

Homocysteine and human reproduction

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Summary

Homocysteine is an amino acid that is capable of disturbing the proper growth of cells. Hyperhomocysteinemia can lead to a non-closure of the neural tube. The underlying basis is a derangement of homocysteine metabolism due to a missense mutation of the MTHFR enzyme that has to catalyze the folate metabolic cycle furnishing sufficient methyl groups for DNA and tRNA synthesis. Folate can overcome the dysfunction of the mutation and the decreased activity of the thermolabile MTHFR.

Homocysteine is also recognized as an independent risk factor for obstetrical vascular disease that can manifest itself in maternal veins (thrombosis), arteries (preeclampsia) or spiral arteries supplying the placenta (placental abruption). Low vitamin status (folic acid, vitamin B6 and B12), hyperhomocysteinemia, the MTHFR gene mutation C677T, and thrombotic factors like Protein C, Protein S, antithrombin III, factor V Leiden and Activated Protein C, are alone or in combination high risk factors for obstetrical vascular disease. Their values can be modulated by B-vitamin status and could be able to prevent disease from occurring or recurring. Placebo-randomized trials have been done in neural tube defects but are urgently needed in the vascular area.

The common denominator of the effect of homocysteine on the embryo and the blood vessels (endothelium) could be sited in the process of proliferation of cells that need proper methyl groups for proper function.

Introduction

Homocysteine and hyperhomocysteinemia are relatively new concepts in Obstetrics and Gynecology. Nevertheless the amino acid homocysteine is known to the obstetrician as one of the eight amino-acids that constitutes octapeptide oxytocin and was synthesized by Du Vigneaud *et al.* (1954).

Thirty-seven years later it became known that folic acid could prevent the recurrence of neural tube defects (MRC 1991). At first glance one wonders what homocysteine and folic acid have in common. The connection of both can now be judged as a derangement of homocysteine metabolism that can be overcome with folates. This derangement is in part the basis for birth defects, vascular disease like thrombosis, preeclampsia and placental abruption and some forms of oncology.

Causes of birth defects

Neural tube defects (NTD) are serious defects and are caused by genetic and environmental influences. Factors establishing the genetic role include a preponderance in females, ethnic differences persisting after migration, parental consanguinity, increased rate in concordance in monozygotic twin pairs, and increased incidence in siblings and children of affected patients.

Environmental influences are diabetes, hyperthermia, the use of valproic acid and other anticonvulsants, alcohol abuse or familial NTD history.

Also geography, month of conception, maternal age, birth order, socio-economic class and maternal diet contribute to increased risk.

Folic acid alone or embedded in a multivitamin preparation prevents neural tube defects

There is considerable evidence from observational and intervention studies that the periconceptional administration of multivitamins (containing folic acid) or folic acid alone prevents the occurrence and recurrence of neural-tube defects. These studies have been reviewed and extensively analyzed (De Bree *et al.* 1998).

Fortunately the Medical Research Council in the United Kingdom later on mounted a multicenter, double-blind, randomized trial to investigate the possible effect of multivitamins and folic acid on the recurrence rate of neural tube defects (MRC 1991). This study fulfilled all the criteria for an excellent randomized investigation. Daily supplementation with folic acid (4 mg/d), folic acid (4 mg/d) with other vitamins, other vitamins without folic acid and placebo, led to a 72% reduction of the recurrence rate in the folic acid groups. The rationale for the choice of such a high dose of folic acid was to avoid the risk of an ineffective low dose and the impossibility to repeat such a long-lasting and costly study. The trial was closed prematurely because of the achieved significance before the calculated numbers were reached.

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The Hungarian randomized trial (Czeizel and Dudás, 1991) provided evidence that also the occurrence of neural tube defects could be prevented. Subjects took a daily multivitamin containing 800 mcg folic acid per day. The control group used a placebo containing trace elements. It could not be disclosed if the significant result was due to folic acid alone or to folic acid embedded within the multivitamin preparation.

Periconceptional intake of 400 micrograms of folic acid daily can reduce the risk of neural tube defects in areas with high rates of these defects and in areas with low rates as demonstrated in a large population study in China (Berry *et al.*, 1999).

