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Androgen Receptor Coregulator *CTBP1-AS* Is Associated With Polycystic Ovary Syndrome in Chinese Women: A Preliminary Study

Zhenteng Liu, MD[1,2], Cuifang Hao, PhD[2], Dehua Song, MD[2], Ning Zhang, PhD[2], Hongchu Bao, PhD[2], and Qinglan Qu, MD[2]

Abstract

Polycystic ovary syndrome (PCOS) is currently considered a predominantly hyperandrogenic syndrome. In theory, hyperandrogenism can be caused by high level of testosterone (T) as well as by enhanced androgen receptor (AR) activity. C-Terminal binding protein 1 antisense (*CTBP1-AS*) was a novel long noncoding RNA (lncRNA) to regulate AR activity. In this study, we found that expression level of *CTBP1-AS* in peripheral blood leukocytes was significantly higher in women with PCOS than that in controls after adjustment for age and body mass index (BMI). Individuals having higher expression of *CTBP1-AS* had significantly greater disease risk than those having lower expression. We also identified expression of *CTBP1-AS* as an independent risk factor for PCOS. A positive correlation was observed between the *CTBP1-AS* expression and the total T (TT) concentration either unadjusted or after adjusting for age, BMI, and homeostatic model assessment insulin resistance. Taken together, our current study presented the first evidence that the lncRNA *CTBP1-AS*, a novel AR modulator, is associated with PCOS in Chinese population and established the possibility that abnormal *CTBP1-AS* expression is a risk factor for PCOS and it is a predictor of variability in serum TT level in Chinese women with PCOS.

Keywords

androgen receptor, lncRNA, *CTBP1-AS*, polycystic ovary syndrome, hyperandrogenism

Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent metabolic and reproductive diseases affecting approximately 10% of reproductive-aged women worldwide.[1] Although the clinical manifestations of the syndrome are highly variable including reproductive, metabolic, and psychological abnormalities, androgenic effects constitute the common mechanism responsible for its phenotype.[2] Although the pathophysiology of PCOS is complex and remains largely unclear, the disorder is currently considered a predominantly hyperandrogenic syndrome.[3]

Androgenic effects are exerted via the androgen receptor (AR), a nuclear transcription factor and member of the steroid receptors subgroup, which controls the expression of a large number of downstream target genes.[4] In theory, hyperandrogenism can be caused by high level of testosterone (T) as well as by enhanced AR activity. Notably, a hyperactive AR at the levels of the gonadotropin-releasing hormone pulse generator in the hypothalamus[5] and at the granulosa cells in the ovary,[6,7] skeletal muscles, or adipocytes[8,9] can make initially normal T and dihydrotestosterone as biochemically hyperandrogenic.

In the past decades, various clinical studies established that the transcriptional activity of AR is mainly mediated by AR gene CAG repeat polymorphism and the epigenetic effect of X chromosome inactivation (XCI).[10-14] However, other existing studies have produced conflicting and inconsistent results[15-21] and even yielded paradoxical result.[22] Therefore, we must evaluate AR as a dynamic heterocomplex because there are a large number of AR coactivators and corepressors, which influence androgen action via cross talk with AR.[23] In addition, it has been suggested that abnormalities in the expression of these factors may be involved in the development and progression of some diseases such as prostate cancer,hirsutism,

[1] Medical College of Qingdao University, Qingdao, China
[2] Department of Reproductive Medicine, Yantai Yuhuangding Hospital, Affiliated Hospital of Medical College of Qingdao University, Yantai, Shandong, China

Corresponding Author:
Cuifang Hao, Department of Reproductive Medicine, Yantai Yuhuangding Hospital, Affiliated Hospital of Medical College of Qingdao University, 20 Yuhuangding East Rd, Yantai, Shandong, 264000, China.
Email: cuifanghao@outlook.com

and androgen insensitivity syndrome.[24-26] In theory, it is plausible for the hypothesis that variation in expression or function of these modulators might modify the activity of AR and be involved in the pathogenesis of hyperandrogenism and thus contribute to development of PCOS. However, until now, there have been no studies on the association between AR modulators and PCOS.

