A novel SNP at exon 17 of INSR is associated with decreased insulin sensitivity in Chinese women with PCOS

Li Jin*, Xiao-Ming Zhu*, Qiong Luo, Yuli Qian, Fan Jin and He-Feng Huang1

Department of Reproductive Endocrinology, Women’s Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

To whom correspondence should be addressed at: Department of Reproductive Endocrinology, Women’s Hospital, Zhejiang University School of Medicine, 2 Xueshi Road, Hangzhou, Zhejiang 310006, China. E-mail: huanghefg@hotmail.com

To investigate the association of single-nucleotide polymorphisms (SNPs) in exon 17 of the insulin receptor (INSR) gene with insulin resistance and INSR β-subunit expression in polycystic ovary syndrome (PCOS) patients, a case-control study was carried out in an academic endocrinology clinic of China. One hundred and nine Chinese patients with PCOS and 107 healthy Chinese women as control were recruited. Their leukocytes and red blood cells were separated from blood samples, for SNP analysis with single-stranded conformation polymorphism and for the INSR β-subunit expression. A novel T/C SNP at codon Cys1008 (position 3128 of chromosome 19p13.3 in the INSR) was found to be significantly associated with decreased insulin sensitivity index in the women with each of the two mutant genotypes was revealed (CC: 0.335 ± 0.027 versus TT: 0.367 ± 0.029, P < 0.05). No relationship was found between the novel SNP and the INSR β-subunit expression. We concluded that the novel T/C SNP at codon Cys1008 of INSR is associated with decreased insulin sensitivity in Chinese women with PCOS and that the association is not by the change of synthesis or secretion of INSR β-subunit, but most possibly by the effects of this novel SNP on the function of INSR β-subunit.

Key words: insulin receptor/insulin resistance/polycystic ovary syndrome/single-nucleotide polymorphism

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction. Its cardinal features are hyperandrogenism and polycystic ovary morphology (Laven et al., 2002). Approximately 50–70% of all women with PCOS have some degree of insulin resistance and this hormone insensitivity probably contributes to the hyperandrogenism that is responsible for the signs and symptoms of PCOS (Legro et al., 2004). In addition to playing an important role in the pathogenesis of the reproductive abnormalities of PCOS, insulin resistance has major metabolic consequences (Azziz, 2002; Legro et al., 2004; Ehrmann, 2005), as the so called insulin–resistance metabolic syndrome (Legro et al., 2004; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

Postbinding defects in insulin receptor (INSR) signalling is now considered one of the major molecular pathogenesis for insulin resistance in PCOS (Dunaif, 1997; Azziz, 2002) and the mechanism of insulin resistance in at least some women with PCOS appears to be a defect in which autophosphorylation of tyrosine residues on the INSR is decreased (Dunaif, 1997; Azziz, 2002).

Strong evidence also indicates that PCOS is familial (Legro et al., 1998; Azziz and Kashar-Miller, 2000; Ehrmann, 2005). The familial nature of PCOS clearly indicates a significant genetic component, and this genetic component probably comprises multiple gene variants each contributing a small effect (Diao et al., 2004; Roldan et al., 2004; San Millan et al., 2004; Ehrmann, 2005). Previous studies have shown the evidence that a susceptibility gene for PCOS was located on chromosome 19p 13.3 in the INSR region, which suggested that INSR itself might be the susceptibility gene for PCOS (Tucci et al., 2001).

INSR comprises 22 exons spanning 120 kilobases on chromosome 19 (Seino et al., 1990). The region of exons 17–21 encodes the tyrosine kinase domain of the receptor, which is necessary for insulin signal transduction. Mutation in exons 17–21 has been shown to cause severe insulin resistance and hyperinsulinemia (Krook et al., 1994).

Given the wide variability of insulin resistance among patients with PCOS, it is unlikely that a major mutation in INSR would lead to PCOS; as Southern blot analysis experiments have excluded gross gene deletions, insertions, or rearrangements in INSR (Moller et al., 1994; Talbot et al., 1996). Rather, polymorphisms in INSR that induce mild changes in INSR function may contribute to the development of PCOS (Siegel et al., 2002).

