Endocr (2010) 37:71–75
DOI 10.1007/s12020-009-9268-x

ORIGINAL ARTICLE

Interleukin 1 beta (IL-1β) promoter C [−511] T polymorphism but not C [+3953] T polymorphism is associated with polycystic ovary syndrome

Yuan Mu · Jingjing Liu · Binbin Wang · Qiaolian Wen · Jing Wang · Jinting Yan · Sirui Zhou · Xu Ma · Yunxia Cao

Received: 19 May 2009 / Accepted: 18 September 2009 / Published online: 10 November 2009

Abstract PCOS is a complex multifactorial disorder involving a number of genetic and environmental factors. One of the genetic factors that has been associated with PCOS is Interleukin 1 beta (IL-1β), is an important inflammatory cytokine that plays a regulatory role in both the body's immune and the inflammatory responses. All these responses appear to be are also affected in at least some women with PCOS. To investigate the possible association of polymorphisms of the *IL-1β* gene with the occurrence and clinical characteristics of PCOS, we evaluated two common polymorphisms of the *IL-1β* gene (promoter C [−511] T and exon 5 position [+3953]) in 200 Chinese women with PCOS and 177 healthy Chinese controls. We found the frequency of *IL-1β* C/C [−511] genotype in PCOS was significantly higher than that in the controls ($\chi^2 = 15.48$, df = 1, $P < 0.001$ OR = 2.73 95% CI: 1.64–4.56 by genotype; $\chi^2 = 10.21$, df = 1, $P = 0.001$ by allele). However in contrast, no association between genotype and relative allele frequencies was observed for the C [+3953] T polymorphism for Chinese women with PCOS when compared to that for a similar group of Chinese women without PCOS ($P = 0.35$).

Keywords Polycystic ovary syndrome · Polymorphism · Interleukin 1 beta

The authors Yuan Mu and Jingjing Liu contributed equally to the work.

J. Liu · Y. Cao (✉)
Reproductive Medicine Center, The First Affiliated Hospital, Anhui Medical University, Hefei, China
e-mail: wbbahu@163.com

Y. Mu · B. Wang · Q. Wen · J. Wang · J. Yan · S. Zhou · X. Ma
Graduate School, Peking Union Medical College, Beijing, China

Y. Mu · B. Wang · Q. Wen · J. Wang · J. Yan · S. Zhou · X. Ma (✉)
Center for Genetics, National Research Institute for Family Planning, 12, Dahuisi Road, Haidian, Beijing 100081, China
e-mail: ADgenetics@gmail.com

X. Ma
World Health Organization Collaborating Centre for Research in Human Reproduction, Beijing, China

Introduction

Polycystic ovary syndrome (PCOS) is the most common heterogeneous hormonal disorder endocrinopathy in females and that affects 4–12% premenopausal women [1]. Women with PCOS have reproductive disorders due to anovulation, hyperandrogenism, polycystic ovaries, obesity, and a subsequent increased risk for type 2 diabetes [2]. To date, no study has convincingly established a mode of inheritance for the disorder [3]. Multiple genetic factors including mutations and polymorphisms have been reported to be associated with PCOS [4, 5].

Although the inheritance mode of PCOS is still uncertain, multiple genetic factors including mutations and polymorphisms to within several genes which have been associated with the established PCOS state to the PCOS risk [6].

The presence of elevated C-reactive protein (CRP) levels, inflammatory cytokines, and increased leucocyte count provides evidence of low-grade chronic inflammation in at least a subgroup of women with PCOS [7].

