ORIGINAL ARTICLE

Journal of Pre-Clinical and Clinical Research 2024, Vol 18, No 2, 109-113
www.jpccr.eu

MTHFR gene polymorphisms and serum homocysteine concentration in women with recurrent miscarriages

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Budzyńska E, Olszak-Wąsik K, Borowiec M, Moczulska H. *MTHFR* gene polymorphisms and serum homocysteine concentration in women with recurrent miscarriages. J Pre-Clin Clin Res. 2024; 18(2): 109–113. doi: 10.26444/jpccr/189443

Abstract

Introduction and Objective. The significance of MTHFR gene polymorphisms in the course of pregnancy has not been definitively resolved. Variants c.665C>T and c.1286A>C in the MTHFR gene leads to increased concentration of homocysteine. The aim of the study was to evaluate homocysteine serum concentrations in Polish women with recurrent pregnancy loss, depending on the presence of the MTHFR gene variants c.665C>T and c.1286A>C in the MTHFR gene.

Materials and Method. 137 women with recurrent miscarriages, normal karyotype and normal molecular tests results for the variants c.1691G>A of F5 gene and c.97*G>A of F2 gene were qualified for the study. The relationship between the presence of the MTHFR gene polymorphisms c.665C>T and c.1286A>C in gene MTHFR and homocysteine serum concentration was stadied.

Results. There were no significant differences found in homocysteine levels in the study group of women with recurrent miscarriages. There were no differences in homocysteine concentration in patients with MTHFR gene c.655C>T polymorphism (heterozygous nor homozygous), compared to patients without the MTHFR gene polymorphism (CC wild type). Similarly, no differences were noted between patients with AA-wild type and patients with the MTHFR variant c.1286A>C (heterozygous nor homozygous). There was no also correlation between homocysteine concentration and women's age, regardless of the patients' group (control vs. study groups).

Conclusions. The c.655C>T and c.1286A>C variants of the MTHFR remain without significant impact on homocysteine concentration in Polish women with recurrent pregnancy loss.

Key words

homocysteine, MTHFR, miscarriages

INTRODUCTION

In recent years there has been a great deal of debate concerning the determination and interpretation of 5,10-methylenetetrahydrofolate reductase (MTHFR) and homocysteine in the context of thrombophilia and pregnancy loss. Due to the many studies conducted, it seems that MTHFR problem has been finally recognised as polymorphism – not as a mutation. However, the role of other factors engaged in the folate and methionine cycles needs more research.

The *MTHFR* gene is located on chromosome 1 (1p36.22), and is one of the enzymes involved in the folate cycle and the regulation of homocysteine. In the wild type in the c.c.655 position of the *MTHFR* gene, cytosine is present in two chromosomal copies and thymine in the c.c.1286 position [1]. Single-nucleotide polymorphisms of 5,10-MTHFR leads to its reduced activity, thus the increased concentration of homocysteine [2, 3]. The most common polymorphisms in the *MTHFR* gene are c.665C>T and c.1286A>C.

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Received: 21.03.2024; accepted: 28.05.2024; first published: 18.06.2024

In the c.665C>T polymorphism described by Frosst [4], cytosine (C) replaces thymine (T), which changes alanine to valine within the N-terminal catalytic domain. This results in the synthesis of a thermolabile form of MTHFR with the consequence of the reduction in its activity by 40% in heterozygous variant CT, and up to 60%-70% in homozygous variant TT [1, 5]. The second polymorphism (c.1286A>C) causes a less pronounced reduction in MTHFR activity by 10% and 40% in the heterozygous and homozygous forms, respectively. In combined heterozygotes (for c.665C>T and c.1286A>C), MTHFR activity is more reduced [2]. So far, no individuals have been found to be homozygous for both polymorphisms [1].

Figure 1 presents methionine and homocysteine metabolism and the role of MTHFR in the folate cycle.

