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GENETICS

# Single nucleotide polymorphisms in the *TGF-β1* gene are associated with polycystic ovary syndrome susceptibility and characteristics: a study in Korean women

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**Abstract**
*Purpose* Although many hypotheses regarding the pathogenesis of polycystic ovary syndrome (PCOS) have been generated, genetic studies have not identified specific genes that play a role in PCOS etiopathogenesis. This study aimed to investigate the relationship between *TGF-β1* gene polymorphism and PCOS in Koreans.
*Method* A total of 51 Korean women with PCOS and 69 healthy women were enrolled. We analyzed 4 single nucleotide polymorphisms (SNPs) of the *TGF-β1* gene (rs11466313, rs1800469, rs2317130, and rs4803457). We also analyzed laboratory measurements, such as free testosterone, glucose, and cholesterol.
*Results* The frequencies of rs1800469*T* allele negativity, rs4803457*T* allele negativity, the rs1800469*CC* genotype, and the rs4803457*CC* genotype showed positive associations with PCOS ($P = 0.003$, $P = 0.027$, $P = 0.009$, and $P = 0.031$, respectively), whereas the haplotypes rs1800469*C*–rs4803457*T* and rs1800469*T*–rs4803457*T* showed negative associations with PCOS. A strong protective effect of the "rs1800469*CT*–rs4803457*TT*" combination (OR = 0.09) and a strong risk effect of "rs1800469*CC*–rs4803457*CC*" (OR = 6.23) for PCOS were observed. The rs1800469*C/T* and rs2317130*C/T* SNPs exhibited associations with several laboratory measurements with various levels of significance.
*Conclusion* The results demonstrated an association of *TGF-β1* gene polymorphisms with the development and/or characteristics of PCOS in the Korean population.

**Keywords** Polycystic ovary syndrome · *TGF-β1* gene polymorphism · Testosterone · Korean

## Introduction

Polycystic ovary syndrome (PCOS) is the leading cause of anovulation and hyperandrogenism in women of reproductive age, with a prevalence of 4–10 % or more according to the several diagnostic criteria. The range of PCOS clinical presentations is wide and generally includes anovulation, cystic ovaries, obesity, hirsutism, and insulin resistance [1, 2]. PCOS is a complex disease originating from the interaction between environmental and genetic factors. Whereas environmental factors related to PCOS have been readily investigated, the individuation of PCOS genetic factors is more difficult. Moreover, inheritance patterns, characterized by complex polygenic traits, further complicate the identification of genetic factors. Genetic susceptibility studies of PCOS have focused on genes related to the metabolism of adipose tissue and steroid hormones and the release and action of gonadotropin and insulin and inflammatory cytokine genes [3–8]. However, it is difficult to establish which genes are involved due to a wide range

*Capsule* This study demonstrates that *TGF-β1* gene SNPs are associated with PCOS development (rs1800469 and rs4803457) and with PCOS characteristics in Korean women (rs1800469 and rs2317130), as assessed by laboratory and clinical measurements.

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of characteristics, including the lack of a male phenotype, absence of an animal model, and variation in the diagnostic criteria.

Cytokines are cell-signaling protein molecules secreted by numerous cells that play a key role in intercellular communication. The imbalance between proinflammatory and anti-inflammatory pathways contributes to the etiology of PCOS. TGF-β is one of the multifunctional cytokine families that function in various physiological and pathological processes in the human body, including wound healing, tissue fibrosis, and embryonic development. TGF-β overexpression in tissue results in overwhelming fibrosis, and TGF-β inhibition extenuates fibrosis via the TGF-β/Smad3 pathway. The level of TGF-β1, a multifunctional cytokine in the human ovary, is increased in the serum and ovaries of PCOS patients compared to normal women [6, 9, 10]. According to recent genetic studies, the TGF-β regulatory pathway appears to play a critical role in the development of PCOS and may be an important therapeutic target for PCOS. PCOS displays all of the hallmarks of increased TGF-β activity, with increased amounts of fibrous tissue and collagen in the ovarian capsule or tunica albuginea and ovarian stroma [11, 12]. The increased TGF-β1 bioavailability due to increased TGF-β1 and decreased levels of its receptor may contribute to the pathogenesis of PCOS and ovarian hyperstimulation. Additionally, variations in fibrillin-3 and the subsequent dysregulation of TGF-β may contribute to the pathogenesis of PCOS. TGF-β pathways operate during ovarian fetal development, and fibrillin-3, which regulates TGF-β activity, is present in the stromal compartments of fetal ovaries [9]. Fibrillin-3 is highly expressed in developing fetal ovaries at a critical early stage when stroma is expanding and follicles are forming. These changes in the fetal ovary could lead to a predisposition to develop PCOS later in life [6]. Fibrillin-3 has a TGF-β binding domain and regulates the expression and activity of TGF-β proteins, and some reports have linked a polymorphism in intron 55 of the fibrillin-3 gene to PCOS [13, 14]. Additionally, TGF-β dysregulation may contribute to cardiovascular and metabolic abnormalities in patients with PCOS because an intact TGF-β-signaling pathway is critical for the normal development and function of multiple organs and tissues [11].

