Hypomethylation of the LH/Choriogonadotropin Receptor Promoter Region Is a Potential Mechanism Underlying Susceptibility to Polycystic Ovary Syndrome

Peng Wang, Han Zhao, Tao Li, Wei Zhang, Keliang Wu, Mei Li, Yuehong Bian, Hongbin Liu, Yunna Ning, Guangyu Li, and Zi-Jiang Chen

Center for Reproductive Medicine (P.W., H.Z., K.W., M.L., Y.B., H.L., Y.N., G.L., Z.-J.C.), Department of Gynecology (T.L.), and Department of Orthopedics (W.Z.), Provincial Hospital Affiliated to Shandong University, Jinan 250100, China; National Research Center for Assisted Reproductive Technology and Reproductive Genetics (P.W., H.Z., K.W., M.L., Y.B., H.L., Y.N., G.L., Z.-J.C.), Jinan 250021, China; The Key Laboratory for Reproductive Endocrinology of Ministry of Education (P.W., H.Z., K.W., M.L., Y.B., H.L., Y.N., G.L., Z.-J.C.), Jinan 250021, China; Shandong Provincial Key Laboratory of Reproductive Medicine (P.W., H.Z., K.W., M.L., Y.B., H.L., Y.N., G.L., Z.-J.C.), Jinan, China; and Department of Obstetrics and Gynecology (Z.-J.C.), Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200001, China

Our previous genome-wide association study identified LH/choriogonadotropin receptor (LHCR) as a susceptibility gene for polycystic ovary syndrome (PCOS). The objective of this study was to determine whether the genetic or epigenetic components associated with LHCR participate in the pathogenesis of PCOS. The exons and flanking regions of LHCR were sequenced from 192 women with PCOS, and no novel somatic mutations were identified. In addition, the methylation statuses of 6 cytosine-phosphate-guanine (CpG) sites in the promoter region of LHCR were measured by pyrosequencing using peripheral blood cells from 85 women with PCOS and 88 control women. We identified 2 hypomethylated sites, CpG

Bisulfite sequencing then was performed to replicate these findings and detect additional CpG sites in the promoter. CpG +17 was significantly hypomethylated in women with PCOS (corrected $P = .02$). Methylation statuses were further evaluated using granulosa cells (GCs), and the region described was hypomethylated as a whole ($P = .004$) with 8 significantly hypomethylated sites (CpG

Transcription of LHCR was elevated in women with PCOS compared with that in control women ($P = .01$). These findings were consistent with the decreased LHCR methylation status associated with PCOS. The tendency of LHCR to be hypomethylated across different tissues and its corresponding expression level suggest that hypomethylation of LHCR is a potential mechanism underlying susceptibility to PCOS. Further studies are needed to evaluate whether a causal relationship exists between LHCR methylation status and PCOS. (Endocrinology 155: 1445–1452, 2014)

Polycystic ovary syndrome (PCOS) has a prevalence of 6% to 8% among women of reproductive age and is regarded as the most common endocrinopathy in this age group (1, 2). Features of PCOS include hyperandrogenemia, oligoovulation, and polycystic ovarian morphology; these commonly lead to anovulatory infertility and a series of metabolic complications, including metabolic syndrome, type 2 diabetes, and cardiovascular disease (3, 4). PCOS jeopardizes a woman’s health throughout her entire lifespan, but the etiology and pathogenesis of this syndrome remain unclear. With the emergence of genome-wide association studies (GWASs), remarkable progress...
has been achieved in genetic studies of PCOS. The first PCOS GWAS identified LH/choriogonadotropin receptor (LHCGR) (locus 2p16.3) as a susceptibility gene for PCOS among the Han Chinese (5). The interaction of LHCGR and its ligand, LH, plays a fundamental role in the folliculogenesis of mammals. Therefore, LHCGR has long been considered a plausible PCOS candidate gene. The significance of LHCGR has been supported further by a second PCOS GWAS and by other replication studies in the Caucasian population (6, 7).

