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# Methylenotetrahydrololate reductase A1298C and C677T polymorphisms and adverse pregnancy outcome in women with PCOS

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## Abstract

**OBJECTIVES:** The aim of this study was to compare SNP C677T and A1298C in the MTHFR gene and pregnancy outcome in PCOS women.
**STUDY DESIGN:** We investigated 76 PCOS and 56 non-PCOS women. Among PCOS patients 63 were women with a history of recurrent pregnancy loss (RPL) and 13 women were infertile. In non-PCOS group 40 women were RPL and 16 were infertile. We investigated the relationship between SNP in the MTHFR gene and pregnancy loss, homocysteine and AMH concentration in the study groups.
**RESULTS:** DNA analysis of the PCOS and non-PCOS groups for MTHFR C677T and A1298C polymorphism showed no significant association between the groups. We demonstrated an increased miscarriage rate in non-PCOS women with A1298C polymorphism in the MTHFR gene ($p$=0.042).
We found that homocysteine concentration was higher in women with SNP MTHFR A1298C ($p$=0.046). Moreover, we did not observe any association between the level of homocysteine and the pregnancy outcome in the whole study group.
**CONSLUSION:** It seems that the presence of the MTHFR mutation is not associated with PCOS in the Polish population. However, our results may suggest a correlation between the MTHFR A1298C mutation and RPL in the non-PCOS group.

**Abbreviations:**

| | |
|---|---|
| PCOS | - polycystic ovary syndrome |
| MTHFR | - methylenotetrahydrololate reductase |
| SNP | - single-nucleotide polymorphism |
| AMH | - anti-Müllerian hormone |
| PAI1 | - plasminogen activator inhibitor-1 |

ORIGINAL ARTICLE

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is estimated at around 4–12% of women of reproductive age and is associated with increased risk of pregnancy loss (Chang 2014). As much as 30–50% of pregnancies end in spontaneous abortion. It is believed that hyperinsulinemia is the immediate cause of pregnancy loss, leading to disturbances in the fibrinolysis system mediated by high activity of PAI1 (plasminogen activator inhibitor-1) (Speroff & Fritz 2007). However, the cause of miscarriage remains unknown in as much as 50% of cases (ACOG 2002). In the recent years a lot of attention has been focused on the analysis of genetic polymorphisms that might constitute background for abnormalities leading to pregnancy loss.

Many literature reports confirm the association between the presence of a mutation in the methylenetetrahydrofolate reductase (MTHFR) gene and increased risk of congenital abnormalities as well as the risk of miscarriage (Isotalo *et al.* 2000; Nelen 1998; Parveen *et al.* 2013). However the result is still controversial and inconclusive (Dutra *et al.* 2014; Rai 2014). MTHFR mutation is thought to result in accumulation of homocysteine in blood. Maintaining proper homocysteine levels is particularly important for development of early pregnancy. Hyperhomocysteinemia increases the production of proinflammatory cytokines, disrupts the process of folliculogenesis and damages the embryos (Gmyrek *et al.* 2005; Szymański *et al.* 2003). Recent data suggest that hyperhomocysteinemia is a risk factor for thrombosis, placental insufficiency and pregnancy loss (Nelen *et al.* 2000; Unfried *et al.* 2002). The exact pathomechanism of damaging action of homocysteine still remains unresolved.

Presence of MTHFR enzyme SNPs (single-nucleotide polymorphisms) is the most common genetic cause of homocysteine metabolism abnormalities. MTHFR enzyme plays an important role in the conversion of 5,10-methylenotetrahydrofolate into 5-methylenotetrahydrofolate, which provides a single carbon to homocysteine in methionine synthesis (Kobashi *et al.* 2005; Wu *et al.* 2012). MTHFR polymorphism usually involves cytosine-to-thymine conversion at the 677 position (C677T) and an adenine-to-cytosine conversion at the 1298 position (A1298C). This SNP leads to thermolability of MTHFR, resulting in decreased enzyme activity. The activity of MTHFR enzyme is reduced by 35% in 677CT carriers and by 70% in 677TT carriers. The effect of A1298C polymorphism also results in a decrease in enzyme activity (Jacques *et al.* 1996). Moreover, there is growing evidence suggesting an impact of MTHFR polymorphisms on antimüllerian hormone (AMH) concentration (Thaler 2014; Pavlik *et al.* 2011).

It is currently not known whether SNP in the MTHFR affects reproductive failure in PCOS. Glueck *et al.* (1999) was the first to report an association between MTHFR C677T polymorphism and PCOS. However, other reports did not confirm this relationship.