Hyperandrogenism is a diagnostic component of PCOS, and free testosterone is the most sensitive marker for the excessive androgen production with numerous reports demonstrating a positive correlation of the polycystic ovary with serum free testosterone concentrations [1, 15, 16]. Therefore, we have focused on the association of free testosterone levels with *TGF-β1* gene polymorphisms in patients with PCOS. In the Korean population, several studies have been conducted on genetic susceptibility to PCOS, such as those concerning steroidogenic enzyme genes, steroid hormone genes, insulin genes, and fat metabolism genes, with variable results [17–21]. However, the roles of cytokine genes in the pathogenesis and clinical features of PCOS in the Korean population have not been widely investigated.

This study investigates the relationship between *TGF-β1* gene polymorphisms (rs2317130, rs4803457, rs11466313, and rs1800469) and PCOS in Korean women. In this context, we compared the *TGF-β1* genotype distributions of Korean women with PCOS and controls and investigated the association of *TGF-β1* gene polymorphisms with several clinical and laboratory measurements, including free testosterone levels, a biochemical marker of hyperandrogenism.

## Materials and methods

1. Subjects

A total of 51 Korean PCOS patients who had reached menarche were enrolled in this study. Their diagnoses were confirmed at the obstetrics and gynecology clinic of the Seoul National University Boramae Medical Center or the Healthcare System Gangnam Center. The diagnosis of PCOS was made according to the "2003 ESHRE/ASRM guidelines" if more than two of the following criteria were met: (i) oligo-ovulation or anovulation (irregular menstruation or amenorrhea), in which oligomenorrhea was defined as less than eight periods per year or menstrual cycles longer than 35 days and amenorrhea was defined as the absence of menstruation for more than 3 months without pregnancy; (ii) clinical or biochemical evidence of hyperandrogenism; and (iii) polycystic ovaries on ultrasonography (USG): the presence of ≥12 follicles measuring 2–9 mm in diameter or increased ovarian volume (>10 mL) in the follicular phase without follicles >10 mm in diameter. Clinical hyperandrogenism was defined by a modified Ferriman and Gallwey score (mF-G score) of more than 8 [22]. Biochemical hyperandrogenism was defined as an elevation of serum androgen levels beyond the 95 % confidence limits measured in 89 ovulatory, nonhirsute controls in our population who did not show polycystic ovary on ultrasonography (total testosterone >0.68 ng/mL, free testosterone >1.72 pg/mL, free androgen index >5.36) [23]. The control group consisted of 69 healthy Korean women who visited the same clinics for regular or premarriage medical evaluations with a regular menstrual cycle range of 21–35 days, normal ovaries on USG, and no hirsutism. All of the subjects provided informed consent and participated in the study voluntarily. This study, including subject enrollment, was approved by the

Institutional Review Board of Seoul National University Boramae Medical Center (16-2015-144).

2. *TGF-β1* gene analysis

Genomic DNA was prepared from anticoagulated venous blood using the Puregene® DNA purification kit (Gentra Systems, Minneapolis, MN, USA) at the time of donation and was cryopreserved at −80 °C until analysis. Four SNP sites for the *TGF-β* gene, rs11466313 (*−1550AGG/deletion*), rs1800469 (*−509C/T*), rs2317130 (*−1886G/A*), and rs4803457 (*−1571A/G*), were analyzed. Polymerase chain reaction (PCR) amplification was performed with sequence-specific primers (Table 1) and h-Taq (Solgent Co., Ltd., Daejeon, Korea) according to the following protocol: 94 °C for 5 min, 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 50 s for 35 cycles, followed by 72 °C for 10 min. The purified PCR products were Sanger-sequenced using the BigDye terminator v3.1 sequencing kit and a 3730xl automated sequencer (Applied Biosystems, Foster City, CA, USA).

3. Clinical and biochemical measurements

Clinical variables, such as body weight (BW) (kg), height (m), body mass index (BMI, body weight/ height$^2$), and waist circumference (WC), and biochemical variables, such as thyroid-stimulating hormone (TSH), cholesterol, triglyceride (TG), high-density lipoprotein (HDL) cholesterol, free testosterone (FT), and fasting glucose, were assessed in all subjects. Measurements of total testosterone (TT), 17-hydroxyprogesterone (17-OHP), dehydroepiandrosterone (DHEAS), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), prolactin (PRL), insulin (fasting and 2-h), and 2-h postprandial glucose (PP2-GLC) were analyzed only in patients with PCOS.

