in the *MTHFR* gene were published. The children developed catastrophic neurologic outcomes after being anesthetized with nitrous oxide.[12,13] Based on these reports and the apparent importance of the *MTHFR* genotype for plasma homocysteine, we hypothesized that patients carrying *MTHFR* 677C>T and/or 1298A>C mutations develop higher plasma homocysteine concentrations compared with noncarriers.
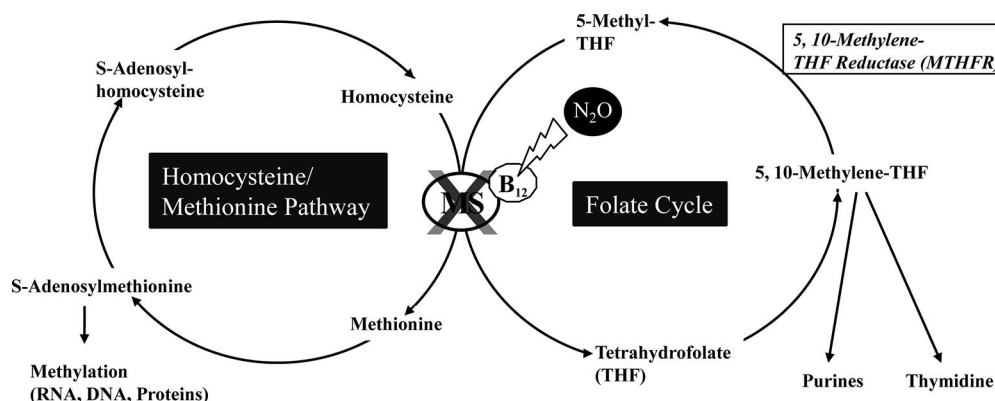
To test this hypothesis, we conducted a prospective cohort study in which all patients received 66% nitrous oxide. Patients were unaware of their *MTHFR* genotype before the study. We measured plasma total homocys-

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Fig. 1. Inhibition of methionine synthase (MS) by nitrous oxide (N_2O) occurs at a critical juncture of two pathways: the folate cycle and the remethylation of methionine from homocysteine. Nitrous oxide oxidizes the cobalt atom of vitamin B_{12} (cobalamin), thereby inactivating vitamin B_{12} as a necessary cofactor for methionine synthase function. The subsequent accumulation of 5-methyl-tetrahydrofolate due to inactive methionine synthase is called "folate trapping." THF = tetrahydrofolate.

teine concentrations as the main outcome variable before and after nitrous oxide anesthesia.

Materials and Methods

In this prospective, observational cohort study, all patients received nitrous oxide. Because genotyping was performed after completion of the study, patients and research staff were blinded to the *MTHFR* genotype. Our research design used a population-based randomization of the two *MTHFR* polymorphisms, which is also known as mendelian randomization (randomization according to the naturally occurring genotype frequencies).[14]

The study was approved by the Ethics Committee at the Medical University of Vienna, and every patient gave written, informed consent before participating.

Study Population

Between November 2004 and March 2007, 140 healthy patients (American Society of Anesthesiologist status I or II) who were scheduled to undergo orthopedic surgery during general anesthesia were recruited to participate in this study. Surgical procedures included mostly arthroscopic surgery and some prosthetic procedures.

Inclusion criteria were American Society of Anesthesiology status I or II, age older than 18 yr, and surgery scheduled for 2 h or longer. Exclusion criteria were pregnancy, age younger than 18 yr, contraindication against the use of nitrous oxide (*e.g.*, pneumothorax, mechanical bowel obstruction, middle ear occlusion, laparoscopic surgery, elevated intracranial pressure), and preoperative use of supplemental vitamin B_{12} or folate.

Study Protocol

All patients received the same standardized general anesthetic regimen including 66% nitrous oxide. After premedication with 2 mg midazolam and application of standard monitoring (electrocardiogram, noninvasive

blood pressure, pulse oximetry), general anesthesia was induced with fentanyl (1–3 μg/kg) and propofol (2–3 mg/kg). Muscle relaxation was achieved by administration of 0.6 mg/kg rocuronium, and all patients were orally intubated. For maintenance of anesthesia, patients received 1–2 vol% sevoflurane in a nitrous oxide:oxygen mixture of 66%:33%.

