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The rs16944 SNP in *IL-1B* and risk of polycystic ovarian syndrome

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrine condition in females during the reproductive years. Several genetic factors have been shown to impact the normal ovulation and be involved in the pathogenesis of PCOS. Genes regulating immune responses are among these factors. In the current investigation, we genotyped a variant in *Interleukin* (*IL*)*-1B* gene (rs16944) in 270 patients with PCOS (including 154 infertile cases (PCOS-I) and 116 cases with history of recurrent abortion (PCOS-A)) and 112 healthy controls. There was no significant difference in frequencies of rs16944 alleles/genotypes between PCOS-I subjects and healthy controls. However, the G allele of the rs16944 SNP was less frequent among PCOS-A cases compared with controls (OR (95% CI) = 0.58 (0.4–0.84), *P* value = 0.004). The rs16944 SNP was associated with PCOS-A in co-dominant (GG vs. AA: OR (95% CI) = 0.37 (0.18–0.76), *P* value = 0.02), dominant (AG + GG vs. AA: OR (95% CI) = 0.51 (0.28–0.91), *P* value = 0.02) and recessive (GG vs. AG + AA: OR (95% CI) = 0.50 (0.27–0.92), *P* value = 0.02) models. Taken together, the mentioned SNP can be regarded as a risk locus for a certain type of PCOS in Iranian population.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine condition in females during the reproductive years (Teede et al., 2010). This condition is characterized by hyperandrogenism, ovulatory defects and polycystic ovarian appearance (ESHRE and Group, 2004). Genetic studies have revealed a complex form of inheritance for this condition (Kahsar-Miller et al., 2001; Vink et al., 2006). The severe defects in the ovulation in women with PCOS is at least partly explained by the tonic raise in LH levels, lack of an LH surge, and the untimely stop in follicular growth (Adams et al., 1986). However, some studies point to abnormal inflammatory responses in PCOS women (Adams et al., 2016). Chronic low-level inflammatory responses have been detected in patients with PCOS (Duleba and Dokras, 2012). Moreover, ovulation is considered as a multifaceted immune-related process that encompasses release of inflammatory cytokines by follicles, production of prostaglandins, and the involvement of immune cells (Norman and Brannstrom, 1996; Smolikova et al., 2012). Consequently, dysregulation of such immune activities might be involved in the pathogenesis of PCOS. Several single nucleotide polymorphisms (SNPs) within cytokine coding genes have been associated with PCOS in different populations (de Alencar et al., 2016). A certain SNP within *Interleukin* (*IL*)*-1B* gene (rs16944) is located in the promoter of this gene and is possibly involved in the regulation of IL-1b levels (Iacoviello et al., 2005; Rogus et al., 2008). Moreover, this SNP might regulate expression of this cytokine in PCOS patients (Yang et al., 2012). Researchers have also shown correlations between IL-1b levels and obesity in PCOS patients (Yang et al., 2012). The rs16944 SNP has been associated with risk of

Abbreviations: PCOS, polycystic ovary syndrome; SNPs, single nucleotide polymorphisms; *IL*, *Interleukin*; RFLP, restriction fragment length polymorphism; CI, confidence intervals

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Table 1
The general information about the rs16944 SNP.

SNP	Position	Minor allele	MAF	MAC	Type
rs16944	Chr 2:112832813	G	0.49	2457	Intergenic variant

PCOS in Chinese women (Mu et al., 2010; Yang et al., 2010), but not in Caucasians (Kolbus et al., 2007). This SNP has been associated with diverse human disorders in different populations (Harrison et al., 2008; Falfán-Valencia et al., 2012; Yao et al., 2019), so it might be regarded as a genomic hot spot for human diseases. Moreover, this SNP is the most common studied variant of IL-1β in recurrent pregnancy loss (Zhang et al., 2017). Based on the lack of data regarding the association between this SNP and PCOS in Iranian population, in the current case-control study, we genotyped this SNP in a population of Iranian patients with PCOS and healthy women to unravel the role of this genetic locus in conferring risk of PCOS in Iranian population.

2. Materials and methods

2.1. Study participants

The current study was conducted on 270 patients with PCOS (including 154 infertile cases (PCOS-I) and 116 cases with history of recurrent abortion (PCOS-A)) and 112 healthy controls. Female subjects aged between 15 and 35 years, entered the study. The following criteria were considered for diagnosis of PCOS: oligoovulation/anovulation, hyperandrogenemia and the presence of polycystic ovaries in ultrasonography. None of the PCOS patients enrolled in the study had successful pregnancy. Recurrent abortion was defined as the presence of three or more spontaneous abortions. Control subjects were females with no history of infertility or recurrent abortion and no signs of hyperandrogenemia (as assessed by biochemical tests and clinical examination) who had normal appearing ovaries (as assessed by transvaginal ultrasonography). Subjects with alcohol abuse, diabetes mellitus or cardiac, endocrine or metabolic disorders were excluded from the study. All study participants signed the informed consent form. The study protocol was approved by ethical committee of Shahid Beheshti University of Medical Sciences.

