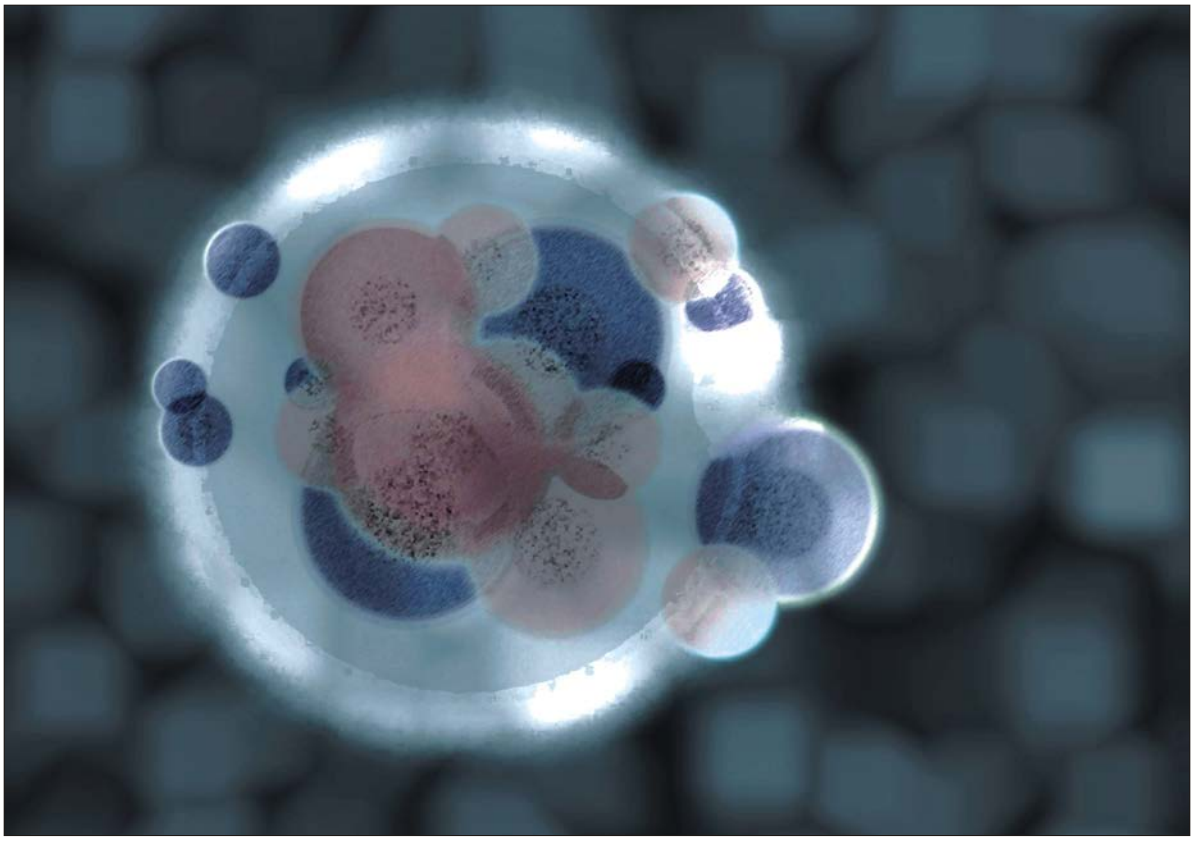


Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 5 (6): 832-836, 2005
ISSN 1727-3048

Relationship Between the C/T Single Nucleotide Polymorphism in Exon 17 of the Insulin Receptor Gene and Polycystic Ovary Syndrome