Blood samples for the main outcome variable total homocysteine were collected at three different time points: preoperatively (in the holding area), after 2 h of general anesthesia, and at the end of surgery. Homocysteine samples were immediately put on ice after drawing and then transported to the laboratory within 15 min and analyzed after the end of the study.

Vitamin B_{12}, holotranscobalamin II, and folate samples were drawn before and at the end of surgery. In addition, plasma creatinine and albumin concentrations were tested because they influence total plasma homocysteine concentrations. Blood for *MTHFR* genotyping was drawn before surgery, but samples were not analyzed before the end of the study.

Biochemical Methods

EDTA blood for homocysteine, folate, and holotranscobalamin II assays was immediately placed on ice and centrifuged at 2,000g at 4°C (20 min) within 60 min. Plasma aliquots and 500 μl blood for isolation of DNA were snap-frozen and stored at −70°C. Plasma concentrations of total homocysteine (free plus protein-bound homocysteine)[15] were determined by microparticle enzyme immunoassay (AxSYM homocysteine assay; Abbott GmbH & Co. KG, Wiesbaden, Germany). Holotranscobalamin II plasma concentration was measured by AxSYM HoloTC assay (Abbott). Folate and vitamin B_{12} plasma levels were analyzed with a radioassay (SimulTRAC-SNB; ICN Pharmaceuticals Inc., Costa Mesa, CA). Interassay variability was 4–5% for folate measurements and 4–6% for vitamin B_{12} levels.

Genotyping

Identification of the *MTHFR* 677C>T transition and the *MTHFR* 1298A>C transversion was conducted using restriction fragment length polymorphism analysis and the polymerase chain reaction primers and restriction enzymes *Hinf* I and *Fnu4* HI as previously described.[7,16] Furthermore, we also calculated the allele frequencies for both mutations and tested for violations of the Hardy-Weinberg equilibrium assumption.

Sample Size Determination

On the basis of previous trials demonstrating (1) higher plasma total homocysteine concentrations in healthy individuals with the *MTHFR* 677TT genotype (approximately 11 *vs.* 9 μM in CC/CT genotype subjects) and (2) an increase of plasma total homocysteine concentrations of up to 76% during general anesthesia using nitrous oxide as compared with preoperative concentrations, we calculated that 16 patients with a *MTHFR* 677TT genotype would be required to give a statistical power of 80% at a two-sided α level of 5% to detect a mean difference in homocysteine plasma concentrations of 5 μM homocysteine (17 *vs.* 12 μM, SD of 5 μM) *versus* CC/CT genotype patients.

Because the frequency of the *MTHFR* 677TT genotype is approximately 12% in the Caucasian population of Austria, we included 140 patients in this study to expect a minimum of 16 *MTHFR* 677TT genotype patients.

Statistical Analysis

Continuous variables are described as mean ($\pm$ SD) for normally distributed data and with median (minimum–maximum or interquartile range) for skewed data. Binary variables are described as absolute frequencies and percentages. Two-sided *t* tests were used for group comparisons of normally distributed continuous variables, and the Wilcoxon rank sum test was used for not normally distributed continuous variables. For continuous paired observations, the paired *t* test was used for normally distributed differences, and the Wilcoxon signed rank test was used otherwise. The chi-square test was used for binary variables.

Changes over time from baseline to end of surgery, differences between the six combined genotypes, and their interaction were modeled by a linear mixed model accounting for the repeated measurements of the patients by a first-order autoregressive variance–covariance structure. Within this framework, several contrasts were calculated to test specific questions, such as the change from baseline to end of surgery for the six genotype groups. A second linear mixed model was used where the baseline measurements were included as independent factors to account for the differences at baseline to provide a better comparison of the 2-h and end-of-surgery measurements. For specific questions, contrasts were again calculated. The assumptions for both models (normally distributed residuals with equal variances) were checked visually by residual graphs. A logarithmic transformation was performed to homocysteine concentrations because of their skewed distributions.

All calculated *P* values are two-sided, and $P \leq 0.05$ was considered significant. All calculations were performed with the statistical software SAS (version 9.1; SAS Institute Inc., Cary, NC).

Results

Among the 140 patients in the study, the baseline characteristics between patients homozygous for *MTHFR* 677C>T or 1298A>C (n = 25) and heterozygous or wild type (n = 115) were similar except for baseline plasma total homocysteine concentrations, which were significantly higher among homozygotes. This is consistent with the underlying biologic role of the *MTHFR* 677C>T or 1298A>C polymorphism on homocysteine (table 1).