In a very recent study, Takayama et al[27,28] uncovered that *CTBP1-AS* was a novel androgen-regulated long noncoding RNA (lncRNA) associated with the AR signaling pathway, serving as AR modulator. C-Terminal binding protein 1 antisense (*CTBP1-AS*) is located in the antisense (AS) region of *CTBP1*, which is a corepressor for AR. The lncRNA *CTBP1-AS* directly represses the expression of *CTBP1* by recruitment of the RNA-binding transcriptional repressor PTB-associated splicing factor (PSF) and histone deacetylases, which in turn promotes transcriptional activity of AR. Additionally, upregulated *CTBP1-AS* also inhibits other endogenous tumor-suppressor genes and promotes both hormone-dependent and castration-resistant tumor growth in prostate cancer.[29] Thus, the possibility exists that *CTBP1-AS* like CAG repeat polymorphism is involved in the pathogenesis of hyperandrogenism such as PCOS. However, whether the lncRNA *CTBP1-AS* as AR modulator is associated with PCOS is unknown.

In this study, we address the expression profiling of *CTBP1-AS* in women with PCOS and its relation to the key clinical characteristics of PCOS. First, we measured the expression of *CTBP1-AS* in the peripheral blood leukocytes in patients with PCOS and healthy control women. Second, the correlation between *CTBP1-AS* and the disease as well as multiple key endocrine parameters of PCOS was analyzed using multiple statistical methods.

Materials and Methods

Study Patients

The study protocol was approved by the Institutional Ethical Review Board of Yuhuangding Hospital of Yantai, Affiliated Hospital of Qingdao Medical University. Written informed consent was obtained from all patients. All the study evaluations and procedures were conducted in accordance with the guidelines of the Declaration of Helsinki on human experimentation. The study was initiated on March, 2013 and completed in March, 2014. We recruited 23 patients with PCOS at Department of Reproductive Medicine of Yuhuangding Hospital of Yantai from March 2013 to March 2014. The diagnosis of PCOS was based on the 2003 Rotterdam criteria[30] and required 2 or more of the following 3 features: menstrual disorders (oligomenorrhoea or amenorrhea), clinical or biochemical evidence of hyperandrogenism, and polycystic ovaries visible on ultrasound examination (at least 12 follicles measuring 2-9 mm or volume of the ovary >10 cm^3). Hyperandrogenism was assessed by the presence of hirsutism and/or acne and/or by elevated androgen levels. Hirsutism was defined by Ferriman-Gallwey index score >5.[15] We excluded patients with Cushing syndrome, congenital adrenal hyperplasia, and androgen-secreting tumors. The control group consisted of age-, race-, sex-, and BMI-matched 17 healthy women before entering in vitro fertilization program due to male factor infertility. They had no evidence of clinical or biochemical hyperandrogenism, no menstrual cycle irregularities, or other endocrine disorders related to PCOS. None of the patients had taken any hormonal drugs, such as oral contraceptives, antiandrogens, or insulin sensitizers for at least 6 months before the study. All patients and controls were unrelated Han Chinese women from the same geographical area.

Standardized anthropometric measurements for all patients were carried out in the early follicular phase of the menstrual cycle (days 3-5) or randomly in patients with amenorrhea between 09:00 AM and 11:00 AM including body weight, height, waist circumference, and waist/hip ratio (WHR). The body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Blood pressure (BP) was measured in the sitting position after a 10-minute rest. All patients had the ultrasound examinations performed by experienced reproductive gynecologists performing a transvaginal scan on cycle days 12 to 14. Transvaginal scans were reevaluated by one of the authors (CH).

Hormone Assays

After an overnight 12-hour fasting, antecubital intravenous blood samples (5 mL) were collected from all participants at an early follicular phase (days 3-5) of a spontaneous menstrual cycle or progesterone (P)-induced withdrawal bleed between 08:00 AM and 10:00 AM. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), estradiol (E_2), P, total T (TT) concentrations, fasting plasma glucose, and fasting insulin were determined by chemiluminescent immunometric assays (Ortho Clinical Diagnostics, Johnson & Johnson, Rochester, New York). Total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and lipoprotein (a; LP (a)) were measured by colorimetric enzymatic methods (Siemens Advia System, Deerfield, Illinois). The intra-assay and interassay coefficients of variation ranged between 1.8% and 6.8%. Fasting glucose and insulin levels served for homeostasis model assessment of insulin resistance (HOMA-IR), calculated by multiplying fasting insulin (mU/L) by fasting glucose (mmol/L) and dividing this product by 22.5.