Folic acid and homocysteine

Homocysteine is the demethylated derivate of the essential amino acid methionine (Figure 1). Homocysteine and methionine are small sulfur containing amino acids. Homocysteine is methylated to methionine by the transfer of the methyl group of methyltetrahydrofolate (Finkelstein 1990). The transfer of the methyl group is catalyzed by the enzyme called methylenetetrahydrofolate reductase (MTHFR).

The so-called methylation cycle is therefore a methyl-donor cycle and serves "one carbon metabolism". Methyl groups are important for various biochemical systems like DNA and tRNA synthesis, proteins (myelin) and lipids. The transfer of a methyl group is of vital importance for cell functioning. Phospholipid methylation for instance is necessary for the proper function of cell membranes. Methyl groups are necessary for the synthesis and metabolism of neurotransmitters. The synthesis of creatine is important for high energy buffering systems.

Homocysteine is usually determined by high pressure liquid chromatography or HPLC (Te Poel Pothof *et al.* 1995).

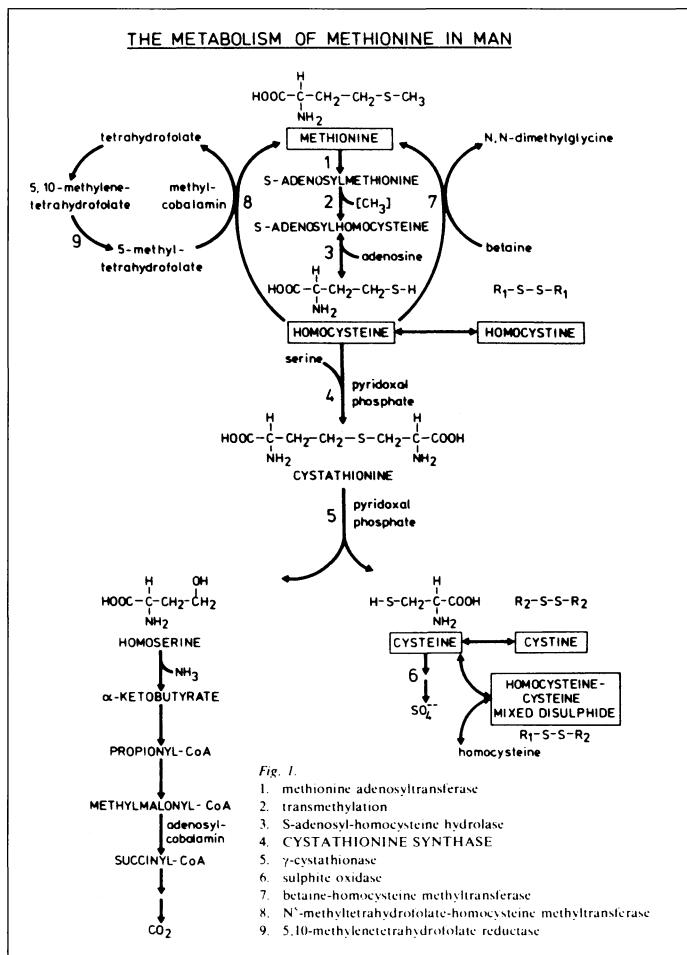


Figure 1. — The essential amino acid methionine is converted to homocysteine via S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). Homocysteine can be remethylated to methionine or transsulphurated to cystathionine and cysteine respectively. The remethylation of homocysteine to methionine is catalyzed by the enzyme 5-methyl-THF-homocysteine methyltransferase or methionine synthase. Methionine synthase requires 5-methyl-THF as a methyl donor and methylcobalamin as a methyl carrier. The irreversible conversion of homocysteine into cystathionine is catalyzed by the pyridoxine-dependent enzyme cystathione synthase. The methyl group delivered by SAM is necessary for DNA methylation and thus for gene expression. SAM is an important regulator of homocysteine metabolism. It inhibits 5,10-methylene THF reductase and stimulates cystathione synthase (after Boers G. H. J., Homocystinuria: "Homozygosity versus heterozygosity". Ph.D. Thesis, University Nijmegen, NL 1985).

The methionine-homocysteine cycle can be challenged by methionine loading (0.1 g/kg body weight). An appreciable amount of patients or control persons (27-50%) with a normal fasting level of homocysteine have hyperhomocysteinemia after methionine loading (Graham *et al.* 1997, Van der Griend *et al.* 1998, Nelen *et al.* 2000b).