Table 1. Baseline Characteristics of the Study Population (n = 140)

	All Patients, n = 140	Homozygous Patients, n = 25	Wild-type or Heterozygous Patients, n = 115	P Value
Age, yr	42 (15)	40 (16)	43 (15)	0.46
Height, cm	173 (9)	174 (9)	173 (10)	0.52
Weight, kg	77 (16)	77 (13)	77 (16)	0.94
Male, n (%)	81 (58)	16 (64)	65 (57)	0.66
Smoking status, n (%)	56 (40)	13 (52)	43 (37)	0.18
Creatinine, mg/dl	0.9 (0.2)	0.9 (0.2)	0.9 (0.1)	0.49
Albumin, g/dl	38.9 (5.4)	38.5 (3.9)	39.0 (5.7)	0.72
Homocysteine, μM	8.2 (6.9–10.1)	9.3 (7.5–15.5)	8.1 (6.7–9.8)	0.007
Vitamin B_{12}, pM	290 (130)	292 (103)	290 (135)	0.95
Folate, nM	21.6 (10.4)	19.1 (9.6)	22.3 (10.5)	0.18
Duration of surgery, h	2.2 (0.5–7.4)	2.5 (1.2–7.4)	2.2 (0.5–6.5)	0.07

Comparisons were made between patients with homozygous methylenetetrahydrofolate reductase (*MTHFR*) genotypes (*677TT* and *1298 CC*; n = 25) and patients with either heterozygous or wild-type *MTHFR* genotypes (*677CC*, *677CT*, *1298AA*, and *1298AC*; n = 115). Continuous variables are described as mean (SD) or as median (interquartile range for homocysteine or minimum–maximum for the duration of surgery). Categorical data are described as frequency (%).

Table 2. Genotype Frequency Distribution of *MTHFR* 677 and 1298 Polymorphisms

MTHFR 677 Genotype	*MTHFR* 1298 Genotype			
	Wild Type, AA	Heterozygous, AC	Homozygous, CC	Total
Wild type, CC	18 (13)	36 (26)	8 (6)	62 (44)
Heterozygous, CT	26 (19)	35 (25)	0	61 (44)
Homozygous, TT	17 (12)	0	0	17 (12)
Total	61 (44)	71 (51)	8 (6)	140 (100)

Data in parentheses are percentages. The observed distribution follows the Hardy-Weinberg equilibrium (chi-square test). Because of rounding error, percentages might not add up to 100%. *MTHFR* = methylenetetrahydrofolate reductase.

Allele Frequency of MTHFR *677C>T or 1298A>C and Combined Genotype Distribution*

After genotyping all patients, six combinations of *MTHFR* 677 and 1298 alleles were found (table 2). In general, a patient could be homozygous (mutations in both copies of the gene), heterozygous (one mutant copy, one wild-type copy), or wild type (two wild-type copies) at either *MTHFR* allele (677 or 1298). Theoretically, 3×3 combinations were possible, but in our patient population, we only observed six. No patient was found with homozygosity for both *MTHFR* polymorphisms (677TT, 1298CC) or with a combination of a homozygous and a heterozygous mutation (table 2). The allele frequency of *MTHFR* 677T was 0.34, and that of *MTHFR* 1298C was 0.31. The observed allele frequency of *MTHFR* 677C>T and 1298A>C in our study sample closely followed the Hardy-Weinberg equilibrium (chi-square test; based on published *MTHFR* allele frequencies) and is comparable to previously published studies.[17]

Homocysteine Concentrations after Nitrous Oxide Anesthesia

Patients with homozygosity for *MTHFR* 677T or *MTHFR* 1298C developed significantly higher plasma total homocysteine concentrations after nitrous oxide anesthesia than wild-type patients (fig. 2). The absolute increase in homocysteine concentrations was tripled in patients homozygous for either mutation compared with wild-type patients (5.6 *vs.* 1.8 μM; $P = 0.03$). Only patients homozygous for either mutation reached median total homocysteine concentrations exceeding 15 μM, reflecting hyperhomocysteinemia. However, plasma total homocysteine concentrations increased after nitrous oxide anesthesia—not unexpectedly—among all *MTHFR* 677/1298 variants. This finding is consistent with the fact that nitrous oxide inhibits vitamin B_{12} in all patients irrespective of the underlying *MTHFR* genotype (table 3).

