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Association of methylenetetrahydrofolate reductase C677T gene polymorphism and polycystic ovary syndrome in the South Indian cohort

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The polycystic ovarian syndrome is the utmost common endocrinopathy state in women. It is related to both reproductive and metabolic disorders. The *MTHFR* gene associated with the ovarian follicular action encodes the 5-*MTHFR* (methylenetetrahydrofolate reductase) enzyme, tangled in folate metabolism. *MTHFR* gene C677T polymorphism declines the enzyme activity and thus folate deficit and increases the level of homocysteine, which affects the progress of oocytes. Here, we evaluated the association of *MTHFR* gene C677T polymorphism with Polycystic ovary syndrome (PCOS) in the South Indian cohort of women. About 129 PCOS women with following Rotterdam criteria and 90 women controls were studied. PCR-RFLP technique was carried out on all PCOS women in this study. Dissimilarities in hormone levels in PCOS patients were detected. *MTHFR* gene polymorphism CC, CT, TT genotype was found to be 74, 16, 9.30% in patients correspondingly. However, in controls, it was 44.4, 24, 31%, respectively. A substantial difference was detected in the genotype frequency distributions among the patients and controls. Also, allele frequency was shown as 82.95% C allele and 17.83% T allele and 56.67%, 43.33% for C, and T allele in controls correspondingly. Our results indicate a possible association and suggest that *MTHFR* C677T polymorphism can be used as a potential biomarker for PCOS progress in the South Indian women.

Keywords: Follicle-stimulating hormone, Luteinizing hormone, Metabolic syndromes, PCOS

Polycystic ovarian syndrome (PCOS) is the utmost common endocrinopathy state in women. It is related to both reproductive as well as metabolic disorders[1]. Nearly, 5 to 10% of women in reproductive age suffer from the symptoms of PCOS[2]. Hirsutism, higher androgen levels, insulin resistance, infertility, and menstrual dysfunction are noticeable features of PCOS[3]. Abnormal folliculogenesis and gonadotropin production, specifically hypersecretion of LH (Luteinizing Hormone), may contribute to the development of PCOS; these irregularities may arise from environmental insults as well as genetic predisposal[4]. PCOS is inherited as a multifaceted genetic characteristic[5]. Women with PCOS are also vulnerable to develop metabolic syndromes, such as hypertension, obesity, diabetes, as well as dyslipidemia, which is a foremost risk cause for cardiovascular disease[6]. Studies on PCOS genes are mostly focused on the involvement of genes in type 2 diabetes, sex hormones, and regulators, cardiovascular disease, steroid metabolism and biosynthesis, and insulin sensitivity. Even if numerous genetic factors comprising mutations and polymorphisms to several genes are linked to PCOS risk, inheritance mode and the molecular genetics mechanisms underlying PCOS risk are not fully understood[7]. Research on PCOS suggests the association of various genes, such as follicle-stimulating hormone receptor gene[8], melatonin receptor gene[9], cytokine gene[10], insulin receptor substrate-1 gene[11], insulin-like factor 3 gene[12], fat mass, and obesity-associated gene[13] are associated with causing of PCOS.

Methylenetetrahydrofolate reductase (*MTHFR*) is reported to be a vital regulatory enzyme in homocysteine metabolism[14]. *MTHFR* gene has two functional polymorphisms, such as C677T (rs1801133) and A1298C (rs1801131), which reduce the enzyme activity by decreasing the concentration of folate in red blood cells (RBCs), serum and plasma, and slightly increase the total plasma homocysteine (tHcy) concentrations[15]. The *MTHFR* alters 5,10 methylenetetrahydrofolate to 5 methyltetrahydrofolates and offers methyl groups for homocysteine in methionine production[16]. Studies on *MTHFR* polymorphisms (C677T and A1298C) in Chinese PCOS women suggest that there may be an association of polymorphisms and PCOS risk[17]. *MTHFR* is connected with the ovarian follicular movement[18]. The 5-*MTHFR* enzyme, which is encoded by gene *MTHFR* is intricate in folate

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metabolism besides reduced enzyme activity, and consequent changes in homocysteine concentration, are associated with C677T polymorphism[15]. Folate deficiency and hyperhomocysteinemia can distress the progress of oocyte[19]. Conferring to studies association of *MTHFR* gene with PCOS established that homocysteine levels were advanced in Turkish patients with PCOS and that homocysteine levels of patients with PCOS were not influenced by *MTHFR* C677T gene polymorphism[20]. Another study on Poland women with metabolic disturbance in PCOS revealed that no association in C677T *MTHFR* gene polymorphism[21]. Qi *et al.*[22] demonstrated that *MTHFR* gene C677T polymorphism is associated with PCOS, for which CT and TT genotypes can upsurge the risk of PCOS and A1298C polymorphism was not associated with the occurrence of PCOS. A Study on Egyptian women has also demonstrated the association between PCOS and *MTHFR* gene C677T polymorphism[23]. A meta-analysis of the association of PCOS and *MTHFR* C677T polymorphism revealed that there is a strong association in Asian Population[24].