[1]Eung-Ji Lee, [1]Kyong-Jai Yoo, [1]So-Jeong Kim, [1]Sook-Hwan Lee, [2]Dong-Hee Choi,
[1]Kwang Yul Cha, [3]Doo-Seok Choi and [1]Kwang-Hyun Baek
[1]Graduate School of Life Science and Biotechnology, Cell and Gene Therapy Research Institute,
Pochon CHA University, CHA General Hospital, Seoul, Korea
[2]Infertility Medical Center, Bundang CHA General Hospital, Bundang, Korea
[3]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract: The Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders in females of reproductive age. The etiology of PCOS has been involved in a combination of genetic and environmental factors. Recently, several studies with different ethnical groups have been demonstrated that the Single Nucleotide Polymorphism (SNP) of *insulin receptor* (*INSR*) gene is associated with susceptibility to the PCOS. This study was undertaken to analyze the influence of the SNP of *INSR* on PCOS in a Korean population. We studied 50 PCOS and 35 normal patients. According to the Body Mass Index (BMI), subjects were classified into two groups (lean and obese based on the number same or higher than 25 kg m^{-2}) and analyzed the association of SNP in the *INSR* gene with PCOS. As shown with controls, PCOS patients were more frequent for the T allele (the CT and TT genotypes) at the exon 17 of the *INSR*. Interestingly, the frequency of the C allele in patients with PCOS was significantly higher than that in normal controls (40% vs. 8.57%, p = 0.001). Various frequencies for the SNP of *INSR* among different ethnical backgrounds suggest that the ethnical background should be considered to determine the relationship between the SNP of *INSR* and PCOS.

Key words: Hirsutism, hyperandrogenism, *INSR*, PCOS

INTRODUCTION

The Polycystic Ovary Syndrome (PCOS) is a heterogeneous hormonal disorder, affecting 5 to 10% of premenopausal women[1]. According to the revised diagnostic criteria announced in 2003 ASRM/ESHRE Rotterdam consensus, PCOS is diagnosed when the phenotypes of patients are satisfied with two standards of three, oligomenorrhea or amenorrhea, clinical or biochemical hyperandrogenism and ultrasonographic polycystic ovarian morphology while other causes like nonclassical congenital adrenal hyperplasia are excluded[2]. This syndrome is caused by a combination of genetic and environmental factors and characterized by hyperandrogenism, anovulation and increased risk of type 2 diabetes and about 50% of patients with PCOS are obese[3-8]. Various Single Nucleotide Polymorphisms (SNPs) expected to be associated with PCOS are investigated by a number of groups[9]. Numerous candidate genes were suggested to affect the susceptibility of PCOS. *CYP17*, encoding for cytochrome P450 17-α hydroxylase, 17/20-lyase has been found to be associated with polycystic ovaries[10]. It has been reported that a steroidogenic gene *CYP11A*, which encodes a cytochrome P450 side-chain cleavage enzyme, is associated with PCOS[11]. A cysteine protease gene, *calpain-10* has been shown to be associated with susceptibility to type 2 diabetes[12]. A recent study showed that 112/121-haplotype of *calpain-10* is associated with the increased risk of PCOS in both African-American and white women[12]. The polymorphism in exon 6 of the *tumor necrosis factor receptor* (*TNF-R*) gene associated with PCOS has been reported[13]. Polymorphisms in *peroxisome proliferator-activated receptor-γ* (*PPARγ*) associated with PCOS also have been identified[14,15]. A previous report showed the association between the Ala allele of Pro12Ala polymorphism in exon 2 of *PPARγ* gene and increased insulin sensitivity in Caucasian women with PCOS[14]. It has been observed that the CT/TT polymorphism in exon 6 of *PPARγ* gene was more frequent in patients with PCOS than in healthy women[15].

Corresponding Author: Dr. Kwang-Hyun Baek, Cell and Gene Therapy Research Institute, Pochon CHA University, CHA General Hospital, 605 Yeoksam 1-Dong, Kangnam-Gu, Seoul 135-081, Korea
Tel: +82 2 3468 3197 Fax: +82 2 3468 3264

As the clinical features of PCOS include the increased risk of type 2 diabetes, *insulin* and *insulin receptor* (*INSR*) genes have been suggested as candidate genes for genetic factors. One of previous studies demonstrated the association between the class III allele at the *insulin* gene 5' VNTR (variable number tandem repeats) in the 5' region of the *insulin* gene and PCOS[16]. It has been suggested that the variation in the exon 17 of *INSR* gene could cause symptoms of PCOS, impaired glucose tolerance and insulin resistance[17]. The exon 17 of *INSR* is located on chromosome 19p13.3 and encodes the partial region of tyrosine kinase domain for *INSR*. Therefore, it is critical for the function of the receptor[18]. A recent report showed that the His 1058 C/T polymorphism at the tyrosine kinase domain of the *INSR* gene is associated with PCOS in the white women[7]. In addition, susceptibility region in the *INSR* gene has been identified[17]. Thus, the polymorphisms in *INSR* gene may be associated with susceptibility of PCOS. In many reported SNP studies, the effects of polymorphisms on clinical features are various in different ethnical backgrounds. For this reason, we analyzed 50 Korean women with PCOS and 35 healthy Korean women to investigate the association between the polymorphism of *INSR* gene and PCOS in a Korean population.