The effect of the homozygous genotypes (*MTHFR* 677TT, *MTHFR* 1298CC) on plasma total homocysteine concentrations after nitrous oxide anesthesia was not uniform: Patients with the 1298CC genotype experienced a 76% increase, whereas patients with the 677TT genotype had a 36% increase. Whether this disparity is due to a true biologic difference or due to random variation because of the small sample size in the subgroups (n = 8 and 17) remains to be determined.

Because inactivation rates of methionine synthase vary substantially in humans after exposure to nitrous oxide,[18] we wanted to determine whether patients with a very long nitrous oxide exposure time exhibit a similar or greater effect on plasma total homocysteine concentrations. In total, 16 patients experienced nitrous oxide anesthesia times longer than 4 h. Plasma homocysteine concentrations increased significantly from 8.1 (7.3–10.0) to 14.6 (13.1–22.8) μM [median (interquartile

Fig. 2. Plasma homocysteine concentrations in the different groups based on methylenetetrahydrofolate reductase (*MTHFR*) 677/1298 genotype at three different time points: preoperative, after 2 h of anesthesia, and at the end of surgery. Because homocysteine was not normally distributed, data are presented as median and lower–upper quartiles as whiskers. Both homozygous groups developed significantly higher homocysteine concentrations than the other groups (*** $P < 0.001$). Genotype combinations (*MTHFR* 677/1298): wt/wt: CC/AA; het/wt: CT/AA; wt/het: CC/AC; het/het: CT/AC; wt/hom: CC/CC; hom/wt: TT/AA.
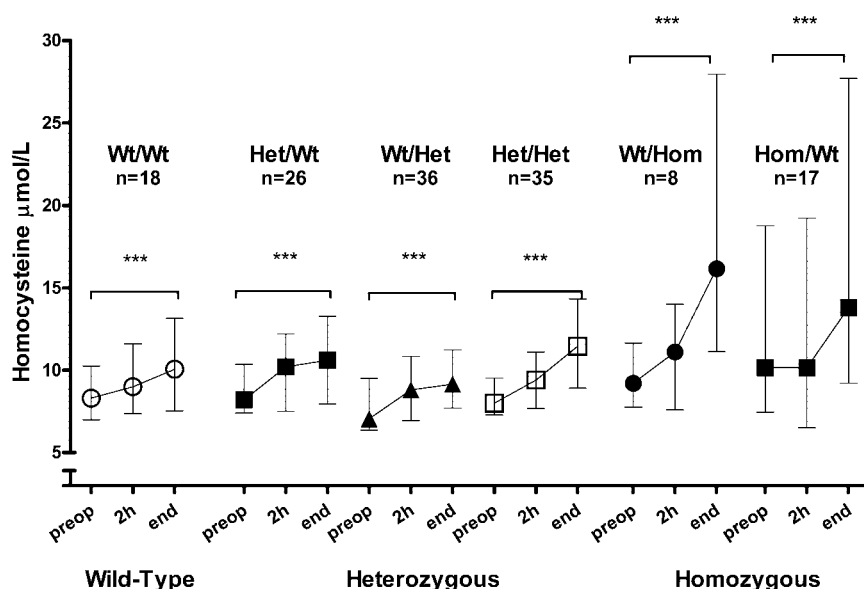
Table 3. Homocysteine, Folate, Vitamin B$_{12}$, and Holotranscobalamin II Concentrations According to Genotype