In addition to positive genome-wide associations between LHCGR and PCOS, sporadic reports have indicated that activating mutations in affected women elicit hyperandrogenism, which is a key feature of PCOS (8). To ascertain all genetic variations and identify candidate causal variants, we sequenced the untranslated regions (UTRs), promoter regions, exons, and exon-intron boundaries of LHCGR in a cohort of Chinese women with PCOS.

Previous research also has reported that the transcription of LHCGR is modulated by the methylation status of its promoter cytosine-phosphate-guanine (CpG) sites (9). LHCGR is demethylated in the ovarian tissue of dehydroepiandrosterone-induced PCOS mouse models (10). Deviations in the methylation status may contribute to PCOS pathogenesis in humans. We evaluated the association between PCOS and the methylation level of LHCGR using human peripheral blood cells (PBCs) and granulosa cells (GCs).

Materials and Methods

Subjects

All of the participants in our study were recruited from the Reproductive Medical Center of Shandong Provincial Hospital, which is affiliated with Shandong University. Anthropometric variables, such as age, height, body weight, and menstrual cycle, and select endocrine and biochemical parameters were recorded. All women in the PCOS group were selected in strict accordance with the Revised 2003 Consensus on Diagnostic Criteria, which requires the presence of at least 2 of the following criteria for a PCOS diagnosis: oligo-ovulation and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovarian morphology (11). Diagnoses of PCOS were made after exclusion of other etiologies for hyperandrogenemia and ovulatory dysfunction (e.g., congenital adrenal hyperplasia, 21-hydroxylase deficiency, androgen-secreting tumors, Cushing syndrome, thyroid disease, and hyperprolactinemia). All subjects in the control group had regular menstrual cycles (26–35 days) and normal ovarian morphology. Total testosterone and modified Ferriman-Gallwey scores were evaluated to exclude hyperandrogenism. Peripheral blood samples were collected from all subjects during days 2 to 4 of spontaneous cycles or after cessation of bleeding after a 12-hour overnight fast. LH and testosterone were tested by chemiluminescence immunization (Beckman Access Health Company). Genomic DNA was obtained from the peripheral blood (QIAamp DNA Blood Mini Kit, QIAGEN). Ethical approval was obtained by the Institutional Review Board of Reproductive Medicine of Shandong University, and informed consent was obtained from all the participants involved.

Sequencing of LHCGR

Exons and their flanking regions of LHCGR were sequenced in a cohort of 192 subjects with PCOS to discover genetic causal variants. Twelve pairs of specific primers for UTR, promoter regions, exons, and exon-intron boundaries of LHCGR were designed from a human sequence (accession no. NM_000233.3). All the LHCGR primers are available in Supplemental Table 1 published on The Endocrine Society’s Journals Online web site at http://end.endojournals.org. The PCR products were then loaded on an ABI3730XL capillary sequencer (Applied Biosystems) for automatic sequencing.

Bisulfite pyrosequencing to measure methylation level

The methylation level of LHCGR initially was detected by pyrosequencing from a larger cohort (85 patients with patients vs 88 control subjects) using DNA extracted from PBCs. Figure 1 indicates the 6 target CpG sites in the PCR products obtained in the pyrosequencing assay with respect to their translation initiation sites (TISs). Sequencing primers were designed in the reverse direction of the 5'-biotin-labeled PCR primer using PyroMark Assay Design software v2.0 (QIAGEN). Primer sequences are available upon request. One microliter of bisulfite-modified DNA (Zymo Research) was amplified in a 25-μL reaction using a PyroMark PCR kit (QIAGEN) and pyrosequencing primers (0.2 mM) were annealed to the purified single-stranded PCR products. Then, 20 μL of PCR product was used for sequencing on a PyroMark Q96 ID pyrosequencing instrument (QIAGEN). Pyrograms were visualized and evaluated for sequence quality, and percent methylation at individual CpG sites was determined using PyroMark ID software (QIAGEN).

