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Original Article

5-Methyltetrahydrofolate reduces blood homocysteine level significantly in C677T methyltetrahydrofolate reductase single-nucleotide polymorphism carriers consulting for infertility

Arthur Clément[a], Yves Menezo[a,b,*], Marc Cohen[c], Dominique Cornet[d], Patrice Clément[a]

[a] Laboratoire Clément, Avenue d'Eylau, 75016 Paris, France
[b] London Fertility Associates, Harley St, London, UK
[c] Clinique Natecia, 69008 Lyon, France
[d] Clinique de La Muette, Rue Nicolo, 75016 Paris, France

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ABSTRACT

Purpose: Methyltetrahydrofolate reductase (MTHFR) C677T (ala222Val) is a single-nucleotide polymorphism (SNP) that affects the formation of 5-methyltetrahydrofolate (5-MTHF), the active folate that allows the recycling of homocysteine (Hcy) to Methionine. Hcy is at the epicentre of oxidative stress and DNA methylation errors. This SNP often increases the circulating Hcy levels and consequently reduces the methylation process, which is involved in the epigenesis and imprinting of markings in gametes. This study aimed to investigate decreases in Hcy levels in MTHFR SNP carriers.
Procedure: Eighty-nine couples with fertility problems for at least 3 years were included in this program. The women were systematically tested for the MTHFR C 677T isoform. If the woman tested positive, testing of the male partner was proposed. All the carriers had well-controlled blood Hcy levels before and after treatment (600 μg of 5-MTHF/day, with a backup of one carbon cycle during at least 3 months).
Findings: As expected, the circulating Hcy level was higher in the homozygous patients than in the heterozygous and wild-type patients. The treatments caused a significant decrease of the circulating Hcy in the SNP carriers group.
Conclusions: Couples with a long history of infertility should be analysed for MTHFR SNP and homocysteine and should be treated with physiological doses of 5-MTHF instead of high doses of folic acid.

Introduction

Methylation is a crucial biochemical process in all aspects of metabolism and its regulation. It is involved in gene expression via imprinting and epigenesis. The targets of this process are lipids, proteins, and DNA. It regulates the level of homocysteine (Hcy), a toxic amino acid affecting health in autoimmune diseases, mental disorders, diabetes, and other renal and liver metabolic pathologies [1]. The reproductive process is strongly "methylation dependent." Gametogenesis [2–5], early embryogenesis [6,7], trophoblast development [8–10], and implantation will be strongly affected by methylation errors. The folates and one-carbon cycles (1-CCs) are "key players" in methylation-mediated regulations (Fig. 1). The folate cycle allows the formation of 5-methyltetrahydrofolate (5-MTHF) necessary for the recycling of Hcy to methionine via the methionine synthase (MS), a major step in the 1-CCs. Hcy is an inhibitor of methylation, as shown in the early embryo [11] and is the epicentre of oxidative stress and DNA methylation errors [7,12].

Numerous mutations affect these two biochemical cycles. The most frequent are the methyltetrahydrofolate reductase (MTHFR) single-nucleotide polymorphisms (SNPs, C677T and A1298C. The two enzymatic isoforms have a weaker capacity, which can reach −75%, to generate 5-MTHF, but C677T can cause worse handicaps. This SNP carries a high risk for generation of neural tube defects (NTDs) [13]. High doses of synthetic folic acids, such as pteroylglutamic acid are generally proposed to patients desirous of pregnancy. This leads to the accumulation of un-metabolised folic acid, the UMFA syndrome, which is not without hazard or at

* Corresponding author at: Laboratoire Clément, 17 Avenue d'Eylau, 75016 Paris, France.
E-mail addresses: aclement@laboclement.com (A. Clément), yves.menezo@gmail.com (Y. Menezo), mcsafe@gmail.com (M. Cohen), dc75@wanadoo.fr (D. Cornet), pclement@laboclement.com (P. Clément).

