Association of the polymorphism of codon 121 in the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 gene with polycystic ovary syndrome in Chinese women

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ABSTRACT

الأهداف: من أجل تحديد إتحاد تعدد الشكل للجين الوراثي 121 في الجين الأول لأكتو– نيوكلوتيد بيروفوسفاتاز / فوسفوديستريس (E–NPP1/PC–1) لدى النساء الصينيات اللواتي يعانين من متلازمة تعدد الأكياس في المبيض (PCOS).

الطريقة: تمت دراسة حالة 51 مريضة تعاني من متلازمة تعدد الأكياس (PCOS)، و61 امرأة سليمة من مقاطعة هان بالصين، في مركز مستشفى جامعة شاندونق، خلال الفترة ما بين يونيو 2005م وحتى يوليو 2006م، واللواتي تلقين العلاج من أجل الجين الأول (E–NPP1/PC–1). تم الحصول على الحامض النووي (DNA) من الكريات الدموية المحيطية للمريضات ومجموعة التحكم. كما أجريت أيضا دراسة النمط الوراثي للجين بواسطة استعمال تفاعل سلسلة الخمائر الناقلة والتي عقبها عملية التكرار.

النتائج: بلغ تكرار الجين 121Q المضاد 13%–18% على التوالي، لدى المريضات اللواتي يعانين من (PCOS) والنساء السليمات، بينما بلغ تكرار مضاد الجين 121K 87% و 82% في كلتا المجموعتين. لم يكن هنالك فرقاً ذو دلالة إحصائية في تعدد الشكل للجين (E–NPP1/PC–1) بين المجموعة المصابة (PCOS) ومجموعة التحكم في النساء الصينيات بمقاطعة هان.

خاتمة: ليس لدى تعدد الشكل للجين (E–NPP1/PC–1) إتحاداً مع متلازمة تعدد الأكياس في المبيض (PCOS). لا زال هنالك الحاجة لإجراء المزيد من الدراسات من اجل توضيح ما إذا كان لدى الجين (E–NPP1/PC–1) دور وظيفي أو لا يوجد له دور في الإصابة بمتلازمة تعدد الأشكال في المبيض (PCOS).

Objective: To determine the association of polymorphism of codon 121 in the ecto-nucleotide pyrophophastase/phosphodiesterase 1 (E-NPP1/PC-1) gene in Chinese women with polycystic ovary syndrome (PCOS).

Methods: A total of 51 PCOS patients and 61 healthy women from the Chinese Han population from the Center Reproductive Medicine of Provincial Hospital affiliated to Shandong University from June 2005 to July 2006 were recruited for the determination of the polymorphism of the E-NPP1/PC-1 gene. Genomic DNA was extracted from peripheral blood monocytes of patients and controls, and genotyping of the gene was performed by using polymerase chain reaction, which was followed by sequencing.

Results: The frequency of the 121Q allele was 13 and 18%, respectively, in PCOS patients and healthy women, while the frequency of the 121K allele was 87 and 82% in the 2 groups. There is no significant difference in the E-NPP1/PC-1 polymorphism between PCOS patients and healthy controls among Chinese Han women.

Conclusion: Ecto-nucleotide pyrophophastase/phosphodiesterase 1 polymorphism has no association with PCOS. Further studies are still needed to elucidate whether or not the E-NPP1/PC-1 gene has a functional role in PCOS.

Saudi Med J 2008; Vol. 29 (8): 1119-1123

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Received 1st January 2008. Accepted 6th July 2008.

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Polycystic ovary syndrome (PCOS) is a common disorder in premenopausal women, is characterized by signs, and symptoms of hyperandrogenism and oligo- or amenorrhea, and is frequently associated with insulin resistance.[1,2] At present, PCOS, similar to other common metabolic disorders such as type 2 diabetes mellitus, is considered a complex metabolic disorder of inheritance and genetics. Polycystic ovary syndrome affects 5% to 10% of women of reproductive age.[3] Approximately 50% of patients with PCOS are obese, and up to 70% have enlarged ovaries with multiple subcapsular cysts 8 to 10 mm in size.[4] There have been many studies on the molecular genetics of PCOS. Ecto-nucleotide pyrophosphatase (E-NPP) comprises a family of 3 closely related proteins: E-NPP1 (also named as phosphodiesterase 1 [PC-1]), E-NPP2 (PDNP2/PD-1ß/autotoxin), and E-NPP3, which are expressed in various cells or at different locations even in the same type of cells. cto-nucleotide pyrophosphatase 1, originally described as a cell-surface antigen of differentiated antibody-secreting B cells, has, however, been found to be expressed in non-lymphoid tissues.[5-7] Ecto-nucleotide pyrophosphatase-1 is one of 5 cell membrane proteins with an extracellular active site catalyzing the release of nucleoside 5'-monophosphatase from nucleotides and their derivatives. The gene encoding for E-NPP1 has 25 exons and is located on human chromosome 6q22-23.[8] It has been reported that the E-NPP1/PC-1 gene plays an important role in insulin resistance.[5] The K121Q polymorphism in exon 4 of the E-NPP1/PC-1 gene has been shown to be associated with features of insulin resistance and type 2 diabetes.[9-11] Recently, it has been reported that this polymorphism is associated with PCOS in Caucasian women.[12] However, little is known concerning the association between the polymorphism within exon 4 of the E-NPP1/PC-1 gene and PCOS in Chinese women. Since the strong evidence implicating the E-NPP1/PC-1 gene in insulin resistance, the polymorphism of the E-NPP1/PC-1 gene was investigated in Chinese PCOS patients and healthy volunteers.