In the interpretation of homocysteine values one has to take into account a number of variables that can explain low or high values. Factors like age, gender, the phase of the menstrual cycle, the use of oral contraceptives, pregnancy, race, renal insufficiency, folate status, vitamin B6 and B12 concentrations, genetic mutations and antifolate medication have to be known for the clinical interpretation of homocysteine values.

Neural tube defect (NTD): embryology and prevalence.

The neural tube is formed in the human embryo by a neurulation process of the ectodermal neural plate. The closure of the neural tube occurs from day 21 until day 28 after conception. This is a period that women just think that they are pregnant being one week overdue. If closure of the neural tube fails rostrally anencephaly occurs and when that closure fails caudally spina bifida will be the end result.

The majority of NTDs are caused by interaction of the environment and the susceptible genes of the embryo. The minority of NTDs is due to chromosomal or single gene defects. The prevalence of NTD varies among different countries and ethnic groups and is reported to be between 2 per 1,000 infants (Mexico and Ireland) and 0.2 per 1,000 infants (Finland, Japan) (Papapetrou *et al.* 1996, Franco *et al.* 1998). The recurrence rate is 2%. Although the prevalence is low, one has to realize that spina bifida is a very disabling disease and the highest in ranking of birth defects in general. Furthermore women carrying a NTD fetus are confronted with numerous aspects of prenatal diagnostics including the medico-ethical issue of pregnancy interruption.

The history of research on neural tube defects.

Neural tube defects were already known to the Egyptians and drew continuous attention in every century because of the enigma of birth defects.

One of the first detailed pictures of spina bifida was made in 1641 by Nicholas Tulp, a disciple of Rembrandt.

The history of research in regard to the neural tube is also an example of serendipity by the finding that a derangement of homocysteine metabolism was the pathogenic basis for neural tube defects (Eskes 1998).

Among the various causal factors for spina bifida, nutrition was one of the first. After the second world war a higher incidence of spina bifida was reported in 18-year-old males conceived during the famine period in 1945 who were drafted for military service in the Netherlands.

A further refinement of nutritional deficiency was made by the gynecologist Hibbard and the pediatrician Smithells from the United Kingdom (Hibbard and Smithells 1965). They observed vitamin deficiencies in women who had offspring with an open neural tube defect. After multivitamin supplementation, also containing folic acid, the recurrence rate of neural tube defects was markedly reduced (Smithells *et al.*, 1980-1983).

Credit has to be given to the first attempt to perform a randomized trial by Laurence *et al.* (1981) in the United Kingdom and the pioneering work of Smithells in Leeds (UK). The protocol of the Leeds group containing a placebo double-blind controlled study was rejected by three hospital research ethics committees. Therefore the final study had an observational character.

Studies on folate status in serum, plasma or red blood cells – as a possible predictor of neural tube defects – were not conclusive nor predictive (Mooij *et al.* 1993) and motivated researchers to find new leads in the field of prevention of these serious birth defects.

Derangement of homocysteine metabolism is the possible basis for neural tube defects.

Steegers-Theunissen *et al.* (1991) were the first to report a possible derangement of folate-dependent homocysteine metabolism in women who had NTD offspring. This finding was extended and confirmed (Steegers-Theunissen *et al.* 1994, Mills *et al.* 1995, Van der Put *et al.* 1997b).

Mills *et al.* (1995) could add that mothers of children with a neural tube defect had higher homocysteine values than did vitamin B12 matched controls. The difference was highly significant ($p=0.004$) in the lower half of the B12 distribution after adjusting for plasma folate.

When folate intake is inadequate – as demonstrated in controlled studies – plasma homocysteine concentrations will rise. Plasma homocysteine concentrations are inversely associated with red cell and serum folate concentrations (O'Keefe *et al.* 1995) and are a sensitive indicator of the folate status.

Van der Put *et al.* (1997a) found lowered plasma folate and elevated plasma homocysteine levels in 60 out of 63 mothers with spina bifida offspring at the 25th percentile of plasma folate and at the 75th percentile of plasma homocysteine values. At the 5th and 95th percentiles vitamin B12 values were significantly lower in mothers with affected spina bifida offspring. This was also found in spina bifida patients and their fathers. This finding underlines the functional importance of homocysteine remethylation to methionine.

