# MTHFR C677T polymorphism is associated with hyperlipidemia in women with polycystic ovary syndrome

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## ABSTRACT

**CONTEXT:** Women with polycystic ovary syndrome (PCOS) are prone for coronary artery disease (CAD), and hyperhomocysteinemia is an independent risk factor for CAD. MTHFR deficiency is the most common cause of hyperhomocysteinemia, thereby provoking a possible association between PCOS and MTHFR C677T polymorphism. **AIMS:** The aim of this study was to investigate an association of MTHFR C677T polymorphism with PCOS. **SETTINGS AND DESIGN:** 92 women with PCOS (Rotterdam criteria) and 95 age-matched controls were compared with respect to MTHFR C677T polymorphism. The 2 genotypes (CC and CT) obtained were compared with clinical and laboratory parameters in women with PCOS. **MATERIALS AND METHODS:** In a case-control study, clinical, biochemical, hormonal and genetic analysis (PCR-RFLP of peripheral leucocytes) was carried out on all women with PCOS as well as controls. **STATISTICAL ANALYSIS:** Student "t" test for quantitative and Chi-square test for nominal variables was used. For estimation of risk, odds ratio and 95% confidence interval were calculated. **RESULTS:** The odds ratio of bearing a heterozygous genotype (CT) was 1.32 in women with PCOS as compared to controls ($P = 0.48$). No homozygous mutation (TT) was found in the study population. Serum cholesterol was more in heterozygous (CT) genotype ($215.48 \pm 25.56$ mg/dl) as compared to normal (CC) genotype ($203.29 \pm 16.35$ mg/dl) in women with PCOS ($P = 0.01$). Similarly, serum triglyceride was more in heterozygous (CT) genotype ($95.86 \pm 37.34$ mg/dl) as compared to normal (CC) genotype ($82.36 \pm 20.88$ mg/dl) in women with PCOS ($P = 0.04$). **CONCLUSIONS:** Although not statistically significant, there is a slightly higher prevalence of heterozygous (CT) genotype in women with PCOS. MTHFR C677T polymorphism when present may confer an increased susceptibility to develop hyperlipidemia in women with PCOS. More prospective studies are needed to confirm whether this hyperlipidemia due to MTHFR C677T polymorphism clinically manifests into CAD in long term in women with PCOS.

**KEY WORDS:** CAD, coronary artery disease, hyperlipidemia, MTHFR C677T polymorphism, PCOS, polycystic ovary syndrome

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is considered to be the most common endocrine disorder affecting women.[1] It is the most common cause of anovulatory infertility and hirsutism.[2,3] About 15% women of reproductive age group are affected.[4] Women with PCOS in long term are known to exhibit adverse cardiovascular risk profile – obesity, dyslipidemia, hypertension, insulin resistance and hyperinsulinemia[5] and an increased risk of premature coronary artery disease (CAD).[6]

Hyperhomocysteinemia is associated with hyperlipidemia[7] and is an independent risk factor for CAD.[8] Moreover, hyperhomocysteinemia has been positively associated with hyperinsulinemia and insulin resistance in a number of studies.[9-13] In general population, the most common cause of hyperhomocysteinemia is reduced activity of MTHFR, an enzyme involved in folate-dependent remethylation of homocysteine to methionine.[14] This may either be due to dietary deficiency of folate or genetic MTHFR deficiency. Importantly, MTHFR gene C677T polymorphism encodes a thermolabile

variant of the enzyme characterized by cytosine to thymine transition at nucleotide 677, characterized by alanine to valine substitution at codon 222 and 30% reduction in enzyme activity in heterozygous and 70% in homozygous genotypes.[14] Furthermore, MTHFR deficiency and resulting hyperhomocysteinemia can lead to impaired DNA methylation,[15-17] accumulation of dUMP with excessive incorporation of uracil into DNA and activation of repair mechanisms resulting in increased risk of chromosomal breakage,[18,19] decreased nitric oxide formation,[20,21] increased reactive oxygen species formation[22,23] and proinflammatory cytokine release.[24] Female reproductive functions, especially folliculogenesis and oogenesis are very sensitive to the above-mentioned changes. Experimental data are in favor that hyperhomocysteinemia can be involved in maturity of oocytes,[25] ovulation,[26] proliferation and differentiation of granulosa cell and steroidogenesis.[27] All the above facts point towards a possible correlation between MTHFR and PCOS.