One of the most prominent mediators of inflammation is from the interleukin-1 (IL-1) family. The *IL-1* gene cluster on chromosome 2q12-q13 contains three related genes *IL-1α*, *IL-1β*, and *IL-1RN*, encoding the pro-inflammatory

cytokines IL-1α, IL-1β, and their endogenous receptor antagonist. IL-1β and IL-1α are potent pro-inflammatory cytokines and play a central role in many inflammatory cascades [8]. IL-1β, a single nuclear factor, which is produced mainly by the monocytes, giant macrophages, and dendritic cells during the intake process of antigen–antibody complex or antigen presentation [9]. IL-1β is also a potent pro-inflammatory cytokine released by macrophages in systemic inflammatory responses. It not only has important biologic effect but also regulates inflammatory reaction and immune response through promoting other cytokines expressions, such as IL-6 and IL-12 [10]. Furthermore, IL-1β has been implicated in inflammatory episode. Previous studies suggested that IL-1β plays an important role in inflammatory-linked mechanisms in the ovary. Hurwitz et al. [11] demonstrated that in vitro treatment of rat ovarian cells with IL-1β leads to the accumulation in the culture medium of a 92 kDa gelatinase, which could be involved in the ovulatory process. And several results lead us to conclude that IL-1β intervenes in PG production, mainly by acting on cyclooxygenase-2 (COX-2) synthesis [12–15]. In addition, Davis et al. [16] demonstrated that IL-1β is able to restore ovulation in mice carrying a null mutation for COX-2, and which thus fails to ovulate. Finally, *IL-1β* mRNA and proteins have been localized in human embryos at the time of fertilization, suggesting their presence in the mature oocyte [17].

IL-1β is mapping in 2q13-21. There are two common polymorphisms (C [−511] T and C [+3953] T in exon 5) in the *IL-1β* gene. Kolbus et al. [18] carried out the first association analysis of the two *IL-1β* gene polymorphisms and PCOS development in Caucasian women (105 cases and 102 controls), which failed to establish an association between these polymorphisms and PCOS.

However, to the best of our knowledge, no study has addressed the potential correlation of specific polymorphisms of the *IL-1β* gene with the established PCOS state in Asian women.

To investigate a possible association between the two common polymorphisms of the *IL-1β* gene and PCOS in Asians, we examined the frequency of occurrence of genotype and alleles for C [−511] T and C [+3953] T in Chinese women.

Results

Clinical and laboratory variables

The clinical characteristics (Mean ± SD) of women enrolled in the study are summarized in Table 1. Comparing with the healthy controls, PCOS patients have significant differences in BMI, E2 levels, and LH levels ($P < 0.05$).

Genotype and allele frequencies

The distribution of the different C [−511] T and C [+3953] T genotypes in the study population is shown in Table 2.

There were significant differences in C [−511] T genotypic and allelic frequencies between PCOS cases and controls.

Table 1 Demographic and clinical characteristics of the study population

Parameter	PCOS ($n = 200$)	Control ($n = 177$)	P
Age (year)	26.91 ± 4.02	31.14 ± 4.22	NS
Menarche age (year)	14.38 ± 1.94	14.33 ± 1.38	NS
BMI (kg/m^2)	23.22 ± 4.19	21.37 ± 2.47	$P < 0.001$
E2 levels (pg/ml)	232.04 ± 209.91	176.1 ± 124.6	$P < 0.001$
FSH levels (mIU/ml)	5.45 ± 2.47	6.71 ± 2.13	NS
LH levels (mIU/ml)	12.89 ± 6.89	4.98 ± 3.12	$P < 0.001$
Prolactin levels (ng/ml)	17.78 ± 33.32	18.07 ± 19.46	NS
Total testosterone (ng/ml)	3.47 ± 8.55	2.07 ± 6.96	NS

BMI body mass index, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone

Table 2 Genotype distribution and relative allele frequencies of C [−511] T polymorphism of *IL-1b* gene in Chinese with PCOS ($n = 200$) and controls ($n = 177$)

Group	No.	Genotype frequency (%)			Allele frequency (%)	
		C/C	C/T	T/T	C	T
PCOS	200	64(32)	76(38)	60(30)	204(51)	196(49)
Controls	177	26(14.69)	87(49.15)	64(36.16)	139(39.38)	214(60.62)
		$\chi^2 = 15.57$, df = 2, $P < 0.001$, OR = 2.73			$\chi^2 = 10.21$, df = 1, $P = 0.001$	

Table 2 presents the distribution of the C [−511] T and C [+3953] T genotypes in women with PCOS and the control group. There were significant differences in C [−511] T genotypic and allelic frequencies between these two groups with significantly higher C/C genotype and C allele frequencies associated with the C [−511] T polymorphism in the PCOS cases ($\chi^2 = 15.48$, df = 1, $P < 0.001$ OR = 2.73 95% CI: 1.64–4.56 by genotype; $\chi^2 = 10.21$, df = 1, $P = 0.001$ by allele).