2.2. Genotyping

Table 1 shows the general information about the rs16944 SNP.

The genotypes of rs16944 were determined using PCR-restriction fragment length polymorphism (RFLP) techniques. Genomic DNA was extracted using standard salting out method. The 5′-TGGCATTGATCT GGTTCATC-3′ (forward primer) and 5′-GTTTAGGAATCTTCCCACTT-3′ (reverse primer) primers were used for amplification of DNA. Reactions were made using the Taq DNA Polymerase Master Mix RED (Amplicon, Denmark). PCR products were incubated with *Ava*I restriction enzyme (Roche diagnostic GmbH, Mannheim, Germany) based on the manufacturer's instruction. The resulting bands were analyzed on 2% agarose gel run in electrophoresis buffer. Amplicons having T allele were not digested by the enzyme, while those having T allele were digested leading to production of two bands with 190 and 340 bp lengths.

Table 3
Exact test for Hardy-Weinberg equilibrium.

Groups	Genotypes			*P*-value
	AA	AG	GG	
PCOS-I	30	81	43	0.46
PCOS-A	42	51	23	0.30
Healthy controls	25	50	37	0.31

2.3. Statistical methods

Genotyping data was analyzed using the SNP Analyzer 2.0 online tool (Yoo et al., 2008). Accordance of the observed genotypes frequencies and expected frequencies was tested using Chi-square test. Associations between rs16944 genotypes and PCOS-I/PCOS-A were assessed in allelic, co-dominant, dominant and recessive models. *P*-value, odds ratio (OR) and 95% confidence interval (CI) were calculated. *P*-values < 0.05 were considered as significant.

3. Results

3.1. General features of enrolled subjects

Table 2 summarizes the demographic and clinical data of study participants.

3.2. Genotyping

There was no significant difference between the observed frequencies of rs16944 genotypes and the expected ones based on the supposition of Hardy-Weinberg equilibrium (Table 3).

No association was found between the rs16944 genotypes and BMI either in cases or controls ($P > 0.05$). There was no significant difference in frequencies of rs16944 alleles/genotypes between PCOS-I subjects and healthy controls (Table 4).

However, the G allele of the rs16944 SNP was less frequent among PCOS-A cases compared with controls (OR (95% CI) = 0.58 (0.4–0.84), *P* value = 0.004). The rs16944 SNP was associated with PCOS-A in co-dominant (GG vs. AA: OR (95% CI) = 0.37 (0.18–0.76), *P* value = 0.02), dominant (AG + GG vs. AA: OR (95% CI) = 0.51 (0.28–0.91), *P* value = 0.02) and recessive (GG vs. AG + AA: OR (95% CI) = 0.50 (0.27–0.92), *P* value = 0.02) models (Table 5).

4. Discussion

In the current study, we appraised association between a putative functional variant in the promoter region of *IL-1b* and risk of PCOS in Iranian population. IL-1b has an acknowledged role in the process of ovulation. Animal studies have shown the role of this cytokine in induction of ovulation and oocyte maturation, enhancement of

Table 2
Demographic and clinical data of study participants.

Variable	PCOS-I (n = 154)	PCOS-A (n = 116)	Control (n = 112)
Age (mean ± SD, Y)	29.78 ± 4.40	30.04 ± 4.26	33.36 ± 4.51
BMI (mean ± SD, kg/m^2)	26.79 ± 4.05	27.0 ± 4.30	25.84 ± 4.22
LH (mean ± SD, IU/L)	8.59 ± 5.85	5.76 ± 2.71	6.07 ± 2.21
FSH (mean ± SD, IU/L)	6.16 ± 2.20	7.15 ± 4.74	8.05 ± 2.10

The data are presented as mean ± standard deviation (SD). PCOS: polycystic ovarian syndrome; PCOS-I: PCOS infertile; PCOS-A: PCOS abortion: BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone. ANOVA was used to determine the differences of variables between tree groups. $P < 0.05$ was considered statistically significant.

Table 4
Association between rs16944 SNP and PCOS-I.

Model		PCOS-I number (%)	Control number (%)	OR (95% CI)	*P* value
Allelic	A vs. G	141 (46)	100 (45)	1.05 (0.74–1.48)	0.80
		167 (54)	124 (55)		
Co-dominant	AA vs. GG	30 (19.5)	25 (22.3)	0.97 (0.49–1.92)	0.44
	AG vs. GG	81 (52.6)	50 (44.6)	1.39 (0.79–2.44)	
Dominant	AG + AA vs. GG	111 (72.1)	75 (67)	1.27 (0.75–2.16)	0.37
		43 (27.9)	37 (33)		
Recessive	AA vs. AG + GG	30 (19.5)	25 (22.3)	0.84 (0.46–1.53)	0.57
		124 (80.5)	87 (77.7)		

OR, odds ratio; CI, confidence interval.