Isolation of Peripheral Blood Leukocytes

Blood samples were obtained from patients with PCOS (n = 23) and normal healthy donors (n = 17). The blood in heparin tubes (5 mL per patient) was diluted with equal volumes of phosphate-buffered saline and then overlaid on a Ficoll-Paque Plus kit (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania) at a 1:1 ratio and centrifuged at 800*g* for 30 minutes at 20°C. The peripheral blood mononuclear cell layer was harvested and washed with phosphate-buffered saline 2 times to remove plasma and Ficoll (Axis-Shield, Oslo, Norway). Then, these samples were stored at −80°C until further analyses.

RNA Isolation and Real-Time Polymerase Chain Reaction

Total RNA was harvested from the above-mentioned peripheral blood leukocytes using the RNeasy Mini kit (QIAGEN, Hilden, Germany) according to the manufacturers' instructions. After evaluation of concentration and purity of RNA using the ND-1000 NanoDrop spectrophotometer (NanoDrop, Wilmington, Delaware) and denaturing gel electrophoresis, the RNA reverse-transcribed into complementary DNA using PrimeScript RT Reagent Kit (TaKaRa, Shiga, Japan) in the Rotor-Gene 3000 Real-time PCR system (Corbett Research, Australia), according to the manufacturers' protocol.

Measurement of CTBP1-AS Gene Expression

In this process, the levels of *CTBP1-AS* were quantified by quantitative real-time polymerase chain reaction (qRT-PCR) using the SYBR Premix Ex Taq II (TaKaRa) following the manufacturer's protocol. In brief, reactions were conducted using the Rotor-Gene 3000 Real-time PCR system (Corbett Research) with the following reaction profile: predenaturation for 30 seconds at 95°C and PCR amplification for 45 cycles with 5 seconds at 95°C and 60 seconds at 60°C. The PCR was followed by a melt curve analysis to determine the reaction specificity. Agarose gel electrophoresis was performed to confirm the size of PCR product. The levels of *CTBP1-AS* were analyzed by relative quantification using the comparative threshold (Ct) cycle method relative to the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression level.[31] It is best to calculate the mean $\pm$ standard error of the mean (SEM) for the change in gene expression in each group as individual data points using equation $2^{-\Delta Ct}$.[32] Each set of qRT-PCR reactions was repeated 3 times.

The following primers were used to quantify the lncRNA *CTBP1-AS* levels: *CTBP1-AS* (GenBank: NR_104331.1), 5′-ACAACACAAAGCCCCGGAA-3′ (forward) and 5′-AGTGAAGAATGGTCTCGCCC-3′ (reverse), fragment length: 121; GAPDH RNA was quantified as a control to normalize differences in total RNA levels using the following primers (GenBank: NM_002046): 5′-GGGAAACTGTGGCGTGAT-3′ (forward) and 5′-GAGTGGGTGTCGCTGTTGA-3′ (reverse), fragment length: 202.

Statistical Analysis

The results are expressed as means $\pm$ SEM or median (interquartile range). Normal distribution of the continuous variables was tested by the Shapiro-Wilk statistic. The expression level of *CTBP1-AS* was natural logarithm-transformed (-ln) because of a skewed distribution. Characteristics of patients with PCOS and healthy controls were compared using either Student *t* test for quantitative data with Gaussian distribution or Mann-Whitney *U* test for data with non-Gaussian distribution. The distributions of the *CTBP1-AS* expression were divided into binary groups among the patients with PCOS and control participants. Limits for the binary groups of this lncRNA expression were derived from the control group as follows: <3.757 for the low expression group and ≥ 3.757 for the high expression group. In a backward stepwise binomial logistic regression analysis, continuous parameters independently predicting PCOS were identified. Predictors that did not contribute to the model were excluded (exclusion threshold $P > .04$). Separate Pearson or Spearman rank correlation coefficients were calculated to analyze whether there were linear relations between the expression of *CTBP1-AS* and certain clinical biochemical traits. To explore the effect of the *CTBP1-AS* expression on TT concentration, a multiple linear repression model was constructed with TT as a dependent variable and age, BMI, HOMA-IR, and *CTBP1-AS* expression level as independent variables. Logistic regression model was conducted to explore the association of the expression of *CTBP1-AS* with hirsutism in patients with PCOS, adjusting for TT as independent variable.

SPSS software version 20.0 (SPSS Inc., Chicago, Illinois) was used for analyses. Data were considered statistically significant at $P < .05$.