In the literature, many authors have described the association of MTHFR activity with various clinical conditions. The disturbances in the folate cycle, MTHFR activity and methionine and homocysteine cycle may influence different levels of human development and further life health conditions, such as cardiovascular disease, thrombosis, osteoporosis, dementia, Alzheimer's disease, migraine, epilepsy, depression and schizophrenia, glaucoma,

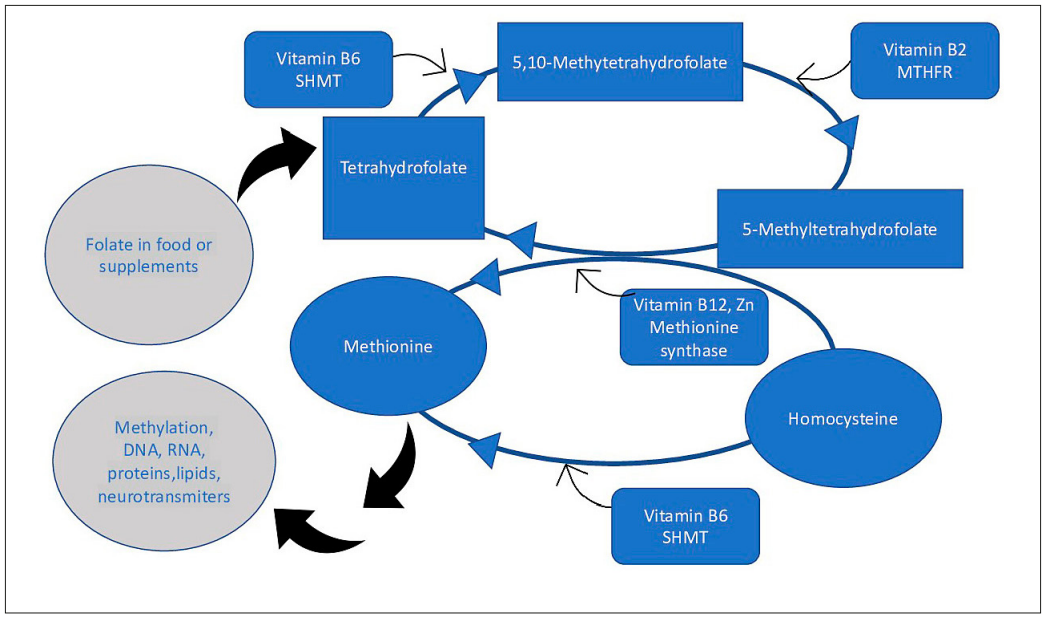

Figure 1. Methionine and homocysteine cycles are strongly dependent on MTHFR activity. If reduced, the formation of 5-methyltetrahydrofolate is insufficient, and methionine synthase cannot restore methionine from homocysteine. Consequently, homocysteine and unmetabolized folic acid accumulate.
SHMT – serine hydroxymethyltransferase, MTHFR- methylentetrahydrofolate reductase

hearing loss, and certain types of cancer. Decreased folate and increased plasma homocysteine (Hcy) levels that occur in the presence of a thermolabile form of MTHFR, may be associated with hypertension and pre-eclampsia in pregnancy, neural tube defects, and cleft lip/palate [5,6].

Although several studies have shown a strong correlation between homocysteine level and recurrent spontaneous abortion (RSA), there are also investigations contrary to these findings [7]. Following the definition given by American Society of Reproductive Medicine (ASRM) and European Society of Reproductive Medicine) ESHRE, recurrent spontaneous abortion refers to the failure of two or more clinically recognised pregnancies. Whereas, according to Royal College of Obstetricians and Gynaecologists (RCOG), recurrence is described as three or more pregnancy losses. The time limit and consecutive losses are additional criteria that vary by guidelines [8]. The etiology of spontaneous pregnancy loss is quite heterogeneous and, in half of the cases, is undiagnosed [9].

Although MTHFR polymorphism is associated with global methylation activity (whole genome), there is still no clear evidence for a link between genetic variants in gene *MTHFR* and DNA methylation status in individuals (within the meaning of cell-tissue depending consequences) [10, 11].

