

Assessment of CYP 17 Gene Polymorphism in Subjects with Polycystic Ovarian Syndrome and Central Obesity in an Indian Subpopulation

Anindya Dasgupta^{1*}, Ushasi Banerjee¹, Pranab Roy², Aparna Khan³,
Mrinalkanti Ghosh⁴ and Kanika M. Chowdhuri⁵

¹Department of Biochemistry, Calcutta National Medical College, Kolkata 14,
West Bengal, India

*E-mail: anindya11@yahoo.com

²Department of Biotechnology, Burdwan University, Burdwan 713104, West Bengal, India
Present Address: Department of Biotechnology, Haldia Institute of Technology,
Haldia, 721 657, West Bengal, India

³Department of Gynecology and Obstetrics, ⁴Department of Radiology,

⁵Department of Biochemistry, Burdwan Medical College, Burdwan 713 104,
West Bengal, India

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ABSTRACT An inconsistent association of the C/T allelic polymorphism at -34 site in the promoter region of the *CYP17 α hydroxylase* gene with polycystic ovarian syndrome (PCOS) and its metabolic complications has been observed throughout the world. The researchers aimed to find out any possible link of this polymorphism with PCOS and central obesity in a subpopulation of Eastern India. Serum testosterone, waist hip ratio (WHR) and body mass index (BMI) were analyzed in 60 PCOS cases against 54 matched control women. From RFLP analysis of the *Msp A1* digest of the PCR product of the target gene, the researchers assessed the association of the C/T polymorphism with PCOS and body fat indices in the case group. Significant increases in serum testosterone value and WHR were observed among the case group ($p < 0.001$) without any definite increase in BMI ($p = 0.08$). Allelic distribution for C/T polymorphism was in Hardy Weinberg equilibrium. The researchers did not find any significant association of C/T polymorphism with PCOS (χ^2 of 1.13 with $p = 0.28$ and Odds ratio of 0.75 with a range of 0.448 – 1.26 at 95% CI) as well as with the WHR (χ^2 of 0.1 with $p = 0.75$ and Odds ratio of 0.89 with a range of 0.426 – 1.85 at 95% CI). The results implicate that *CYP 17 α hydroxylase* gene is not associated with hyperandrogenemia and central obesity in PCOS patients in this study population and therefore suggest search for other candidate genes for this disorder in this region.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the major endocrine problems causing menstrual irregularities and infertility in women worldwide. However, in India its prevalence of 4-11% has been found to be a little higher than world-wide prevalence of 4-7% (Norman et al. 1995). An association of androgen excess with anovulation related symptoms like oligomenorrhoea, amenorrhoea or irregular menstruation remain

the hallmark of this disorder (Zawadski 1992). Although, PCOS is supposed to be heterogeneous in nature regarding its clinical and biochemical features, certain biochemical features are common to all cases with ultrasonographic evidence of polycystic ovaries irrespective of their clinical presentation. Most consistent features in this disorder are an LH excess and hyperandrogenemia, whether the disease is identified as a classical disorder or an incidental observation during ultrasonic examination (Franks 1991).

Accompanied with hyperandrogenemia, several metabolic disorders are found to be associated to PCOS. These herald an increased risk for insulin resistance induced type 2 diabetes mellitus and cardiovascular disorders in women suffering from PCOS. According to some recent hypothesis, PCOS, type 2 diabetes and the metabolic syndrome have been modern phenotypic

Address for correspondence:
Dr. Anindya Dasgupta,
Professor and Head,
Department of Biochemistry,
Calcutta National Medical College,
32, Gorachand Road, Kolkata-14,
West Bengal, India
Phone: (0)9475506658
E-mail: anindya11@yahoo.com.

expressions of a metabolic genotype attuned to the dietary and energetic conditions of the ancient period of severe food crisis, and thereby predict that after attainment of a better nutritional profile for a substantial period, particularly in the economically developed European population, the increasing rates of diabetes and cardiovascular disease, which typically accompany economic development, will be tempered by natural, but particularly fertility, selection against the conserved ancestral genotypes that currently underpin them (Corbett et al. 2009). However, till then hyperandrogenemia and insulin resistance remain significantly related to weight gain, particularly the central obesity (O'Connor et al. 2010; Dasgupta et al. 2013) that is found to be closely linked to the increased cardiovascular mortality in these patients as found in several studies including some recent ones from India (Karoli et al. 2012). These findings put PCOS women at a greater risk for increased mortality and morbidity beyond the reproductive abnormalities and thus notably necessitate the search for precise aetiology of the disease.