Several kinds of polymorphisms have been identified within the coding and noncoding regions of INSR in patients with PCOS (Panz et al., 1996; Talbot et al., 1996; Siegel et al., 2002). Of these polymorphisms, most were silent single-nucleotide polymorphisms (SNPs) (Moller et al., 1994; Talbot et al., 1996) and there was a higher frequency of SNP in exon 17 of INSR (Panz et al., 1996; Talbot et al., 1996).

Among the SNPs in exon 17 of INSR detected to date (Krook et al., 1994; Moller et al., 1994; Panz et al., 1996; Talbot et al., 1996; Siegel et al., 2002), the C/T SNP at His1058 in the tyrosine kinase domain of INSR has been shown to be significantly associated with the development of PCOS most possibly by the resultant effects on

*The authors equally contributed to this work.

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the autophosphorylation of the INSR function in some women with PCOS (Siegel et al., 2002). However, these previous studies had not investigated the relationship between the INSR exon 17 polymorphism and the insulin resistance in the women with PCOS, nor had the relationship between the INSR exon 17 polymorphism and the INSR β-subunit expression in the women with PCOS.

In this study, we analysed the SNPs in exon 17 of INSR with the sensitive single-stranded conformation polymorphism (SSCP) analysis and investigated the association of the exon SNPs with the development of PCOS, especially the association with insulin resistance and the association with the INSR β-subunit expression in Chinese women with PCOS.

Materials and methods

Subjects

One hundred and nine Chinese women with PCOS (age 26.38 ± 4.10 years old and with a BMI of 24.10 ± 4.44 kg/m², mean ± SD) were recruited from the endocrinology clinics of Zhejiang University, Hangzhou, China. A diagnosis of PCOS was according to the ‘Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome’ (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), i.e. any two of the following three criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovary morphology. Patients with any other cause of oligomenorrhea or hyperandrogenism, such as nonclassical 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushing’s syndrome, hypothyroidism, or significant elevations in serum PRL, were excluded. One hundred and seven healthy Chinese women with regular menses and without hyperandrogenemia volunteered as controls. The volunteers’ age (28.36 ± 2.24 years in mean ± SD) was matched with the PCOS patients recruited above; but not their BMI (21.10 ± 1.82 kg/m² in mean ± SD). In addition to meeting these recruiting criteria for this study, no subject had taken steroid preparations (including oral contraceptives) or medications known to alter insulin secretion/action or normal function of the hypothalamic-pituitary-gonadal axis in the last 3 months.

In all subjects, 5 ml heparinized whole-blood samples were obtained for both SNP analysis and INSR β-subunit expression detection. In subjects with PCOS, the fasting blood insulin and fasting blood glucose were measured for insulin sensitivity index (ISI) calculation in QUICKI (quantitative insulin sensitivity check index) (Katz et al., 2000). All blood samples were collected during the first to third day of a spontaneous menstrual cycle, in the morning, after an overnight fast and stored at −20°C for measurements.

The women’s hospital review board of University of Zhejiang approved all studies, and a written informed consent was obtained from each subject.

Assay methods for insulin and glucose

The fasting blood insulin was assayed by an AxSYM insulin assay using commercially available kits (Abbott, Diagnostic Products, Wiesbaden, Germany) with a sensitivity of 1.0 μU/ml. The coefficients of variation of intra-assay and inter-assay for this method were 5.5 and 5.8%, respectively.

The assay method for fasting blood glucose was the glucose oxidase method on a Beckman Glucose Analyzer (Beckman Coulter, Fullerton, CA), with a sensitivity of 0.3 mmol/l. The coefficients of variation of this method both in intra-assay and in inter-assay were less than 2%.

Analysis of the INSR exon 17 SNPs

Genomic DNA was extracted from peripheral blood leukocytes of women with PCOS and the controls by using the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s protocol.

Exon 17 of INSR from each subject was amplified by polymerase chain reaction (PCR) using the same primers and protocol as described by Siegel et al. (2002) with some modifications. Briefly the PCR was conducted in a 50 μl reaction mixture containing 0.5 μg genomic DNA, 0.2 pmol/l primers, 2 mmol/l 1MgCl2, 200 pmol/l each of dNTP, 2.0 IU Taq DNA polymerase. The oligonucleotide of CCAAGGATGCTGTGTTAGATAAG was used as the forward primer and the oligonucleotide of TCAAGGAAAGCCAGCCCATGTC as the reverse primer, which yielded a 317 bp product. The amplification conditions were as follows; 94°C for 10 min, followed by 35 cycles of 1 min at 94°C, 1 min at 61°C, 1 min at 72°C, and ending with a single 10 min extension step at 72°C.