Further replication and expanded measure of methylation level by bisulfite sequencing PCR (BSP)

Because of the limitations associated with pyrosequencing, only 6 CpG sites could be detected in the promoter region of LHCGR.

Figure 1. Promoter of the human LHCGR gene. This schematic diagram displays the promoter of the human LHCGR gene with location of CpG sites (black box), Sp I (oval), and the TIS (the arrow labeled with ATG). The dotted lines represent amplification products of pyrosequencing and BSP, respectively.
Therefore, we also performed BSP to detect 16 CpG sites near the TIS (from CpG – 174 to CpG + 20), including the original 6 CpG sites detected by pyrosequencing. The methylation statuses of the LHCGR promoter CpG sites were measured from the PBCs of 30 patients with PCOS and 30 control subjects. Because methylation displays a tissue-specific pattern, the methylation level of LHCGR also was measured from GCs, which play a pivotal role in folliculogenesis and steroidogenesis and in which LHCGR is maximally expressed during the entire follicular phase in humans (12). GCs were collected from 12 patients with PCOS and 12 control subjects undergoing their first in vitro fertilization/intracytoplasmic sperm injection cycles at our in vitro fertilization center. At 36 hours after the human chorionic gonadotropin trigger, oocytes were retrieved via transvaginal ultrasound–guided aspiration. All of the cumulus-corona oocyte complexes were harvested by an embryologist, and then the follicular fluid that was not heavily blood-stained was pooled from 1 patient. The GC isolation protocol was modified slightly from the method introduced by Matsubara et al (13). In brief, after centrifugation (250 × g, 10 minutes), hyaluronidase (SAGE In Vitro Fertilization) was added to the pellet. After incubation (5% CO2 and air at 37°C for 30 minutes), cell masses were pipetted and then underlayered with Ficoll-Paque (Pharmacia Biotech) and were centrifuged (450 × g, 20 minutes) for cell separation. Specifically, erythrocytes settled to the bottom. The ring-like layer containing GCs was collected, washed with PBS, and stored at −80°C. The LHCR methylation levels then were measured by BSP using genomic DNA from both PBCs and GCs.

Bisulfito conversion (500 ng of DNA) was performed using an EZ DNA Methylation-Gold Kit (Zymo Research), according to the manufacturer’s protocol. Primers specific to the converted DNA were designed using the online program MethPrimer (http://www.urogene.org/methprimer/) (14), as follows: sense, GAGGTTATGGGGTATTTGAT; and antisense, CAACAACTTCAACAACTACAAC. PCR amplification was performed using 2.5 μL of bisulfite-converted DNA in a 25-μL reaction with TaKaRa Taq Hot Start (TaKaRa). The touchdown PCR conditions were as follows: 1 initial denaturation cycle of 95°C for 5 minutes; 10 cycles of 95°C for 25 seconds and 63°C for 1 minute, decreasing by 0.5°C every cycle, and 72°C for 30 seconds; 35 cycles of 95°C for 25 seconds, 60°C for 25 seconds, and 72°C for 30 seconds; and final extension at 72°C for 5 minutes. Two parallel reactions were performed simultaneously, and the products were pooled to avoid bias. PCR products were gel-purified (Promega). Purified PCR products then were ligated into PMD18-T vectors (TaKaRa). Approximately 7 to 9 clones per PBC sample and 20 clones per GC sample were inoculated and grown overnight. Plasmid DNA was extracted and sequenced using a 3730 × 1 DNA analyzer (Applied Biosystems).

### Quantitative real-time PCR

LHCGR mRNA expression in PBCs and GCs was quantified by real-time RT-PCR using actin as housekeeper gene. Primers were designed to target all known transcripts (sense, GGTCCACTCGACTATCCTGC; and antisense, CTCCGGGCTCAATGTATCTCA). Total RNA was extracted from using the TRIzol reagent (Invitrogen). RT was performed using Moloney murine leukemia virus Moloney murine leukemia virus reverse transcriptase (Promega). PCR products were also loaded on 1% agarose gels to confirm specificity of amplification and the absence of primer dimer formation. Real-time PCR was performed in triplicate. The SYBR Green Dye detection system (Roche) was used for quantitative real-time PCR on LightCycler 480 (Roche). The thermal cycling settings were as follows: 45 cycles of 30 seconds of melting at 95°C followed by 15 seconds of annealing and extension at 59°C. Data were analyzed using the ΔΔCt method.