The aetiopathogenesis of PCOS is likely to be multifactorial consisting of both environmental and genetic factors. Polymorphism of several genes like *FSHR*, *CYP17*, *CYP11A1*, *CAPN10*, *INSR*, *SERPINE*, *IL-1 β* and *SHBG* have been found to be associated with hyperandrogenemia and infertility found in PCOS (Hogeveen et al. 2002; Unsal et al. 2009; Xia et al. 2013). It has been hypothesized that interaction of the environmental factors with some important genes involved with production of androgen and synthesis/secretion of insulin can most suitably explain PCOS (Franks et al. 1997). Under or over activity of several important enzymes in the steroidogenic pathway have been attributed to these environmental or genetic changes among these patients. Aromatase activity that converts androgen into estrogen, and the 3 beta hydroxysteroid dehydrogenase activity—a major enzyme for steroid synthesis, have been found to be significantly altered in PCOS women (Erickson et al. 1990; Pierro et al. 1997; Fedorcsak et al. 2000). CYP 450 dependent side chain cleavage enzyme (SCC) and 17 alpha hydroxylase remain other enzymes that have been studied extensively in the PCOS patients (Carey et al. 1994; Franks et al. 1997). A significant increase in *CYP 17* and *CYP 19* mRNA expressions are associated with increased circulating levels of andro-

gens and estrogens respectively in the PCOS patients. All of these enzyme activities along with their corresponding mRNA expression are reported to be dependent on several factors including genetic polymorphisms (Chua et al. 2012; Li et al. 2012). Among genetic polymorphism studies, *CYP 17 α hydroxylase* polymorphism has been studied widely in various parts of the world, but with variable outcomes. Carey et al. (1994) described a change of a single base T into C at -34 point in the promoter region of the gene that created a restriction site for the restriction enzyme *Msp-A1* (Carey et al. 1994). The less common C allele creates an additional Sp1-type (CCACC box) promoter site that is supposed to increase the expression of androgen (Carey et al. 1994). Although, this genetic polymorphism is not the primary aetiology of PCOS, it may significantly contribute to hyperandrogenemia, particularly when homozygosity exists (Diamanti-Kandarakis et al. 1999; Perez et al. 2008). However, in other studies no such association between this genetic polymorphism and PCOS could be found (Unsal et al. 2009). In a study involving 159 Spanish population no such significant association was established (Echiburu et al. 2008). Homozygosity for the 52 -UTR variant was most common in East Asian (32%) and Japanese (22%) populations and was less common among White (mainly European and North American (14%) and Black (mainly African-American (13%) populations, but selection biases were likely to have affected these frequency estimates (Sharp et al. 2004). Studies available from tropical countries also reported incongruent results regarding linkages of genetic polymorphisms to PCOS. Although, SNPs rs 4077582 in *CYP11A1* and rs 2470152 in *CYP 19* were reported to be closely linked to PCOS, the role of *CYP 17 α hydroxylase* polymorphism was ruled out as a causative agent of this syndrome among different groups of Chinese population (Zhang et al. 2012a; Zhang et al. 2012b). Importantly, about 9 percent of the Indian adolescent girls are reported to have PCOS (Nidhi et al. 2011) with an overall prevalence of 4-11% (Norman et al. 1995). Although, India is a country with multiple ethnicities, few studies are available till now regarding the association of this polymorphism and PCOS among the Indian population overall. The researchers could find one study by Pusalkar et al. (2009) that reported a significant association with the T alleles of the

promoter region of the *CYP 17 α hydroxylase* gene in 100 PCOS cases in a defined population in the Western part of the country (Pusalkar et al. 2009). As genetic polymorphism of this allele has been found to vary substantially from region to region, the researchers hypothesized that the *CYP 17 α hydroxylase* polymorphism may not be a conclusive precipitating factor in the PCOS patients as well as their central obesity in the study region. Accordingly, the researchers undertook the present study to analyze the possible association of *CYP 17 α hydroxylase* gene polymorphism with this syndrome and its associated central obesity in a defined population group in Eastern India.