The polymorphism of the PCR product was analysed by the single strand conformation polymorphism (SSCP) analysis. The SSCP protocol of O’Rahilly et al. (1991) was followed with some modifications. Briefly, PCR products were denatured (95°C for 5 min, 95% formamide loading buffer) and immediately cooled on ice before loading (4 μl) on to 9% non-denaturing polyacrylamide gels. The gels were run on ice firstly at 150 V for 4 h and then at 100 V for 2 h in 1 x Tris/borate/EDTA buffer (TBE). After electrophoresis, the gels were stained by 0.5 μg/ml ethidium bromide in 1 x TBE for 20 min and photographed by image system (Kodak EDAS 290, Vilber Lourmat, Marne-la-Vallée, France). During SSCP, DNA fragment samples with mutant and wild-type sequences clarified by sequencing analysis were simultaneously running on each polyacrylamide gel.

For those subjects where SSCP variants were detected, the PCR products were directly sequenced using an ABI PRISM 3100 Genetic Analyzer (ABI, Applied Biosystems, Foster City, CA, USA) and each set of reactions was run with positive and negative controls.

Isolation and presence of the insulin receptor

The RBC membrane fraction was isolated from peripheral blood red cells by centrifugation at 13 362 × g for 20 min. The membrane fraction lysates were obtained using lysis buffer (10 mmol/l HEPES pH 7.9, 10 mmol/l KCl, 1.5 mmol/l MgCl2, 0.1 mmol/l EDTA, 1.0 mmol/l DTT, 100 μg/ml PMSF, 1% NP-40, 200 μM NaVO3, 1 μg/ml Aprotinin) on ice for 30 min followed by centrifuging at 13 362 × g at 4°C for 20 min. The presence of INSR β-subunit (95 kDa) was detected by the western blotting. After treatment with loading buffer and boiling for 5 min, proteins in the membrane fraction lysates were separated by SDS–PAGE on 12% denaturing gels and transferred to nitrocellulose membranes. Then the membrane was blocked with 5% non-fat dry milk (Bio-Rad Laboratories, Hercules, CA, USA) in Tris-buffered saline–Tween 20 (TBS-T) overnight at 4°C and washed three times with TBS-T. The INSR β-subunit protein on the membrane was probed with primary antibody (rabbit polyclonal antibody against human INSR β-subunit diluted 1:100; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) for 3 h at room temperature and then with secondary antibody (goat horseradish peroxidase-conjugated secondary antibody, Santa Cruz Biotechnology) according to the manufacturer’s protocol. The reactions were visualized by enhanced chemiluminescence (ECL) (Amersham Biosciences Europe GmbH, Freiburg, Germany), exposed to Kodak Biomax films and quantified by densitometry and Quantity One software (Bio-Rad Laboratories). The expression level of INSR β-subunit was standardized by simultaneously immunoblotting with a primary antibody against human β-actin (diluted 1:200; Santa Cruz Biotechnology).

Statistical analysis

The data was analysed with the SPSS 11.0 (SPSS, Chicago, IL, USA) package. Two-tailed pooled t tests were used to analyse continuous variables, expressed as mean ± SD. Statistical analyses for genotype frequencies were carried out using χ2 test. A P < 0.05 was considered statistically significant.

Results

The general clinical and biochemical data of women with PCOS are listed in Table I. As shown in Figure 1, a novel T/C SNP at codon Cys1008 (position 3128 in the nucleotide sequence of NM_000208) of INSR was found by using SSCP and clarified with sequencing analysis. There was no any other SNP in the INSR β-subunit protein on the membrane was probed with primary antibody (rabbit polyclonal antibody against human INSR β-subunit diluted 1:100; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) for 3 h at room temperature and then with secondary antibody (goat horseradish peroxidase-conjugated secondary antibody, Santa Cruz Biotechnology) according to the manufacturer’s protocol. The reactions were visualized by enhanced chemiluminescence (ECL) (Amersham Biosciences Europe GmbH, Freiburg, Germany), exposed to Kodak Biomax films and quantified by densitometry and Quantity One software (Bio-Rad Laboratories). The expression level of INSR β-subunit was standardized by simultaneously immunoblotting with a primary antibody against human β-actin (diluted 1:200; Santa Cruz Biotechnology).