### Statistical analysis

Continuous variables were summarized with descriptive statistics (n, mean, and SD). Categorical variables were described with counts and percentages. For independent samples, the Student t test was used to analyze normally distributed quantitative variables, whereas the χ2 test or Fisher test was used to analyze nominal variables. The false discovery rate method was used to correct the multiple testing. The overall methylation level of the region detected by BSP was compared by one-way ANOVA. A P value of <.05 was considered significant for all statistical tests.

### Results

#### Clinical characteristics

The major anthropometric variables and endocrine parameters of the subjects evaluated for methylation statuses are displayed in Table 1. There was no sample overlap among the 3 groups. Subjects with PCOS had statistically elevated body mass indices, LH, and testosterone com-

### Table 1. Major Clinical Characteristics of Subjects With PCOS and Control Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Subject</th>
<th>PCOS</th>
<th>Control</th>
<th>PCOS</th>
<th>Control</th>
<th>PCOS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td>85</td>
<td>88</td>
<td>30</td>
<td>30</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td>27.44 ± 1.71</td>
<td>30.97 ± 2.84^a</td>
<td>28.64 ± 3.11</td>
<td>27.23 ± 2.97</td>
<td>29.38 ± 3.18</td>
<td>27.80 ± 2.31</td>
</tr>
<tr>
<td>Body mass index, kg/m^2</td>
<td>24.67 ± 2.27</td>
<td>22.05 ± 2.8^a</td>
<td>25.98 ± 3.12</td>
<td>22.46 ± 2.42^a</td>
<td>25.27 ± 4.31</td>
<td>21.81 ± 1.98^a</td>
<td></td>
</tr>
<tr>
<td>LH, IU/mL</td>
<td>10.00 ± 6.01</td>
<td>4.88 ± 2.14^a</td>
<td>11.08 ± 5.45</td>
<td>4.72 ± 2.01^a</td>
<td>14.35 ± 5.60</td>
<td>4.67 ± 1.67^a</td>
<td></td>
</tr>
<tr>
<td>Testosterone, ng/dL</td>
<td>68.62 ± 20.92</td>
<td>27.93 ± 7.53^a</td>
<td>78.75 ± 18.12</td>
<td>29.11 ± 10.90^a</td>
<td>55.04 ± 10.21</td>
<td>20.01 ± 6.88^a</td>
<td></td>
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</tbody>
</table>

Group 1 represents PBCs in subjects tested by pyrosequencing. Group 2 represents PBCs in subjects tested by BSP. Group 3 represents GCs in subjects. There was no overlap between the samples from the 3 groups.

^a P < .05.
pared with those of control subjects; this result is consistent with the general features of PCOS.

**LHCGR lacks novel somatic mutations**

No novel somatic mutations were identified when the UTRs, promoter, exons, and exon-intron boundary regions of LHCGR were sequenced. Three known single nucleotide polymorphisms (rs 2293275, rs 11125179, and rs 10176989) were confirmed.

**Women with PCOS harbor hypomethylated LHCGR**

LHCGR methylation levels first were detected by pyrosequencing the PBCs of a cohort of 85 women with PCOS and 88 control women. Because of the overwhelmingly high density of CpG sites in the promoter region, only 6 CpG sites (CpG -174, -148, -141, -139, -116, and -111) (Figure 1) could be analyzed by pyrosequencing. Widespread hypomethylation was observed among the 6 CpG sites, with significant hypomethylation measured for CpG -174 and -111 in women with PCOS compared with control women (CpG -174: 15.66 ± 4.14% vs 16.89 ± 2.39%, corrected $P = .018$; CpG -111: 4.36 ± 2.20% vs 5.17 ± 1.19%, corrected $P = .006$) (Figure 2).