METHODOLOGY

Selection of Cases and Controls

The present study was conducted in the Department of Biochemistry and Gynaecology of Burdwan Medical College and Hospital and Dept. of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India. Cases were selected from the patients attending the outpatient department (OPD) of Gynaecology during the period of 2010-12. A total of 118 non related age matched patients were screened for PCOS during this period on convenience basis. 64 female subjects aged between 15 and 29 years were selected as the case group on the basis of the revised Rotterdam criteria in the presence of at least two of the following: (1) oligomenorrhea and/or anovulation, (2) hyperandrogenism, clinical or biochemical, (3) polycystic ovaries with exclusion of other etiologies (Rotterdam criteria. 2004). All subjects were previously screened to exclude other causes of hyperandrogenism, thyroid function abnormalities, diabetes mellitus, hyperprolactinemia, hypertension and other cardiovascular diseases. 40 age matched healthy volunteer women with normal menstrual cycle, no clinical or biochemical signs for hyperandrogenism, and ultrasound exclusion of any polycystic ovary were selected as control group. None of them had a history of taking oral contraceptives or any other drug during last 3 months that could affect or alter the lipid profile, insulin level, or carbohydrate metabolism. None of them had any history of any drug addiction including alcohol intake and smoking. None of the case or control subject was suffering from

any clinical infection at the time of the study. Total study protocol strictly adhered to the guidelines of Helsinki declaration 1975 as revised in Edinburg 2000 for human studies. Written consents were obtained from all participants and the study was approved by the properly constituted institutional ethical committee.

Methodology of PCR and RFLP

DNA Isolation: Peripheral blood from healthy subjects were collected in EDTA coated vials and stored in -20°C. The genomic DNA was isolated from the blood by the method of Blin and Stafford using standard phenol-chloroform method (Blin and Stafford 1976). The concentration of the DNA was calculated by spectrophotometric method. Extracted DNA 5 μ l is diluted in 1000 μ l of autoclaved distilled water and absorbency of the DNA was measured at 260 nm wavelengths and the concentration was calculated by the following formula: Concentration = absorbance * 50 μ g/ml. The quality of the DNA was verified by ratio of the absorbency at 260nm and 280nm. Integrity of the genomic DNA was assayed by electrophoresing the extracted DNA in 1% agarose gel.

PCR Reaction: The PCR reaction was carried out using the reagents of the GeNei™ DNA amplification core kit from Bangalore GeNei, India. The *CYP 17 α hydroxylase* amplification assay has been done as described previously (Carey et al. 1994). PCR fragment containing the base pair change was generated using the following primers: 1) forward primer: 5'-CATTTCG-CACCTCTGGAGTC-3', and 2) reverse primer: 5'-GGCTCTTGGGGTACTTG-3'. The master mix was prepared by mixing the 10x DNA amplification buffer (500mM KCl, 100mM Tris HCl, pH 8.5, 15mM MgCl₂), 2.5 mM dNTP mix, 20 μ M forward primer, 20 μ M (micromole) reverse primer, Millipore water, Taq DNA polymerase. PCR reactions were carried out in 25 μ M aliquots containing about 50 ng of genomic DNA, 50 pmol of each primer, 1X reaction buffer, 0.25 μ l (microlitre) deoxynucleotide triphosphates, and 1 unit of Taq polymerase. The amplification was continued for 30 cycles with denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min. An initial denaturation step of 5 min at 94°C and a final extension at 72°C for 5 min were used.

RFLP Analysis: The PCR products were digested for 3 h at 37°C using *MspAI* (from GeNei, Bangalore, India) and separated by gel electro-

phoresis on 2% agarose (DNA grade from Hi media, Mumbai, India) followed by staining with ethidium bromide (SRL, Ranbaxy) to identify the base pair change. In addition, all gels were re-read blindly by three persons without any change, and 15% of the analyses were randomly repeated.