However in contrast, there was no statistically significant difference in genotype frequency or allele frequency distribution of the C [+3953] T between PCOS and control subjects.

In addition, to investigate the possible association between *IL-1β* gene polymorphism genotypes and clinical/biochemical parameters in women with PCOS and controls subjects, we determined the mean ages of the menarche, BMI values, and plasma E2 levels, FSH levels, LH levels, PRL levels, and total testosterone levels. Table 3 presents these clinical/biochemical profiles (Mean ± SD) for the PCOS group with the data broken out according to genotypes for both the C [−511] T and C [+3953] T variants of the *IL-1β* gene.

Further, there was no significant association between the C [+3953] T polymorphism and the clinical and biochemical parameters of women with PCOS.

Patients with C/C [−511] genotype in *IL-1β* gene presented a lower BMI value, E2 level, LH level, PRL level, total testosterone level, and LH/FSH, when comparing with patients with C/T and T/T genotypes (shown in Table 4). Further, there was no significant association between the C [+3953] T polymorphism and the clinical and biochemical parameters of women with PCOS.

Discussion

Multiple genetic pathways have been implicated in the pathogenesis of PCOS including steroid hormone metabolism, gonadotropin action, obesity and energy regulation,

Table 3 Biochemical profile (Mean ± SD) of Chinese PCOS women according to genotypes for promoter C [−511] T polymorphism and exon 5 position [+3953] polymorphism of *IL-1β* gene

Parameter	Group							
	IL-1β C [−511] T				IL-1β [+3953]			
	C/C ($n = 64$; 32.00%)	C/T ($n = 76$; 38.00%)	T/T ($n = 60$; 30.00%)	*P*	C/C ($n = 186$; 93.00%)	C/T ($n = 13$; 6.50%)	T/T ($n = 1$; 0.50%)	*P*
Menarche age	14.59 ± 2.12	14.36 ± 1.94	14.18 ± 1.73	NS	14.39 ± 1.95	14.31 ± 1.89	14.00	NS
BMI	23.21 ± 3.41	22.98 ± 4.18	23.54 ± 4.94	NS	23.32 ± 4.20	21.88 ± 4.08	22.60	NS
E2 (pg/ml)	228.8 ± 169.37	232.26 ± 163.28	235.28 ± 289.96	NS	232.29 ± 215.67	220.03 ± 111.68	342.89	NS
FSH (mIU/ml)	5.45 ± 2.91	5.75 ± 2.37	5.07 ± 2.01	NS	5.44 ± 2.49	5.47 ± 2.30	5.71	NS
LH (mIU/ml)	12.22 ± 5.65	13.48 ± 7.98	12.87 ± 6.66	NS	12.84 ± 6.91	13.41 ± 7.14	15.49	NS
Prl (ng/ml)	15.58 ± 14.85	16.28 ± 9.95	22.01 ± 57.93	NS	17.83 ± 34.51	17.33 ± 6.64	12.77	NS
Total testosterone (ng/ml)	2.71 ± 3.69	2.11 ± 1.07	5.99 ± 14.88	NS	3.57 ± 8.86	2.08 ± 0.71	2.26	NS
LH/FSH	2.68 ± 2.13	3.07 ± 4.78	2.73 ± 1.36	NS	2.87 ± 3.36	2.42 ± 1.20	2.71	NS

BMI body mass index, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone

Table 4 Biochemical profile (Mean ± SD) of Chinese PCOS women according to genotypes for C [−511] T polymorphism of *IL-1b* gene