Results

Clinical Characteristics of Patients

The clinical, metabolic, and hormonal features of the controls and patients with PCOS are shown in Table 1. As expected from design, we found no differences in age and BMI between controls and patients with PCOS. Compared to healthy controls, patients with PCOS had higher hirsutism score, higher levels of TT, LH, LH/FSH, fasting insulin, and HOMA-IR ($P < .05$). The differences in WHR, systolic BP and diastolic BP, and the levels of E2, FSH, PRL, fasting glucose, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, and LP(a) between controls and patients with PCOS did not reach statistical significance. These differences persisted after adjustment for BMI.

Expression of CTBP1-AS in Controls and Women With PCOS

As shown in Figure 1, mean *CTBP1-AS* expression level in peripheral blood leukocytes was significantly higher in the women with PCOS than that in the controls (-lnCTBP1-AS, 5.323 ± 0.205 vs 2.059 ± 0.210, $P = .023$) after age and BMI adjustment. The experiment was performed 3 times with similar results.

Association Between CTBP1-AS and the Presence of PCOS

We sought to further analyze the relationship between different expression levels of *CTBP1-AS* and risk of PCOS. Table 2 shows the risk of PCOS in individuals with different *CTBP1-AS* expression levels. Individuals having higher expression had significantly greater disease risk than those having lower expression (odds ratio = 1.822, 95% confidence interval = 1.223-2.225, $P = .005$) after adjustment for BMI.

Table 1. Clinical Characteristic of Controls and Patients With PCOS.[a]

Variables	Controls	Patients With PCOS	*P* Value
Number	17	23	
Age, years	29.71 ± 0.44	28.44 ± 0.37	.051
BMI, kg/m^2	27.01 ± 1.1	28.88 ± 1.56	**.011**
WHR	0.84 ± 0.01	0.88 ± 0.01	**.020**
Hirsutism score	2 (0-3)	4 (1-6)	**<.001**
Systolic BP, mm Hg	119.00 ± 1.56	119.61 ± 3.36	.884
Diastolic BP, mm Hg	76.00 ± 1.57	76.13 ± 2.12	.963
Total T, ng/mL	0.24 ± 0.02	0.41 ± 0.04	**.001**
E_2, pg/mL	43.67 ± 4.76	47.17 ± 3.53	.550
FSH, mIU/mL	6.45 ± 0.37	5.63 ± 0.27	.077
LH, mIU/mL	5.27 ± 0.44	11.43 ± 1.35	**.001**
LH/FSH	0.81 ± 0.07	2.04 ± 0.24	**<.001**
PRL, ng/mL	14.94 ± 1.25	14.54 ± 1.11	.814
Fasting glucose, mg/dL	4.65 ± 0.08	5.07 ± 0.14	**.030**
Fasting insulin, mIU/mL	8.29 ± 0.52	16.00 ± 1.12	**.004**
HOMA-IR	1.72 ± 0.12	4.43 ± 0.89	**.013**
Cholesterol, mmol/L	5.02 ± 0.26	5.06 ± 0.16	.892
Triglycerides, mmol/L	0.66 ± 0.05	1.13 ± 0.14	**.008**
HDL cholesterol, mmol/L	1.62 ± 0.10	1.41 ± 0.06	.075
LDL cholesterol, mmol/L	2.55 ± 0.17	3.02 ± 0.19	.083
LP(a), mg/L	94.60 (46.6-370.8)	167.3 (50.00-598.6)	.092

Abbreviations: BMI, body mass index; WHR, waist to hip ratio; BP, blood pressure; T, testosterone; E_2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; HOMA-IR, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LP(a), lipoprotein(a); SEM, standard error of the mean.

[a]Values are expressed as mean ± SEM or median (interquartile range). *P* values were obtained from unpaired 2-tailed Student *t* test or Mann-Whitney *U* test. Clinical indexes with significant differences ($P < .05$) are in bold.