The frequency of the novel SNP, as revealed in Table II, was very high both in the patients with PCOS and in the control population (51.4 and 38.3%, respectively). However, the distribution of each mutant genotype of the novel SNP was different between them.
A novel SNP at exon 17 of INSR and PCOS

Among the differences, a significantly higher frequency of the mutant homozygous genotype (CC) was found in the women with PCOS than that in the controls (21.1 versus 5.6%, \( P < 0.01 \)).

The INSR \( \beta \)-subunit expression at the protein level in PCOS subjects was determined by western blot analysis. As shown in Table III and Figure 2, no difference was observed among the different genotypes at codon Cys1008 of INSR.

In the present study, the ISI was shown in QUICKI, which now is considered as a superior way in determining insulin sensitivity (Katz et al., 2000; Legro et al., 2004). The calculation equation is: QUICKI = \( 1/\log(I_0) + \log(G_0) \), where \( I_0 \) is the fasting plasma insulin level (microunits per ml) and \( G_0 \) is the fasting blood glucose level (milligrams per dl). As shown in Table III, the women with each of the two mutant genotypes (CC or TC) at codon Cys1008 of INSR revealed a significantly lower ISI in QUICKI than the women with wild-type genotype (TT) \( (P < 0.05) \).

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**Table I.** The clinical and biochemical data of women with polycystic ovary syndrome (PCOS)

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Subject number (n = 109)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 37 years</td>
<td>3</td>
<td>2.75</td>
</tr>
<tr>
<td>Oligo- or anovulation</td>
<td>109</td>
<td>100.00</td>
</tr>
<tr>
<td>Hyperandrogenemia signs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acne</td>
<td>29</td>
<td>26.60</td>
</tr>
<tr>
<td>Hirsutism*</td>
<td>18</td>
<td>16.51</td>
</tr>
<tr>
<td>TTE &gt; 2.5 mmol/l</td>
<td>35</td>
<td>32.11</td>
</tr>
<tr>
<td>Polycystic ovaries</td>
<td>109</td>
<td>100.00</td>
</tr>
<tr>
<td>BMI ≥ 27 kg/m²</td>
<td>25</td>
<td>22.94</td>
</tr>
</tbody>
</table>

BMI, body mass index; TTE, total testosterone.
*Ferriman–Gallwey score of ≥ 8.

**Table II.** Frequency of three genotypes at codon Cys1008 of INSR between the patients with PCOS and the control population

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotypes [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT*</td>
</tr>
<tr>
<td>PCOS patients (n = 109)</td>
<td>53 (48.6)</td>
</tr>
<tr>
<td>Controls (n = 107)</td>
<td>66 (61.7)</td>
</tr>
</tbody>
</table>
*Wide homozygous genotype.
†Mutant heterozygous genotype.
‡Mutant homozygous genotype.
§Compared with the control, \( \chi^2 \) test, \( \chi^2 = 11.151, P < 0.01 \).

**Table III.** The insulin receptor (INSR) \( \beta \)-subunit expression and ISI of PCOS subjects in different genotypes of Cys1008 of INSR

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Relative OD§ for INSR ( \beta )-subunit</th>
<th>ISI in QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT* (n = 53)</td>
<td>0.320 ± 0.063</td>
<td>0.367 ± 0.029</td>
</tr>
<tr>
<td>CT† (n = 33)</td>
<td>0.325 ± 0.054</td>
<td>0.346 ± 0.027†</td>
</tr>
<tr>
<td>CC‡ (n = 21)</td>
<td>0.322 ± 0.033</td>
<td>0.335 ± 0.026‡</td>
</tr>
</tbody>
</table>

ISI, insulin sensitive index; QUICKI, quantitative insulin sensitivity check index.
All data are expressed in mean ± SD.
*Wide homozygous genotype.
†Mutant heterozygous genotype.
‡Mutant homozygous genotype.
§The scanning densitometry values of INSR \( \beta \)-subunit in western blotting divided by the same value of same origin of \( \beta \)-actin.
¶Compared with the wide homozygous genotype TT, \( t \)-test, \( t > 1.96, P < 0.05 \). QUICKI was calculated from the equation: QUICKI = \( 1/\log(I_0) + \log(G_0) \), where \( I_0 \) is the fasting plasma insulin level (microunits per ml), and \( G_0 \) is the fasting blood glucose level (milligrams per dl).

