

ORIGINAL ARTICLE

DISPARATE RELATIONSHIP OF VITAMIN D RECEPTOR CDX2 GENE POLYMORPHISM AND POLYCYSTIC OVARIAN SYNDROME

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Background: Vitamin D regulates various biological processes and its role in the pathogenesis of Polycystic Ovarian Syndrome (PCOS) is still dubious. Vitamin D receptor Cdx2 polymorphism is located in the promoter area of Vitamin D receptor gene, its A allele increases and G allele decreases transcription and might play role in the development of insulin resistance in PCOS. The rationale of present study was to investigate the relationship between Vitamin D receptor Cdx2 gene polymorphism and polycystic ovarian Syndrome among young females. **Method:** A total of 186 subjects (95 cases and 91 controls) were enrolled in this study. Blood samples were collected and genomic DNA was isolated. Genotyping of Vitamin D receptor Cdx2 was carried out using tetra-primer amplification refractory mutation system polymerase chain reaction technique. **Results:** The genotype frequencies of AA, GA, GG for cases were 8.8%, 12.5%, 78.8% and for controls 11.5%, 15.4%, 73.1% respectively. A non-significant association was observed between Polycystic Ovarian Syndrome ($\chi^2:0.707, p=0.702$) and Vitamin D receptor Cdx2 gene polymorphism. However, GG (OR: 1.421, 95% CI: 0.293–3.916) and GA (OR: 1.421, 95% CI: 0.497–4.064) genotypes of vitamin D receptor gene polymorphism were at increased risk for PCOS. **Conclusion:** Vitamin D receptor Cdx2 gene polymorphism may have disparate relationship with PCOS.

Keywords: Vitamin D receptor, Polycystic Ovarian Syndrome, PCOS, Cdx2, PCR, polymorphism, polymerase chain reaction, tetra-primer amplification refractory mutation system

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a metabolically determined multifactorial ovarian endocrine disorder that has major consequences throughout a women's life.¹ Around 20.7% women of reproductive age are reported to have PCOS in Pakistan and 5–10% of women worldwide.² The major characteristic features of PCOS are irregular or anovulatory menstrual cycles accompanied by signs of hyperandrogenism, polycystic ovaries, insulin resistance and obesity.³ Evidence suggests that in females with PCOS decreased vitamin D affects the hormone levels and causes metabolic disturbances.^{2,3}

Vitamin D is a secosteroid which regulates a wide range of metabolic processes that include bone metabolism, immune responses against chronic diseases and multiple cancers.⁴ With such extensive range of activities vitamin D deficiency is believed to be associated with characteristic features related to PCOS.⁵ Krul-Poel and colleagues observed that 67–85% females with PCOS were severely vitamin D deficient. They found an inverse correlation between vitamin D levels and metabolic disturbances in PCOS.⁶ Vitamin D accomplishes its actions by binding to its receptor (vitamin D receptor, VDR) which is located in almost every organ of the body including reproductive tissues. Vitamin D receptor works perfectly fine after binding to its response element, i.e., Vitamin D response element (VDRE) present on the DNA, as long as there is no variant in its sequence. However, its variants (Cdx2,

FokI, ApaI and TaqI) are thought to play a significant role in the development of PCOS.^{7,8} Regarding its role in the hormone functions, VDR gene polymorphism was significantly associated with precocious pubarche by modulating the ovarian steroid secretions particularly estradiol levels in women with PCOS.⁹

The variation of A/G allele on caudal type homeobox-2 (binding site of VDRE) is known as Cdx2 polymorphism. This site serves as a transcription factor, promoting the transcriptional activity in the presence of A allele and decreasing the activity in the presence of G allele. A study suggested significant association between VDR Cdx2 gene polymorphism and PCOS among Indian women with protective odds. However, GA carriers presented higher levels of mean Testosterone and TSH.¹⁰ In another study, Cdx2 AA carriers were associated with the lower plasma insulin and HOMA-IR among Austria PCOS females.¹¹ Considering the probable roles of vitamin D in the causation of PCOS, current study was planned to evaluate the association of VDR gene polymorphism (Cdx2) in females suffering from PCOS.