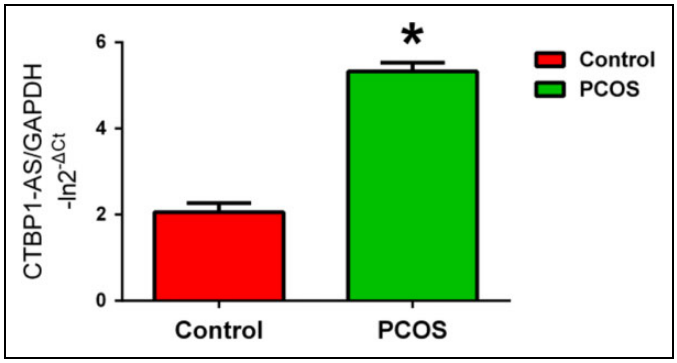

Figure 1. The expression of *CTBP1-AS* in peripheral blood leukocytes in controls and patients with PCOS measured using quantitative real-time PCR after adjustment for age and BMI. Data are expressed as means ± SEM. *$P < .05$, Student *t* test. The experiment was performed 3 times with similar results. *CTBP1-AS* indicates C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome; PCR, polymerase chain reaction; BMI, body mass index; SEM, standard error of the mean.

Expression of CTBP1-AS *as an Independent Risk Factor for PCOS*

We investigated the role of *CTBP1-AS* expression level in context of established risk factors for PCOS (Table 3). To this end, we included BMI, serum LH, HOMA-IR, and expression of *CTBP1-AS* in a backward stepwise binomial regression analysis and identified *CTBP1-AS* expression as an independent risk factor for PCOS ($P < .05$).

Correlation Between CTBP1-AS Level and Clinical Parameters in PCOS

In order to analyze whether there was an association between the expression of *CTBP1-AS* and certain clinical biochemical traits, separate Pearson or Spearman rank correlation coefficients were calculated. We included the expression of *CTBP1-AS* as dependent variables, and clinical characteristics in both controls and patients with PCOS as independent variables. As shown in Table 4, there was a significant correlation between *CTBP1-AS* and TT only in the PCOS groups ($r = .453$, $P = .027$) after adjustment for age and BMI. However, there was no association between *CTBP1-AS* and other clinical characteristics.

The Effect of CTBP1-AS *Expression on TT*

A positive correlation was observed between the expression of *CTBP1-AS* and the TT concentration either unadjusted or after adjusted for age, BMI, and HOMA-IR (Table 5). A model using the expression of *CTBP1-AS*, age, BMI, and HOMA-IR as predictors explained 9.2% (adjusted R^2) of the variability in the serum TT levels ($P = .017$). The same analysis in the control group revealed no significant difference in any biochemical parameter (data not shown).

Association of the CTBP1-AS *Expression With Hirsutism in PCOS*

To explore the association of the *CTBP1-AS* expression with clinical androgen trait, hirsutism, in PCOS, a logistic regression model was constructed with hirsutism (yes/no) as dependent variable, *CTBP1-AS* expression (high/low) as independent variable, and TT as covariate. The result was not significant ($P = .122$). The TT was also not found to have a significant effect on hirsutism ($P = .104$; Table 6).

Discussion

To our knowledge, this is the first study to determine the association of lncRNA *CTBP1-AS*, a novel AR modulator, and PCOS. Patients with PCOS tended to have a significantly higher *CTBP1-AS* expression than healthy controls. Individuals with more elevated *CTBP1-AS* expression had significantly

Table 2. Odds Ratio of PCOS Events by Expression of *CTBP1-AS*.[a]

	Controls (n = 17)		Patients With PCOS (n = 23)				
	Low	High	Low	High	OR	95%CI	*P* Value
No adjustment	8 (47.1%)	9 (52.9%)	8 (34.8%)	15 (65.2%)	1.667	1.163-1.906	**.014**
Adjusted for BMI					1.822	1.223-2.225	**.005**

Abbreviations: BMI, body mass index; *CTBP1-AS*; C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome; OR, odds ratio; 95% CI, confidence intervals.
[a]Data are n (%), unless otherwise indicated. *CTBP1-AS* expression for binary groups cutoffs were <3.757 for the low expression, ≥3.757 for the high expression. The OR, 95% CI, and *P* value were estimated for PCOS events in the high expression group compared to the low expression using logistic regression models. *P* values < .05 are in bold.

Table 3. Expression of *CTBP1-AS* as an Independent Factor for PCOS.[a]

Risk Factor	*P* Value[b]
Elevated TT	**.013**
Elevated LH	**.021**
CTBP1-AS expression	**.015**

Abbreviations: *CTBP1-AS*; C-terminal binding protein 1 antisense; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; TT, total testosterone.
[a]*P* < .05 are in bold.
[b]Backward stepwise binomial regression analysis.