A number of studies found a positive correlation between hyperhomocysteinemia and insulin resistance and/or hyperandrogenemia in women with PCOS[28-33] while many other studies did not.[34,35] Association between PCOS and MTHFR C677T polymorphism has been investigated in various population groups[36-39] with both positive and negative results. Since no study has been done in the Indian population, we designed this study in an effort to investigate a possible correlation between PCOS, its clinical and biochemical parameters, and MTHFR C677T polymorphism.

## MATERIALS AND METHODS

A case-control study was designed taking 92 young women aged 18 - 30 years with PCOS and 95 age-matched healthy controls.

### Inclusion criteria for selection of cases and controls

The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus criteria[40] was used to diagnose PCOS. Women with presence of any 2 of the following 3 features were diagnosed as having PCOS:

1. Oligomenorrhoea (menstrual cycle ≥ 45 days or < 8 cycles per year) and/or amenorrhoea (> 3 months).
2. Clinical hyperandrogenism {presence of acne, hirsutism (Ferriman Gallwey score ≥ 8[41]) or alopecia}.
3. Polycystic ovaries on sonography (≥ 12 follicles in one or both ovaries, 2 - 9 mm in diameter and/or increased ovarian volume ≥ 10 ml).

Controls were defined as healthy age-matched women with regular cycles, absence of clinically apparent hyperandrogenism, obesity, any chronic illness and who were not on medication.

### Exclusion criteria

All patients with diabetes mellitus, hypertension, hyperprolactinemia, thyroid disorder, Cushing's syndrome, acromegaly, premature ovarian failure, virilising adrenal or ovarian tumors, and oral contraceptive pill use within last 6 months were excluded from the study. None of the subjects were alcoholic or smoker.

An informed consent was taken from all subjects. Proper clinical examination including anthropometry was done.

### Biochemical and hormone analysis

Blood samples were drawn on day 2 of normal menstrual cycle or progesterone-induced bleeding after an overnight fast. Plasma glucose was measured by glucose oxidase peroxidase method (Selectra XL analyzer, Vital Scientifics, Holland). Plasma insulin, LH, FSH, prolactin and total testosterone were measured by chemiluminescent enzyme immunoassay using commercially available kits (Immulite 1000 systems, Siemens). Serum cholesterol, triglyceride, LDL and HDL levels were measured using kits by ERBA diagnostic, Mannheim, Germany. TSH levels were measured using IRMA kit (BARC, Mumbai, India).

Insulin resistance was calculated using the homeostasis model assessment-insulin resistance index (HOMA-IR) according to the following formula:[42]

$$\text{HOMA-IR} = \text{Fasting serum insulin } (\mu U/ml) \times \text{Fasting plasma glucose } (mg/dl) \, 405$$

### Sonography

Pelvic sonography (Nemio 30, Toshiba, Japan) was carried out on day 2 of menstrual cycle in both cases and controls.

### Genetic analysis

Blood samples were collected in tubes containing EDTA and stored at 4°C. Genomic DNA was extracted from peripheral leukocytes by salting out procedure.[43] C677T variant of MTHFR gene was amplified using a forward primer 5'AGGCTGTGCTGTGCTGTTG3' and reverse primer 5'CGCTCTGCAAGTTCTGGA3' by polymerase chain reaction (Bio-Rad laboratories Inc., Berkeley, California). The PCR conditions were 30 cycles of 1 min at 94°C, 1 min at 68°C, 1 min at 72°C. The 477bp fragment generated was restricted digested with Hinf1 (Fermentas Life Sciences, Burlington, Canada) at 37°C for 1 hour. Subsequently, separation using 2% agarose gel electrophoresis was done followed by visualization under UV illuminator after ethidium staining (Gel documentation System, Alphalmager, USA).

Genotypes were expressed as CC for homozygous wild, CT for heterozygous and TT for homozygous mutant genotype. In CC genotype, the 477 bp was digested into 425 and 52 bp.

In CT genotype, the 425 bp was further digested into 260 and 165 bp, thus leading to 4 fragments (425, 260, 165, 52). In TT genotype, 3 fragments of 260, 165 and 52 bp were formed.

### Statistical analysis

SPSS 16.0 for Windows was used for statistical analysis. All quantitative variables (Continuous, interval–scaled or ratio scaled) were expressed as mean ± standard deviation while for qualitative variables, a frequency along with their respective percentage was given. The distribution for each variable was tested for normality. The comparison of means was done by Student "t" test. For nominal (categorical) variables, Chi-square ($\chi^2$) test was used. For estimation of risk, Odds ratio (OR) and 95% confidence interval (CI) were calculated. A *P*-value less than 5% ($P < 0.05$) was considered statistically significant.