Biochemical variables were analyzed in the Boramae Medical Center Clinical Laboratory and the reference laboratory (Green Cross Reference Laboratory, Kyunggi-do, Korea). Both laboratories are accredited by the Korean Association of Quality Assurance of Clinical Laboratory (KAQACL). Serum TT, FT, and DHEAS were analyzed using radioimmunoassays. E2, FSH, LH, and PRL were measured using electrochemiluminescence immunoassay. 17-OHP and TSH were analyzed using fluorescence immunoassays and chemiluminescent microparticle immunoassays, respectively. Enzymatic colorimetric assays were used to measure HDL and TG.

4. Statistical analyses

The phenotype frequencies of SNP alleles (frequency of allele-possessing individuals, PFs) and genotype frequencies (GFs) were calculated for the *TGF-β1* gene by direct counting. The $\chi^2$ test or Fisher's exact test was used to compare GF and PF, as appropriate. The odds ratios (ORs) and 95 % confidence intervals (CIs) were used for 2 × 2 comparisons, showing significant *P* values (<0.05). Interval-by-interval correlation coefficients (Pearson's *R*) were used for the linear-by-linear associations. We compared the means of clinical and laboratory measurements in accordance with allele positivity using Student's *t* test. In the comparison of clinical and laboratory measurements in accordance with allele positivity, multiple regression analysis was applied to resolve interference among different SNPs. All analyses were performed using SPSS Statistics version 22.0 (IBM, Armonk, NY, USA).

## Results

1. Demographic characteristics and clinical measurements of PCOS patients and controls

The age of the subjects ranged from 19 to 40 years, and the 51 PCOS patients were significantly younger than the 69 controls (26.5 vs. 30.4 years, $P = 0.00002$). All 120 subjects had reached menarche. Waist circumference, body weight, and body mass index did not differ significantly between the PCOS patients and controls. However, acne was significantly more frequent in patients with PCOS compared to controls (52.0 vs.10.9 %, $P = 0.00003$) (Table 4).

**Table 1** Target gene-specific primer pairs for the *TGF-β1* gene

| SNPs | Description of sequence variants | Primers | Product size (bp) |
|---|---|---|---|
| rs11466313 | *c.–2389_–2388insAGG* | Forward: TGGGCAAAGCTATGGAAGGA<br>Reverse: CCATCATGGGCCTTGTCAG | 201 bp |
| rs1800469 | *c.–1347T>C* | Forward: GGCCCAGTTTCCCTATCTGT<br>Reverse: AGGGTGTCAGTGGGAGGA | 240 bp |
| rs2317130 | *g.41355769C>T* | Forward: TGCTGATCCCTTCTCCTGTG<br>Reverse: CACCCTACCCAGCTCTGTTT | 293 bp |
| rs4803457 | *g.41355454T>C* | Forward: TGGGCAAAGCTATGGAAGGA<br>Reverse: CCATCATGGGCCTTGTCAG | 201 bp |

2. *TGF-β1* gene SNP distribution in accordance with PCOS phenotype

   The *TGF-β1* gene allele and genotype distributions in the PCOS patients and the controls are listed in Table 2. The rs1800469*T* allele and rs4803457*T* allele showed significantly lower frequencies in patients with PCOS compared to the controls (79.6 vs. 97.0 %, $P = 0.003$, OR = 0.12; 68.6 vs. 85.5 %, $P = 0.027$, OR = 0.37). In the genotype comparison, the rs1800469*CC* genotype was present at a higher proportion than the *CT* and *TT* genotypes in PCOS, with a significant linear-by-linear association ($P = 0.009$, $R = -0.2$), and the rs4803457*TT* genotype was observed at a significantly lower proportion than the *CT* and *CC* genotypes ($P = 0.031$, $R = -0.2$).
3. Association of the *TGF-β1* gene haplotype and genotype combination with PCOS