Methods. Fifty-one Chinese women aged 23-36 years (mean age, 29.16 years) with an ethnic Han (accounting for the majority of Chinese population) origin and PCOS were included in the study. All patients were diagnosed according to the Report of the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group.[13] Briefly, women were diagnosed as having PCOS if 2 out of the following 3 criteria were reached: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries after exclusion of other etiological conditions such as congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, and so forth. Patients with insulin-dependent diabetes mellitus were excluded from the study. All patients had not taken hormonal medications, including contraceptive pills, and anti-obesity drugs, for at least 3 months. In addition, a control group composed of 61 Han women with normal menstrual cycles and normal biomedical variables but without obesity, hirsutism, acne, or male-type alopecia, was recruited. These women were diagnosed as healthy, had no endocrine disease history, and were prepared to accept artificial insemination in Shandong Provincial Hospital, due to infertility caused by their husbands' reproduction problems. Their ages ranged from 21-38 years (mean age 30.02 years). The PCOS patients and control group were from the Center Reproductive Medicine of Provincial Hospital, Shandong University, Jinan, China from June 2005 to July 2006. The study was approved by the Institutional Review Boards and by the Ethical Committee of Shandong University, Shandong Provincial Hospital. Written informed consent was obtained from all participants. Clinical and laboratory information was collected and stored in a database.

Study protocol. After a 12-hours overnight fast, peripheral blood samples were obtained from each of the subjects. The blood samples were then centrifuged immediately. The serum and peripheral blood cell samples were separated and frozen at -20^{0}C until further assays. The serum samples were used for measurements of the levels of hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), estradiol (E2), and total testosterone (Total T) by using a competitive electrochemoluminescent immunoassay method by an autoanalyzer (Beckman, Fullerton, USA). Clinical characteristics, such as body mass index (BMI) and waist/hop ratio (WHR), were calculated and recorded in a database. The peripheral blood cell samples were used for DNA extraction and genotype analyses as described below.

DNA extraction and genotype analyses. Genomic DNA was extracted from peripheral blood cells of both the patients and controls by using the phenol-chloroform method and a commercial DNA extraction kit (Scientific Research Center of Shandong Provincial Hospital, Shandong, Jinan, China) according to the manufacturer's instructions. After DNA extraction, a transversion of A-G at the first position of codon 121, resulting in an amino acid change from lysine to glutamine (K121Q) in the E-NPP1/PC-1 gene, was genotyped by a polymerase chain reaction (PCR) method, as previously described.[9] The DNA was amplified by using CTGTGTTCACTTTGGACATGTTG as the forward primer and GACGTTGGAAGATACCAGGTTG as the reverse primer.[9] The forward primer was further used for cycle sequencing. Polymerase chain reaction amplification was performed in a 25-µl reaction

mixture containing 100 ng of total DNA, 200 µmol/L deoxynucleotide triphosphate (dNTPs), 1.5 mmol/L MgCl2, 0.2 µmol/L primers, and 0.05U Taq DNA polymerase. The program for PCR was denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 94°C for 2 minutes, annealing at 55°C for 40 seconds, and extension at 72°C for 40 seconds. Polymerase chain reaction products were separated on 1% agarose gel. The DNA fragments of 208 bp were purified by a commercial gel extraction kit (QIAGEN, Hilden, Germany) and further confirmed by cycle sequencing using the Big Dye Terminator (Applied Biosystems, Foster city, USA) on an ABI-3100 genetic analyzer (ABI, Applied Biosystems).