In this study, we examined the effects of the His 1058 C/T polymorphism in the *INSR* on Korean patients with PCOS using the Restriction Fragment Length Polymorphism (RFLP) analysis. We divided the subjects into two groups by Body Mass Index (BMI) and inquired into the association between the exon 17 C/T polymorphism of *INSR* and PCOS.

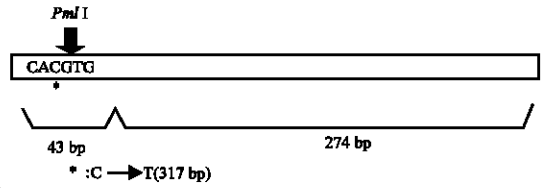
MATERIALS AND METHODS

Patients: We recruited 50 women who have been diagnosed for PCOS based on their clinical symptoms, ultrasonographic examination and biochemical data. Another 35 healthy women were enrolled and considered as a control group. According to the redefinition of adult obesity in the Asian Pacific Perspective proposed at the Hong Kong meeting in 2000[19], the subjects were divided into two groups, lean group (BMI <25 kg m^{-2}) and obese group (BMI ≥25 kg m^{-2}). Clinical and biochemical characteristics of women with PCOS and controls are given in Table 1. Blood samples were obtained from subjects for biochemical assay and DNA analysis. Blood samples for molecular genetic studies were collected in tubes containing EDTA as an anticoagulant and stored at 4°C. All patients and controls in this study were Korean women.

RFLP analysis for the exon 17 of *INSR*: We extracted the genomic DNA from the blood of patients with PCOS and normal women. RFLP analysis was performed to characterize the His 1058 C/T polymorphism in the *INSR* gene. Exon 17 was amplified by Polymerase Chain Reaction (PCR), using 5'-CCAAGGATGCTGTGTAGATAAG-3' as the forward primer and 5'-TCAGGAAAGCCAGCCCATGTC-3' as the reverse primer (Fig. 1) in a total volume of 25 μL. 100 ng of DNA was contained in a reaction mixture. Cycling parameters were denaturation at 94°C for 2 min, 30 cycles with 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and 72°C for 10 min. Following PCR amplification, the DNA was purified from PCR products by PCR purification kit (Accuprep, Bioneer, Daejeon, Korea) and digested with *Pml* I (New England Biolabs, Beverly, MA, USA) for 3 h at 37°C. Digested DNA fragments were electrophoresed on a 2% agarose gel containing ethidium bromide and visualized by Fluor-S MultiImager (Bio-Rad, Hercules, CA, USA). The C allele is recognized and restricted by *Pml* I. Hence, a single 317 bp band indicated homozygosity for the T allele. The presence of two fragments, 274 and 43 bp, indicated homozygosity for the C allele. And the presence of three fragments, 317, 274 and 43 bp bands,

tcaggaaagccagcccatgtcccacccccactggactcaccac/gtgatg
gcaggtgaagcccttcatgaccgaggcctcattgaggaactcaatccgctc
tcggagactggctgactcgttgaccgtcttcaccgccacgcgggtctctgc
ctcacccttgatgatgtccctggcattgccctatacaccatgccgaaggag
ccctgccccagctctcgaaggagggtgatcttctctcgagacacctcccac
tcgtccggcacgtacacagagcatggaaacactacttcttacttatctacac
agcatccttgg

(A)



(B)

Fig. 1: The analyzed sequence of exon 17 in human *INSR* gene (NCBI, Accession No.: NM_000208). The sequences of forward and reverse primers are underlined. The sequence in bold indicates the recognition site of *Pml* I. (B) The structure of the exon 17 region for *INSR*. An arrow indicates the restriction site of *Pml* I. The site of the His 1058 C/T polymorphism is marked with an asterisk. When the sequence has the C allele, *Pml* I restrict the site and two fragments are produced (43 and 274 bp) whereas the T allele makes one fragment (317 bp)