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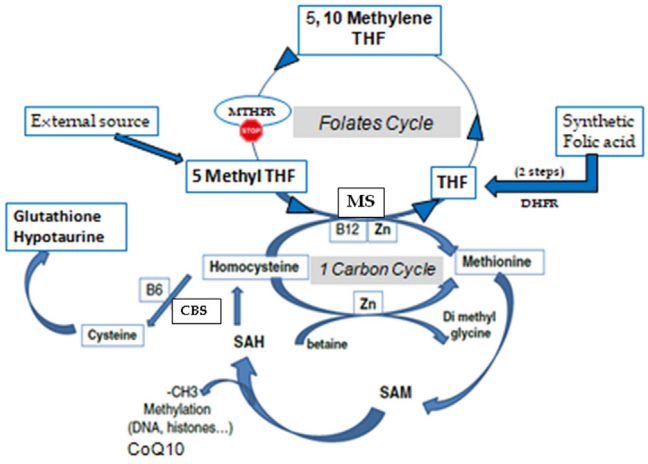

Fig. 1. The One-carbon and the folates cycles. Synthetic folic acid must undergo 2 metabolic steps to enter the folate cycle. The human liver has a poor dihydrofolate reductase (DHFR) activity. MTHFR SNPs severely reduce the formation of 5-MTHF, the active folate. At the end, the quantity of 5-MTHF provided by synthetic folic acid is very low. CBS: cystathionine beta synthase; MS: methionine synthase; MTHFR: methyltetrahydrofolate reductase; THF, tetrahydrofolate.

least recurrent issues [14–17]. It affects immune responses and cause flare-up of tumour genesis, accelerating leukaemia, and colorectal and prostate cancers [18]. In addition, the un-metabolised folic acid competes with the natural folates and block the binding and transport of all folates to receptors, resulting in downregulation. This leads to a pseudo MTHFR syndrome that increases, in turn, the Hcy level even in the wild-type (WT) patients. As the need for methyl groups is of major importance in the reproductive process and the UMFA syndrome must be avoided, we have treated the MTHFR SNP (SNP) C677T carriers who consulted for infertility with 5-MTHF supported with a 1-CC backup [19]. 5-MTHF showed good efficacy to reduce blood Hcy levels in a normal population [20,21]. It provided a solution to long-lasting infertility problems in MTHFR SNP carrier couples [22] Blood Hcy level was tested before and after treatment to control the efficacy of the treatment before starting assisted reproductive technology (ART) attempts.

Materials and methods

Patients

By law, the consent of the patients is required for any genetic testing: forms have to be filled before any genetic testing and they require the signature of the doctor, the laboratory in charge of the testing and the patients.

All the treatments are available without prescription and did not involve any new molecule. The advice of the French Agence de Biomedicine in charge of all the supervisions is so not required.

In our units, all patients with repeated miscarriages (>3) or who were facing infertility for >3 years and had at least 3 ART failures were tested for MTHFR SNP C677 T. The control for C677T SNP only was proposed for the male partner when the woman tested positive (homozygous (HMZ) or heterozygous (HTZ). The HTZ or HMZ tested and WT partner(s) among the couples received a blood Hcy dosage to determine the background values.

Genetic testing

The presence of the isoform was determined from a blood sample by real-time polymerase chain reaction with the Real Fast assay (Vienna Lab Diagnostic GMBH, Vienna, Austria). The presence of WT and SNP probes in the reagent mixture allowed the determination of the following 3 types: WT, C677 T (heterozygote HTZ), and T677 T (homozygote HMZ).

Circulating Hcy determination

The total Hcy + homocystine (oxidised form of Hcy) level was measured using the VYTROS kit. Briefly, all homocysteine was reduced with tris(2-carboxyethyl) phosphine to form Hcy. All reduced Hcy was transformed to cystathionine in the presence of cystathionine beta synthase (CBS). The cystathionine is then hydrolysed by cystathionine lyase to form Hcy, ammonia, and pyruvate. After reduction with lactic dehydrogenase and NADH to form lactate, the amount of NAD + produced was measured at 340 nm, which was proportional to the Hcy levels present in the sample.