   Because the rs1800469*C/T* and rs4803457*C/T* alleles exhibited an association with PCOS at the allele and/or genotype level, we investigated the frequency of the rs1800469–rs4803457 haplotype in different subjects. In the comparison of haplotypes (whether *cis*- or *trans*-location), the haplotype frequencies of rs1800469*C*–rs4803457*T* and rs1800469*T*–rs4803457*T* were markedly lower in patients with PCOS than in the controls (62.7 vs. 81.2 %, $P = 0.024$; and 64.7 vs. 85.5 %, $P = 0.008$), and the haplotype frequencies of rs1800469*C*–rs4803457*C* were higher in PCOS patients (92.2 vs. 76.8 %, $P = 0.026$, OR = 3.55). The proportion of individuals possessing both rs1800469*CC* and rs4803457*CC* genotypes ("rs1800469*CC* and rs4803457*CC*" combination) was significantly higher in patients with PCOS compared to the controls (15.7 vs. 2.9 %, $P = 0.018$, OR = 6.23), and the proportion of individuals possessing both the rs1800469*CT* and rs4803457*TT* genotypes ("rs1800469*CT* and rs4803457*CC*" combination) was significantly lower in patients with PCOS compared to the controls (2.0 vs. 18.8 %, $P = 0.004$, OR = 0.09) (Table 3).
4. *TGF-β1* gene SNP distribution in accordance with clinical and laboratory measurements

   The serum free testosterone concentration was significantly higher in patients with PCOS than in the controls (1.25 vs. 0.77 pg/mL, $P = 0.0001$). The mean value of the free testosterone concentration did not differ significantly between individuals possessing each allele and individuals without the allele. Individuals without the rs1800469*T* allele showed a higher free testosterone level than individuals with the rs1800469*T* allele, although this difference did not reach significance (1.25 vs. 0.93 pg/mL, $0.05 \le P < 0.1$). We compared the clinical and laboratory measurements between the PCOS patients and controls and compared them in accordance with allele positivity. TSH, cholesterol, and fasting glucose were significantly higher in patients with PCOS compared to the controls (2.38 vs. 1.79 μIU/mL, $P = 0.048$; 192.9 vs. 167.6 mg/dL, $P = 0.0004$ and 89.3 vs. 84.8 g/dL, $P = 0.002$, respectively), and acne was observed with significantly higher frequency in patients with PCOS compared to the controls (52.0 vs. 10.9 %, $P = 0.00003$). An analysis of subjects positive for different alleles revealed that individuals possessing the rs11466313*del* allele, rs1800469*C* allele, or rs2317130*C* allele showed higher levels of cholesterol (175.3 vs. 158.6, $P = 0.028$; 173.4 vs. 147.3 mg/dL, $P = 0.0002$; 175.0 vs. 159.2, $P = 0.043$, respectively); subjects with the rs1800469*T* allele or without the rs2317130*T* allele had markedly decreased fasting

**Table 2** Comparison of the *TGF-β*1 allele phenotype (allele-possessing individuals) and genotype frequencies between the PCOS patients and controls

| SNP | Association of allele PF | | | | | Linear-by-linear association of GF | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Phenotype | PCOS ($n = 51$) | Control ($n = 69$) | *P* value | OR [95 % CI] | Genotype | PCOS ($n = 51$) | Control ($n = 69$) | *P* value (Pearson's *R*) |
| rs11466313 | *deletion* | 76.5 % | 81.2 % | NS | | *deldel* | 35.7 % | 64.3 % | NS |
| | *AGG(+)* | 90.2 % | 87.0 % | NS | | *AGGdel* | 42.0 % | 58.0 % | |
| | | | | | | *AGGAGG* | 48.0 % | 52.0 % | |
| rs1800469 | *C(+)* | 94.1 % | 95.7 % | NS | | *CC* | **83.3 %** | **16.7 %** | **0.009** |
| | *T(+)* | **80.4 %** | **97.1 %** | **0.003** | **0.12 [0.03–0.59]** | *CT* | **37.3 %** | **62.7 %** | **(−0.2)** |
| | | | | | | *TT* | **50.0 %** | **50.0 %** | |
| rs2317130 | *C(+)* | 74.5 % | 78.3 % | NS | | *CC* | 9.8 % | 23.2 % | NS |
| | *T(+)* | 86.3 % | 78.3 % | NS | | *CT* | 66.7 % | 59.4 % | |
| | | | | | | *TT* | 23.5 % | 17.4 % | |
| rs4803457 | *C(+)* | 76.5 % | 82.6 % | NS | | *CC* | **61.5 %** | **38.5 %** | **0.031** |
| | *T(+)* | 68.6 % | 85.5 % | **0.027** | **0.37 [0.15–0.91]** | *CT* | **40.8 %** | **59.2 %** | **(–0.2)** |
| | | | | | | *TT* | **22.2 %** | **77.8 %** | |

Significant results ($P < 0.05$) are listed with *P* value and odds ratio (95 % confidence interval) for 2 × 2 comparisons and with *P* value and interval-by-interval correlation coefficient (Pearson's *R*) in parentheses for the linear-by-linear associations. Bold type: significant results with $P < 0.05$