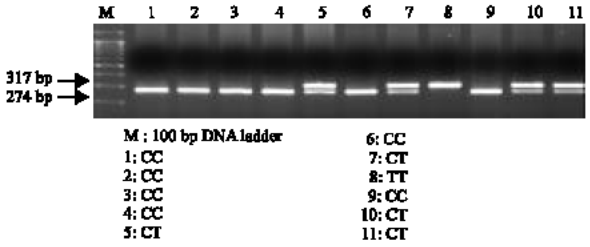


Fig. 2: RFLP analysis of the His 1058 C/T polymorphism in the *INSR* gene. The T allele makes restriction site of *Pml* I. Agarose gel (2%) electrophoresis with ethidium bromide staining following *Pml* I digestion of PCR product is shown. Single band of 317 bp indicates TT genotype, two bands, 317 and 274 bp, represent CT genotype and homozygosity of C allele has a band with 274 bp

indicated heterozygosity for the C allele or the T allele. The small 43 bp band has run out from the gel (Fig. 1 and 2).

Statistical analysis: The statistical analysis was performed using Epistat. χ^2 test was used to analyze the association between two groups. A $p<0.05$ value was considered statistically significant.

RESULTS AND DISCUSSION

One of the symptoms showed in PCOS patients is insulin resistance[17]. Therefore, genes related with insulin action have been suggested to be candidate genes for PCOS. According to the previous reports, the SNP in *INSR* gene was found to be associated with PCOS in various ethnical groups[7,20]. In this study, we analyzed the association between the His 1058 C/T polymorphism in the exon 17 of the *INSR* and PCOS in a Korean population. All subjects were examined age, BMI, waist/hip ratio (WHR) and hormone levels including FSH, LH, E2, Prolactin, TSH and DHEA-S. There is no difference between the PCOS patient group and the control group except the obese/lean ratio. The rate of obese subjects in the PCOS patient group is three times higher than that of the control group (Table 1). The frequency of the CC, CT, TT genotypes of the exon 17 C/T polymorphism for *INSR* in patients with PCOS and controls (Table 2). The rate of CC genotype in PCOS patient was significantly higher than that of control group (patient group = 40%, control group = 8.57%). And the frequency of T allele (the CT + TT genotypes) was remarkably increased in a control group (patient group = 60%, control group = 91.43%). After classification of subjects with BMI, the rates of genotypes were analyzed (Table 3). In the lean subjects, the CC genotype was presented more frequently in PCOS patients than control patients (37.14 and 9.68%, respectively). In addition, the frequency of T allele was clearly higher in the control group than the PCOS patient group (90.32 and 62.86%, respectively). Therefore, we, concluded that the frequency of the C allele was significantly increased in patients with PCOS compared with controls ($p = 0.001$). However, the frequency of the C and T alleles did not differ significantly between lean and obese patients with PCOS. Interestingly, all obese controls (N = 4) showed the genotype of CT heterozygous allele (Table 3).

A previous report showed that the His 1058 C/T polymorphism at the tyrosine kinase domain of the *INSR* gene is associated with PCOS in the white women, especially in lean patients (BMI 27 kg m^{-2})[7]. In addition, a recent report demonstrated that this SNP of *INSR* gene is correlated with PCOS in Chinese patients[20].

Table 1: Clinical and hormonal characteristics of subjects

	PCOS patient group	Control group
	50	35
Age (years)	32.47±3.04	33.09±4.36
BMI (kg m^{-2})	23.18±4.04	21.42±2.93
Waist/hip ratio (WHR)	00.82±0.06	00.82±0.04
Obese/Lean (%)	42.86	12.9
FSH levels (m IU mL^{-1})	04.90±1.42	06.54±2.09
LH levels (m IU mL^{-1})	06.24±4.1	03.09±1.38
E2 levels (pg mL^{-1})	36.84±19	30.86±13.9
Prolactin levels (pg mL^{-1})	18.47±11.66	13.09±8.14
TSH levels (μ LU mL^{-1})	02.74±2.03	02.58±1.29
DHEA-S levels (μg dL^{-1})	185.93±80.54	181.99±50.3