## RESULTS

The demographic, anthropometric, biochemical and hormonal parameters of the study population (cases and controls) were compared [Table 1].

Genotype distribution and allele frequency of MTHFR C677T polymorphism were compared in cases and controls [Table 2]. CT genotype was 1.32 times more in women with PCOS as compared to controls ($P = 0.48$). No homozygous mutant (TT) genotype was found in the study population.

In women with PCOS, the demographic, anthropometric, biochemical and hormonal parameters were compared in relation to the 2 genotypes (CC and CT) [Table 3]. Serum cholesterol was more in heterozygous (CT) genotype (215.48 ± 25.56 mg/dl) as compared to normal (CC) genotype (203.29 ± 16.35 mg/dl) in women with PCOS ($P = 0.01$). Similarly, serum triglyceride was more in heterozygous (CT) genotype (95.86 ± 37.34 mg/dl) as compared to normal (CC) genotype (82.36 ± 20.88 mg/dl) in women with PCOS ($P = 0.04$). Fasting insulin levels were also increased in CT genotype (20.33 ± 3.47 μU/ml) as compared to normal (CC) genotype (19.0 ± 2.24 μU/ml) but did not reach statistical significance ($P = 0.05$).

## DISCUSSION

C677T polymorphism of MTHFR gene causes decreased activity of MTHFR enzyme[14] resulting in hyperhomocysteinemia, which is associated with hyperlipidemia.[7] As noted by Choi *et al.*,[38] development of therapeutic approaches is very necessary for women with PCOS, and the genetic association studies will

### Table 1: Demographic, anthropometric, biochemical and hormonal parameters of the study population (PCOS cases and controls)

| Parameters | PCOS (*n* = 92) | Control (*n* = 95) | *P* value |
|---|---|---|---|
| Age (yrs) | 24.19 ± 4.27 | 24.31 ± 2.45 | 0.81 |
| BMI (kg/m²) | 28.13 ± 1.99 | 23.18 ± 1.70 | < 0.001* |
| Waist circumference (inches) | 36.28 ± 2.32 | 31.15 ± 1.74 | < 0.001* |
| LH (mIU/ml) | 8.60 ± 4.80 | 2.68 ± 0.77 | < 0.001*† |
| FSH (mIU/ml) | 4.85 ± 1.56 | 4.56 ± 0.88 | 0.11 |
| LH/FSH ratio | 1.89 ± 1.04 | 0.59 ± 0.15 | < 0.001*† |
| TSH (IU/ml) | 1.49 ± 0.81 | 1.53 ± 0.79 | 0.72 |
| Prolactin (ng/ml) | 10.50 ± 6.20 | 9.18 ± 3.88 | 0.54† |
| Total testosterone (ng/dl) | 33.34 ± 27.03 | 12.44 ± 6.75 | < 0.001*† |
| Fasting glucose (mg/dl) | 92.73 ± 3.74 | 79.46 ± 4.33 | < 0.001* |
| Fasting insulin (mU/ml) | 19.23 ± 2.53 | 11.73 ± 1.65 | < 0.001* |
| HOMA-IR | 1.12 ± 0.16 | 1.35 ± 0.21 | < 0.001* |
| Cholesterol (mg/dl) | 205.41 ± 18.70 | 181.51 ± 23.29 | < 0.001* |
| Triglyceride (mg/dl) | 84.71 ± 24.81 | 68.71 ± 17.91 | < 0.001* |
| LDL (mg/dl) | 72.64 ± 25.27 | 48.86 ± 7.83 | < 0.001* |
| HDL (mg/dl) | 48.65 ± 7.84 | 71.62 ± 25.05 | < 0.001* |

*$P < 0.05$ was considered statistically significant. †Mann Whitney U test was used

### Table 2: Genotype distribution and allele frequency of MTHFR C677T polymorphism in study population (PCOS women and Controls) along with Odds ratio and 95% CI

| Polymorphism | PCOS (*n* = 100) No. (%)* | Control (*n* = 100) no. (%) | OR (95% CI)† | *P* value |
|---|---|---|---|---|
| CC | 76 (82.6) | 82 (86.31) | 1.0‡ | - |
| CT | 16 (17.39) | 13 (13.68) | 1.32 (0.59 - 2.94) | 0.48 |
| C | 168 (91.3) | 177 (93.15) | 1.0‡ | |
| T | 16 (8.69) | 13 (6.84) | 1.29 (0.60 - 2.77) | 0.50 |