OBJECTIVE

Many studies have revealed that an increased homocysteine concentration might be associated with miscarriage and pregnancy complications. The aim of this study was to evaluate the homocysteine concentrations in women with recurrent pregnancy loss, depending on the presence of the *MTHFR* gene polymorphisms c.665C>T and c.1286A>C in gene *MTHFR*.

MATERIALS AND METHOD

A group of women with at least two consecutive pregnancy losses (from six to 20 weeks of gestation) who were referred to the Department of Clinical Genetics, Central Clinical Hospital of the Medical University in Lodz, Poland, were enrolled into the study. All patients were Caucasian. The patients were first examined for congenital thrombophilia (polymorphism c.1691G>A of *F5* gene and c.97*G>A of *F2* gene), and chromosomal abnormalities (karyotype). Their male partners' karyotypes revealed no abnormalities. Finally, 137 patients were selected for inclusion in the study. All pregnancies had been conceived naturally, and there were no cases of couple infertility treatment (male and female factors excluded).

Among the 137 patients, two groups were distinguished: 38 women without *MTHFR* gene polymorphisms (MTHFR 665 CC wild type and MTHFR 1286 AA wild type – control group), and 99 women with *MTHFR* gene polymorphism (c.665C>T/c.1286A>C combined genotype – study group). The mean age of patients enrolled on the study was 31 (ranging between 21 – 44 years (Fig. 2).

Serum homocysteine concentration was measured using immunochemiluminescence assay in Immulite 2000 XPi analyzer (Siemens Healthcare Diagnostics Inc.). DNA was isolated from a venous blood sample using an automated DNA isolation station (Promega Maxwell RSC).

MTHFR gene polymorphisms were detected using FV-PTH-MTHFR Strip Assay (ViennaLab, Vienna, Austria), based on the reverse hybridisation principle. Statistical analysis was performed using R software version 3.5.3 (R Foundation for Statistical Computing).

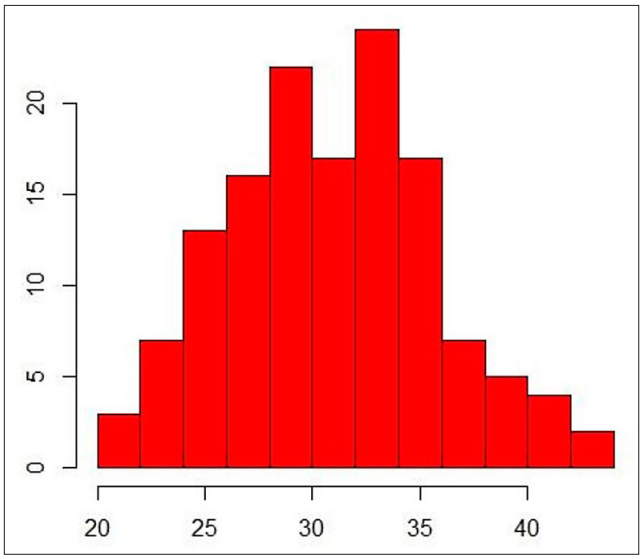

Figure 2. Age distribution among the 137 patients

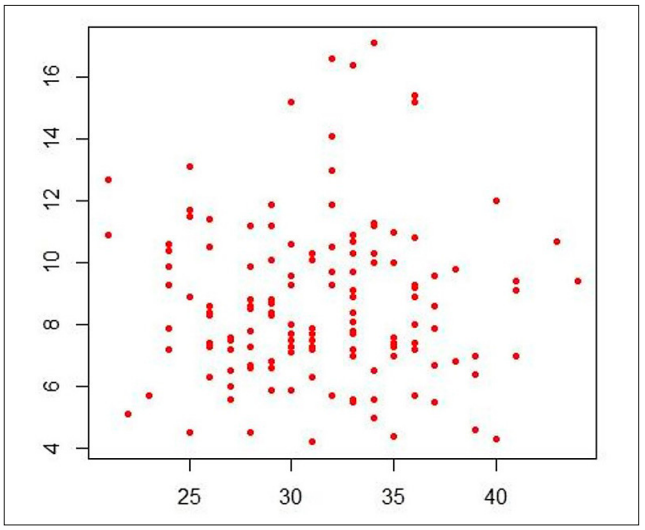

Figure 3. Homocysteine concentration in the 137 patients showed non-parametric distribution, mean concentration – 8,4 mmol/l (rang – 4,2 mmol/l-17,1 mmol/l). No correlation existed between patients' age and homocysteine concentration