Table 2: Allele frequencies of C/T polymorphism of exon 17 of *INSR* gene in PCOS patient group (N=50) and control group (N=35)

Genotypes	PCOS patient group (N=50) (%)	Control group (N=35) (%)
CC	20 (40)	3(8.57)*
CT	19 (38)	25(71.43)**
TT	11 (22)	7(20.00)

*p = 0.001, **p = 0.002

Table 3: Allele frequencies of C/T polymorphism of exon 17 of *INSR* gene in classified PCOS patient and control groups into lean and obese groups by BMI

Body Mass Index (kg m^{-2})	Groups	Genotypes CC	CT	TT
Lean (BMI <25 kg m^{-2})	PCOS patients (N=35) (%)	13 (37.14)	12 (34.29)	10 (28.57)
	Controls (N=31) (%)	3 (9.68)*	21 (67.74)**	7 (22.58)
Obese (BMI≥25 kg m^{-2})	PCOS patients (N=15) (%)	7 (46.67)	7 (46.67)	1 (6.66)
	Controls (N=4)	All have CT genotype		

*p = 0.009, **p = 0.007

They found significantly different frequency of T allele between in non-obese patients and obese patients (52.2 and 25.5%, respectively, $p<0.01$)[20]. These results indicate that a C/T SNP at the exon 17 of *INSR* affects more non-obese patients with PCOS than obese patients. Different from two reports[7,20], there was a report that SNP at the His 1058 of the exon 17 for *INSR* gene is unlikely to play a direct role in the pathogenesis of human disorders with insulin resistance in a Chinese population[21].

In this study, the frequencies of CC genotype and CT/TT genotypes are significantly different between PCOS patients and controls. And, it is opposed to previous results that the rate of T allele is higher in the PCOS patient group than the control group[7,20]. We found the increased rate of T allele in the control group both in total subjects and lean subjects. It is suggested that the C/T SNP of exon 17 in *INSR* gene is not associated with pathogenesis of PCOS in a Korean population. The subjects in this study generally had normal value of BMI (23.18 kg m^{-2} in the PCOS patient group and 21.42 kg m^{-2} in the control group, respectively) (Table 1), indicating that the obese subjects were rarely included in this study. Therefore, a large number of obese patients should be included to delineate the association of insulin signaling and PCOS. In conclusion, we demonstrated that the His 1058 C/T polymorphism of the exon 17 in human *INSR* gene is not associated with PCOS in a Korean population.

ACKNOWLEDGMENTS

We would like to thank members of the Infertility Medical Center and Cell and Gene Therapy Research Institute at Pochon CHA University and CHA General Hospital. This study was supported by a grant from Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ10-PG6-01GN13-0002).

REFERENCES

1. Futterweit, W., 1999. Polycystic ovary syndrome: Clinical perspectives and management. Obstet. Gynecol. Surv., 54: 403-413.
2. Balen, A.H., J.S. Laven, S.L. Tan and D. Dewailly, 2003. Ultrasound assessment of the polycystic ovary: International consensus definitions. Hum. Reprod. Update, 9: 505-514.
3. Ehrmann, D.A., R.B. Barnes and R.L. Rosenfield, 1995. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Endocr. Rev., 16: 322-353.
4. Legro, R.S., A.R. Kunselman, W.C. Dodson and A. Dunaif, 1999. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: A prospective, controlled study in 254 affected women. J. Clin. Endocrinol. Metab., 84: 165-169.
5. Calvo, R.M., D. Telleria, J. Sancho, J.L. San Millan and H.F. Escobar-Morreale, 2002. Insulin gene variable number of tandem repeats regulatory polymorphism is not associated with hyperandrogenism in Spanish women. Fertil. Steril., 77: 666-668.
6. Ehrmann, D.A., X. Tang, I . Yoshiuchi, N.J. Cox and G.I. Bell, 2002. Relationship of Insulin receptor substrate-1 and -2 genotypes to phenotypic features of polycystic ovary syndrome. J. Clin. Endocrinol. Metab., 87: 4297-4300.
7. Siegel, S., W. Futterweit, T.F. Davies, E.S. Concepcion, D.A. Greenberg, R. Villanueva and Y. Tomer, 2002. A C/T single nucleotide polymorphism at the tyrosine kinase domain of the insulin receptor gene is associated with polycystic ovary syndrome genes. Fertil. Steril., 78: 1240-1243.
8. San Millan, J.L., M. Corton, G. Villuendas, J. Sancho, B. Peral and H.F. Escobar-Morreale, 2004. Association of polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus and obesity. J. Clin. Endocrinol. Metab., 89: 2640-2646.
9. Amato, P. and J.L. Simpson, 2004. The genetics of polycystic ovary syndrome. Best Pract. Res. Clin. Obstet. Gynaecol., 18: 707-718.
10. Carey, A.H., D. Waterworth, K. Patel, D. White, J. Little, P. Novelli, S. Franks and R. Williamson, 1994. Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. Hum. Mol. Genet., 3: 1873-1876.
11. Gharani, N., D.M. Waterworth, S. Batty, D. White, C. Gilling-Smith, G.S. Conway, M. McCarthy, S. Franks and R. Williamson, 1997. Association of the steroid synthesis gene CYP11a with polycystic ovary syndrome and hyperandrogenism. Hum. Mol. Genet., 6: 397-402.
12. Ehrmann, D.A., P.E. Schwarz, M. Hara, X. Tang, Y. Horikawa, J. Imperial, G.I. Bell and N.J. Cox, 2002. Relationship of calpain-10 genotype to phenotypic features of polycystic ovary syndrome. J. Clin. Endocrinol. Metab., 87: 1669-1673.