*PF* phenotype frequency, *GF* genotype frequency, *OR* odds ratio, *CI* confidence intervals, *NS* not significant

**Table 3** Association of rs1800469–rs4803457 haplotype and rs1800469–rs4803457 genotype combinations with the PCOS phenotype

| Haplotypic association with PCOS | | | | | Additive association of genotype combinations with PCOS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| rs1800469–rs4803457[a] | PCOS ($n = 51$) | Control ($n = 69$) | *P* value | OR [95 % CI] | rs1800469 genotype | rs4803457 genotype | PCOS ($n = 51$) | Control ($n = 69$) | *P* value | OR [95 % CI] |
| ***C–C*** | **92.2 %** | **76.8 %** | **0.026** | **3.55 [1.11–11.36]** | ***CC*** | ***CC*** | **15.7 %** | **2.9 %** | **0.018[b]** | **6.23 [1.26-30.75]** |
| | | | | | *CC* | *CT* | 5.9 % | 18.8 % | BS | |
| ***C–T*** | **62.7 %** | **81.2 %** | **0.024** | **0.39 [0.17–0.90]** | *CC* | *TT* | 0.0 % | 0.0 % | NS | |
| | | | | | *CT* | *CC* | 15.7 % | 14.5 % | NS | |
| *T–C* | 72.5 % | 76.8 % | NS | | ***CT*** | ***TT*** | **2.0 %** | **18.8 %** | **0.004** | **0.09 [0.01-0.68]** |
| | | | | | *TT* | *CC* | 0.0 % | 0.0 % | NS | |
| ***T–T*** | **64.7 %** | **85.5 %** | **0.008** | **0.31 [0.13–0.75]** | *TT* | *CT* | 0.0 % | 2.9 % | NS | |
| | | | | | *TT* | *TT* | 5.9 % | 1.4 % | NS | |
| | | | | | *CT* | *CT* | 56.9 % | 62.3 % | NS | |

*NS* not significant, *OR* odds ratio, *CI* confidence intervals. Bold type: significant results with $P < 0.05$

[a] No information about *cis*–or *trans*- location of each allele

[b] Fisher's exact test

glucose levels (86.5 vs. 92.6 g/dL, $P = 0.014$; 82.9 vs. 87.7, $P = 0.01$); and subjects with the rs2317130*C* allele exhibited a larger WC (76.6 vs. 70.9 cm, $P = 0.020$). TSH was lower among rs1800469*T* allele-positive subjects (1.93 vs. 2.98, $P = 0.034$). TG, BW, BMI, and HDL cholesterol were not significantly different between different SNPs in all subjects (not listed in Table 4). In the PCOS group, subjects with the rs1800469*T* allele had markedly decreased 2-h postprandial glucose (95.4 vs. 122.3 g/dL, $P = 0.034$), and the remaining laboratory measurements (FT, TT, 17-OHP, DHEAS, LH, FSH, E2, fasting glucose, and fasting insulin) did not differ significantly between the PCOS subjects with different SNPs (not listed in Table 4). No differences were observed in FT level and other laboratory measurements in subjects with different haplotype and genotype combinations (Table 4). Variables with significance ($P < 0.05$) in univariate analysis of laboratory measurements (rs11466313*del*, rs1800469*C*, rs2317130*C*, rs1800469*T*, rs2317130*T*) were used for the multivariate analysis. rs1800469*C* and rs2317130*T* showed significant associations with the cholesterol level and fasting glucose level, respectively ($P = 0.044$ and $P = 0.009$, respectively) (Table 5).

## Discussion

As the most frequent cause of female infertility, PCOS is clinically important because it is associated with metabolic defects that increase the risk for cardiovascular disease. Although many hypotheses concerning the pathogenesis of PCOS have been proposed, none fully explain the heterogeneous characteristics of the syndrome [24–26].

In this study, we examined the *TGF-β1* gene polymorphism in women with PCOS and compared the rate of allele frequencies with those of healthy women without PCOS. The PCOS patients were younger than the controls, likely because such patients visit clinics in young adulthood for irregular menstrual cycles, hirsutism, and acne, whereas the control subjects tended to visit clinics for regular physicals or pre-marriage medical evaluations. However, we excluded minor (<18 years of age) patients in this study because some "PCOS-like" symptoms observed in adolescents may be normal physiological phenomena. The rs1800469*T* phenotype (individuals possessing the *T* allele) showed a significant association with PCOS and a borderline association with free testosterone level; however, it was not associated with free testosterone concentration in patients with PCOS. rs1800469*C/T* allele positivity showed an association with other laboratory measurements, suggesting that the *TGF-β1* gene polymorphism (rs1800469*C/T*) is associated with the development of PCOS and/or metabolic disorders. Regarding haplotype, the rs1800469*C*–rs4803457*T* and rs1800469*T*–rs4803457*T* haplotypes displayed a negative association with PCOS development, resulting in a strong protective effect of the rs1800469*CT* and rs4803457*TT* combination on PCOS (OR approximately 0.1). Additionally, the rs1800469*CC* and rs4803457*CC* combination was a strong risk factor for PCOS with a high OR. We predicted that the alleles associated with susceptibility to PCOS would also be associated with the free testosterone level. As expected, the rs1800469*T* allele that showed a significant negative association with PCOS also showed a borderline negative association with free testosterone level. Moreover, this phenotype was negatively associated with fasting glucose, TSH, and PP2-GLC with statistical