**Replication of hypomethylation by BSP**

The amplification products of BSP spanned 16 CpG sites adjacent to the TIS (from CpG -174 to CpG +20), including the 6 CpG sites measured by pyrosequencing (Figure 1). Thirteen CpG sites were upstream of the +1 ATG, and the others were downstream of the ATG. The methylation statuses of patients with PCOS and control subjects were measured in PBCs. Specifically, 248 clones derived from 30 patients with PCOS and 213 clones derived from 12 control subjects were sequenced. Although the 16 CpG sites of the promoter region were largely hypomethylated in patients with PCOS, the overall methylation levels did not significantly differ between patients and control subjects by one-way ANOVA (Figure 3A). We attribute this finding to our limited sample size. Analysis of single CpG sites revealed that CpG +17 was significantly hypomethylated in women with PCOS (2.02% vs 8.45%, corrected $P = .02$) (Figure 3B).

**Further validation of hypomethylation in GCs**

Methylation is known to exhibit tissue or cell-type specificities. Therefore, we also evaluated GCs for LHCGR methylation status to validate the results observed in PBCs. We sequenced 250 clones derived from 12 women with PCOS and 237 clones derived from 12 control women. The overall methylation level in the promoter region was significantly lower in GCs retrieved from women with PCOS than in GCs from control women ($P = .004$) (Figure 3, C and D). Analysis of single CpG sites revealed that CpG -174, -148, -61, -43, -8, +10, +17, and +20 were significantly hypomethylated in women with PCOS compared with those in control women (CpG -174: 14.00% vs 19.41%, corrected $P = .048$; CpG -148: 9.60 vs 15.19%, corrected $P = .048$; CpG -61: 5.60% vs 10.13%, corrected $P = .048$; CpG -43: 5.60% vs 10.13%, corrected $P = .048$; CpG -8: 9.60% vs 14.77%, corrected $P = .046$; CpG +10: 6.00% vs 10.13%, corrected $P = .047$; CpG +17: 4.80% vs 10.13%, corrected $P = .050$; CpG +20: 4.80% vs 10.13%, corrected $P = .050$). Notably, CpG -61 is adjacent to an activating Sp (l) site, which binds transcription factors Sp1 and Sp3 and contributes to the basal promoter activity of LHCGR (15).

**Lower transcription of LHCGR in GCs from patients with PCOS**

Transcription of LHCGR in peripheral blood samples was measured using quantitative real-time PCR, and no significant difference was observed between patients with PCOS and control subjects. Subsequent transcription analysis in GCs revealed significantly higher LHCGR levels in patients with PCOS than in control subjects ($P < .01$) (Figure 4).

**Discussion**

In the “post-GWAS” era, the major challenge for researchers has been to interpret the functional effects of individual genetic variations that confer susceptibility to specific diseases. We examined genetic and epigenetic factors associating LHCGR with PCOS. We determined that no novel somatic mutations were detectable in PCOS subjects, but the promoter region was hypomethylated. The level of
Epigenetic information in mammals can be transmitted in multiple forms, with methylation being the most common and extensively discussed. Methylation is the conversion of cytosine into 5-methylcytosine, which has been described as the fifth base in DNA. In vertebrates, this phenomenon typically occurs at CpG sites. Normal cellular function relies on the maintenance of epigenomic homeostasis; this is shown by an abundance of reported associations between epigenomic perturbations and human diseases, notably cancer and complex diseases (16). Regarding PCOS, emerging research is directed at the exploration of the uncharted territory beyond genetics (17). Evidence indicates that an excess of prenatal testosterone could program PCOS-like phenotypes in female mammals, including rhesus monkeys, sheep, mice, and rats, suggesting a role for the intrauterine environment in PCOS pathogenesis (18, 19). Previous epigenetic studies of PCOS in humans have primarily focused on DNA methylation. Skewed X-chromosome inactivation was observed in PCOS, but the results were not consistent across studies (20–22). In a pilot study, no difference was reported regarding total methylation statuses of peripheral blood leukocyte DNA in patients with PCOS vs matched control subjects (23). In a quantitative methylation survey, the methylation levels of CpG sites in the follistatin promoter and 5*-UTR were not associated with PCOS (24). The results of these previous methylation studies are either negative or vague.