Methylenetetrahydrofolate reductase (MTHFR) and neural tube defects.

In 1988 Kang *et al.* described a thermolabile variant of MTHFR that is associated with decreased enzyme activity and mildly elevated plasma homocysteine levels.

The derangement of homocysteine metabolism in mothers with neural tube defect offspring is based on gene mutations of the methylenetetrahydrofolate reductase (MTHFR) enzyme. MTHFR catalyses the reduction of 5,10-methylene THF to 5-methyl THF. The methyl group (CH₃) is then available to a variety of methylation reactions. Crucial for the proper development of the embryo is the methylation of homocysteine to methionine after which the methyl group serves the synthesis of DNA and tRNA.

When it became clear that a derangement of homocysteine metabolism was one of the possibilities for the pathogenesis of NTD, research efforts focused on possible enzyme deficiencies and underlying polymorphisms in the metabolic methionine-homocysteine chain in which folic acid and vitamin B12 play an essential role. One common polymorphism (C677T) was reported (Frosst *et al.* 1995). A change of alanine to valine at the 225th amino acid of MTHFR decreased the activity of this enzyme by 35% (Engbersen *et al.* 1995). The mutated MTHFR was indeed a risk factor for spina bifida offspring (Van der Put *et al.* 1995). The mutation predisposes to mild hyperhomocysteinemia in the presence of a low folate status.

A meta-analysis of the MTHFR mutation demonstrated a two-fold increase of the risk of neural tube defects in a Dutch population (Van der Put *et al.* 1997a). Among the 1,067 population-based controls 117 individuals were identified as homozygous for the mutation, giving an overall frequency of 11.0% (95% CI 9.2-12.9%). This homozygote frequency does not differ between men and women and compares with the reported frequencies of Australian and American controls.

The distribution of the MTHFR genotypes among the population is in accordance with the Hardy-Weinberg equilibrium.

Direct role of methionine and homocysteine in the pathogenesis of NTD in whole embryo culture.

The common denominator seems to be an inadequate SAM/SAH (S-adenosylmethionine/S-adenosylhomocysteine) ratio and therefore inadequate methylation.

The role of homocysteine and folic acid in the pathogenesis of NTDs is understood almost exclusively from animal studies.

The method of whole embryo culture has been extensively studied in analyzing the mechanisms of neural tube closure. A complex panorama of cellular and molecular mechanisms is now unfolding.

Methionine can prevent cranial NTD in rat embryos cultured in cow or human serum (Coelho *et al.* 1989). Homocysteine can disturb embryonic development or prevent defects depending upon the milli- or microgram doses. It seems likely that homocysteine reduces the S-adenosylmethionine (SAM)-(SAH) ratio thus inhibiting methyl group donation (van Aerts *et al.* 1994).

Methylation of contractile proteins in the cells of the neural epithelium is an important step in the closing process of the neural tube (Moephuli and Klein 1995). Because the enzyme cystathione-beta-synthase only appears when the fetal liver is present, the homocysteine moiety is conserved in the embryonic homocysteine-methionine cycle. Methionine synthase and S-adenosyl homocysteine hydrolase are present in all embryonic tissues throughout the neurulating period (van Aerts *et al.* 1995).

The studies of Rosenquist *et al.* (1996) in avian embryos in vitro support the direct role of homocysteine and folic acid in the closure mechanism of the neural tube. Homocysteine acts as an agonist at the N-methyl-D-aspartate receptor and as a coagonist at the glycine site.

The studies of Zhao *et al.* (1996) suggest that the mutant Cart 1 mouse, which causes abnormal apoptosis in the forebrain and subsequent absence of forebrain mesenchyme cells, is a homologue of one of the human genes involved in the development of NTD. It is noteworthy that prenatal treatment with folic acid of these mouse embryos suppresses the development of NTD.

Other studies report evidence that many folate-resistant NTDs can be prevented by the supplementation of the complex B-vitamin myoinositol at least in the curly tail mouse (Greene and Copp 1997). Previously these authors showed that neural tube defects in curly tail mutants resulted from a cell proliferation defect in the hindgut endoderm that is causally related to down-regulation of retinoic acid receptor beta expression.