**Table 4** Comparison of clinical and laboratory measurements according to the *TGF-β1* genotypes and PCOS phenotype

| Phenotype | Total subjects | | | | | | PCOS | |
|---|---|---|---|---|---|---|---|---|
| | FT (pg/mL) | TSH (μIU/mL) | CHOL (mg/dL) | FBS (mg/dL) | WC (cm) | Acne | PP2-GLC (mg/dL) | Insulin (PP2) (μU/mL) |
| PCOS | **1.25 ± 0.85** | **2.38 ± 1.61** | **192.9 ± 23.6** | **89.3 ± 8.1** | 77.9 ± 8.1 | **52.0 %** | | |
| Controls | **0.77 ± 0.44** | **1.79 ± 1.13** | **167.6 ± 24.1** | **84.8 ± 6.9** | 75.4 ± 6.6 | **10.9 %** | | |
| | ***P* = 0.001** | ***P* = 0.048** | ***P* = 0.0004** | ***P* = 0.002** | NS | ***P* = 0.00003** | | |
| rs11466313*del*(+) | 0.97 ± 0.63 | 1.93 ± 1.37 | **175.3 ± 25.5** | 87.0 ± 7.4 | 76.3 ± 6.9 | 21.6 % | 97.2 ± 25.9 | 34.1 ± 25.9 |
| rs11466313*del*(−) | 0.98 ± 0.90 | 2.37 ± 1.25 | **158.6 ± 24.1** | 85.7 ± 8.9 | 73.1 ± 6.9 | 26.7 % | 113.1 ± 40.8 | 73.3 ± 50.3 |
| | NS | NS | ***P* = 0.028** | NS | NS | NS | NS | NS |
| rs11466313*AGG*(+) | 0.97 ± 0.72 | 2.04 ± 1.35 | 171.7 ± 27.2 | 87.1 ± 8.0 | 75.8 ± 6.9 | 19.5 % | 102.8 ± 60.1 | 42.8 ± 33.9 |
| rs11466313*AGG*(−) | 1.00 ± 0.45 | 1.86 ± 1.40 | 175.9 ± 17.3 | 84.1 ± 5.3 | 75.9 ± 7.0 | 41.7 % | 73.5 ± 19.1 | 16.6 ± 1.0 |
| | NS | NS | NS | NS | NS | NS | NS | NS |
| rs1800469*C*(+) | 0.97 ± 0.69 | 2.05 ± 1.36 | **173.4 ± 25.9** | 86.7 ± 7.8 | 75.9 ± 7.0 | 23.3 % | 105.5 ± 30.4 | 42.8 ± 33.9 |
| rs1800469*C*(−) | 1.09 ± 0.65 | 1.23 ± 0.87 | **147.3 ± 4.9** | 87.5 ± 6.1 | 74.0 ± 7.7 | 0.0 % | 76.3 ± 14.4 | 16.6 ± 1.0 |
| | NS | NS | ***P* = 0.0002** | NS | NS | NS | NS | NS |
| rs1800469*T*(+) | 0.93 ± 0.67 | **1.93 ± 1.31** | 171.5 ± 26.0 | **86.5 ± 7.7** | 75.8 ± 7.0 | 21.4 % | **95.4 ± 24.3** | 34.1 ± 25.9 |
| rs1800469*T*(−) | 1.25 ± 0.78 | **2.98 ± 1.50** | 188.8 ± 20.4 | **92.6 ± 6.6** | 77.6 ± 5.5 | 40.0 % | **122.3 ± 41.8** | 73.3 ± 50.3 |
| | BS | ***P* = 0.034** | NS | ***P* = 0.014** | NS | NS | ***P* = 0.034** | NS |
| rs2317130*C*(+) | 0.95 ± 0.63 | 1.91 ± 1.31 | **175.0 ± 25.9** | 86.9 ± 7.7 | **76.6 ± 6.8** | 20.0 % | 97.2 ± 25.9 | 34.1 ± 25.9 |
| rs2317130*C*(−) | 1.04 ± 0.89 | 2.50 ± 1.45 | **159.2 ± 22.0** | 86.3 ± 7.9 | **70.9 ± 5.5** | 35.7 % | 113.1 ± 40.8 | 73.3 ± 50.3 |
| | NS | BS | ***P* = 0.043** | NS | ***P* = 0.02** | NS | NS | NS |
| rs2317130*T*(+) | 0.99 ± 0.71 | 2.06 ± 1.36 | 172.1 ± 26.9 | **87.7 ± 7.7** | 76.3 ± 7.1 | 22.5 % | 102.8 ± 30.1 | 42.8 ± 33.9 |
| rs2317130*T*(−) | 0.91 ± 0.58 | 1.82 ± 1.31 | 173.3 ± 23.0 | **82.9 ± 6.7** | 74.6 ± 6.3 | 22.2 % | 73.6 ± 19.1 | 16.6 ± 1.0 |
| | NS | NS | NS | ***P* = 0.01** | NS | NS | NS | NS |
| rs4803457*C*(+) | 0.98 ± 0.72 | 2.08 ± 1.36 | 172.1 ± 27.0 | 87.1 ± 7.9 | 76.2 ± 6.9 | 20.0 % | 103.5 ± 30.4 | 42.8 ± 33.9 |
| rs4803457*C*(−) | 0.92 ± 0.43 | 1.68 ± 1.29 | 173.6 ± 20.8 | 84.9 ± 6.7 | 74.3 ± 6.8 | 35.7 % | 76.3 ± 14.4 | 16.6 ± 1.0 |
| | NS | NS | NS | NS | NS | NS | NS | NS |
| rs4803457*T*(+) | 0.95 ± 0.61 | 1.91 ± 1.36 | 173.2 ± 26.2 | 87.2 ± 7.5 | 75.9 ± 7.0 | 23.3 % | 97.2 ± 25.9 | 34.1 ± 25.9 |
| rs4803457*T*(−) | 1.06 ± 0.91 | 2.43 ± 1.25 | 169.0 ± 25.1 | 85.3 ± 8.5 | 75.5 ± 6.5 | 18.8 % | 113.1 ± 40.8 | 73.3 ± 50.3 |
| | NS | NS | NS | NS | NS | NS | NS | NS |