Statistical analysis. Population frequencies were derived from the same geographical area, and they were based on 112 tested normoglycemic subjects in the general population. Biomedical variables obtained from this study are expressed as the mean ± SEM. The data were analyzed using SPSS 11.0 software (SPSS Inc., Chicago, IL). The significance association was determined by Pearson chi-square and t-test. A significance of $p<0.05$ was considered to be statistically significant.

Results. The comparison of clinical, biochemical, and hormonal variables between PCOS patients and the healthy controls are shown in **Table 1**. Body mass index of women with PCOS was greater than those of women in the control group (25.25±4.51 versus 21.87±4.16, p=0.00), and thus women with PCOS were significantly more likely to be obese as demonstrated by having higher BMIs, as compared with the controls (25.25±4.51 versus 21.87±4.16, p=0.000). In addition, PCOS patients presented with higher LH levels than the controls (11.57±8.05 versus 4.36±1.61, p=0.000).

The frequency of the 121Q allele was 13% among PCOS cases and 18% among healthy women (**Table 2**), as determined by PCR (**Figure 1**), sequencing (**Figure 2**) with the overall frequency of the 121Q allele being 16%. Correspondingly, the frequency of the 121K allele was 87% among PCOS patients and 82% among

Table 1 - Comparisons of clinical and biochemical variables between polycystic ovary syndromeis (PCOS) patients and healthy controls.

Characteristics	PCOS patients N=51	Healthy women N=61	*P*-value
Age (y)	29.16±4.18	30.02±4.31	0.31
Body mass index (kg/m²)	25.25±4.51	21.87±4.16	0.000
Waist/hop ratio	0.85±0.07	0.83±0.06	0.062
Follicle-stimulating hormone (IU/L)	6.75±1.53	7.93±2.75	0.12
Luteinizing hormone (IU/L)	11.57±8.05	4.36±1.61	0.000
Prolactin	13.48±5.88	14.32±5.74	0.49
Total testosterone	56.77±23.66	27.09±12.16	0.086
Estradiol	63.27±30.40	52.90±25.71	0.190

Data are means± SEM. Data were analyzed by t-test.

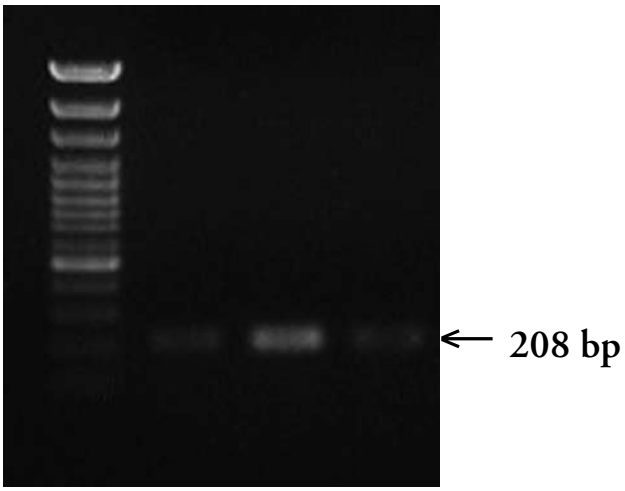

Figure 1 - Polymerase chain reaction products were separated on 1% agarose gel.

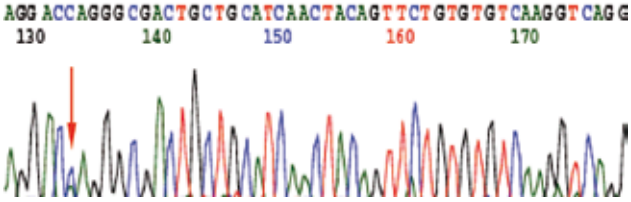

Figure 2 - Polymerase chain reaction products of 208 bp were purified and further confirmed by cycle sequencing using the Big Dye Terminator (Applied Biosystems, Foster, USA) on an ABI-3100 genetic analyzer (ABI, Applied Biosystems).

Table 2 - Frequencies of the 121Q and 121K alleles in polycystic ovary syndromeis (PCOS) patients and healthy controls.

Frequency	PCOS patients N=51	Healthy women N=61	Overall N=112
Genotype	44 (87)	50 (82)	94 (84)
K121K	7 (13)	11 (18)	18 (16)
K121Q	0 (0)	0 (0	0 (0)
Q121Q			
Allele frequency			
121Q	7 (13)	11 (18)	18 (16)

Data were analyzed using the Pearson chi-square test.

control women, with the overall frequency of the 121K allele being 84% (**Table 2**). There was no statistically significant difference in the distribution of the K121Q polymorphism in the E-NPP1/PC-1 gene between PCOS patients and healthy women among Chinese Han (p=0.0781).