Statistical Analysis: For analysing the association of C/T allele distribution among PCOS patients and their central obesity, chi square tests and Odds ratio (OR) ranges at 95% confidence interval were performed. Differences in the values of serum testosterone and WHR between the case and control groups were calculated by independent t test. Any possible difference among the WHRs of the CC, TT and CT allelic variants were tested by ANOVA with post hoc modification with Bonferroni correction. One of the major aims in the present study was to search for any possible relationship between the *Cyp 17 α hydroxylase* polymorphism and the WHR in the PCOS patients. For this, the researchers obtained a more realistic and accurate cut off value of the WHR between the case and control population groups by performing the receiver operator characteristic (ROC) curve analysis. Through this ROC curve the target cut off value would correspond to its left upper most corner with maximum sensitivity and minimum false positivity (1-specificity). Thereafter, with the help of chi square test the researchers assessed

whether the distribution of the WHR above and below this cut off value differed significantly or not among the PCOS cases.

For all tests p value was considered significant at $p < 0.05$. All tests were performed with the help of SPSS software version 17.0 for Windows.

RESULTS

RFLP pattern of *Msp A1* digest of *CYP 17 α hydroxylase* gene in the PCOS patients is shown in the Figure 1. Homozygotes of TT alleles were not cleaved by the *MspA1* and showed one band at the region of 459 base pairs (lane number 2 and 4). Heterozygotes for CC alleles, on the other hand, generated two bands (lane numbers 6, 7 and 8) at the region of 335 and 124 bp due to generation of the cleavage site to *Msp A1* due to T>C mutation. Heterozygotes, who had both of the alleles showed three bands (lane number 5) at the regions of 459 bp, 335 bp and 124 bp.

Distribution of different allelic forms and their corresponding values of significance are enumerated in the Table 1. It is evident that there is no significant difference between the distribution of the polymorphic C alleles and the wild T alleles of the homozygote and heterozygote groups between our PCOS subjects and normal control women ($\chi^2 = 1.05$, $p = 0.30$). Although, the total number of C alleles is higher than the T

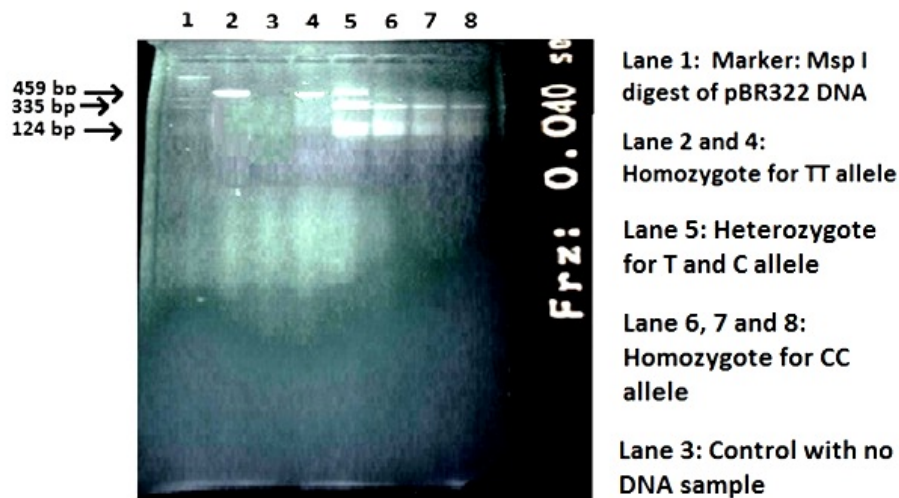


Fig. 1. RFLP pattern of *MspA1* digest of *Cyp17 alpha hydroxylase* gene

Table 1: Distribution of different alleles for SNP rs743572 at CYP17: -34 T/C promoter regions among the case and control group

	Cases (n = 60)	Controls (n = 54)	χ^2 value	Significance ^a
Homozygote for TT allele	15	18	1.05	p = 0.30*
Heterozygote for TC allele	26	22		
Homozygote for CC allele	19	14		
Total number of T alleles	56	58	1.13	p = 0.28*
Total number of C alleles	64	50		