13. Peral, B., J.L. San Millan, R. Castello, P. Moghetti and H.F. Escobar-Morreale, 2002. Comment: The methionine 196 arginine polymorphism in exon 6 of the TNF receptor 2 gene (TNFRSFIB) is associated with the polycystic ovary syndrome and hyperandrogenism. J. Clin. Endocrinol. Metab., 87: 3977-3983.
14. Hara, M., S.Y. Alcoser, A. Qaadir, K.K. Beiswenger, N.J. Cox and D.A. Ehrmann, 2002. Insulin resistance is attenuated in women with polycystic ovary syndrome with the Pro12Ala ploymorphism in the PPARγ gene. J. Clin. Endocrinol. Metab., 87: 772-775.
15. Orio, F.Jr., G. Matarese, S. Di Biase, S. Palomba, D. Labella, V. Sanna, S. Savastano, F. Zullo, A. Colao and G. Lombardi, 2003. Exon6 and 2 peroxisome proliferator–activated receptor-gamma polymorphisms in polycystic ovary syndrome. J. Clin. Endocrinol. Metab., 88: 5887-5892.
16. Waterworth, D.M., S.T. Bennett, N. Gharani, M.I. McCarthy, S. Hague, S. Batty, G.S. Conway, D. White, J.A. Todd, S. Franks and R. Williamson, 1997. Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. Lancet, 349: 986-990.
17. Urbanek, M., R.S. Legro, D. Driscoll, J.F. 3rd Strauss, A. Dunaif and R.S. Spielman, 2000. Searching for the polycystic ovary syndrome genes. J. Pediatr. Endocrinol. Metab., 13: 1311-1313.
18. Tucci, S., W. Futterweit, E.S. Concepcion, D.A. Greenberg, R.B. Villanueva, T.F. Davies and Y. Tomer, 2001. Evidence for association of polycystic ovary syndrome in Caucasian women with a marker at the insulin receptor gene locus. J. Clin. Endocrinol. Metab., 86: 446-449.
19. WHO/IASO/IOTF: The Asia-Pacific Perspective: Redefining obesity and its treatment, Hong Kong, Health Communications Australia Pty Ltd, 2000.
20. Chen, Z.J., Y.H. Shi, Y.R. Zhao, Y. Li, R. Tang, L.X. Zhao and Z.H. Chang, 2004. Correlation between single nucleotide polymorphism of insulin receptor gene with polycystic ovary syndrome. Zhonghua Fu Chan Ke Za Zhi, 39: 582-585.
21. Wang, L., J. Mi, X.Y. Zhao, J.X. Wu, H. Cheng, Z.K. Zhang, X.Y. Ding, D.Q. Hou and H. Li, 2004. Polymorphisms of exon 17 of insulin-receptor gene in pathogenesis of human disorders with insulin resistance. Biomed. Environ. Sci., 17: 418-425.