MTHFR C677T polymorphism has been studied in several populations with positive and negative outcomes. Since no such studies were carried previously in the South Indian population, here, we investigated the potential correlation of PCOS with *MTHFR* C677T polymorphism in South Indian Women.

Material and Methods

Study population

Our study includes native South Indian cohort, PCOS patients (N=129) following 2003 Rotterdam Criteria[25,] and controls (N=90) selected by excluding the diagnosis of PCOS according to the 2003 Rotterdam Criteria exhibiting normal menstrual cycles (b32 days), aged group 15-40 years enrolled from Sandhya Hospital, Vellore. This study was approved by the VIT Institutional Human Ethical Committee. Inform consent was taken from the patients and controls before involving them in this study. Blood samples from each patient were taken on day 2 of their normal menstrual cycle or progesterone-induced flow after an overnight fast. All the women in the control group were healthy and without any clinical symptoms of PCOS.

Genotype determination of *MTHFR* gene C677T polymorphism

DNA was extracted based on our lab protocol[26] by collecting 2 mL venous blood from each patient, and the samples were subjected to store at –20°C. Amplification of DNA sample was carried using primers such 5′-AGG ACG GTG CGG TGA GAG TG-3′ as the forward primer and 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ as a reverse primer by PCR in thermal cycling (Eppendorf Master Cycler gradient). The volume of each PCR mixture is of 20 µL (10 pmol from each primer, 5 µL water, master mix (Amplicon) 10 µL. DNA sample (3 µL) was amplified for 35 cycles with initial denaturation for 5 min at 95°C followed by denaturation at 95°C for 45 s, annealing at 60°C for 50 s, the extension for one min at 72°C and final extension for 10 min at 72°C). The PCR products were run on electrophoresis on 2% agarose gel having 5 µL ethidium bromide (50 µg/µL), and final separated bands were visualized using a UV Transilluminator (Medox) which resulted in a 198 bp product. These products were digested using restriction enzyme (one unit) *Hinf I* for 2 h at 37°C. Digested PCR products then finally imperiled to electrophoresis on 3% agarose gel, then these bands were visualized under UV transilluminator (Medox), and bands were photographed by Gel Dock software. The expected genotypic polymorphism of the C allele is CT, CC, and TT were concluded in our study.

Results and Discussion

A major percent of the patient's samples obtained for this study belonged to the age group between 21-25 years (Fig. 1A). The patient's clinical report showed that among all age groups, the PCOS patients aged between 21-25 years were observed to be overweight after calculating their BMI index (Fig. 1B). The majority of the population were diagnosed with the symptoms between the 21-25 age group and treatment for PCOS began after marriage due to infertility and patients diagnosed before that would be if any irregularities in the menstrual cycle (Fig. 1 C & D). No significant hereditary pass on of the syndrome can be stated with the data obtained from the survey of the patient symptoms of a syndrome-like Insulin problem, cardio problem, obesity, reproductive problems (Fig. 2). The hormone level of the patients involved in the study showed no major variation in the hormone profile from the normal range. The lower FSH value in few patients might be a possibility of ovarian failure. The FSH and LH ratio should be 1:1 for normal functioning as this gives an idea about a normal number of eggs present in the ovaries (Fig. 3). Elevation in the prolactin levels is