Antifolate drugs like methotrexate, trimethoprim, and aminopterin act as potent inhibitors of dihydrofolate reductase. Among the anticonvulsants valproic acid causes a 5-20 fold increase of NTD. In a murine model valproic acid decreases the embryonic levels of formyltetrahydrofolate and also induces methionine deficiency.

Homeobox genes and paired box (Pax) genes dictate the development of the central nervous system. Several intrinsic and extrinsic factors that can disturb essential cellular events have to be elucidated to discover and finally prevent potential teratogens.

More mutations found.

Recently another mutation in the MTHFR gene was found: A 1298 C (Weisberg *et al.* 1998, van der Put *et al.* 1998). This mutation reduces the MTHFR activity to a lesser extent than the C677T mutation. The pathology risk for neural tube defects for the C677T and A1298C mutations together had an Odds Ratio of 2.4 (95% CI 1.1-5.5). Both mutations can explain 30-50% of the clinical effect of folic acid (Van der Put *et al.* 1999). Folate status and folic acid supplementation modulate plasma homocysteine concentrations and MTHFR genotypes (Jacques *et al.* 1996). Homozygotes have elevated homocysteine and have an enhanced response to the homocysteine lowering effect of folates (Malinow *et al.* 1997).

It can be concluded that the common MTHFR C-T mutation occurs in about 10% of the white populations conferring an enhanced risk of NTD (Wilcken 1997).

Birth defects other than NTD.

Women who experience repeated unexplained early pregnancy loss have a two to three-fold increased risk for recurrence due to low folate status, hyperhomocysteinemia and a higher prevalence of the C677T mutation.

With the start of homocysteine research in relation to neural tube defects and the genetic mutations found, it was logical to explore also this fascinating field of early embryonic development.

Hibbard *et al.* 1964 (cited by Nelen 2000b) was the first to suggest a possible relationship between miscarriage and folate deficiency. An increased FIGLU excretion was found after histidine loading in 32% of women with an isolated unexplained "abortion" and in 60.5% of women with two or more recurrent events.

Sutterlin *et al.* (1997) did not find significant differences in serum concentrations of folate and cobalamin in 29 patients with a history of three or more consecutive early losses. Patients with at least four previous events showed a significant negative correlation with the number of miscarriages and serum folate concentration.

Mild hyperhomocysteinemia has been suggested in patients with recurrent early pregnancy loss (Steegers-Theunissen *et al.* 1992, Wouters *et al.* 1993, Nelen *et al.* 1997a, Quere *et al.* 1998, Coumans *et al.* 1999). The Odds Ratio (OR) of these studies was 2.7 (95% CI: 1.4-5.2) for fasting homocysteine and 4.2 (95% CI: 2.0-8.6) for homocysteine values after methionine loading.

The common mutation C677T was found (Nelen *et al.* 1997b; 2000a, b) in 16% of 185 Dutch women with unexplained recurrent early loss and in 5% of 113 case controls [OR: 3.3; (95% CI: 1.3-10.1)] and 1,250 population controls [OR 2.0: (95% CI: 1.2-3.2)].

This was confirmed in a small French retrospective study (Quere *et al.* 1998).

Ray and Laskin (1999) calculated a pooled Odds Ratio of 3.4 (95% CI: 1.2-9.9) for folate deficiency, 3.7 (0.96-16.5) for hyperhomocysteinemia following methionine loading and 3.3 (1.2-9.2) for the MTHFR mutation.

The higher prevalence of the C677T mutation in women with recurrent early pregnancy loss was confirmed by Guttormsen *et al.* (1996) in the Norwegian Hordaland study.

Homozygotes for the mutant gene of thermolabile MTHFR were sensitive to 0.5 mg of folic acid per day and normalized their plasma homocysteine concentrations (Nelen *et al.* 1998).

One of the factors recently found for early miscarriage is a defect in the vascularization of the chorionic villi. Nelen *et al.* (2000c) found that women with elevated homocysteine concentrations also after methionine loading showed significant smaller vascular areas and perimeters. This suggests that the vascular influence of homocysteine is also apparent in the vessels of the early placenta.