Table 4. Partial Pearson or Spearman Rank Correlation Coefficients of the Expression of *CTBP1-AS* and Patients' Characteristics.[a]

	Controls		Women With PCOS	
	r	*P* Value	*r*	*P* Value
Age, years				
BMI, kg/m^2				
WHR	−.191	.464	−.079	.721
Hirsutism score	−.361	.154	−.064	.773
Systolic BP, mm Hg	−.101	.699	−.077	.726
Diastolic BP, mm Hg	−.029	.912	−.108	.623
Total T, ng/mL	.415	.057	.453	**.027**
E_2, pg/mL	−.059	.822	−.046	.836
FSH, mIU/mL	−.277	.102	−.306	.156
LH, mIU/mL	.294	.252	−.073	.742
LH/FSH	−.267	.301	.059	.788
PRL, ng/mL	.005	.984	.270	.213
Fasting glucose, mg/dL	.179	.492	.023	.918
Fasting insulin, mIU/mL	−.247	.339	−.032	.883
HOMA-IR	−.155	.552	.284	.167
Cholesterol, mmol/L	−.180	.490	.018	.933
Triglycerides, mmol/L	−0.091	.728	.127	.563
HDL, mmol/L	.091	.729	.106	.630
LDL, mmol/L	−.041	.877	−.050	.822
LP(a), mg/L	−.408	.067	−.101	.647

Abbreviations: BMI, body mass index; *CTBP1-AS*; C-terminal binding protein 1 antisense; WHR, waist to hip ratio; BP, blood pressure; T, testosterone; E_2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LP(a), lipoprotein(a).
[a]The correlation coefficient (*r*) and *P* value were adjusted for age and BMI. *P* values <.05 are in bold.

greater disease risk than those having the lower expression after adjustment for BMI, indicating that *CTBP1-AS* seems to be a major determinant of PCOS. Our results are consistent with a previous functional study, which found upregulated *CTBP1-AS* can promote AR-mediated transcriptional activity to facilitate the expression of androgen-responsive genes.[27]

There are several lines of evidence demonstrating that hyperandrogenism, which is a central feature for PCOS, determines the characteristic phenotype of the syndrome, that is, menstrual cycle dysfunction, hirsutism, and polycystic ovarian morphology.[10,33,34] In theory, hyperandrogenism can be caused by high level of TT as well as by enhanced AR activity. Therefore, more active AR can cause a hyperandrogenic phenotype in absence of markedly elevated androgens. In view of this, a number of studies have reported that increased androgenic activity could amplify IR as well as T in physiological concentrations in differentiated rat skeletal muscle myotubes and cultured subcutaneous adipocytes of women.[8,9] Amplified transcriptional activity of AR promotes intraovarian androgenic microenvironment which presumably stimulates early follicular growth and contributes to mechanisms of follicular arrest found in PCOS.[35]

Although the association of AR activity with CAG polymorphism and XCI has been extensively studied, they have produced conflicting results.[10,11,13,15,17-19,21,22,36,37] There is little information on AR coregulators in the pathogenesis of PCOS, although recent advances in molecular biology have identified several AR coregulators that modulate the androgen action via cross talk with AR.[35,38] The dependency of the AR on its coregulators suggests a key role for these regulatory molecules in the development and maintenance of disorders associated with hyperandrogenism in humans.

It is noteworthy that Takayama et al[27] recently reported the identification of a novel androgen-dependent lncRNA *CTBP1-AS* as AR activity modulator, whose expression is inversely correlated with the expression of CTBP1, a novel AR corepressor. More importantly, epigenetic modifications of *CTBP1-AS* provide new insight into the regulated mechanism of AR activity without changing the DNA code.[39] Notably, *CTBP1-AS* as an important modulator of AR activity appears to be clinically relevant in some diseases closely associated with abnormal AR activity. Thus, it is possible that *CTBP1-AS* might play a role in the pathogenesis of PCOS-related hyperandrogenism.

Table 5. Multiple Linear Regression Models With TT as the Dependent Variable in PCOS.[a]

Variable	Standardized β coefficient	*P* Value
Unadjusted model ($R^2 = .051$)		
CTBP1-AS expression	.231	**.018**
Adjusted model ($R^2 = .081$)		
CTBP1-AS expression	.209	**.023**
Age	−.140	.129
BMI	.101	.319
Adjusted model ($R^2 = .092$)		
CTBP1-AS expression	.239	**.017**
Age	−.138	.131
BMI	.125	.213
HOMA-IR	−.102	.317

Abbreviations: BMI, body mass index; *CTBP1-AS*, C-terminal binding protein 1 antisense; HOMA-IR, homeostasis model assessment of insulin resistance; PCOS, polycystic ovary syndrome.
[a]$P < 0.05$ are in bold.