Since abnormal homocysteine metabolism and PCOS are both associated with thrombosis and pregnancy complications, while the pathophysiology remains uncertain and some proposed mechanism of thrombosis and pregnancy complications are similar for both conditions, our objectives were to compare single nucleotide polymorphism (SNP) involving C677T and A1298C in the MTHFR gene, homocysteine levels and risk of miscarriages among women with PCOS.

## MATERIAL AND METHODS:

This study included 132 patients aged 27–46 years, who had been diagnosed and treated between 2012 and 2014. The study group consisted of 76 women diagnosed with polycystic ovary syndrome (PCOS). In that group, 63 women had a history of recurrent miscarriages and 13 were infertile.

The control group included 56 non-PCOS women. Polycystic ovary syndrome was excluded in those patients. In that group, 40 patients had a history of recurrent miscarriages and 16 were infertile. All information was obtained from the patients' medical records. All patients gave informed consent to use of their medical records provided that their data will be kept confidential and anonymous. This study design was also approved by the Institutional Review Board of the Military Institute of Medicine in Warsaw, Poland.

PCOS was diagnosed according to the European Society of Human Reproduction and Embryology/ American Society for Reproductive Medicine (ESHRE/ ASRM) consensus workshop in Rotterdam in patients who presented with phenotypes of any two of the three criteria, including oligo- or amenorrhea, clinical or biochemical hyperandrogenism and polycystic ovaries. Irregular ovulation manifested as oligomenorrhea (reduction in the frequency of menses with intervals between 40 days and 6 months) and amenorrhea (no menstrual periods for 1 year). Hyperandrogenism was defined clinically as hirsutism. Ovarian morphology was assessed with transvaginal ultrasound between the 3$^{rd}$ and 5$^{th}$ day of menstrual cycle. Polycystic ovaries were defined as having at least 12 or more follicles (2–9 mm in diameter) and/or increased ovarian volume (>10 cm$^3$) (Balen *et al.* 2003).

The recurrent pregnancy loss was defined as two or more consecutive spontaneous miscarriages before the 20$^{th}$ week of gestation (Rai & Regan 2006). Diagnosis of infertility was made according to WHO as a failure to achieve clinical pregnancy after 12 months or longer of regular unprotected sexual intercourse.

In order to assess the subjects for the presence of MTHFR mutation a cheek swab or collected peripheral blood samples were taken into EDTA tubes from each patient. Genomic DNA was extracted using a standard procedure. Polymerase chain reaction assays were

performed for C677T and A1298C mutations of the MTHFR gene. Moreover, each study participant was evaluated for fasting serum homocysteine levels. Homocysteine concentrations in a range of 5–14 µmol/L were considered as normal. The initial routine infertility work-up also included assessment of serum AMH concentration for the purpose of determining the ovarian reserve between the 3rd and 5th day of menstrual cycle. We considered AMH concentrations of 1–2.5 ng/mL as normal.

### *Statistics*

Statistical analysis was performed on R version 3.1.2. Data were reported using descriptive statistics. The distribution of continuous variables was first analyzed with the Shapiro-Wilk test of normality and then continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range (IQR)) respectively. Categorical variables were reported as frequencies and percentages. Continuous variables were compared with t-Student or Mann-Whitney test respectively. Categorical variables were compared using Fisher's exact test. The influence of homocysteine level on miscarriage versus primary infertility was assessed with logistic regression. Odds ratio with 95% confidence interval and *p*-value were reported on. The association between AMH and homocysteine level was analyzed with Spearman's rank correlation coefficient. The significance level was set at 0.05.

## RESULTS

### *MTHFR polymorphism in women with and without PCOS*

The patients enrolled in the study were assigned into two groups basing on the history of polycystic ovary syndrome (PCOS) in order to analyze single nucleotide polymorphism (SNP), involving C677T and A1298C in the MTHFR gene. Therefore, 76 women were assigned to the PCOS group and 56 to the non-PCOS group. Table 1. shows the characteristics of patients included in this study.

Data concerning the MTHFR (A1298C and C677T) polymorphisms in women from the study and control group were presented in Table 2.

**Tab. 1.** Characteristic of patient included in the study.

| | PCOS | | Non-PCOS | | |
|---|---|---|---|---|---|
| | RM | Infertility | RM | Infertility | *p*-value |
| AGE | 33 | 36 | 36 | 35 | 0.004 |
| Homocysteine (µmol/L) | 9.0 | 8.6 | 8.6 | 8.3 | 0.939 |
| AMH (ng/ml) | 9.7 | 8.2 | 1.3 | 1.9 | <0.001 |

*p*-value PCOS vs non-PCOS.