^aFor 95% confidence interval

* p value non significant at 95% confidence interval.

alleles in the case group the difference is not significant ($\chi^2 = 1.13$, $p = 0.28$). The Odds ratio (OR) calculated for this allelic distribution was 0.75 with a range of 0.448-1.26 for 95% confidence interval. Hardy Weinberg equilibrium analysis revealed Chi-square values for cases, control, and all study people to be 1.08, 1.69, and 2.84 respectively. These chi square statistics for the Hardy Weinberg equilibrium of the above parameters are less than the critical value 3.84 for 1 degree of freedom that signify that all of these polymorphisms are stabilized in the population according to the Hardy Weinberg equilibrium rule.

Results of Table 2 indicate that central or abdominal obesity is significantly more in the case group ($p < 0.001$). BMI, the indicator of overall body fat distribution, show an increase in the case group that cannot be considered significant statistically at 95% CI ($p = 0.084$). Serum

testosterone level, on the other hand exhibits a definite rise in the patient group in comparison to the controls ($p < 0.001$). All these observations indicate considerable increase in abdominal fat deposition and hyperandrogenemia in this PCOS group without a significant increase in overall body fat distribution.

To study whether the distribution of abdominal fat varied significantly among the different allelic polymorphs in the PCOS cases, the researchers carried out both the one way ANOVA as well as its post hoc model with Bonferroni correction. Results in the Table 3A showed that there was no significant difference in the overall distribution of WHR among the CC, TT and CT allelic polymorphs ($F = 0.819$, $p = 0.445$). Moreover, when the ANOVA was extended to its post hoc model with Bonferroni correction, no significant difference could be observed between either of any allelic pairs (Table 3B). As a stronger

Table 2: Mean values of anthropometric parameters and testosterone levels in PCOS patients and normal healthy control subjects

	Cases(n = 60)	Controls(n = 54)	t value	Significance ^a
WHR (Mean $\pm$ SD)	0.82 $\pm$ 0.07	0.75 $\pm$ 0.03	6.52	p < 0.001**
BMI (Mean $\pm$ SD)	24.97 $\pm$ 3.25	24.11 $\pm$ 1.72	1.73	p = 0.084*
Serum testosterone in μ g/l (Mean $\pm$ SD)	1.25 $\pm$ 0.46	0.42 $\pm$ 0.19	12.29	p < 0.001**

^a For 95% confidence interval

*p value non significant at 95% confidence interval

**p value significant at 95% confidence interval.

Table 3A: ANOVA showing the overall significance of difference between the WHR among the CC, TT and CT allelic variants for the Cyp 17 α hydroxylase in PCOS patients

	Sum of squares	df	Mean square	F	Sig. (p)
Between groups	.008	2	.004	.819	.445
Within groups	.303	61	.005		
Total	.311	63			

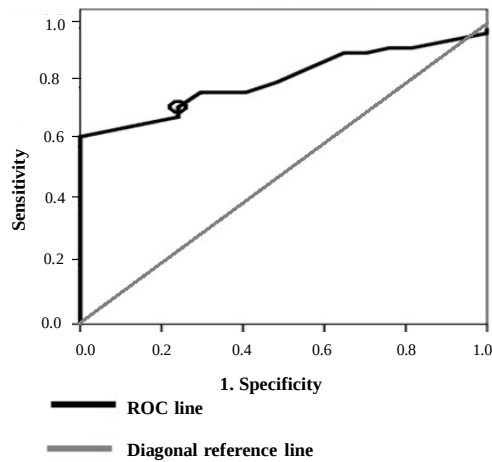
p value considered to be significant at the level of $p < 0.05$ at 95% confidence interval

Table 3B: Post hoc ANOVA with Bonferroni correction showing multiple comparisons between the WHR among the CC, TT and CT allelic variants for the *Cyp 17 α hydroxylase* in PCOS patients