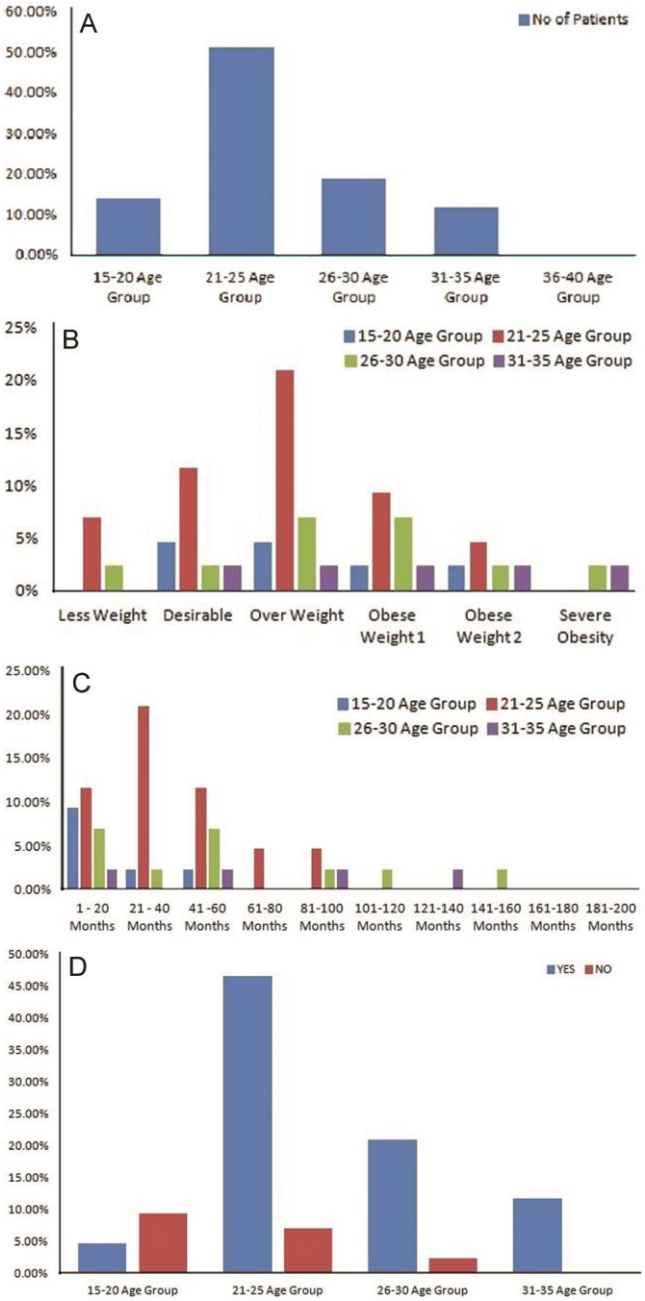

Fig.1 — (A) Age group distribution of PCOS patients; (B) Comparison of body mass index; (C) Comparison of the time since the patients were diagnosed with PCOS; and (D) Marital status of the patients when PCOS was diagnosed

a clear sign of amenorrhea. It is observed that levels are getting elevated with an increase in the age group (Fig. 3). It can be stated as the increased levels might be due to stress caused in the patients due to the Syndrome outcome as it is a stress hormone too. The elevated TSH levels observed in a few might be a cause of decreased thyroid function (Fig. 3). Distributions of

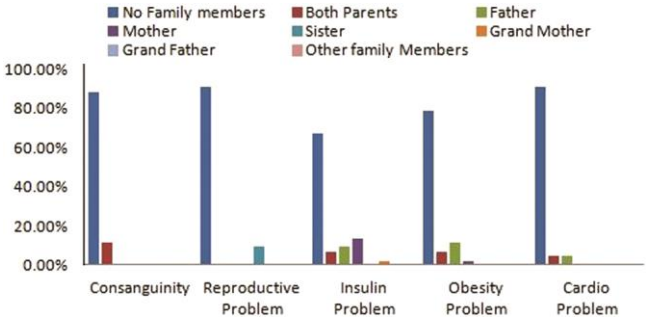

Fig. 2 — Consanguinity and comorbidity associated with PCOS patients family members.

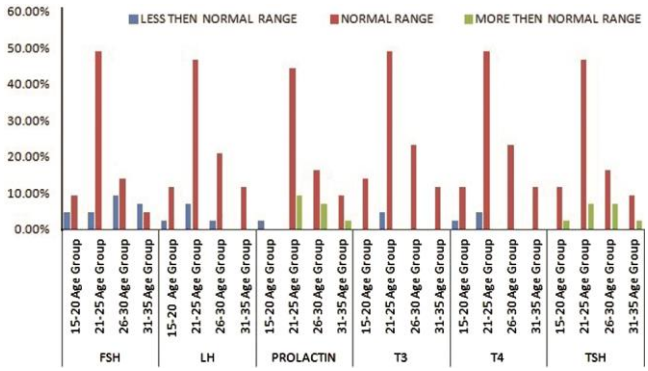

Fig. 3 — Hormone profiling of PCOS patients

Table 1 — Genotype distribution and allele frequencies of *MTHFR* gene C677T polymorphism between PCOS patients and controls