### *MTHFR polymorphism and pregnancy failure*

The prevalence of MTHFR mutation and pregnancy outcome are shown in Figure 1. The A1298C SNP was significantly more frequent in non-PCOS patients with a history of miscarriage than in those who had never been pregnant before (50% vs 12.5%; $p$=0.042). Moreover, no statistically significant difference was found with regard to the C677T prevalence, between recurrent pregnancy loss (RPL) and infertility in non-PCOS subjects.

### *MTHFR polymorphism, homocysteine and AMH concentration*

In the present study the relationship between the MTHFR polymorphism, homocysteine concentration and AMH level was evaluated. It was observed that the plasma homocysteine concentration was higher in women with A1298C SNP compared to the normal genotype (8.9 (IQR 3.3) vs 7.8 (IQR 1.6); $p$=0.046). No statistically significant relationship between C677T SNP and homocysteine level was reported either of the study groups ($p$=0.308). The homocysteine level had no impact on pregnancy outcome in any of the patients enrolled in the study (OR 1.030 (95%CI 0.92–1.24), $p$=0.683).

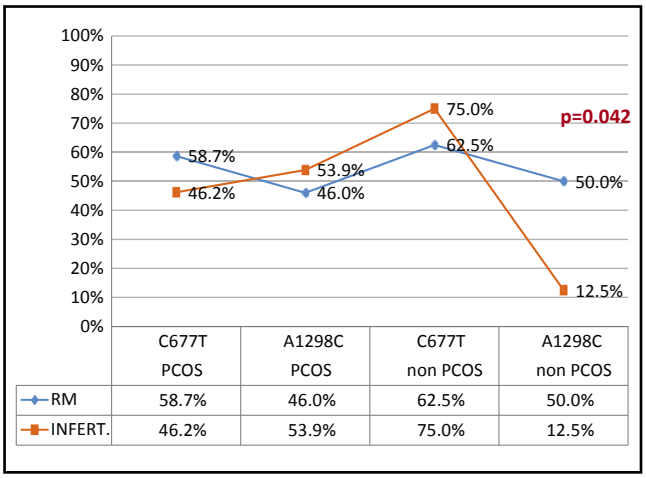


**Fig. 1.** The prevalence of MTHFR mutation and pregnancy outcome in the study groups. (RM – recurrent miscarriages; INFERT – infertility).

**Tab. 2.** Gene polymorphism comparison in women with PCOS and without PCOS.

| Variable | SNP | Total | PCOS | Non-PCOS | *p*-value |
|---|---|---|---|---|---|
| A1298C | AA | 74 (56.1%) | 40 (52.6%) | 34 (60.7%) | 0.605 |
| | AC | 43 (32.6%) | 26 (34.2%) | 17 (30.4%) | |
| | CC | 15 (11.4%) | 10 (13.2%) | 5 (8.9%) | |
| C677T | CC | 52 (39.4%) | 33 (43.4%) | 19 (33.9%) | 0.264 |
| | CT | 69 (52.3%) | 39 (51.3%) | 30 (53.6%) | |
| | TT | 11 (8.3%) | 4 (5.3%) | 7 (12.5%) | |

We observed that the AMH level was much lower in individuals with homozygous 677TT than in patients with a normal genotype (1.2 (IQR 1.1) vs 3.5 (IQR 5.7); $p$=0.078. The study results suggest that the prevalence of SNP A1298C in the MTHFR gene does not correlate with the AMH level. Moreover, there was no statistically significant relationship between homocysteine and AMH level in either of the study groups (Spearman's rank correlation coefficient 0.063; $p$=0.286).

## DISCUSSION

In this study we analyzed the prevalence of the polymorphisms (C677T and A1298C) in the MTHFR gene, homocysteine concentration, AMH level and pregnancy outcome in 132 PCOS and non-PCOS Polish women. Current literature contains different data regarding the incidence of the MTHFR mutation in women with PCOS and with a history of reproductive failure.

The present study did not demonstrate any significant association between C677T and the A1298C MTHFR polymorphism and polycystic ovary syndrome in Polish women. In many reports it was even considered that the MTHFR polymorphism is one of the genetic factors predisposing to PCOS. Glueck *et al.* (1999) were the first to introduce the association between the C677T polymorphism and polycystic ovary syndrome. Karadeniz *et al.* (2010) revealed that the C677T MTHFR polymorphism tends to be more frequent in Turkish women with PCOS. Similarly, basing on the meta-analysis, Fu LY *et al.* (2014) proved that the C677T polymorphisms in the MTHFR gene is associated with altered susceptibility to PCOS in European women. On the other hand current literature convincingly demonstrates no distinct correlation between MTHFR SNP and PCOS. YH Lee *et al.* (2014) based on a meta-analysis, which included eight studies and showed no association between PCOS and MTHFR 677T allele prevalence in either of the patients. Moreover, even after stratification of ethnicity, he did not find any association between PCOS and MTHFR SNP in the European population. Chan *et al.* (2012) obtained a similar negative result. The relationship between SNP in the MTHFR gene and other genetic predisposition for polycystic ovary syndrome requires further research.