Parameter	Group		*P*
	C/C ($n = 64$; 32%)	C/T + T/T ($n = 136$; 68%)	
Menarche age	14.59 ± 2.12	14.28 ± 1.85	NS
BMI	23.21 ± 3.41	23.22 ± 4.52	NS
E2 levels (pg/ml)	228.8 ± 169.37	233.59 ± 227.07	NS
FSH levels (mIU/ml)	5.45 ± 2.91	5.45 ± 2.24	NS
LH levels (mIU/ml)	12.22 ± 5.65	13.21 ± 7.40	NS
Prolactin levels (ng/ml)	15.58 ± 14.85	18.81 ± 39.11	NS
Total testosterone (ng/ml)	2.71 ± 3.69	3.83 ± 10.05	NS
LH/FSH	2.68 ± 2.13	2.92 ± 3.67	NS

and insulin action. There might be association between IL-1 family genes and ovarian function for their act as inflammatory control and host defense responses. IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells, and might be implicated in the immunobiology of both acute and chronic graft rejection [19]. It might also influence ovarian function and the processes of ovulation, fertilization, and implantation [18]. IL-1β is an important inflammatory cytokines in the body's immune and inflammatory response in both of which play the regulatory roles. IL-1β participates in the main type II Des Voeux and helps the T-lymphocytes (Th2) response [20]. Previous study showed that IL-1β might be the promoter of a pancreatic islet cell oxygen B of nitrogen (NO) generation and apoptosis caused by selective destruction of B cells, which also induced insulin resistance.

Kolbus et al. [18] carried out first association analysis between *IL-1β* gene polymorphisms and PCOS developing, which showed that the *IL-1β* gene is not associated with the presence of PCOS in Caucasian.

To investigate a possible association between the *IL-1β* ([−511] and [+3953]) polymorphisms and PCOS in Chinese, we examined 200 PCOS patients and 177 healthy controls. Our findings suggested that the C/C genotype of C [−511] T may represent a genetic risk factor for PCOS in Chinese. But the C [+3953] T polymorphism was not associated with PCOS in Asian.

The C [−511] T polymorphism and IL-1β-31 sites of the T to C SNP complete the linkage disequilibrium [21]. The latter is related with *IL-1β* in the promoter region of the TATA box. By changing of the DNA and protein interactions, T allele enhances the transcription factors, thus affecting *IL-1β* production. And it showed that C [−511] T polymorphism may have the effect of the adoption of its linkage disequilibrium with the TATA box polymorphism IL-1β-31 mediated [22, 23]. In addition, [−511] allele T showed a modest increase in transcriptional activity when compared with allele C [24]. Therefore, the C [−511] T polymorphism may be involved in the PCOS developing by influencing IL-1β production.

As well, no statistical significant difference was discovered between clinical and biochemical parameters (BMI, e2, LH, FSH, PRL levels, mean total testosterone levels) and genotypes.

In summary, we found there was an association between C [−511] T variant of the IL-1β and PCOS, which conflicted to Kolbus's findings.

There are several possible reasons for the conflicting results.

First of all, it should be noticed that, Kolbus' study was performed in a limited sample (105 cases plus 102 controls).This might cause false negative results.

Secondly, the frequency of C/C [−511] genotype in our study is much lower than that in Kolbus' study (22.2% vs. 39.2%), which suggested an obvious ethnic difference. This ethnic difference may be a possible reason for the confused results.

In conclusion, our finding suggested that the C/C genotype of *IL-1β* C [−511] T polymorphism may represent a genetic risk factor for PCOS in Chinese. To our knowledge, the present study firstly established an association between *IL-1β* gene polymorphisms and PCOS developing.

Materials and methods

Subjects

A total of 200 patients with PCOS and 177 unrelated healthy controls were recruited from the First Affiliated Hospital, Anhui Medical University, China. Women with PCOS were diagnosed following the criteria of Rotterdam Revised 2003 (two out of three) diagnosis: oligomenorrhea or amenorrhea for at least 6 months; clinical and/or biochemical signs of hyperandrogenism; polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased volume 10 ml). Congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumor, hyperprolactinemia, and thyroid dysfunction were excluded. Controls were individuals of proven fertility, with normal menstrual cycles and ovary morphology, and without the history of treatment for subfertility.

The study was approved by the Ethics Committee of the National Research Institute for Family Planning and informed consent was obtained from all participants.

Biochemical and hormonal measures

Each blood sample's plasma concentrations of total testosterone, prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) were determined, respectively. Body mass index (BMI) for each participant was calculated to assess obesity.