It is important to realize that the possibly preventive effect of folic acid on the recurrence of early pregnancy loss cannot be investigated anymore in a placebo-randomized fashion because of the evidence-based prevention of neural tube defects with folic acid (MRC 1991), a preventive approach that has to start around conception. Therefore more in depth research into the mechanisms that interfere with embryonic development is necessary.

The C677T mutation and hyperhomocysteinemia are also possibly involved in some birth defects other than NTD.

There is starting evidence that folic acid, homocysteine and the MTHFR mutation are involved in the pathogenesis and/or prevention of congenital heart disease (Kapusta *et al.* 1999) and orofacial clefts (Wong *et al.* 1999).

An interesting observation is an abnormal folate metabolism and a mutation in the MTHFR gene in mothers of children with Down's Syndrome (James *et al.* 1999, Rosenblatt 1999).

Plasma homocysteine values can be lowered by folic acid supplements.

Folic acids supplements are clearly capable of lowering plasma homocysteine concentrations, as demonstrated in a meta-analysis of randomized controlled trials (Clarke *et al.* 1998). These studies were performed in cardiovascular patients, volunteers and elderly people.

It is interesting to note that the use of folate or folic acid in healthy volunteers is also followed by a decrease in plasma homocysteine concentrations (Brouwer *et al.* 1999).

Dietary intake patterns also relate to plasma folate and homocysteine concentrations as demonstrated in the Framingham study (Malinow *et al.* 1998).

Folic acid and vitamin B6 supplementation lowered homocysteine loading values in patients with preeclampsia, fetal growth restriction and hyperhomocysteinemia (Leeda *et al.* 1998).

A substantial minority of people may have increased folate needs: 5 to 15% of the general population are homozygous for the thermolabile variant of the thermolabile variant of 5,10-MTHFR (C677T) which causes hyperhomocysteinemia. Red cell folate and plasma folate are significantly lower in these homozygous individuals. These results suggest that a substantial minority of people may have increased folate needs (Molloy *et al.* 1997).

Homocysteine and obstetrical vascular disease in women.

Homocysteine is an independent risk factor for vascular disease. There is substantial evidence that homocysteine is a strong and independent risk factor for vascular disease (Clarke *et al.* 1991). The evidence can be found in more than 80 epidemiologic studies with more than 10,000 subjects. The risk for cardiovascular disease is dose-dependent without a cut-off level. Venous thrombosis data from case-control studies support hyperhomocysteinemia as a risk factor (Den Heijer *et al.* 1997).

In women one has to recognize hyperhomocysteinemia when thrombo-embolic episodes occur during the use of oral contraceptives, postmenopause, in a postoperative period or during pregnancy and the postpartum period.

A randomized controlled trial of the effect of vitamins (including folic acid) on the risk of vascular disease still has to be undertaken.

Homocysteine and preeclampsia.

Rajkowich *et al.* (1997 and 1999) reported elevated homocysteine levels in pregnant nulliparous American women with preeclampsia and case controls tested at the time of delivery. The hematocrit values were not different between the groups, excluding therefore hemoconcentration. Folic acid concentration did not differ.

This finding was confirmed by Powers *et al.* (1998). Furthermore these authors found evidence of endothelial activation as demonstrated by an increase of cellular fibronectin, a marker of oxidative stress.

Sorensen *et al.* (1999) found elevated levels of serum homocysteine in pregnant women with preeclampsia in the second trimester. The risk of developing preeclampsia was calculated as 3.2 (95% CI: 1.1-9.2). Nulliparous women with elevated homocysteine levels experienced a 9.7-fold increased risk of preeclampsia (95% CI: 2.1-14.1) compared with multiparous women without homocysteine elevations. These risk values are comparable to the study by Raikowich *et al.* (1999).

In patients with severe early-onset preeclampsia, hemostatic and metabolic disturbances, associated with a tendency toward vascular thrombosis, were found in 79 women tested postpartum: Protein S deficiency (24.7%), activated protein C resistance (16.0%) and hyperhomocysteinemia in plasma fasting and after methionine loading (17.0%) (Dekker *et al.* 1995).

Pooled data on homocysteine and preeclampsia showed an Odds Ratio of 20.9 (95% CI: 3.6-121.6) (Ray and Laskin 1999).

Methylenetetrahydrofolate reductase (MTHFR) polymorphism also plays a role in preeclampsia.