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**Figure 1.** The three patterns of INSR exon 17 in SSCP. Subjects with the pattern seen in lane TT were wide homozygous. Subjects with the pattern in lane TC were mutant heterozygous. Subjects with the pattern in lane CC were mutant homozygous. Subsequent direct sequencing of amplified DNA revealed that the pattern in lane TT represented the normal sequence (TGC/TGC) at codon Cys1008 of INSR and that in lanes TC and CC represented a missense SNP (TGC/CGC or CGC/CGC) at the same codon from Cys1008 to arginine.

**Figure 2.** The representative insulin receptor (INSR) \( \beta \)-subunit expression of PCOS subjects in western blotting in different genotypes of Cys1008 of INSR. TT: wide homozygous genotype; TC: mutant heterozygous genotype; CC: mutant homozygous genotype. Figure 2 shows the three representative INSR \( \beta \)-subunit expression results in western blotting from three separate subjects with PCOS. Beta-actin was used to standardize the expression level of INSR \( \beta \)-subunit.

Among the differences, a significantly higher frequency of the mutant homozygous genotype (CC) was found in the women with PCOS than that in the controls (21.1 versus 5.6%, \( P < 0.01 \)).

The INSR \( \beta \)-subunit expression at the protein level in PCOS subjects was determined by western blot analysis. As shown in Table III and Figure 2, no difference was observed among the different genotypes at codon Cys1008 of INSR.

In the present study, the ISI was shown in QUICKI, which now is considered as a superior way in determining insulin sensitivity (Katz et al., 2000; Legro et al., 2004). The calculation equation is: QUICKI = \( 1/\log(I_0) + \log(G_0) \), where \( I_0 \) is the fasting plasma insulin level (microunits per ml) and \( G_0 \) is the fasting blood glucose level (mg per dl). As shown in Table III, the women with each of the two mutant genotypes (CC or TC) at codon Cys1008 of INSR revealed a significantly lower ISI in QUICKI than the women with wild-type genotype (TT) \( (P < 0.05) \).
Discussion
This is a first report about a novel T-to-C substitution at codon Cys1008 (position 3128 in the nucleotide sequence of NM_000208) of INSR. The novel SNP is within exon 17 of INSR, exactly at the ATP binding site of the tyrosine kinase domain of INSR β-subunit, resulting in a missense mutation from cysteine to arginine in two allele genotypes, i.e. the homozygous CC and the heterozygous TC.

Previous studies of exon 17 INSR have shown that a C/T SNP at codon His1058 was obviously associated with PCOS (Siegel et al., 2002). There was no report, to our knowledge, on the T/C SNP at codon Cys1008 of INSR presented here, let alone the report on the association of the novel SNP with PCOS. The present study definitely showed the existence of the novel SNP in two mutant allele genotypes. And, furthermore, as shown in the present study, although the frequency of the novel SNP was very high both in the patients with PCOS and in the controls, the distribution of each mutant genotype of the novel SNP was different between them. Compared with the control women, a significantly higher frequency of the mutant homozygous genotype (CC) was found in the women with PCOS (Table II). In contrast with the women with wild type (TT) at codon Cys1008 of INSR, a significantly lower ISI in QUICKI in the women with each of the two mutant genotypes was observed (Table III). No relationship was found in the present study between the INSR β-subunit expression and the T/C SNP at codon Cys1008 of INSR. All of this evidence strongly suggests that the T/C SNP at codon Cys1008 of INSR, like the C/T SNP at codon His1058 (Siegel et al., 2002), is significantly associated with PCOS, especially with a decreased insulin sensitivity in PCOS patients, and that the significant association is not by the change of synthesis or secretion of INSR β-subunit but most possibly by the effects of this novel SNP on the function of INSR β-subunit.