Hypomethylation of LHCGR promoter CpG sites was observed in patients with PCOS in the present study. This phenomenon also has been described in studies using dehydroepiandrosterone-induced PCOS mouse models (10). Previous in vitro experiments have demonstrated that LHCGR transcription largely depends on coordinated changes in DNA methylation and histone modification at the LHCGR transcription in GCs from patients with PCOS was consistent with this deviated methylation status. This is the first study to suggest that LHCGR, the candidate gene described for PCOS, may participate in the pathophysiology of PCOS by deviations in the methylation statuses of its promoter CpG sites.

Figure 3. Methylation status of the promoter CpG sites of LHCGR in PBCs and GCs tested by bisulfite sequencing. A, Illustrated methylation profile of LHCGR in PBCs. Fifteen clones were randomly selected from PCOS and control groups to represent the overall methylation profile. Each line represents an individual clone allele, with open circles for unmethylated CpG sites and filled circles for methylated CpG sites. B, Methylation level of each CpG site in PBCs shown in percentage form. C, Illustrated methylation profile of LHCGR in GCs. D, Methylation level of each CpG site in GCs shown in percentage form. *, Corrected P < .05.
CGR promoter. Demethylation of promoter CpG sites is necessary for maximal stimulation of this gene. This type of control is independent of the pathway involving unliganded nuclear receptors (9). Although the histone code was not investigated in the present study, our results indicate elevated LHCGR transcription in GCs from patients with PCOS compared with that in control subjects. This corresponds, in theory, to the fact that promoter CpG sites were hypomethylated as a whole in PCOS. The LHCGR promoter is driven by 2 transcription factor binding sites, Sp1 sites I and II, which occur in the human gene at −79 and −119 bp from the TIS, respectively. These sites bind transcription factors and contribute similarly to LHCGR basal promoter activity. They are of central importance to the transcription of the LHCGR gene. The regions adjacent to Sp1 sites are rich in CpG sites and may operate as a transcription factor–directed transcriptional complex (25). We demonstrated in the present study that promoter CpG sites in LHCGR were hypomethylated as a whole in GCs from patients with PCOS compared with that in control subjects. This corresponds, in theory, to the fact that promoter CpG sites were hypomethylated as a whole in PCOS. The LHCGR promoter is driven by 2 transcription factor binding sites, Sp1 sites I and II, which occur in the human gene at −79 and −119 bp from the TIS, respectively. These sites bind transcription factors and contribute similarly to LHCGR basal promoter activity. They are of central importance to the transcription of the LHCGR gene. The regions adjacent to Sp1 sites are rich in CpG sites and may operate as a transcription factor–directed transcriptional complex (25). We demonstrated in the present study that promoter CpG sites in LHCGR were hypomethylated as a whole in GCs from patients with PCOS compared with that in control subjects, and CpG −61 (adjacent to the Sp1 site) was significantly hypomethylated. Differences between overall methylation levels and absolute differences at most CpG sites compared between patients with PCOS and control subjects were more obvious in GCs than in PBCs. This could explain why LHCGR transcription was significantly elevated in the GCs, but not the PBCs, of patients with PCOS.

