

# Association of Genetic Polymorphisms in DNA Repair Genes in Polycystic Ovary Syndrome



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## Abstract

**Introduction:** Polycystic ovary syndrome (PCOS) involves expression of chronic anovulation and hyperandrogenism. Role of environmental and genetic factors in PCOS is strongly supported but the genes that are positively involved in the etiology of the PCOS have not been fully investigated until now.

**Material & Methods:** A total of 127 patients with PCOS and 140 healthy controls were included in the present study. DNA was isolated from 4mL of blood samples from all the enrolled subjects. The polymorphisms of selected genes (XRCC1, XPD and hMSH2) were carried out by ARMS-PCR and PCR-RFLP.

**Results:** GA genotype of XRCC1 gene were found to be predominant in the PCOS compared to controls (57.5%, 32.9% respectively,  $p = <0.0001$ ). There was not much difference observed in the frequency of A allele of XPD among the controls and PCOS (0.76 and 0.62 respectively). Heterozygotes of hMSH2 gene were found to be predominant in the PCOS group compared to controls with 2.64 folds increased risk for PCOS, which was statistically significant (OR 2.64, 95% CI 1.59–4.39,  $p=0.0001$ ).

**Conclusion:** Polymorphisms in XRCC1, XPD and hMSH2 genes were found to be predominant in patients with PCOS. Since different populations have distinct genetic backgrounds, it is necessary to validate or replicate such associations from other ethnic populations.

**Keywords:** DNA Repair; XRCC1; XDP; hMSH2; Polymorphisms; PCOS

**Abbreviations:** PCOS: Polycystic Ovary Syndrome; AP: Apurinic/Apyrimidinic; NER: Nucleotide excision repair; hMSH2: Human MutS Homolog 2; ARMS-PCR: Amplification Refractory Mutation System-Polymerase Chain Reaction; PCR-RFLP: Polymerase Chain Reaction- Restriction Fragment Length Polymorphism; OR: Odds Ratio; CI: Confidence Interval

## Introduction

Polycystic ovary syndrome (PCOS) is an endocrine-influenced pathology mostly seen in women of reproductive age [1]. PCOS is characterized by polycystic ovaries, hyperandrogenism and menstrual irregularity. PCOS is an inflammatory disease that usually develops as a consequence of a complex interaction between susceptible genes, the environment and the immune system [2]. Number of candidate genes have been identified which is susceptibility with PCOS [3,4]. Few of these genes have been identified to play an important role in the pathogenesis of the PCOS [3,4]. Many genes have been identified that their changed expression indicating thus that the genetic instability in PCOS disturb the signal transduction ruling steroidogenesis, gonadotrophin action and regulation, steroid hormones action, energy homeostasis, insulin action and secretion, chronic inflammation and others.

Efficient DNA-repair mechanisms help to remove the lesions that cause DNA breaks during replication and prevent propagation of mutations [5]. Previous reports have been demonstrated that polymorphisms in the DNA repair genes modify the clinical

outcome [6-8]. However, there is still a paucity of data on the association of genetic polymorphisms in DNA repair genes with the etiopathogenesis of PCOS. The base excision repair (BER) is one of the important pathways that repairs spontaneous and endogenously produced DNA damage [9]. Though many proteins are involved in BER, the X-ray repair cross-complementing group 1 (XRCC1) and apurinic/apyrimidinic (AP) endonuclease (APE1) genes play a key role in the repair pathway.

Nucleotide excision repair (NER) is a highly versatile and sophisticated DNA damage repair pathway that counteracts the deleterious effects of a multitude of DNA lesions [10]. NER is a process by which the cells prevent unwanted mutation by removing the vast majority of DNA damage [11]. The XPD Lys751Gln (Lysine to Glutamine) substitution is attributed to a (A>C) transversion in exon 23 at 751 codon [12]. Several studies revealed that polymorphisms in this gene may have an effect on prostate cancer [13], age related macular degeneration [14].