	CC/AA, wt/wt, n = 18	CT/AA, het/wt, n = 26	CC/AC, wt/het, n = 36	CT/AC, het/het, n = 35	CC/CC, wt/hom, n = 8	TT/AA, hom/wt, n = 17	All hom, n = 25	All wt/het, n = 115
Hcy, μM								
Preoperative	8.3 (7.1–10.1)	8.2 (7.4–10.4)	7.1 (6.4–9.5)	8.0 (7.3–9.5)	9.2 (8.1–11.1)	10.2 (7.5–18.8)	9.3 (7.5–15.5)	8.1 (6.7–9.8)
Postoperative	10.1 (7.6–12.7)‡	10.9 (8.1–13.3)‡	9.2 (7.7–11.2)‡	11.5 (8.9–14.3)‡	16.2 (11.2–25.0)‡	13.8 (9.2–27.7)‡	14.9 (10.0–26.4)‡	10.2 (8.1–12.9)‡
Change (%)	1.8 (22)	2.7 (33)	2.1 (30)	3.5 (44)	7.0 (76)	3.6 (35)	5.6 (60)	2.1 (26)
Folate, nM								
Preoperative	26.1 ± 13.0	21.1 ± 8.1	23.7 ± 11.4	19.7 ± 9.0	22.5 ± 9.0	17.4 ± 9.6	18.3 ± 9.6	22.4 ± 10.2
Postoperative	26.9 ± 11.4	25.8 ± 7.5†	28.1 ± 10.82†	25.3 ± 8.2‡	27.3 ± 6.9	21.3 ± 9.7†	23.2 ± 9.2‡	26.6 ± 9.7‡
Change (%)	0.8 (3)	4.7 (22)	4.4 (19)	5.6 (28)	4.8 (21)	3.9 (22)	4.9 (27)	4.2 (19)
B$_{12}$, pM								
Preoperative	275 ± 128	291 ± 105	291 ± 154	297 ± 143	309 ± 93	283 ± 110	292 ± 108	287 ± 134
Postoperative	273 ± 120	256 ± 106*	281 ± 154	294 ± 137	264 ± 117*	256 ± 113	259 ± 112†	276 ± 135*
Change (%)	−2 (−1)	−35 (−12)	−10 (−3)	−3 (−1)	−45 (−15)	−27 (−10)	−33 (−12)	−11 (−4)
Holo-TC, pM								
Preoperative	54.4 ± 24.2	52.0 ± 17.5	55.9 ± 24.1	55.2 ± 25.3	60.0 ± 19.0	53.9 ± 20.3	55.8 ± 19.7	54.6 ± 23.0
Postoperative	56.0 ± 24.8	53.1 ± 17.3	61.4 ± 29.6	58.0 ± 23.1	59.5 ± 22.2	54.9 ± 23.6	56.4 ± 22.7	57.7 ± 24.5†
Change (%)	1.6 (3)	1.1 (2)	5.5 (10)	2.8 (5)	−0.5 (−1)	1.0 (2)	0.6 (1)	3.1 (6)

Data are presented as mean ± SD, median (lower–upper quartile), or frequency (%).

* $P < 0.05$.   † $P < 0.01$.   ‡ $P < 0.001$.

All hom = all patients homozygous for either methylenetetrahydrofolate reductase allele; all wt/het = all patients either wild type or heterozygous; Hcy = homocysteine; holo-TC = holotranscobalamin.

range); $P < 0.001$], a relative increase of approximately 80% (fig. 3). This increase was substantially larger than during shorter nitrous oxide exposure times. Although four patients in this group were homozygous for one *MTHFR* genotype, the effect of long nitrous oxide exposure time on plasma total homocysteine concentrations was independent of the *MTHFR* genotype.
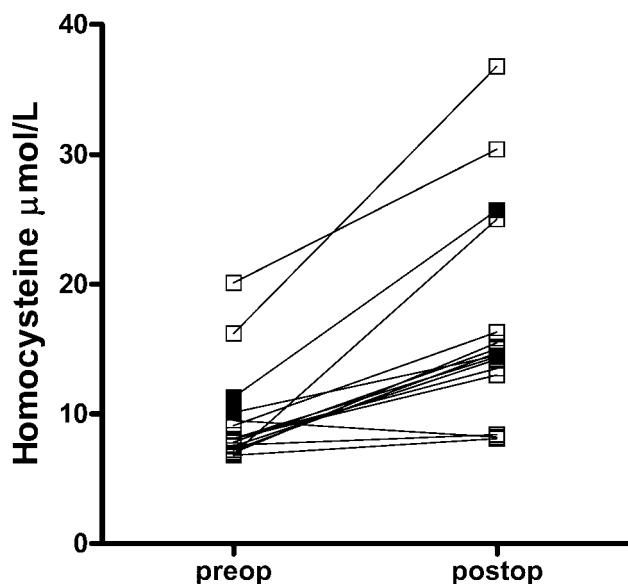
Fig. 3. Plasma homocysteine concentration in patients with very long nitrous oxide exposure (> 4 h) (n = 16). Postoperative homocysteine concentrations were significantly higher compared with preoperative: 8.1 (7.3–10.0) *versus* 14.60 (13.1–22.8) μM [median (lower–upper quartile)], $P < 0.001$, Wilcoxon signed rank test. A solid black box (■) indicates the two patients who were double wild type, indicating that the effects of very long exposure to nitrous oxide are also operant in patients without *MTHFR* mutation.