Laboratory methods

Blood samples from PCOS patients and controls were collected and stored at −20°C. Genomic DNA was extracted from peripheral blood leukocytes using QIAamp genomic DNA kits under the brief protocol listed below.

Add 20 μl Proteinase K and 200 μl Buffer AL to the 200 μl blood sample into a 1.5 ml microcentrifuge tube, mix by pulse-vortexing for 15 s and incubate at 56°C for

10 min or more. Add 200 μl 100% ethanol to the sample, and mix again for 15 s. Then apply the mixture to the QIAamp Spin Column (in a clean 2 ml collection tube) and centrifuge at 8000 rpm for 1 min. Then add 500 μl Buffer AW1 and centrifuge at 8000 rpm for 1 min and add 500 μl Buffer AW2 and centrifuge at 14,000 rpm for 3 min. Place the QIAamp Spin Column in a clean 1.5 ml microcentrifuge tube and add 200 μl Buffer AE. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 8000 rpm for 1 min.

Statistical analysis

The allelic and genotypic distributions of C [−511] T and C [+3953] T were estimated by allele counting and compared in the PCOS and control groups by χ^2 test. Logistic regression analysis was performed to examine the effect of C [−511] T polymorphism and [+3953] polymorphism on the risk for PCOS using Statistic Package for the Social Science (SPSS10.0). The criterion for significance was set at $P < 0.05$. ANOVA and post test was used to compare data of the clinical parameters (age at menarche, E2 levels, FSH levels, LH levels, PRL levels, and total testosterone levels) between different genotypes.