Human mutS homolog 2 (hMSH2) genes are integral components of the DNA mismatch repair pathway. One of the two

other mismatch repair proteins, hMSH6 or hMSH3 binds with hMSH2 and can form a heterodimer to perform repair mechanisms [15]. Formation of heterodimer with other proteins can affect by substitution at codon 322 (Gly322Asp) of hMSH2 and which may lead to deficiency in repair mechanisms.

Some studies have shown an association between these polymorphisms and PCOS while some have not [16]. Since many of these studies have emphasized for further studies in different population groups, the present study was undertaken to find out the association of DNA repair genes polymorphism in women susceptibility to develop PCOS.

## Materials & Methods

### Study population

Total of 127 patients and 146 controls were recruited in the present study. Written consent was taken from the entire subject. The study was approved Institutional Review Board. Detailed information on clinical diet was recorded through Performa. Our sample size of 273 is large enough and exceeds the estimated number of samples (~200 cases + controls) required to obtain a 90 % statistical power.

### Molecular analysis

2mL of peripheral blood sample was collected from each participant. DNA was extracted from peripheral blood samples and genotyping were done for XRCC1, XPD and hMSH2 genes. Genotyping of XRCC1 Arg399Gln (G>A) and XPD Lys751Gln (A>C) polymorphism was performed using the Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). PCR-RFLP (Polymerase chain reaction- Restriction fragment length polymorphism) was performed for hMSH2 Gly322Asp (G>A) polymorphisms. All amplifications were repeated twice and were analyzed using agarose gel electrophoresis system.

**Table 2:** XRCC1 genotypic frequency distribution in controls and PCOS.

Model	Genotype	Controls	Patients	OR (95% CI)	P-value
Codominant	G/G	90 (61.6%)	44 (34.6%)	1	<0.0001
	G/A	48 (32.9%)	73 (57.5%)	<b>3.11 (1.86-5.19)</b>	
	A/A	8 (5.5%)	10 (7.9%)	2.56 (0.94-6.93)	
Dominant	G/G	90 (61.6%)	44 (34.6%)	1	<0.0001
	G/A-A/A	56 (38.4%)	83 (65.3%)	<b>3.03 (1.85-4.97)</b>	
Recessive	G/G-G/A	138 (94.5%)	117 (92.1%)	1	0.43
	A/A	8 (5.5%)	10 (7.9%)	1.47 (0.56-3.86)	
Over dominant	G/G-A/A	98 (67.1%)	54 (42.5%)	1	<0.0001
	G/A	48 (32.9%)	73 (57.5%)	<b>2.76 (1.69-4.52)</b>	

**Table 3:** XPD allele frequency distribution in controls and PCOS.

Allele	Controls		Patients	
	Count	Frequency	Count	Frequency
A	221	0.76	158	0.62
C	71	0.24	96	0.38

### Statistical analysis

The genotypic distribution of XRCC1, XPD and hMSH2 gene was performed using c2-test. Distribution of genotypes and alleles between PCOS and control groups were tested using Fishers exact test. Since differences between conditional logistical regression and unconditional logistical regression were small, unconditional logistical regression was used to estimate odds ratio (OR) and 95% confidence interval (CI). The above statistical analysis was performed using Graph pad prism version 5.0 (GraphPad Software, Inc., San Diego, California, USA).

### Results

The frequency of 'A' allele of XRCC1 gene was found to be predominant in PCOS group compared to controls (37% vs 22% respectively) (Table 1). Heterozygotes (GA) were found to be predominant in the PCOS group compared to controls (57.5%, 32.9% respectively,  $p = <0.0001$ ) with 3.11 folds increased risk for PCOS, which was statistically significant (OR 3.11, 95% CI 1.86-5.19,  $p = <0.0001$ ) (Table 2). Based on the dominant model, combination of GA+AA genotypes were also observed to be associated with high risk for PCOS (OR 3.03, 95% CI 1.85- 4.97,  $p = <0.0001$ ). In recessive model the AA genotype (compared with GG+GA) did not reveal any risk to PCOS (OR 1.47, 95% CI 0.56-3.86,  $p = 0.43$ ) (Table 2). Variation in the distribution of allele frequency is revealed in the present study (Table 3). There was not much difference observed in the frequency of A allele among the controls and PCOS (0.76 and 0.62 respectively).