RESULTS

There were no significant differences in homocysteine levels in the study group of women with recurrent miscarriages. No correlation was found between homocysteine concentration and women's age, regardless of the patients' group (control vs. study group). There were no differences in homocysteine concentration in patients with *MTHFR* gene c.655C>T polymorphism (heterozygous nor homozygous), compared to patients without *MTHFR* gene polymorphism (CC wild type). Similarly, no differences were noted between patients with the AA-wild type and patients with the *MTHFR* variant c.1286A>C (heterozygous nor homozygous). Multivariate analysis demonstrated no associations between patients' age and homocysteine concentration, nor both analysed *MTHFR* gene polymorphism (c.655C>T nor c.1286A>C) (Fig. 2 and Table 1). (Figures 3–5 show no significant differences in homocysteine concentration in distinguished *MTHFR* gene variants).

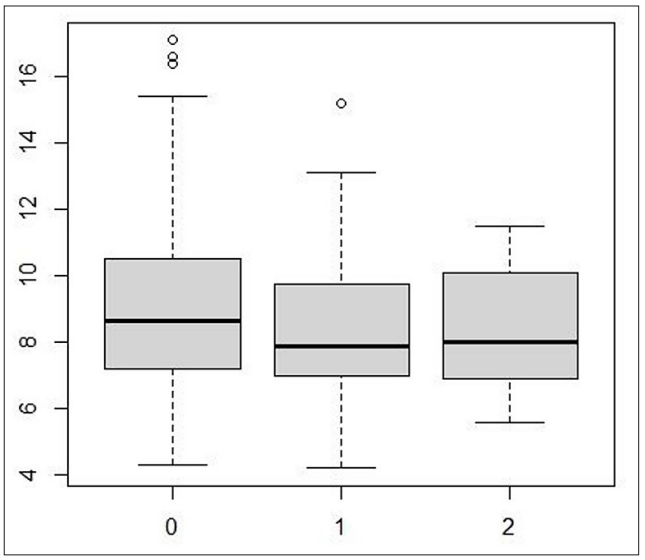

Figure 4. Homocysteine concentration in c.655C>T *MTHFR* genotype

Table 1. Detailed results of homocysteine concentration according to MTHFR genotype variants

MTHFR variant	Wild type *MTHFR* 665CC	*MTHFR* 665 CT	*MTHFR* 665 TT
Homocysteine concentration	Median = 8,7 (Range 4,3–40,3)	Median = 7,9 (Range 4,2–15,2)	Median = 8,0 (Range 5,6–11,5)

MTHFR variant	Wild type *MTHFR* 1286 AA	*MTHFR* 1286 AC	*MTHFR* 1286 CC
Homocysteine concentration mmol/l	Median = 8,45 (Range 4,4–40,3)	Median = 7,95 (Range 4,2–16,4)	Median = 9,65 (Range 7,2–17,1)

MTHFR variants	CC 665 and AA 1286 n=39	CT 665 and AC 1286 n=18	CC 665 and CC 1286 n=14	CT 665 and AA 1286 n=30	CT 665 and AC 1286 n=22	TT 665 and AA 1286 n=20
Homocysteine concentration mmol/l	Median = 8,6 (Range 5,0–40,3)	Median = 7,9 (Range 4,3–16,4)	Median = 9,65 (Range 7,2–17,1)	Median = 7,65 (Range 4,4–15,2)	Median = 8,15 (Range 4,2–13,0)	Median = 8,0 (Range 5,6–11,5