In the ovary, the interaction of LH and LHCGR promotes the formation of preovulatory follicles and corpora lutea and enhances steroidogenesis in luteal cells and GCs. Previous research on human ovarian theca cells and GCs suggested that the transcription of LHCGR was premature and increased in theca cells and GCs from polycystic ovaries compared with that normal controls (26). LHCGR is expressed during the entire follicular phase, from antral follicles to ovulatory follicles. According to the present study, LHCGR transcription remained elevated in GCs from ovulatory follicles of polycystic ovaries. The methylation statuses of promoter CpG sites are independent determinants of LHCGR transcription, so we deduced that the phenomenon of hypomethylation contributed to elevated LHCGR transcription in PCOS, possibly in antral follicles as well as ovulatory follicles.

Methylation exhibits tissue-specific patterns (27). In the current study, similar tendencies toward hypomethylation were observed in the PBCs and GCs of patients with PCOS compared with those in PBCs and GCs of control subjects. These cell types are not functionally related and are derived from different embryological tissues (hemangioblast and intermediate mesoderm, respectively). The similar trends of hypomethylation across different tissues indicate that hypomethylation occurs early, possibly before the onset of PCOS (28, 29). Follicular-phase hypertranscription of LHCGR in theca cells and GCs of PCOS may be due, at least in part, to hypomethylation of promoter CpG sites.

High circulating LH levels and elevated LH pulse amplitudes and frequencies are typical biochemical features of PCOS (30). The premature hypertranscription of LHCGR in both theca cells and GCs induces follicle hypersensitivity to LH impulses. LH hypersecretion combined with premature hypersensitivity to LHCGR results in hyperfunctioning of the LH/LHCGR interaction. This could contribute to the pathogenesis of PCOS by affecting 2 important cells in folliculogenesis, GCs and theca cells.

Classic studies have indicated that GCs exhibit a clear, developmentally regulated response to LH during folliculogenesis (12, 31–33). GCs responded to LH in ovulatory subjects once follicles reached 9.5/10 mm in diameter. In contrast, GCs from follicles as small as 4 mm responded to FSH and LH in anovulatory women with polycystic ovaries (34). Given the hyperfunctioning of the LH/LHCGR interaction, GCs from anovulatory women with PCOS are significantly more responsive to LH than GCs from ovulatory women with normal ovaries or polycystic ovaries. This may lead to the premature development of multiple small antral follicles, a hallmark of PCOS (34). However, according to the “LH ceiling” hypothesis, inappropriately high LH levels during the follicle development stage are detrimental to subsequent follicle maturation and ovulation (35, 36). Premature exposure of GCs to LH inhibits their proliferation such that follicle development is arrested (37, 38). Thus, hyperfunctioning of the LH/LHCGR interaction can lead to premature arrest of follicle growth and thus anovulation.

Our study did not scrutinize the possible effects of clinical characteristics of patients with PCOS on methylation patterns. Age may affect methylation level, so patients with PCOS and control subjects were age-matched in the current study. Obesity also has a potential influence on methylation patterns. However, there is no published ev-
idence of an association between obesity and the methylation level of LHCGR. This is a complicated question that requires a large sample size and longitudinal observation to address adequately. Finally, the relationship between the endocrine profiles and LHCGR methylation patterns of participants remains unknown. We intend to explore this relationship in future studies.

In summary, the present study contributes to post-GWAS research in PCOS by identifying a tendency to LHCGR hypomethylation across different tissues and a correspondingly elevated LHCGR transcription level in patients with PCOS compared with that in controls. Hypomethylation of LHCGR is highly relevant and may even be central to the etiology of anovulation in PCOS. Our findings highlight the importance of epigenetic studies of PCOS.

Acknowledgments

We acknowledge the very helpful participation of the study subjects.

Address all correspondence and requests for reprints to: Zi-Jiang Chen, Shandong Provincial Key Laboratory of Reproductive Medicine, Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, 324 Jingwu Road, Jinan 250021, China. E-mail: chenzijiang@hotmail.com.

This work was supported by the National Basic Research Program of China (973 program) (2012CB944700 and 2011CB944502), the National Natural Science Foundation of China (81000238 and 81200409), and the Scientific Research Foundation of Shandong Province of Outstanding Young Scientist (2012BSE27089).

Disclosure Summary: The authors have nothing to disclose.

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