Treatments

All SNP carriers were treated for at least 3 months with 5-MTHF at a dose of 600 μg per day, complemented with components of the one-carbon cycle (1-CC; B3, B6, and B12 vitamins and zinc: Impryl®, Parthenogen, Lugano Switzerland or Tetrafolic®, Nurilia, Lyon, France) [19]. The doses are fixed according to the ENSES/EFSA recommendations (EFSA, European Food Safety Authority), 2017. Dietary reference values for nutrients: Summary report. EFSA supporting publication 2017:e15121. 92 pp. doi:10.2903/sp.efsa.2017.e15121)

Serum homocysteine was measured at the end of the 3 months treatment.

Statistical analysis

Inferential statistics for qualitative variables were performed using the chi-square test or the Fisher exact test if available. Inferential statistics for quantitative variables were performed using the Student *t* test or its non-parametric equivalent if necessary.

Results

As our laboratory is the French reference centre since February 2018 for MTHFR SNP patients consulting for fertility problems (871 patients tested from February to July 2018), the following C677 T distribution for women was observed: WT, 44.4%; HTZ, 44.2%; and HMZ, 11.4%. It is close to that generally observed in Europe [6]. In this specific population of 89 couples, the percentage of HMZ carriers was overrepresented: 24% vs 11.4%.

The whole population (89 couples) included 50 HTZ (21 men and 29 women), 39 HMZ (11 men and 28 women), and 72 WT patients. For the SNP carriers, the mean (SD) Hcy concentration was 14.9 (8.2) μmol/L before treatment, significantly higher ($p = 3 \times 10^{-4}$) in HMZ (18.4 (11.2) μmol/L) than in HTZ (12.2 (2.6) μmol/L) patients. These values in the two groups of SNP carriers are significantly higher than those observed in the WT group: 7.9 (2.3) μmol/L, p = 0.0000)

The treatment decreased the Hcy level from 14.9 μmol/L to 10.1 (2.4) μmol/L in the mixed carrier population (−32.3%, $p = 3 \times 10^{-5}$). A similar significant ($p = 7 \times 10^{-7}$) decrease from 12.2 μmol/L to 9.8 (1.9) μmol/L was also obtained for the HTZ patients (−20.1%). The decrease in the HMZ population was the highest at −42.7% from 18.4 to 10.5 μmol/L ($p = 5.9 \times 10^{-5}$): Fig. 2. At the end of the treatment, no significant difference (p = 0.12) can be observed between HMZ and HTZ (10.5 μmol/L *vs.* 9.8 μmol/L)

Discussion

The concentration of circulating Hcy was <8 μmol/L in our WT group of 72 patients. The C677 T SNP in the MTHFR gene increases