The C677T mutation of the MTHFR gene was significantly increased in Japanese preeclamptic women (24%) compared with normal pregnant women (11%) and healthy adults (11%). The Odds Ratio was calculated as 2.5 (95% CI: 1.3-4.8) for the homozygous genotype (Sohda *et al.* 1997).

The effect of MTHFR polymorphism and the genetic susceptibility to preeclampsia can be attenuated by the presence of factor V Leiden (Grandone *et al.* 1997). In this Italian population TT homozygotes were found in 29.8% of cases and 18.6% of controls [OR 1.8 (95% CI: 1.0-3.5)]. In cases where also factor V Leiden was present, an illustrative case history demonstrated a high risk for a complicated pregnancy (Grandone *et al.* 1997).

Kupferminc *et al.* (1999) reported a prevalence of the genes of factor V Leiden, MTHFR and prothrombin of 53% among Jewish women with preeclampsia [OR 5.4 (95% CI: 2.3-12.4)]. Van Pampus *et al.* (1999) and De Groot *et al.* (1999) found no differences in the prevalence of genetic risk factors (factor V Leiden, prothrombin 20210 A allele, protein C and S and antithrombin deficiency) in women with preeclampsia compared with control subjects.

Three reports (Powers *et al.* 1999, Zusterzeel *et al.* 1999, O'Shaughnessy *et al.* 1999) could not confirm an increased risk for eclampsia due to the 677 C-T polymorphism.

A meta-analysis of the studies mentioned above comprised 518 patients and 1,100 controls (Zusterzeel *et al.* 1999). The Odds Ratio was 2.0 (95% CI: 1.4-2.9). In the interpretation one has to keep in mind confounding factors like folate status, the population involved, the number of patients and the type of controls.

Nevertheless the homozygous 677 TT genotype seems to be a moderate risk factor for preeclampsia. This finding is in line with research in the general vascular area.

Maternal hyperhomocysteinemia is a risk factor for placental abruption.

Steegers-Theunissen *et al.* (1992) from the homocysteine research group at Nijmegen (NL) noted that homocysteine could be a risk factor for placental abruption.

From the same group Goddijn-Wessel *et al.* (1996) found hyperhomocysteinemia in 26 out of 84 Dutch patients (31.0%) who experienced placental abruption, placental infarcts and fetal growth retardation – all in a combination summarized as placental vasculopathy – and in 9% of 46 controls. The difference had an Odds Ratio of 4.7 (95% CI: 1.4-17.3). Serum and red cell folate, serum vitamin B12 and B6 were significantly lower in the study group as compared with the controls.

This finding was confirmed in a small study from South Africa (Owen *et al.* 1997) without differences in vitamin profiles.

In extended studies on placental abruption in the Dutch population van der Molen *et al.* (2000a) found a significantly higher plasma homocysteine in 175 women with placental vasculopathy compared with 141 matched controls: OR 3.3 (95% CI: 1.4-7.9). The OR for folic acid was 2.1 (95% CI: 1.1-4.0) for vitamin B12: 2.6 (95% CI: 1.3-4.9) and 2.9 (95% CI: 1.5-5.5).

Endothelial cells in vitro may reflect the natural in vivo feature.

Homocysteine metabolism in endothelial cells is dependent on folic acid. When endothelial cells from the human umbilical vein are studied in vitro homocysteine concentrations increase by constant amounts under standard culture conditions. Folic acid supplementation lowers the homocysteine export in a dose-dependent manner. Methyltetrahydrofolate and folic acid, its precursor, are ten times more active than folic acid. Additions of vitamin B6 or B12 do not show any effect on the in vitro homocysteine cellular export (Van der Molen *et al.* 1996).

Among the many hypotheses for the atherosclerotic action of homocysteine the demonstrated dual action of homocysteine by promoting growth of smooth muscle cells of blood vessels while inhibiting endothelial cell growth is very attractive (Tsai *et al.* 1994, 1996).

The MTHFR gene mutation (C677T) is likely to be a risk factor for placental abruption (vasculopathy).

The C677T mutation of the MTHFR gene was found in 19/165 (12.0%) Dutch women with vasculopathy, in 7/139 (5%) matched controls [OR 2.5 (95% CI: 1.0-6.0)] and 106/1,250 population based controls (Odds Ratio: [1.4 (95% CI: 0.8-2.4)]) of the study of Van der Molen *et al.* (2000b).