Mean ± SD and *P* values are listed. Bold type: significant results with $P < 0.05$

*FT* free testosterone, *TSH* thyroid-stimulating hormone, *CHOL* cholesterol, *TG* triglyceride, *FBS* fasting blood sugar, *WC* waist circumference, *BW* body weight, *PP2-GLC* 2-h postprandial glucose, *Insulin (PP2)* 2-h postprandial insulin, *NS* not significant, *BS* borderline significance with $0.05 \leq P < 0.1$

significance. This series of findings suggests that the rs1800469*T* allele is associated with the development and clinical presentation of PCOS. By contrast, the rs4803457*T* allele showed a significant association with PCOS but was not associated with the clinical presentation of PCOS. Among the analyzed SNPs, rs1800469*C* and rs2317130*T* showed significant associations with laboratory measurements, including in the multiple regressions. Those various associations may be

**Table 5** Multiple regression analysis of *TGF-β* 1 genotypes in the comparison of clinical and laboratory measurements

| Dependent variable | Independent variable | B | Beta | *t* value | *P* value | VIF |
|---|---|---|---|---|---|---|
| Cholesterol | rs11466313*del* | 12.589 | 0.187 | 1.380 | 0.172 | 1.562 |
| | **rs1800469*C*** | **29.952** | **0.222** | **2.044** | **0.044** | **1.010** |
| | rs2317130*C* | 9.415 | 0.136 | 1.003 | 0.319 | 1.561 |
| FBS | rs1800469*T* | −4.270 | −0.165 | −1.804 | 0.074 | 1.000 |
| | **rs2317130*T*** | **4.787** | **0.243** | **2.653** | **0.009** | **1.000** |

Variables with significance ($P < 0.05$) in the *t* test are shown. Bold type: significant results with $P < 0.05$

*FBS* fasting blood sugar, *VIF* variance inflation factor

due to the small number of subjects in our study and to the heterogeneous phenotypes of PCOS, whereby milder types without hyperandrogenism are more prevalent in Koreans than in other ethnic populations [27]. Many different primary disturbances could result in this pathological outcome. For example, weight gain might promote the PCOS phenotype in a susceptible population [28]. Furthermore, the susceptible cytokine gene may exist within each subgroup of PCOS; however, we could not divide the patients into different subgroups due to the limited number of patients.