Discussion. Polycystic ovary syndrome is a disorder characterized by signs and symptoms of hyperandrogenism and oligo- or amenorrhea, and it is frequently associated with insulin resistance.[14-16] The familial aggregation of the functional hyperandrogenism and the metabolic traits associated with PCOS strongly suggests an inherited basis for PCOS.[14] Polycystic ovary syndrome is considered to be a complex metabolic disorder of inheritance and genetics. Women with PCOS have been shown to have a higher prevalence of the T45G polymorphism in the adiponectin gene compared with healthy controls.[17] The possible association of PCOS with 15 genomic variants previously described to influence insulin resistance, obesity, and/or type 2 diabetes mellitus has been evaluated in previous reports.[18] The paraoxonase -108 C→T variant and the Apal polymorphism in the IGF2 gene are demonstrated to be associated with PCOS and might contribute to increased oxidative stress, insulin resistance, and hyperandrogenism in this prevalent disorder.[18] The E-NPP1/PC-1 gene belongs to the ectophosphodiesterase family.[19] The exact physiological function of this gene is still unclear, although its content has been reported to be increased in fibrotic, muscle, and adipose tissues of insulin-resistant patients.[19] It has been demonstrated that overexpression of the E-NPP1/PC-1 gene in transfected cells results in decreased insulin-induced tyrosine kinase activity in vitro.[20] Previous studies have also shown that the K121Q polymorphism in exon 4 of the E-NPP1 gene is associated with features of insulin resistance and type 2 diabetes.[9-11] Polycystic ovary syndrome confers an up to 7-fold increased risk of type 2 diabetes mellitus, and almost all women with PCOS have insulin resistance independent of obesity.[21] Therefore, the K121Q polymorphism in exon 4 of the E-NPP1 gene may be also associated with the development of PCOS. Indeed, a recent clinical study carried out on Caucasian women showed that the 121Q allele of the E-NPP1/PC-1 gene predisposed women to developing PCOS.[12] There was also another study demonstrating that the 121Q allele of the ENPP1/PC-1 gene was not associated with PCOS in Caucasian women.[18] However, the association between the E-NPP1/PC-1 gene polymorphism and PCOS has not been investigated in Asian populations, including Chinese women. Therefore, we carried out this study to test the hypothesis that the K121Q polymorphism of the E-NPP1/PC-1 gene may also be associated with the development of PCOS in Chinese women. In the present study, biomedical variables of patients and controls were measured and analyzed. The most characteristic feature of PCOS was chronic hyperandrogenic anovulation. It has been demonstrated that this hyperandrogenism may be caused by elevated LH levels resulting from dysregulation of the hypothalamic-pituitary axis.[22] Indeed, PCOS patients in our study also showed significantly elevated LH levels (**Table 1**). Moreover, it has been reported that a rapid and major weight gain often occurs in girls with PCOS during adolescence.[23] In the present study, obesity was more frequently observed in PCOS patients than in healthy controls, although all of our study subjects were between 20 and 38 years old. Moreover, the K121Q polymorphism in the E-NPP1/PC-1 gene was investigated in 51 PCOS patients and 61 healthy volunteers from the Chinese Han population in the present study. The frequency of the 121Q allele was found to be 13% in PCOS cases and 18% in healthy controls, while the frequency of the 121K allele was 87% and 82% in the 2 groups, respectively. Thus, there was no significant difference in the allele frequencies of codon 121 in exon 4 of the E-NPP1/PC-1 gene between PCOS patients and healthy controls, suggesting that the K121Q polymorphism in the E-NPP1/PC-1 gene is not associated with PCOS in Chinese Han women. This finding was not in accordance with a previous study by Heinonen et al who addressed the same issue in Caucasian women.[12] Several factors may account for the discrepant results. First, the distribution of E-NPP1/PC-1 may be different across different ethnic populations.[24] Secondly, we used DNA sequencing, while Heinonen et al used restriction enzyme analysis with Eco471 to confirm the PCR results. The difference in the methodologies used in the 2 studies might lead to the different findings. Finally, the sample sizes in both studies were small, which resulted in inconsistent findings, and thus further studies with large sample sizes are required to replicate our findings. Nevertheless, even if the 121Q allele of the E-NPP1/PC-1 gene is associated with PCOS, it may play a limited role in the development of PCOS, since it has been reported that PCOS is associated with other genomic variants that relate to insulin resistance, type 2 diabetes mellitus and obesity.[18]

In conclusion, the polymorphism in exon 4 of the E-NPP1/PC-1 gene is not associated with PCOS in Chinese Han women. However, further studies are still needed to confirm our observation and to elucidate whether or not the E-NPP1/PC-1 gene has a functional role in PCOS.

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