Lots of studies have shown that the insulin resistance in PCOS is secondary to a postbinding defect in INSR signaling (Dunaif, 1997; Aziziz, 2002), which means that the molecular pathogenesis of insulin resistance in PCOS is mainly situated at INSR β-subunits, either in number or in function, not at INSR α-subunits that bind insulin. Evidence for this arose from earlier in vitro and in vivo mutagenesis experiments where one or two critical amino acid residues, such as Lys1018, Lys1030, Tyr1162, Tyr1163, etc., located in the tyrosine kinase domain of INSR β-subunits, were changed to one of several other amino acids. Although insulin binding to these mutant INSRs was unaffected, the tyrosine kinase was completely inactive, along with no response of these kinase-deficient INSRs to insulin (Ellis et al., 1986; Chou et al., 1987; Ebina et al., 1987; McClain et al., 1987; Stumpo and Blackshear, 1991). The T/C SNP at codon Cys1008 of INSR presented here was also located in the tyrosine kinase domain of INSR, exactly at the presumed ATP binding site of the domain (Ebina et al., 1985; Ulrich et al., 1985). The presumed ATP binding site is one of the highly conserved functional sequences in INSR (Ebina et al., 1985; Ulrich et al., 1985; Cheatham and Kahn, 1995). The novel SNP may interfere with the ATP binding of tyrosine kinase and, further, with the autophosphorylation of tyrosine residues in the INSR β-subunits and with the preceding insulin’s pleiotropic actions (Cheatham and Kahn, 1995; Dunaif, 1997). That is the most possible mechanism for the association of the novel SNP with PCOS, especially with lower insulin sensitivity in those PCOS women as shown in this study. It is reasonable to say here that it is the impaired function, not the synthesis or secretion, of INSR β-subunit that the novel SNP at codon Cys1008 of INSR is associated with.

However, it is to our surprise that, in our screening results, there was no other SNP in exon 17 INSR to be screened, including the absence of silent C/T SNP at codon His1058, which has been previously found to have a significant association with PCOS (Siegel et al., 2002). The reasons for this difference in screening results between the present and previous studies may be that the present one may be a relative small sample in the present study, the difference in screening methods, the subject differences in ethnic origins (Williamson et al., 2001; Kaufman et al., 2002; Wijeyaratne et al., 2002), or some others. Thus, it is possible that there exists a T/C SNP at codon Cys1008 of INSR in PCOS but the absence of any other SNPs in exon 17 of INSR in some populations like those in the present study.

To date, lots of SNPs of INSR exon 17 gene have been found (Krook et al., 1994; Moller et al., 1994; Panz et al., 1996; Talbot et al., 1996; Siegel et al., 2002). However, no overt effects of most of these SNPs, especially those synonymous silent SNPs, have been reported. If the alteration in the codon necessitated the use of a different and scarce transfer RNA molecule, especially those missense SNPs at the key region of INSR, like the novel SNP described in this study, it might be difficult to maintain a normal translation and a normal gene function (Kinnaird et al., 1991).

Nonetheless, we should note that PCOS is a heterogeneous disorder, most likely a complex trait with an oligogenic basis. Currently, the polygenic trait of PCOS is considered to result from the interaction of susceptibility and protective genomic variants under the influence of environmental factors (Roldan et al., 2004). From the results of the present study, we can not conclude that the appearance of the novel SNP at codon Cys1008 of INSR only may result in PCOS or insulin resistance in the corresponding women. There are abundant other factors, either genetic or environmental, that could affect the onset or the development of PCOS. We should also note that most of the studies showing a correlation with PCOS mentioned above, including this study, are based on small samples that are often insufficient to adjust for significant confounding factors (Cibula, 2004). Hence, further corroborative studies are required in different populations of various ethnic and/or environmental backgrounds in a larger sample.

In conclusion, a novel T/C SNP at codon Cys1008 (NM_000208) of INSR was found, in which the mutant homozygous genotype CC is significantly associated with PCOS, especially with decreased insulin sensitivity in PCOS patients, but not with the change of INSR β-subunit expression. The significant association is not by change of synthesis or secretion of INSR β-subunit but most possibly by the effects of this novel SNP on the function of INSR β-subunit. Sequencing of this novel SNP may predispose to the development of PCOS, especially those Chinese women with PCOS with decreased insulin sensitivity.

References

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