Plasma Folate Concentrations after Nitrous Oxide Anesthesia

Plasma folate concentrations increased in all groups after nitrous oxide exposure, on average by 20%, except for double-wild-type patients. No difference between homozygous patients and others was found. Because the plasma folate assay measures predominantly 5-methyl-tetrahydrofolate, the folate substrate upstream of methionine synthase, the observed increase in folate indicates inhibition of methionine synthase and subsequent folate trapping.

Plasma Vitamin B$_{12}$ and Holotranscobalamin II Concentrations after Nitrous Oxide Anesthesia

Vitamin B$_{12}$ concentrations decreased uniformly across all groups; the effect was slightly more pronounced in homozygous patients (12% decrease *vs.* 4%). This effect of nitrous oxide on vitamin B$_{12}$ was unexpected as endogenous vitamin B$_{12}$ stores usually last several months. Therefore, we did not expect *a priori* to find any changes in vitamin B$_{12}$ plasma concentrations after nitrous oxide anesthesia. To corroborate these findings, we decided to additionally measure plasma holotranscobalamin II concentrations. Holotranscobalamin II is the fraction of cobalamin that is bound to transcobalamin II and represents the biologically active vitamin B$_{12}$ that is available to DNA-synthesizing cells.[19] The results show that holotranscobalamin II concentrations remained essentially unchanged after exposure to nitrous oxide (table 3), indicating that in fact no actual change in blood vitamin B$_{12}$ concentrations occurred.

Discussion

This study shows that patients who are homozygous for *MTHFR* 677C>T or 1298A>C develop significantly

higher homocysteine concentrations after nitrous oxide anesthesia than noncarriers. This increase was nearly tripled in homozygous patients and reached concentrations defined as hyperhomocysteinemia ($> 15 \mu M$).

The inhibition of methionine synthase by nitrous oxide has two consequences because the enzyme is located at a critical juncture between two pathways (fig. 1).[20] First, as clearly shown in this study as well as previous studies, inhibition of methionine synthase by nitrous oxide leads to an increase in homocysteine because it cannot be remethylated to methionine.[5,21–23] Second, the inactivation causes a process called "folate trapping," where the folate substrate upstream of the enzyme block, 5-methyltetrahydrofolate, accumulates and lack of biologically available methyl groups ensues.[24] The lack of activated folate and methyl groups can ensue quickly and subsequently severely limits the *de novo* synthesis of DNA resulting in apoptosis of rapidly dividing cells.[25] This process is the cause for the megaloblastic changes observed in blood cells and bone marrow after nitrous oxide exposure. Therefore, the increase in serum folate concentrations after nitrous oxide anesthesia found in this study is not surprising and is consistent with trapping of methylated tetrahydrofolate.

The observed decrease of vitamin B_{12} concentrations after nitrous oxide exposure was unexpected because endogenous vitamin B_{12} stores usually last several months before being depleted. The results from our subsequent holotranscobalamin II tests showed that this was a spurious finding: Serum vitamin B_{12} concentrations apparently do not change after nitrous oxide exposure. A possible cause for this finding is that nitrous oxide might interfere with some commercial vitamin B_{12} assay, perhaps by altering binding of cobalamin substrates to intrinsic factor, the most commonly used method of vitamin B_{12} detection, and thus leading to falsely low vitamin B_{12} concentrations. However, there is currently no evidence, and this hypothesis should be studied formally in biochemical studies.

What are the clinical implications of our finding? To answer this question, two separate issues arise: (1) Is there evidence for nitrous oxide toxicity related to homocysteine and *MTHFR* genotype, and (2) is acute hyperhomocysteinemia associated with adverse outcomes?