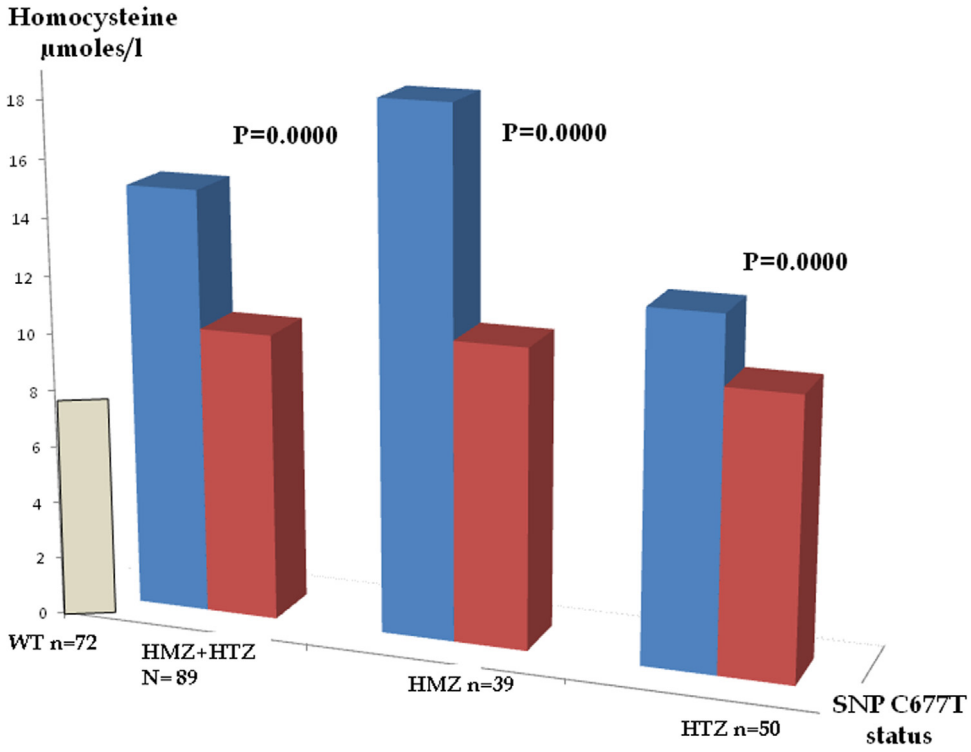

Fig. 2. Variations in Hcy concentrations before and after treatment: blue, before treatment and red, after treatment (WT: wild type).

Hcy concentrations; this increase is higher in HMZ patients. 5-MTHF, associated with components of the 1-CC, reduced rapidly the Hcy level in our patients, with a similar efficacy for the HMZ and HTZ patients. In previous studies, we demonstrated [19,22,23] that support of the 1-CC components improves the chance of conception for both the male and female patients. Moreover, decrease in Hcy level with 5-MTHF allows high pregnancy and delivery rates in patients with repeated miscarriages (RM) and ART failures [22,24,25]. The toxicity of Hcy has been elucidated: It is particularly relevant and Hcy levels have been studied in obstetrics (generation of NTDs), cardiology, psychiatry, and urology [1,26]. Hcy has been neglected in fertility, though not completely. Associated with a low efficacy of the 1-CC, Hcy has a negative impact on gametes and embryo quality [11,2,3,27,28]. Treatment with high doses of synthetic folic acid: pteroyl glutamic acid to reduce Hcy levels and improve the methylation process is not a solution for MTHFR SNP carriers [29], as they poorly metabolise this compound. First, synthetic folic acid has to be transformed in the liver in a 2-step manner by the dihydrofolate reductase to form tetrahydrofolate (THF). However, whatever the population, human liver has a very poor conversion capacity [30]. Then, THF is transformed to 5,10-methylene THF, which has to be converted by MTHFR to form 5-MTHF, the active compound necessary to recycle Hcy to methionine via the MS (Fig. 1). This means that un-metabolised folic acid increases in the blood, competing for the receptors and transporters of the active 5-MTHF for entry into the cells. This leads to erratic responses toward Hcy reduction and methylation in these patients [31,32]. In human and mouse testes, high doses of synthetic folic acid induce DNA hypomethylation in the sperm of WT patients and, even to a higher extent, in C677 T SNP carriers [33,29]. It the general population, it leads to the accumulation of un-metabolised folic acid; UMFA is not without risk: it may induce a flare-up in some cancers (especially prostate and colorectal).

The main limitation of our study is that we did not test the second most common genetic variant of MTHFR, the A1228C mutation. This is also associated to decrease MTHFR function and to lightly elevated Hcy [34], and may occur together with the C677 T mutation [35]. But the C677 Timpact is, by far, the most deleterious [26]. Thus, our WT group might have been slightly over-estimated. A second possible limitation of the study is that we did not test our patients for blood folates. Indeed, the common and usual fluorescence-based folate detection is centred on the pteroyl nucleus of the molecule. Accordingly, it does not distinguish between soluble and bioactive folates and UMFA, and is of little clinical value in this setting. Better precise tests such as mass spectrometry associated with liquid chromatography (LC/MS/MS) are complex and expensive, and we are not using them in the clinical practice.

In case of long lasting infertility and repeat pregnancy losses (RPLs), both members of a couple should be tested for MTHFR SNPs, especially before oocyte donation. Failure to do so could be considered poor practice. Carriers' supplementation with 5-MTHF before attempting an ART or conception project should be also recommended.

AC, YM, DC, and PC have no conflict of interest to disclose. MC is a manager of a nutraceutical company (Nurilia).

Declaration of Competing Interest

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