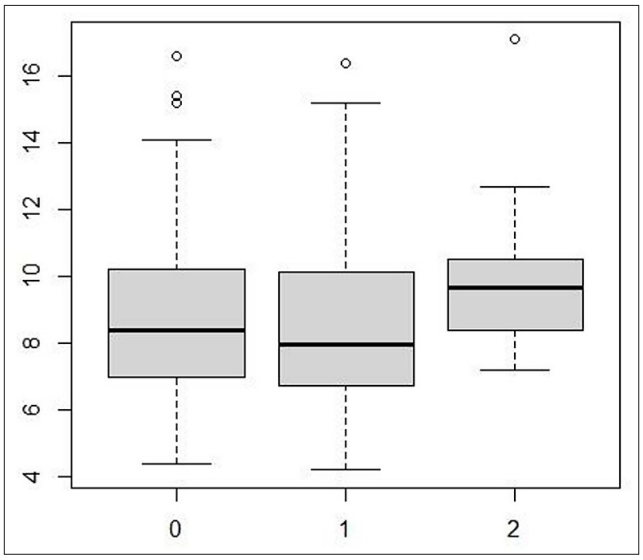

Figure 5. Homocysteine concentration in c.1286A>C *MTHFR* genotype

DISCUSSION

In the late 1990s, Nelen et al. concluded that *MTHFR* c.655C>T homozygous mutation was associated with a 3-fold risk of recurrent spontaneous pregnancy loss [12]. Since then, many studies have been conducted among different populations with conflicting results.

The current study concurs with the findings of Dell'Edera et al. who reported that the c.655C>T and c.1286A>C variants of the *MTHFR* gene did not appear to influence the predisposition to miscarriage in the first or second trimester of pregnancy [13].

Apart from the lack of correlations concerning *MTHFR* polymorphism, in the current study, no relations were found with respect to the Hcy concentration. In non-pregnant women, the concentration of Hcy is established between 5,8 mmol/l – 14,9 mmol/l. In the first trimester of pregnancy, its concentration decreases significantly, reaching the lowest values in the second trimester. This may be associated with increasing levels of estrogens or a higher need for methionine because of maternal and foetal consumption [14]. In the current study, the Hcy concentration ranged from 4,2 mmol/l – 17,1 mmol/l, which suggests that the presence of the *MTHFR* gene polymorphism remains without significant impact on Hcy concentration.

A study by Zen et al. among infertile couples showed that the clinical pregnancy rate, miscarriage rate and live birth rate at the first embryo transfer cycle, were not significantly different between the wild type, homozygous, nor heterozygous *MTHFR* genotype, regardless of the stimulation protocol. However, they reported that the *MTHFR* 665TT genotype was associated with a decreased number of transferable embryos, decreased number of good-quality embryos, and decreased cumulative live birth rate in the first complete cycle in patients undergoing the GnRHa short protocol [15]. Although the study by Zen et al. involved infertile couples, is in opposition to the current study, they suggested that the outcome might be associated with homocysteine participation in fertilisation and very early stages of embryo development, mainly with the quality of the follicular fluid and possible interaction with genes related to DNA methylation [16, 17].

All pregnancies lost by patients in the current study had been conceived naturally. Because of the lack of possibility to observe early embryo development, the *MTHFR* 665TT genotype polymorphisms influence cannot be excluded, even if the results of this study do not support these findings.

Limitations of the study. The limitations may indirectly explain the differences between many studies that have already raised the issue of MTHFR, Hcy and pregnancy loss. The authors of this study have received only partial information about preconception multivitamin supplementation. Thus, there was a lack data concerning folic acid concentration, vitamin B12 concentration, and vitamin B6 concentration.