Bonferroni (I) grouping	(J) grouping	Mean difference (I-J)	Std. error	Sig.(p)	95% Confidence interval	
					Lower bound	Upper bound
CC	TT	-.02648	.02086	.628	-.0778	.0249
	CT	-.01770	.02264	1.000	-.0734	.0380
TT	CC	.02648	.02086	.628	-.0249	.0778
	CT	.00878	.02179	1.000	-.0449	.0624
CT	CC	.01770	.02264	1.000	-.0380	.0734
	TT	-.00878	.02179	1.000	-.0624	.0449

p value considered to be significant at the level of $p < 0.05$ at 95% confidence interval

indicator of this outcome, the researchers proceeded to compare the distribution of these allelic polymorphs above and below a more realistic cut off value of WHR obtained by the ROC curve analysis (Fig. 2). From the ROC curve we found this cut off value to be 0.795 at its left upper most corner (noted with a circle in Fig. 2) against a sensitivity of 0.700 and false positivity

**Fig. 2. ROC curve to delineate the cut off value of WHR between the case and control groups**

(1-specificity) of 0.241. Utilising this cut off value, we performed chi square test to analyse any possible link between different alleles of *CYP 17 α hydroxylase* gene and this PCOS patients having WHR more than the obtained cut off value of 0.795 (Table 4). Results from the chi square test indicate that there is no significant difference in the WHRs among different alleles in the homozygote and heterozygote groups in the PCOS subjects in this study population ($\chi^2 = 0.227$, $p = 0.13$). Although the total number of C alleles is higher than the T alleles in the patients having WHR above the critical value of 0.795, the difference is not significant throughout the overall study population range ($\chi^2 = 0.1$, $p = 0.75$; OR = 0.89 with a range of 0.426-1.85 for 95% confidence interval).

DISCUSSION

In the present study the researchers made an effort to find out whether there was any association between the allelic polymorphism at the *CYP 17 alpha hydroxylase* gene with PCOS and its major metabolic complication, the abdominal obesity. Earlier, several studies encompass-

Table 4: Distribution of allelic polymorphism of *CYP 17 α hydroxylase* gene above and below the critical value of WHR in our PCOS population

	WHR > 0.795	WHR < 0.795	χ^2	Significance ^a
Homozygote for TT allele	9	6	0.227	$p = 0.13^*$
Heterozygote for TC allele	11	15		
Homozygote for CC allele	12	7	0.1	$p = 0.75^*$
Total number of T alleles	29	27		
Total number of C alleles	35	29		

^a For 95% confidence interval

*p non significant at 95% confidence interval

ing these parameters came out with different results that based mainly on different population groups in different areas of the world. In this study the researchers found that although the total number of C alleles were higher than that of their T counterparts in the case group along with an opposite trend in the control group, the differences were not significant statistically ($p = 0.28$, Table 1). Furthermore, the difference in the allelic variation was not also significant when considered separately among the homozygote and heterozygote groups for both alleles ($p = 0.30$, Table 1). These findings suggest that although the genetic polymorphism exists in this study population according to the Hardy Weinberg equilibrium, it is not associated with the PCOS in these people.

As the prevalence of PCOS is supposed to vary with ethnic variations throughout the world, several studies have been undertaken to find out specific genetic polymorphisms related to it. One such linkage with the follistatin gene region was found recently to be inconsistent in the South Indian population (Dasgupta et al. 2012a), while the polymorphism of IRS-1 and PPAR γ seemed to provide a protective role (Dasgupta et al. 2012b). Furthermore, significant association of C/T polymorphism at His1058 of INSR with PCOS in the lean rather than obese Indian women strengthen the concept that pathogenesis of PCOS is different in lean and obese women (Mukherjee et al. 2009). Although hyperactivity of *CYP 17 alpha hydroxylase* gene is associated with hyperandrogenemia, no overt relationship could be found between the C/T polymorphism of its promoter region and PCOS in the present study. The researchers' results are in close agreement with the large meta-analytical study recently that suggested no association between this polymorphism and PCOS (Li et al. 2012). Keeping several views together it can be suggested that this polymorphism has a minor role in development of PCOS but could be used as a genetic marker for this disease in some areas.