**Table 1:** XRCC1 allele frequency distribution in controls and PCOS.

Allele	Controls		Patients	
	Count	Frequency	Count	Frequency
G	228	0.78	161	0.63
A	64	0.22	93	0.37

AC genotypic frequency was found to be predominant in PCOS group (53.5%) compared to controls (34.9%) with the difference being statistically significant ( $p = 0.0002$ ). Based on the dominant model, combination of AC+CC genotype frequency were 64.4% in PCOS and 41.8% in control (OR 2.54, 95%CI 1.56-4.15,  $p = 0.003$ ). Over dominant model revealed AC (Compare with AA+CC genotype) genotype was observed to be associated with PCOS

(OR 2.15, 95%CI 1.32–3.49,  $p=0.0019$ ) (Table 4). The frequency of 'A' allele was found to be predominant in PCOS group compared to controls (38% vs 25% respectively) (Table 5). Heterozygotes (GA) were found to be predominant in the PCOS group compared to controls (63.8%, 43.1% respectively,  $p = 0.0001$ ) with 2.64 folds increased risk for PCOS, which was statistically significant (OR 2.64, 95% CI 1.59–4.39,  $p=0.0001$ ) (Table 6). Based on the dominant model, combination of GA+AA genotypes was also

observed to be associated with high risk for PCOS (OR 2.69, 95% CI 1.63– 4.43,  $p= 0.001$ ). In recessive model no such variation was observed (compared with GG+GA) (OR 1.90, 95% CI 0.60– 5.95,  $p= 0.27$ ) (Table 6). Whereas in the over dominant model, GA (Compared with GG+AA genotype) genotype found to be associated with a 2.32 folds increased risk for PCOS (OR 2.32, 95% CI 1.42-3.78,  $p = 0.004$ ), further confirming the risk of 'A' allele in PCOS.

**Table 4:** XPD genotypic frequency distribution in controls and PCOS.

Model	Genotype	Controls	Patients	OR (95% CI)	P-value
Codominant	A/A	85 (58.2%)	45 (35.4%)	1	0.0002
	A/C	51 (34.9%)	68 (53.5%)	<b>2.52 (1.51-4.20)</b>	
	C/C	10 (6.8%)	14 (11%)	<b>2.64 (1.09-6.43)</b>	
Dominant	A/A	85 (58.2%)	45 (35.4%)	1	0.003
	A/C-C/C	61 (41.8%)	82 (64.6%)	<b>2.54 (1.56-4.15)</b>	
Recessive	A/A-A/C	136 (93.2%)	113 (89%)	1	0.22
	C/C	10 (6.8%)	14 (11%)	1.68 (0.72-3.94)	
Over dominant	A/A-C/C	95 (65.1%)	59 (46.5%)	1	0.0019
	A/C	51 (34.9%)	68 (53.5%)	<b>2.15 (1.32-3.49)</b>	

**Table 5:** hMSH2 allele frequency distribution in controls and PCOS.

Allele	Controls		Patients	
	Count	Frequency	Count	Frequency
G	219	0.75	157	0.62
A	73	0.25	97	0.38

**Table 6:** hMSH2 genotypic frequency distribution in controls and PCOS.

Model	Genotype	Controls	Patients	OR (95% CI)	P-value
Codominant	G/G	78 (53.4%)	38 (29.9%)	1	0.0001
	G/A	63 (43.1%)	81 (63.8%)	<b>2.64 (1.59-4.39)</b>	
	A/A	5 (3.4%)	8 (6.3%)	<b>3.28 (1.01-10.72)</b>	
Dominant	G/G	78 (53.4%)	38 (29.9%)	1	0.001
	G/A-A/A	68 (46.6%)	89 (70.1%)	<b>2.69 (1.63-4.43)</b>	
Recessive	G/G-G/A	141 (96.6%)	119 (93.7%)	1	0.27
	A/A	5 (3.4%)	8 (6.3%)	1.90 (0.60-5.95)	
Over dominant	G/G-A/A	83 (56.9%)	46 (36.2%)	1	0.004
	G/A	63 (43.1%)	81 (63.8%)	<b>2.32 (1.42-3.78)</b>	