MATERIAL AND METHODS

This case control study was conducted at the Department of Physiology, University of Karachi from Jan 2013 to Dec 2014. Sample size as calculated using online sample size calculator, OpenEpi with 5% significance level and 90% of statistical power. The study was approved by Board of Advanced Studies and Research University of Karachi. A written informed

consent was obtained from study subjects included on the basis of Rotterdam criterion¹² for cases, i.e., PCOS subjects, and the healthy females with no diagnosed endocrine and non-endocrine abnormality served as controls, while all other were excluded. A total of 186 subjects (95 cases + 91 controls) with an age range of 12–45 years. Genotyping of the VDR-Cdx2 (rs11568820)¹³ was performed through tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR)¹⁴ on genomic DNA samples extracted from venous blood by proteinase-K method. PCR was carried out using Go-Taq green master mix (Promega-USA) and reactions were amplified on an automated thermal cycler (Veriti TM, Applied BioSystems, USA). All the PCR products were run on 2.0% agarose gel and visualized through ChemiDoc-It2 (UVP, UK) using Vision works LS software version 7.1.

Control subjects were tested for Hardy Weinberg Equilibrium (HWE) whereas; Pearson’s χ^2 was calculated to determine association between VDR Cdx2 gene polymorphism and PCOS. Odds ratio along with 95% confidence intervals were calculated using

binary logistic regression analysis to assess the risk of disease at $p < 0.05$. All data was analyzed on SPSS-22.

RESULTS

The present study included 95 cases diagnosed with PCOS and 91 controls recruited from tertiary hospitals in Karachi, with a mean age in years of 25.93±6.07 and 23.09±5.19 for cases and control respectively.

The controls were inconsistent with the HWE distribution ($p=0.000$). Genotype frequency of AA 8.8%, GA 12.5% and GG 78.8% among cases whereas the genotype frequency of AA 11.5%, GA 15.4% and GG 73.1% among controls were observed. This study showed no association (χ^2 : 0.707, $p=0.702$) between Cdx2 polymorphism and PCOS among cases and controls. However, GG (OR: 1.421, 95% CI: 0.293–3.916) and GA (OR: 1.421, 95% CI: 0.497–4.064) genotypes of vitamin D receptor gene polymorphism were increases the risk of PCOS but the results were non-significant (Table-1).

Table-1: Cdx2 VDR Gene Polymorphism and PCOS

Cdx2 variant	Cases n (%)	Controls n (%)	HWE*** (p)	χ^2	p	OR** (95% CI)
AA	7 (8.8)	9 (11.5)	19.8 (0.000)	0.707	0.702	1
GA	10 (12.5)	12 (15.4)				1.421 (0.497–4.064)
GG	63 (78.8)	57 (73.1)				1.421 (0.293–3.916)
Total	80*	78				
A	24 (15)	30 (19.2)	---	0.998	0.318	1
G	136 (85)	126 (80.7)				1.349 (0.749–2.431)
Total	160	156				

*Out of 95 cases, 80 were amplified. **OR along with 95% CI were calculated using unconditional binary logistic regression analysis. ***Hardy-Weinberg equilibrium is calculated for controls only.

DISCUSSION

This study was designed to evaluate the association between VDR-Cdx2 gene variant among healthy controls and women with PCOS. The Cdx2 polymorphism is a G>A sequence variation on VDR gene in its 1a promoter region associated with transcriptional activity of the VDR gene. The VDR gene further controls the expression of about 3% of the human genome along with the genes that are of significant importance in glucose metabolism.

In this study, the VDR-Cdx2 genotype frequencies of controls were inconsistent with HWE distribution which may be due to the multiple factors that include genotype misclassification, migration, ethnic diversity and inbreeding by consanguinity, as Pakistan almost tops the ranking of consanguineous marriages in the world.¹⁵ Moreover, Indian population had also showed same observations of HWE probably due to similar ethnic and cultural diversities.¹⁰

A non-significant difference was observed in the genotypic and allelic frequency distribution of

Cdx2 between the PCOS cases and the controls. This observation is in agreement to a previous study conducted in Austria¹¹, however, the findings are contrary to study carried out by Dasgupta *et al*¹⁰.

This study showed disparate relationship between Cdx2 polymorphism and PCOS which is not in accordance with a study conducted in 2011 by Wehr and colleagues.¹¹ In another study AA genotype increased the PCOS risk among Indians¹⁰, which is also not in agreement with our study and does not support the investigational approach as 78.8% population in our data also has GG genotype. Although strong relationship of VDR Cdx2 gene polymorphism has been found with bone mineral density¹³ and fracture risk, the association of Cdx2 with PCOS related metabolic features were rarely reported.

Moreover, genetic studies involving haplotypes, other candidate gene variants, genetic linkage assessment with a large sample size are needed for further evaluation regarding the relationship between VDR gene polymorphisms and PCOS.

CONCLUSION

VDR Cdx2 gene polymorphism may have disparate relationship with PCOS. Vitamin D receptor gene polymorphism and vitamin D levels should be considered during PCOS therapy.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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