Table 5
Association between rs16944 SNP and PCOS-A.

Model		PCOS-A number (%)	Control number (%)	OR (95% CI)	*P* value
Allelic	G vs. A	97 (42)	124 (55)	0.58 (0.4–0.84)	0.004
		135 (58)	100 (45)		
Co-dominant	GG vs. AA	23 (19.8)	37 (33)	0.37 (0.18–0.76)	0.02
	AG vs. AA	51 (44)	50 (44.6)	0.61 (0.32–1.14)	
Dominant	AG + GG vs. AA	74 (63.8)	87 (77.7)	0.51 (0.28–0.91)	0.02
		42 (36.2)	25 (22.3)		
Recessive	GG vs. AG + AA	23 (19.8)	37 (33)	0.50 (0.27–0.92)	0.02
		93 (80.2)	75 (67)		

OR, odds ratio; CI, confidence interval.

fertilization, and improvement of further steps of embryonic development (Takehara et al., 1994). Moreover, IL-1 has been among cytokines whose expressions have been reported in the uterus during peri-implantation period (De et al., 1993). Consistent with the presence of a low-level inflammatory response in PCOS patients, a human study has shown higher median of serum levels of IL-1b in PCOS patients compared with healthy subjects (Zafari Zangeneh et al., 2017). The selected SNP in the current study is located in the promoter region of the *IL-1b* gene. This SNP has been associated with numerous human disorders such as coronary artery disease (Momiyama et al., 2001), breast cancer (Kaarvatn et al., 2012) and prostate cancer (Yencilek et al., 2015).

We reported higher frequency of A allele and AA genotype of the rs16944 SNP among PCOS-A cases compared with controls. Our result was in accordance with the results of Xia et al. study which reported higher frequency of AA genotype among Chinese PCOS patients compared with control group. They also detected higher frequency of A allele in patients (Xia et al., 2013). However, Yang et al. have shown higher presence of AG genotype of this SNP among Chinese PCOS patients compared with controls, but they did not show any significant difference in the distribution of AA and GG genotypes between cases and controls (Yang et al., 2010). On the other hand, Mu et al. detected over-representation of the GG genotype of this SNP in PCOS than in controls (Mu et al., 2010). Finally, a single study in Caucasians reported no association between this SNP and PCOS (Kolbus et al., 2007).

The GG genotype of this SNP has been associated with higher levels of the proinflammatory cytokine IL-1b and C-reactive protein in Caucasians (Rogus et al., 2008). Moreover, mononuclear cells from volunteers carrying the A allele displayed lower production of IL-1b after stimulation with lipopolysaccharide (LPS) compared with GG homozygotes (Iacoviello et al., 2005). However, Hall et al. have determined a specific haplotype, including the A allele of the rs16944 and certain allele of another promoter SNP which was considerably associated with higher IL-1b production following induction with LPS (Hall et al., 2004). Others have reported no functional influence for this SNP and related the associations between rs16944 variants and clinical conditions to another concordant SNP (El-Omar et al., 2000). Consistent with the latter studies, Chen et al. have shown that the impact of each SNP on reporter gene transcription is different in relation with other alleles of the SNPs which exist in the promoter construct (Chen et al., 2006). Taken together, the observed association between the rs16944 SNP and PCOS-A in the current study must be interpreted based on the haplotype structure of the assessed population. Consequently, we propose genotyping of other SNPs within the promoter region of this gene in the Iranian population to unravel the definite risk locus for this condition.

Based on the results of the current study, the mentioned SNP was not associated with PCOS-related infertility in spite of its association with the recurrent abortion. This observation might imply different underlying mechanisms for PCOS-associated phenotypes. This SNP might also modulate only some aspects or risk factors of PCOS. Consistent with this speculation, a previous study reported association between the mentioned SNP and risk of obesity in PCOS patients (Yang et al., 2012). Alternatively, this SNP might be associated with risk of abortion in an independent manner from its putative roles in PCOS. In line with this hypothesis, a meta-analysis has shown consistent association between the rs16944 genotypes and recurrent pregnancy loss (Zhang et al., 2017).

The current study shows association between an immune-related SNP and risk of PCOS and further supports the previously detected interplay between immune and reproductive systems (Sukhikh and Vanko, 1999). However, the data presented above indicate that there is no consensus on defining the risk allele of the mentioned SNP. Such discrepancy highly proposes the presence of another functional variant in linkage disequilibrium with this SNP which modulate risk of PCOS.

Declaration of competing interest

The authors declare they have no conflict of interest.

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