## Discussion

PCOS is a chronic inflammatory disease with unknown aetiology. The pathophysiology of PCOS relates to a dysregulated immune response on a background of genetic susceptibility. The last few decades have seen a discernible shift in the global prevalence of PCOS. PCOS may represent a polygenic disorder [17-19]. Several genes have been identified which play important role in the pathogenesis of PCOS and the presence of their polymorphisms have been explored [20]. Serum adiponectin plays a vital role in the pathogenesis of insulin resistance of PCOS women [21]. Investigator demonstrated that patients with PCOS have a 7.8-fold higher frequency of CYP1A1 Ile/Val genotype and a 7.4-fold CYP1A1 any Val genotype (Ile/Val or Val/Val) [22].

Our study demonstrated that heterozygotes (GA) of XRCC1 gene were found to be predominant in the PCOS group compared to controls (57.5%, 32.9% respectively,  $p = <0.0001$ ) with 3.11 folds increased risk for PCOS, which was statistically significant (OR 3.11, 95% CI 1.86-5.19,  $p = <0.0001$ ). Our results are in contrast to those obtained by Gulbay et al. [16] revealed that there was no difference between women with PCOS and controls in terms of XRCC1 Arg194Trp and XRCC1 Arg399Gln genotypes (OR= 1.15, 95% CI= 0.57-2.32,  $p= 0.69$  for XRCC1 Arg194Trp and OR= 1.14, 95% CI= 0.87-1.48,  $p= 0.32$  for XRCC1 Arg399Gln). Gulbay et al. [16] also reported that the frequency of variant alleles for XRCC1 Arg194Trp and XRCC1 Arg399Gln in the study population were 7.02% and 32.90% respectively and similar to controls. Several epidemiological studies have assessed bladder cancer risk

and Arg399Gln XRCC1 polymorphism [23,7,24]. Many of them have suggested a decreased risk for individuals with the variant homozygote genotype (AA) [7,24]. The homozygote variant genotype (AA) was inversely associated with bladder cancer risk [25].

In 2003, Mort and colleagues, suggested lack of association between XPD gene polymorphisms and cancer. AC genotypic frequency was found to be predominant in PCOS group (53.5%) compared to controls (34.9%) with the difference being statistically significant ( $p = 0.0002$ ). Our study also demonstrated that combination of AC+CC genotype frequency were 64.4% in PCOS and 41.8% in control (OR 2.54, 95%CI 1.56–4.15,  $p=0.003$ ). Gln allele was reported to be associated with poor DNA repair capacity in a study conducted on XPD Lys751Gln polymorphism [26]. Gulbay et al. [16] revealed that no statistical differences were observed between PCOS women and the control group in terms Lys751Gln XPD polymorphism. Wang et al. [27] also demonstrated that AC genotype was not associated with breast cancer. In another study, Sanyal et al. [28] also reported that variant allele in XPD did not show any significant difference between the patients with bladder cancer and healthy control. Fontana et al. [25] demonstrated that the CC and AC genotype of XPD were associated with a decreased risk of bladder cancer, but these results were not significant. Stern et al. [29] also found a small but non-significant decrease in risk for the CC genotype when compared to subjects with the AA or AC genotypes. Most of the published studies did not reveal any association between XPD polymorphisms and bladder cancer risk [30-32].

Heterozygotes (GA) of hMSH2 gene were found to be predominant in the PCOS group compared to controls (63.8%, 43.1% respectively,  $p = 0.0001$ ) with 2.64 folds increased risk for PCOS, which was statistically significant (OR 2.64, 95% CI 1.59–4.39,  $p=0.004$ ) which is in absolute conformity with data of Poplawski et al. [33] Significant association of Gly322Asp polymorphism of the hMSH2 gene with breast cancer and colorectal cancer was reported [34]. The frequency of A allele was found to be predominant in PCOS group compared to controls.

Since this variation was observed to be statistically significant in the patient group compared to the controls, the possibility of this variation in the pathogenicity of the disease under the influence of other genes and environmental factors cannot be ruled out. The reasons for the disparity in results needs an in depth analysis of the sequel of events that leads to PCOS.

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