TGF-β1 is a multifunctional cytokine that plays an important role in human ovary function by facilitating the differentiation of granulosa cells, stimulating progesterone production and maintaining the corpus luteum, and inducing follicular atresia [12]. PCOS patients exhibit higher ovarian levels of TGF-β1 than normal women do [6, 9, 10]. Therefore, normal levels of TGF-β1 are necessary to maintain ovarian function, and abnormal levels or dysregulation of TGF-β1 may cause pathological disorders. Although all these data demonstrate that TGF-β1 participates in PCOS development, the precise mechanisms underlying the association of PCOS with the *TGF-β1* gene are uncertain, and several hypotheses have been generated. One hypothesis involves a direct relationship of a certain *TGF-β1* gene allele in the pathogenesis of the disease, and another involves linkage disequilibrium between the responsible gene and the susceptible allele. In support of the direct relationship hypothesis, a potential role of T lymphocytes in the local pathological mechanisms of PCOS has been reported [29], and the *TGF-β1* gene is thought to play a role in this process and to modulate the immune response. Although recent genetic studies have demonstrated that the *TGF-β1* gene is associated with the development and clinical features of PCOS and that dysregulation in TGF-β signaling may contribute to the pathogenesis of PCOS [5–7, 11], no compelling evidence exists for actual changes in TGF-β in the ovaries of patients with PCOS.

This study has several limitations. Most importantly, the small sample size decreased the statistical power of the study, and we could not stratify subjects into subgroups according to PCOS phenotype, such as disease severity or the presence of a metabolic disorder. Due to the lack of functional studies, we could not conclusively demonstrate the role of *TGF-β1* genes in the pathogenesis of PCOS. Second, an evaluation of potential interactions, such as gene–environment and gene–gene interactions, was not performed. Although we investigated the independent and additive association of each *TGF-β1* SNP and *TGF-β1* gene and HLA genes with PCOS, we cannot exclude the possibility that other genes located close to the candidate genes also play a role. Although the exact functions and effects of *TGF-β1* genetic polymorphisms on the development or clinical features of PCOS in different populations are not yet clear, we predicted an association with PCOS based on the known functions of *TGF-β1* and several reports of *TGF-β1* association with other immune responses. Several studies have reported that the SNPs in the functional regions of the *TGF-β1* gene, such as −509C/T, +869T/C, positions −800, codon 25, and codon 263, may affect TGF-β1 expression and production by influencing the transcriptional activity of TGF-β1, steady-state concentrations of *TGF-β1* mRNA in peripheral blood mononuclear cells, and serum TGF-β1 levels [30–33]. A previous study that analyzed the *TGF-β1* gene SNP at codon 10 (rs1800470) showed no association with PCOS in Korean women [34]. However, this is the first report of the four SNP sites of the *TGF-β1* gene (rs11466313, rs1800469, rs2317130, and rs4803457) in Korean women with PCOS, and it is partially consistent with the results of a study on Chinese women [5]. The benefits of identifying genes that mediate susceptibility to PCOS range from an increased understanding of the nature of the disease to clinical applications, as well as the reduction of long-term complications and overall morbidity. Although the association between *TGF-β1* genes with PCOS and/or free testosterone level was not strong in this study, these results provide immunogenetic data on the development and clinical features of PCOS in Korean women.

In conclusion, rs1800469*T* and rs1800469*TT* have strong protective effects on PCOS development and characteristics, such as metabolic disorders. rs4803457*T* and rs4803457*TT* have a protective effect on PCOS development but not on the clinical features of the disease. Haplotypes rs1800469*C*–rs4803457 and rs1800469*T*–rs4803457*T* have a negative association with PCOS development, resulting in a strong protective effect of the rs1800469*CT* and rs4803457*TT* combination on PCOS. Without any protective genotype, the rs1800469*CC* and rs4803457*CC* combination is a strong risk factor for the development of PCOS. Among the SNPs showing associations with clinical features, rs1800469*T/T* and rs2317130*C/T* were associated with clinical features even in multiple regression analysis. Our work demonstrates that the rs1800469*C/T* and rs4803457*C/T* SNPs are associated with PCOS risk. Because rs1800469*C/T* and rs4803457*C/T* are located in the promoter region and coding regions of the *TGF-β1* gene, these polymorphisms may also influence serum TGF-β1 levels and lead to PCOS. Further investigation in a larger number of subjects with classification according to the metabolic abnormality (severity and characteristics) will be required to clarify their consistent and/or variable association in patients with PCOS.

**Compliance with ethical standards** This study complied with ethical standards. Study design, including subject enrollment and informed consent, were approved by the Institutional Review Board of Seoul National University Boramae Medical Center (16-2015-144).

**Conflict of interest** The authors declare that they have no conflict of interest.

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