References

1. P. Acién, F. Quereda, P. Matallín, E. Villarroya, J.A. López-Fernández, M. Acién, M. Mauri, R. Alfayate, Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. Fertil. Steril. **72**, 32–40 (1999)
2. E.-J. Lee, B. Oh, A novel single nucleotide polymorphism of INSR gene for polycystic ovary syndrome. Fertil. Steril. **89**(5), 1213–1220 (2008)
3. P. Amato, J.L. Simpson, The genetics of polycystic ovary syndrome. Best Pract. Res. Clin. Obstet. Gynaecol. **18**, 707–718 (2004)
4. E. Diamanti-Kandarakis, C. Piperi, Genetics of polycystic ovary syndrome: searching for the way out of the labyrinth. Hum. Reprod. Update **11**, 631–643 (2005)
5. M. Haap, F. Machicao, N. Stefan, C. Thamer, O. Tschritter, F. Schnuck, D. Wallwiener, M. Stumvoll, H.U. Häring, A. Fritsche, Genetic determinants of insulin action in polycystic ovary syndrome. Exp. Clin. Endocrinol. Diabetes **113**, 275–281 (2005)
6. P. Valdés, A. Cerda, C. Barrenechea, M. Kehr, C. Soto, L.A. Salazar, No association between common Gly972Arg variant of the insulin receptor substrate-1 and polycystic ovary syndrome in Southern Chilean women. Clin. Chim. Acta **390**(1–2), 63–69 (2008)
7. E. Diamanti-Kandarakis, T. Paterakis, H.A. Kandarakis, Indices of low-grade inflammation in polycystic ovary syndrome. Ann. N.Y. Acad. Sci. **1092**, 175–186 (2006)
8. J. Karasneh, A.H. Hajeer, J. Barrett, W.E. Ollier, M. Thornhill, A. Gul, Association of specific interleukin 1 gene cluster polymorphisms with increased susceptibility for Behçet's disease. Rheumatology **42**, 860–864 (2003)
9. X. He, L. Jiang, B. Fu, X. Zhang, Relationship between Interleukin-1B and Interleukin-1 receptor antagonist gene polymorphisms and susceptibility to gastric cancer. J. Jiangsu Univ. **16**, 339–341 (2006)
10. D. Zhang, H. Zheng, Y. Zhou, X. Tang, B. Yu, J. Li, Association of IL-1beta gene polymorphism with cachexia from locally advanced gastric cancer. BMC Cancer **7**, 45 (2007)
11. A. Hurwitz, M. Dushnik, H. Solomon, A. Ben-Chetrit, Z. Finci-Yeheskel, A. Milwidsky, M. Mayer, E.Y. Adashi, S. Yagel, Cytokine-mediated regulation of rat ovarian function: interleukin-1 stimulates the accumulation of a 92-kilodalton gelatinase. Endocrinology **132**(6), 2709–2714 (1993)
12. E.E. Wallach, R. Bronson, Y. Hamada, K.H. Wright, V.C. Stevens, Effectiveness of prostaglandin F2 alpha in restoration of HMG-HCG induced ovulation in indomethacin-treated rhesus monkeys. Prostaglandins **10**, 129–138 (1975)
13. L. Ainsworth, B.K. Tsang, B.R. Downey, R.D. Baker, G.J. Marcus, D.T. Armstrong, Effects of indomethacin on ovulation and luteal function in gilts. Biol. Reprod. **31**, 115–121 (1979)
14. E.D. Watson, P.L. Sertich, Concentrations of arachidonate metabolites, steroids and histamine in preovulatory horse follicles after administration of human chorionic gonadotrophin and the effect of intrafollicular injection of indomethacin. J. Endocrinol. **129**, 131–139 (1991)
15. M. Brännström, Inhibitory effect of mifepristone (RU 486) on ovulation in the isolated perfused rat ovary. Contraception **48**, 393–402 (1993)
16. B.J. Davis, D.E. Lennard, C.A. Lee, H.F. Tiano, S.G. Morham, W.C. Wetsel, R. Langenbach, Anovulation in cyclooxygenase-2-deficient mice is restored by prostaglandin E2 and interleukin-1B. Endocrinology **140**, 2685–2695 (1999)
17. M.J. De Los Santos, A. Mercader, A. Frances, E. Portolés, J. Remohí, A. Pellicer, C. Simón, Role of endometrial factors in regulating secretion of components of the immunoreactive human embryonic interleukin-1 system during embryonic development. Biol. Reprod. **54**, 563–574 (1996)
18. A. Kolbus, K. Walch, F. Nagele, R. Wenzl, G. Unfried, J.C. Huber, Interleukin-1 alpha but not interleukin-1 beta gene polymorphism is associated with polycystic ovary syndrome. J. Reprod. Immunol. **73**, 188–193 (2007)
19. H. Lee, B. Clark, H.C. Gooi, J. Stoves, C.G. Newstead, Influence of recipient and donor IL-1α, IL-4, and TNF-α genotypes on the incidence of acute renal allograft rejection. J. Clin. Pathol. **57**(1), 101–103 (2004)
20. Y. Yang, J. Qiao, M. Li, Study on the correlation between interleuKin 18, InteHeukin 1β and pathogenesis of polycystic ovary syndrome. Chin. J. Pract. Gynecol. Obstet. **24**, 38–40 (2008)
21. F.S. Giovine, E. Takhsh, A.I. Blakemore, G.W. Duff, Single base polymorphism at −511 in the human interleukin-1 beta gene (IL-1 beta). Hum. Mol. Genet. **1**, 450 (1992)
22. Y. Wang, N. Kato, Y. Hoshida, H. Yoshida, H. Taniguchi, T. Goto, M. Moriyama, M. Otsuka, S. Shiina, Y. Shiratori, Y. Ito, M. Omata, Interleukin-1 beta gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. Hepatology **37**, 65–71 (2003)
23. E.M. El-Omar, M. Carrington, W.H. Chow, K.E. McColl, J.H. Bream, H.A. Young, J. Herrera, J. Lissowska, C.C. Yuan, N. Rothman, G. Lanyon, M. Martin, J.F. Fraumeni Jr., C.S. Rabkin, Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature **404**, 398–402 (2000)
24. H. Chen, L.M. Wilkins, N. Aziz, C. Cannings, D.H. Wyllie, C. Bingle, J. Rogus, J.D. Beck, S. Offenbacher, M.J. Cork, M. Rafie-Kolpin, C.M. Hsieh, K.S. Kornman, G.W. Duff, Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. Hum. Mol. Genet. **15**(4), 519–529 (2006)