*Values are given as number (%) unless otherwise indicated. †OR with 95% CI for PCOS with CT genotype. ‡Reference category

**Table 3: Demographic, anthropometric, biochemical and hormonal of women with PCOS according to the genotypes CC and CT of MTHFR gene C677T polymorphism**

| Parameters | CC ($n = 76$) | CT ($n = 16$) | P value |
|---|---|---|---|
| Age (yrs) | 23.97 ± 4.01 | 25.25 ± 4.89 | 0.26 |
| BMI (kg/m²) | 28.82 ± 1.87 | 28.15 ± 2.57 | 0.96 |
| Waist circumference (inches) | 36.22 ± 2.25 | 36.53 ± 2.72 | 0.64 |
| LH (mIU/ml) | 8.36 ± 4.43 | 9.77 ± 6.59 | 0.77[†] |
| FSH (mIU/ml) | 4.80 ± 1.53 | 5.10 ± 1.72 | 0.49 |
| LH/FSH ratio | 1.86 ± 1.00 | 2.03 ± 1.24 | 0.74[†] |
| TSH (IU/ml) | 1.54 ± 0.83 | 1.26 ± 0.79 | 0.20 |
| Prolactin (ng/ml) | 10.10 ± 5.68 | 9.18 ± 3.88 | 0.33[†] |
| Total testosterone (ng/dl) | 31.91 ± 24.64 | 12.43 ± 8.20 | 0.84[†] |
| Fasting glucose (mg/dl) | 92.59 ± 3.14 | 94.40 ± 5.94 | 0.43 |
| Fasting insulin (µU/ml) | 19.0 ± 2.24 | 20.33 ± 3.47 | 0.05 |
| HOMA-IR | 1.13 ± 0.17 | 1.07 ± 0.14 | 0.25 |
| Cholesterol (mg/dl) | 203.29 ± 16.35 | 215.48 ± 25.56 | 0.01* |
| Triglyceride (mg/dl) | 82.36 ± 20.88 | 95.86 ± 37.34 | 0.04* |
| LDL (mg/dl) | 70.49 ± 21.28 | 82.86 ± 38.38 | 0.07 |
| HDL (mg/dl) | 49.10 ± 7.12 | 46.53 ± 10.60 | 0.23 |

*$P < 0.05$ was considered statistically significant. †Mann Whitney U test was used

provide insights into the role of MTHFR gene in the pathological milieu of PCOS. Present study shows a relative risk of 1.32 in Indian population for presence of CT genotype in women with PCOS ($P = 0.33$). Most studies done till date[36-38] did not find any significant correlation ($P \geq 0.05$) between PCOS and MTHFR C677T polymorphism. Tsanadis *et al*.[36] found a relative risk of 1.2 ($P = 0.83$), while Karadeniz *et al*.[39] found a relative risk of 4.34 ($P < 0.001$). Although higher plasma homocysteine levels were found in women with PCOS ($P < 0.05$) as compared to controls, MTHFR C677T polymorphism was not found to influence these homocysteine levels ($P = 0.19$).[39] The differences in sample size as well as in difference in ethnicity between different population groups may explain the differences in result.

The results of our study show a novel relation between MTHFR C677T polymorphism and laboratory parameters of PCOS. CAD and type 2 DM being common in PCOS patients, the results of this study assumes importance. The finding of increased risk of MTHFR C677T polymorphism in PCOS together with the finding that heterozygous genotype (CT) is associated with hyperlipidemia signify that this can be a cause of women with PCOS developing CAD in later life. Simultaneous estimation of serum homocysteine and folate levels would have given better insight into the relation between dietary MTHFR deficiency, MTHFR C677T polymorphism, hyperhomocysteinemia, and PCOS. More studies need to be carried out in Indian and other population groups to see for correlation between MTHFR C677T polymorphism and PCOS and the clinical and biochemical findings of PCOS to further verify our

results. Furthermore, long term prospective case-control studies taking larger population groups in different parts of the world would clarify whether the increased serum cholesterol and triglyceride levels manifest clinically into CAD in women with PCOS having this polymorphism, thus further clarifying the role of MTHFR gene. Taking plasma homocysteine and folate levels into consideration will give a better insight into pathogenesis of late sequelae of PCOS and its relation to MTHFR gene.

## ACKNOWLEDGEMENT

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