Several lines of evidence support the hypothesis of homocysteine and *MTHFR* genotype being associated with nitrous oxide toxicity. During the past 30 yr, more than a dozen case reports have been published where nitrous oxide anesthesia led to neurologic complications including myelopathy, peripheral neuropathy, and even hemiparesis.[26–28] In most cases, nitrous oxide–induced folate trapping in conjunction with chronic vitamin B_{12} deficiency was responsible for the neurologic damage. How catastrophic nitrous oxide–induced hyperhomocysteinemia and folate trapping can be when they develop on top of an inherited *MTHFR* enzyme defect was

highlighted by two recent reports, one of which described the death of an infant after being anesthetized twice with nitrous oxide.[12,13] In their report, Selzer *et al.*[13] postulated a "double-hit" hypothesis to explain the pathophysiology of the fatal neurologic outcome. This hypothesis links nitrous oxide–induced hyperhomocysteinemia and inhibition of folate metabolism with an underlying *MTHFR* gene defect. Our results are consistent with this "double-hit" hypothesis and might be applicable to other patients with a deficiency in vitamin B_{12}/folate metabolism who are exposed to nitrous oxide, *e.g.*, patients with other common *MTHFR* gene polymorphisms.

Polymorphisms in genes within the folate cycle or homocysteine–methionine pathway that go in hand with reduced enzyme activity are common and affect the population at large.[9] As in our study, the cumulative prevalence of homozygous *MTHFR* 677C>T and 1298A>C mutations approaches 20%, rendering these patients susceptible for a "double hit" when they are exposed to nitrous oxide. Although evidence is currently lacking, these genetically "at-risk" patients might develop hypomethylation of DNA and defective DNA synthesis with apoptosis and megaloblastic anemia and sequelae related to hyperhomocysteinemia.

The neurologic damage caused by nitrous oxide occurs often in combination with hematologic changes such as megaloblastic anemia and bone marrow disorders.[29,30] These toxic side effects of nitrous oxide have been recognized for decades and are directly associated with nitrous oxide's inhibition of methionine synthase and folate metabolism.

Nitrous oxide has been linked in a recent study by Myles *et al.*[31] to an increased risk for adverse postoperative outcomes, including a higher trend for adverse cardiovascular outcomes including myocardial infarction. In a smaller study, patients receiving nitrous oxide had higher rates of postoperative myocardial ischemia.[32] Although the inhibition of vitamin B_{12} and the subsequent hyperhomocysteinemia due to nitrous oxide lasts for only a few days,[33] this effect coincides with a period of increased vulnerability for the postoperative patient, highlighted by the fact that the highest risk for major cardiovascular complications after surgery occurs on the second and third postoperative day.[34,35] For both studies, nitrous oxide–induced hyperhomocysteinemia and impaired folate metabolism could serve as a potential pharmacogenetic explanation to account for the increased risk.

The second question regarding the clinical implications of our findings, whether acute nitrous oxide–induced hyperhomocysteinemia is associated with adverse outcomes, is difficult to answer. Although homocysteine has emerged from numerous studies as an established risk factor for long-term cardiovascular morbidity and mortality,[9,36,37] the clinical impact of an acute elevation

of plasma homocysteine caused by nitrous oxide is currently unclear. Recent experimental studies, however, investigated the effects of acute hyperhomocysteinemia and showed that in humans and animals, acute hyperhomocysteinemia impairs myocardial substrate use and causes endothelial dysfunction in blood vessels indicating a profound effect on cardiovascular function.[38–40] It also causes enhanced platelet aggregation, which might be causative for its procoagulatory effects.[41]

Limitations

Our study was designed to measure biochemical markers after nitrous oxide anesthesia and not to detect clinical outcomes. Therefore, any inference about a possible link between nitrous oxide, elevated homocysteine, and adverse outcomes cannot be made. The sample size in our study was large enough to include a sufficient number of homozygous patients, although we did not observe double homozygous patients (*MTHFR* 677TT, 1298CC) or the combination of homozygous and heterozygous. In addition, because all patients in our study were exposed to nitrous oxide, we could not observe the effects of a nitrous oxide–free anesthetic regimen on homocysteine concentrations in the different *MTHFR* genotype populations. Although unlikely, there is a small chance that patients with homozygous *MTHFR* genotypes would develop higher homocysteine concentrations even without being exposed to nitrous oxide.

Conclusions

In summary, the results of our study show that patients with reduced 5,10-methylenetetrahydrofolate reductase enzyme activity caused by a homozygous *MTHFR* 677C>T or 1298A>C gene polymorphism are at higher risk to develop hyperhomocysteinemia after nitrous oxide anesthesia. It seems that this subgroup of patients with a combined prevalence in the general population of approximately 20% may be particularly susceptible to the toxic effects of nitrous oxide on vitamin B_{12} and folate metabolism. The question of whether this biochemical effect translates into a higher risk for adverse clinical outcomes requires large-scale clinical trials.

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