Group	Distribution of genotypes			Allele frequencies	
	CC	CT	TT	C	T
Patients	74%	16%	12%	82.95%	17.83%
Controls	44.40%	24%	31%	56.67%	43.33%

genotypic and allele frequency of *MTHFR* C677T polymorphism were associated with patients and controls. The allelic frequency designated for the C allele and T allele was 82.95 and 17.83% in patients, respectively, whereas it was 56.67 and 43.33%, respectively, in control groups (Table 1).

In this study, we hypothesized that the association of *MTHFR* C677T polymorphism with PCOS in the South Indian cohort might be the risk feature for PCOS. Homozygous genotypes CC of *MTHFR* C677T gene polymorphism were found among patients. The proportion of genotypic distribution of *MTHFR* gene C677T polymorphism was suggestively different among patients and controls. In patients, CC genotype was found to be 74%, CT (16%), and TT (9.30%) correspondingly, and CC genotype was found to be 44.4%, CT (24%), and TT (31%) statistically in controls. The allelic frequencies seem to be higher in

patients as compared to controls. When the outcomes were assessed from the aspect of personal traits in patient groups, the prolactin rate was higher than FSH and LH, Similarly, for BMI values were seems to be higher among patients of age group 21-25 years. Karadeniz *et al.*[20], who studied 30 PCOS patients and 28 healthy controls, concluded that *MTHFR* gene C677T polymorphism is not associated with PCOS in the Turkish population. Also, Sung-Woo Choi *et al.*[27] perceived that the C677T polymorphism of the *MTHFR* gene is not interrelated to PCOS among Korean women population and concluded that the C677T polymorphism in *MTHFR* may have different impacts in among various ethnic groups. Rosen *et al.*[18] concluded that the higher basal levels of FSH are linked to *MTHFR* C677T polymorphism may not distress ovarian stimulation. Bagos *et al.*[28] revealed that *MTHFR* genotypes do not have a noteworthy correlation with the plasma Hcy (Homocysteine) levels in PCOS patients. Nevertheless, higher homocysteine levels in PCOS groups were witnessed than controls. In a study based on a meta-analysis, no evidence for association of *MTHFR* gene polymorphism C677T with PCOS[28] was observed. Palep-Singh *et al.*[29] also compared *MTHFR* gene C677T polymorphism in South Asian and Caucasian populations with PCOS patients and concluded that the occurrence of homozygosity of TT677 and CC1298 (7.2%) and (4.9%) in the Caucasians and (0%) and (16.6%) among South Asians. Also, in one of the other studies carried out by Madhu Jain *et al.*[30] on 92 PCOS patients and 95 controls, they found a marginally higher prevalence of heterozygous (CT) genotype among PCOS patients. The association of *MTHFR* with ovarian cancer shows highly significant (P <0.001) variance in heterozygous (CT) condition of the C677T allele by computing odd ratio signifying that three-time increase the risk factor for the genetic predisposition of *MTHFR* "T" allele for the progress of ovarian carcinoma[31]. Wu *et al.*[17], too established that the *MTHFR* C677T and A1298C polymorphisms might have an association with polycystic ovary syndrome in Chinese women; however, again, this study also suffers from the smaller sample size. Another study in the Chinese population reported that C677T nucleotide change is associated with Polycystic ovary syndrome in which CT and TT genotypes have increased risk for developing PCOS[22].

Conclusion

In this study, CC Homozygous genotype was found to be higher when compared with heterozygous CT and Homozygous TT of *MTHFR* C677T gene polymorphism in PCOS Patients. The genotype distribution and allele frequencies of the C allele were found to be remarkably higher in patients than controls. Hence, *MTHFR* gene C677T polymorphism can be a possible genetic marker of PCOS development in the South Indian cohort. This finding may help the clinician for evaluation of PCOS. The different geographic area varies in gene pools of population. Therefore, additional studies can be done in diverse geographical regions. PCOS is a multi-factorial disorder; it mainly has the associated with environment and lifestyle factors, and further research should confirm the association of *MTHFR* gene polymorphisms and environmental and lifestyle factors in PCOS cause.

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Conflict of interest

Authors declare no conflict of interests.

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