Reduction of fasting and post-methionine load plasma homocysteine values could be lowered by the administration of 250 mg Vitamin B6, 5.0 mg folic acid per day or vit B12 (Bostom *et al.* 1997, Bronstrup *et al.* 1998). This opens perspectives for the prevention of recurrence of placental abruption, to be validated in a proper clinical placebo-randomized trial.

The combination of homocysteine and thrombotic factors increase the risk for placental vasculopathy three to seven times.

A combination of thrombotic risk factors (homocysteine, MTHFR mutation, Activated Protein C resistance, Protein-C) raised the Odds Ratio for two risk factors to 3.40 (95% CI: 1.80-6.42) and for three risk factors to 6.83 (95% CI: 1.52-30.7), respectively (van der Molen *et al.* 2000b).

Homocysteine and oncology.

The possible relationship between folates and cancer cells dates back at least four decades. Massey and Rubin (1954) described the persistence of abnormal gastric columnar cells in the stomach of patients with pernicious anemia even after treatment with vitamin B12. Since the cytologic appearance of these cells exhibited some characteristics of both megaloblastic cells and cancer cells and since an increased incidence of gastric cancer in cases with pernicious anemia, these investigators postulated that these abnormal cells might represent a transitional cell type between the cells of atrophic gastric epithelium in pernicious anemia and gastric cancer cells.

Gynecologic studies on the relationship between megaloblastosis due to folate deficiency and cancer of the uterine cervix was observed by Van Niekerk (1966). Several cytologic similarities were seen between epithelial cells of the uterine cervix from folate deficient women and cervical dysplastic cells.

The studies published in the seventieth till the eighties were too conflicting to make any statement about whether alterations in folate status truly modulate the process of carcinogenesis in the cervix (Mason 1995).

More promising and interesting are the recent findings on coloncarcinoma.

An association between diminished folate status and enhanced risk of colorectal adenomas or cancer was first noted by Lashner *et al.* (1989). In a case control study of patients with ulcerative colitis. These authors also determined that individuals who had not taken folate supplements had a 2.5-fold greater risk of colon neoplasia than those who did not.

Furthermore chronic administration of sulfasalazine-a drug commonly used for the treatment of ulcerative colitis and known to inhibit folate absorption- and metabolism was associated with a 50% increase in the risk of dysplasia (Franklin and Rosenberg 1973, Selhub *et al.* 1978).

Direct evidence from two cohort studies suggests that a diet low in folate and methionine and high in alcohol is associated with increased risk of coloncarcinoma and adenoma.

An inverse association of the polymorphism in the MTHFR gene (667 C-T) with colorectal cancer was observed by Ma *et al.* (1997) and Chen *et al.* (1996, 1999). Men with the homozygous MTHFR mutation had half the risk of colorectal cancer compared with the homozygous normal or heterozygous genotypes [OR 0.49 (95% CI: 0.27-0.87)]. Men with adequate folate levels had a 3-fold decrease in risk [OR 0.32 (95% CI: 0.15-0.68)]. The protection due to the mutation was absent in men with folate deficiency.

The observation that the polymorphism can be protecting in case of colorectal cancer, but also provocative in case of a risk factor of neural tube defects (Van der Put *et al.* 1995) and endometrial cancer (Esteller *et al.* 1997) deserves an explanation.

MTHFR irreversibly converts 5,10-methylenetetrahydrofolate, the major form of intracellular folate, to 5-methyl tetrahydrofolate, the major form of circulating folate in plasma. The former donates its one-carbon moiety to deoxyuridate thymidilate, which is the rate-limiting nucleotide in DNA synthesis. The latter is the primary methyl donor for the remethylation of homocysteine to methionine and subsequently to S-adenosylmethionine (SAM). Declines in SAM levels enhance MTHFR activity and direct folate cofactors away from DNA synthesis and DNA methylation. Next to that, folate deficiency results in deficient methylation of dUMP to dTMP causing misincorporation of uracil into DNA and subsequent chromosomal breaks (Blount *et al.* 1997). Therefore the place of demethylation in the folate cycle can determine dysregulation of gene expression causing disturbances in development or prevent chromosomal breaks causing cancer.

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