The link between PCOS and the metabolic derangements is well known (Barber et al. 2007). Several factors like hyperandrogenemia and insulin resistance have been noted as precipitating factors in these derangements, particularly the central obesity (O'Connor et al. 2010; Dasgupta et al. 2013). In this study this has been reflected by a significantly increased serum tes-

tosterone level and WHR in the case group ($p < 0.001$, Table 2). Although the case group showed relatively higher BMI, it was not significant statistically ($p = 0.08$, Table 2). This observation signified a more important association of the central obesity with PCOS as suggested in some other studies also, particularly in the Indian population (Gill et al. 2012). This close association between central obesity and PCOS prompted us to explore any possible relationship between the WHR and C/T polymorphism in the *CYP 17 alpha hydroxylase* gene in this case group. The results of the ANOVA tests clearly indicated no significant association between abdominal obesity and PCOS in this region (Table 3A and 3B). For further confirmation the researchers determined the cut off value of WHR between the PCOS case group and non PCOS control group by ROC to obtain a more realistic demarcating value of WHR between the case and control groups among this study population. The researchers found it to be 0.795 which is very close to the recommended value of 0.8 for females (Lean et al. 1995). From the chi square test it was evident that there was no significant association found between the central obesity and T/C polymorphism among this PCOS patients ($\chi^2 = 0.227$, $p = 0.13$; Table 4). The same trend was found when total number of T and C alleles were compared with each other in the case group ($\chi^2 = 0.1$, $p = 0.75$). An Odds ratio of 0.89 (range of 0.426 -1.85 for 95% CI) also suggested that the risk of central obesity in the whole range of this PCOS patients was not directly related to the T/C genetic polymorphism. Although central obesity has been reported to be associated with the polymorphism of apo E in Chinese population (Liu et al. 2013), Myostatin gene variants in Asian Indians recently (Bhatt et al. 2012), 1519T > C polymorphism in GABRA6 in Swedish men (Rosmond et al. 2002) and the T allele at the FTO rs3751812 locus (Moore et al. 2012), few studies have suggested the role of T/C polymorphism in the -34 promoter region of the *CYP 17 alpha hydroxylase* gene. Echiburu et al. (2008) described a definite association between this polymorphism and obesity in Chilean population but suggested an associated insulin resistance as its explanation (Echiburu et al. 2008). On the other hand, the A2/A2 *CYP 17* genotype, traditionally regarded as unfavourable, was found to be associated with low insulin level and C peptide in a Russian study (Berstein et al. 2002). From all

these observations it can be suggested that among the PCOS patients, polymorphism of *CYP 17 α hydroxylase* gene might not have any worldwide relationship with central obesity or any metabolic parameter. Keeping in track with this principle the researchers did not observe any association between this polymorphism and central obesity in the PCOS cases in this region.

However, limitations of the present study need to be mentioned. The major limitation is the small sample size due to the limited study period of two years. Furthermore, India is a country full of ethnic variation and genetic studies of PCOS face several practical problems (Legro 1995). As this disease is diagnosed mainly in the reproductive age group, it is difficult to perform segregation studies involving more than one generation. In addition there is no commonly accepted male phenotype.

CONCLUSION

The present study implicate that *CYP 17 α hydroxylase* gene is not the limiting factor in increased androgen production in PCOS and therefore, its polymorphism may not be the sole factor responsible for hyperandrogenemia found in PCOS. Considering PCOS as a multigenetic disorder, the researchers propose a minor role of C/T polymorphism at -34 site in the promoter region of *CYP 17 α hydroxylase* gene in this population group and henceforth put forward the need of further explorations for other genetic polymorphisms linked to PCOS in this region. Similarly, the relationship between the androgen induced local accumulation of visceral fat and abdominal obesity in PCOS subjects with this polymorphism remains inconclusive.

RECOMMENDATIONS

Lack of association of *CYP 17 α hydroxylase* gene polymorphism and PCOS in the present study suggests that the allelic variation of this gene is not a limiting factors development of PCOS in this region and hence, the researchers suggest search for other candidate genes related to this disorder. Furthermore, it can be recommended that central obesity should be dealt as a confounding factor for the metabolic complications of this syndrome rather than having a close genetic association with it.

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