In the present study we demonstrated an increased miscarriages rate in non-PCOS women with A1298C polymorphism in the MTHFR gene. Similarly to our result Idali *et al.* (2012) revealed an important correlation between the A1298C mutation and recurrent miscarriages in Iranian women with or without PCOS as compared to normal women. Also Parven *et al.* showed a significant influence of MTHFR C677T and A1298C polymorphism on recurrent miscarriages. According to Cao *et al.* (2013) the prevalence of the C677T polymorphism correlates with a higher risk of recurrent miscarriages in the East Asian population. On the other hand, in the same study these authors found no association between C677T MTHFR and recurrent pregnancy loss in the Caucasian population. Yildiz *et al.* (2012) observed that the incidence rate of MTHFR C677T in Turkish women with a history of RP is comparable to the general population. The diversity of outcomes is probably caused by ethnicity differences among the enrolled patients. However, most of the studies suggest that the correlation between mutations and RPL exists. In the present study we did not demonstrate any significant association between C677T, A1298C SNP and RPL in polycystic ovary syndrome patient.

There is a large number of studies suggesting that women with PCOS have higher concentration of homocysteine in serum and therefore may be at a higher risk of cardiovascular diseases (CVD) (Wijeyaratne *et al.* 2002; Loverro *et al.* 2002; Mohamadin *et al.* 2010). It has been reported that homocysteine damages the endothelium causing local thrombosis, but the mechanism is still unknown. However we did not find an association between homocysteine concentration and polycystic ovary syndrome.

Interestingly, in our study we found an increased homocysteine level among women with A1298C SNP in the MTHFR gene only. In contrast to numerous reports, the present study did not show any influence of the C677T mutation on the homocysteine level (Cao *et al.* 2013; Callejón *et al.* 2007). Our findings regarding the correlation between SNP in the MTHFR gene and homocysteine level are partly consistent with existing literature reports. Palep-Singh *et al.* (2008), suggested that polymorphisms in the MTHFR gene may have no impact on plasma homocysteine levels in young women.

Almost 30% of women with recurrent pregnancy loss was diagnosed with hyperhomocysteinemia (Wouters *et al.* 1993; Steegers-Theunissen *et al.* 1992). Some studies proved that a high homocysteine concentration may lead to a decrease in cell division and high embryo fragmentation. Therefore, hyperhomocysteinemia affects the quality of oocytes and embryos (Aitken *et al.* 1992; Berker *et al.* 2009). High homocysteine levels can result in defective vascularization of chorionic villous, placental abruption and infarction leading to early recurrent pregnancy loss in pregnant women (Nelen *et al.* 2007; Obwegeser *et al.* 1999). Moreover, hyperhomocysteinemia induces trophoblast apoptosis and reduces the secretion of HCG, which can also lead to RPL (Di Simone *et al.* 2004). Nevertheless, results of the current study suggest that there is no correlation between homocysteine concentration and incidence of miscarriages or infertility in the entire evaluated group.

There are several reports suggesting that MTHFR polymorphism may have a significant influence on AMH concentration. Pavlik P *et al.* (2011) observed that women with 677TT polymorphism in the MTHFR gene were found to have higher AMH concentration. However, the presence of this polymorphism had a negative influence on the number of oocytes retrieved

in the IVF procedure (Thaler *et al.* 2014; Pavlik *et al.* 2011). The mechanism by which this mutation exerts this effect is hardly to explain. Furthermore, Rosen MP *et al.* (2007) assumed that MTHFR polymorphisms may disturb the activity of granulosa cells in a grooving follicle and decrease ovarian reserve. He revealed that only the A1298C polymorphism (not the C677T polymorphism) was associated with a higher basal FSH level and lower response to ovarian stimulation. On the other hand, it has been recently suggested that women with the C677T mutation of the MTHFR gene may have an earlier onset of menopause (Thaler *et al.* 2006). In our study, we found no statistically significant correlation between the A1298C and C677T mutation and the AMH level.

The results of the current study provide preliminary evidence that single nucleotide polymorphism in the MTHFR gene is related to recurrent pregnancy loss in non-PCOS patients. However, the outcomes did not suggest any association between SNP A1298C and C677T in the MTHFR gene and miscarriages in PCOS patients. Our study was limited by a small number of patients. It is believed that the findings regarding recurrent miscarriages, PCOS and ovarian reserve assessed using AMH may encourage further investigation on how MTHFR mutations influence ovarian and endometrial physiology at a molecular level. However, further studies are needed to individualize the therapy in the near future.

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