A study by Kjaergaard et al. analysed genes involved in homocysteine (*MTHFR*, *MTR*, and *CBS*) and vitamin B12 metabolism (*MMACHC*, *CUBN*, *FUT2*, and *MUT*) combined, as well as genes involved only in homocysteine (*MTHFR*, *MTR*, and *CBS*) metabolism alone. In their multiple-instrument approach, no association was found between elevated Hcy with female fertility, pregnancy loss, and offspring birthweight [18]. Another study revealed a significant difference between Hcy, folate, and vitamin B12, which existed in the three analysed genotypes [19]. Although this study was not conducted among women with recurrent pregnancy loss, it delivered significant information. Xiang et al. noted that for females, the mean values of Hcy level for female genotypes: CC, CT and TT were 8,85±3,53, 9,22±3,34, and 11,39±4,98 μmol, respectively. Among women with the TT genotype, the peak level of Hcy was observed at the early age of 20–40 years, with a slight change in the later years.

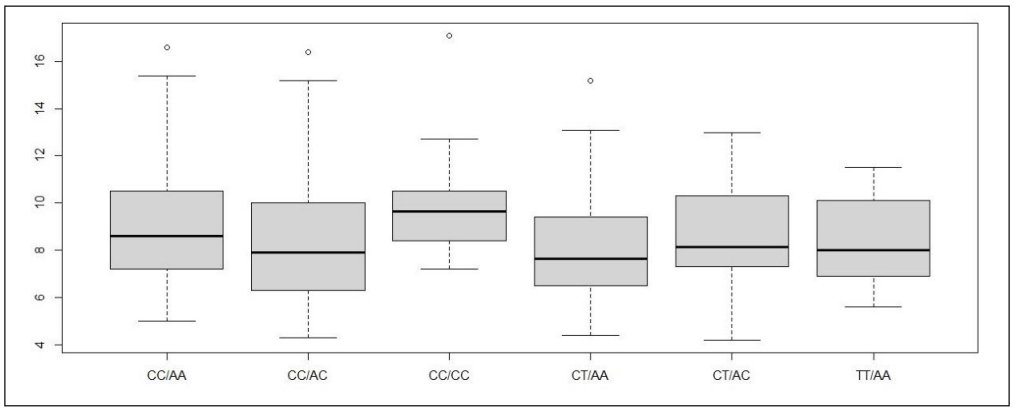

Figure 6. Homocysteine concentration in c.655C>T /c.1286A>C genotype

The authors speculated that the activity of the enzyme could be mostly lost in the TT genotype at an early age, while the function was gradually damaged with ageing by the nutritional and health status in the CC and CT groups [19]. In the groups in the current study -with and without *MTHFR* polymorphisms – no differences regarding women's age were noticed, mean age of the women – 31 years.

In the current study, the patients were not tested for vitamin D concentration. Ota et al. showed the possible influence of vitamin D and folic acid on Hcy decrease as preconception care for women with recurrent pregnancy loss [20]. They speculated the possible regulatory effect of vitamin D and folic acid on uterine NK cells. Interestingly, immunological parameters of peripheral blood, such as the proportion of CD3þ T, CD19þ B and CD56þ NK cells and tumour necrosis factor (TNF)-alpha/IL-10 and interferon-gamma/IL-10 expressing CD3þ/CD4þ T helper (Th) cell), were not significantly different among patients with *MTHFR* C665T genotypes. [20]

The next issue is *MTHFR* polymorphism a male partner. The current study does not contain this data. In couples where both parents carry the 'mutant' alleles, the fact of foetus inheritance (autosomal recessive type of inheritance) may influence its ability to metabolise folic acid and Hcy transported from the mother through the placenta [21].

For years, the role of folic acid supplementation (dose and form – methylated or not) has been raised in the context of prophylaxis. One should not underestimate the effect of the accumulation of unmetabolised folic acid, the UMFA syndrome, with the consequences such as disturbances of immune responses and a flare-up of tumour genesis, acceleration of leukaemia, and colorectal and prostate cancers. In early pregnancy, the fact that unmetabolised folic acid accumulates and competes with natural folates may lead to pseudo-MTHFR syndrome. Thereby, Hcy levels increase even in wild-type patients [3].

CONCLUSION

The results obtained in this study indicate that the c.655C>T and c.1286A>C variants of the *MTHFR* remain without significant impact on Hcy concentration in women with recurrent pregnancy loss.

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