Table 6. Association of Expression of *CTBP1-AS* With Hirsutism in PCOS.[a]

	Low Expression	High Expression			
Hirsutism (yes)	2	9	OR	95% CI	*P* value
Hirsutism (no)	6	6	0.222	0.033 -1.493	.122
Adjusted for TT			0.213	0.026 -1.485	.104

Abbreviations: CI, confidence interval; *CTBP1-AS*, C-terminal binding protein 1 antisense; OR, odds ratio; PCOS, polycystic ovary syndrome; TT, total testosterone.
[a]A logistic regression model was constructed with hirsutism (yes/no) as dependent variable, *CTBP1-AS* expression (high/low) as independent variable, and TT as covariate.

According to our data, there is a significant elevation in *CTBP1-AS* expression in patients with PCOS compared to healthy controls. Furthermore, higher expression of *CTBP1-AS* was more frequent in PCOS possibly enhancing androgenic effects in these patients, while lower expression of the modulator was more frequent in the control group, presumably exerting a protective effect. This indicated that women with higher expression of *CTBP1-AS* have greater disease risk which was consistent with a role for androgen hyperactivity in the development of the PCOS. Additionally, we report novel evidence that *CTBP1-AS* influenced the PCOS phenotype by serving as an independent risk factor for PCOS, comparable to established markers of PCOS, for example, elevated LH and increased BMI (Table 3). Yeh et al[40] had shown that the AR coactivator had been shown to enhance AR activity up to 10-fold, a level that AR alone cannot reach. Hence, we would infer that even a modest change in *CTBP1-AS* level could have a large or cumulative effect on AR sensitivity. The mechanisms in the background of these findings remain to be elucidated.

Moreover, the more detailed analysis showed that the *CTBP1-AS* expression was associated with a linear increase in serum TT level only in the group with PCOS. To obtain a reliable conclusion, a multiple linear regression model was performed. A positive correlation was observed between *CTBP1-AS* expression and serum TT, either unadjusted or after the adjustment for age, BMI, and HOMA-IR. Testosterone is the major circulating androgen in women. It is likely that change in androgen sensitivity could result in altered androgen production, which mimics altered insulin secretion when insulin resistance is present. This trend is in agreement with Ibanez et al's observation which reported that the increased androgen activity has a stimulatory effect on ovarian androgen production.[41] Intriguingly, our findings are consistent with that of many prior genetic studies that found an association between the CAG repeats and serum T in patients with PCOS,[13,19,21,22] suggesting *CTBP1-AS* might modulate the transcriptional activity of AR by interacting CAG polymorphism. Hsiao et al[42] demonstrated that ARA 24, an AR coactivator, can interact with the AR N-terminal polyglutamine region (CAG) and enhance AR transactivation. They concluded that the interaction of AR coactivator with the CAG repeat could cooperate to modulate the AR activity. Accordingly, we speculated that *CTBP1-AS* might modulate AR transcriptional activity alone but also functionally interact with CAG polymorphism to regulate AR activity, which remains to be elucidated.

Although we have found a significant positive correlation between *CTBP1-AS* and TT, it is only a modest correlation ($r = .453$, $P = .027$), and the adjusted model explains only 9.2% of the variability in serum TT. Theoretically, hyperandrogenism in PCOS results from the interaction of multifactors, therefore other factors might confound the true effect of *CTBP1-AS* on TT in our study. The transcriptional activity of AR is affected not only by AR coregulators such as *CTBP1-AS*[27] but also by polymorphisms in the AR gene,[43] E_2,[44] and insulin-like growth factor.[45] Thus, it may highlight that multiple mechanisms affect the results.

In addition, it is expected that because of a higher receptor activity, women with higher *CTBP1-AS* will express a significantly clinical androgen trait such as hirsutism. However, it was not borne out in our study. Level of TT was not found to be the predictor of hirsutism as well. Regarding the hirsutism of PCOS, some reported that short CAG repeat was associated with hirsutism and acne.[46] Thus, hirsutism in patients with PCOS may be affected by the multiple and complex factors, and this could explain why hirsutism was not correlated with *CTBP1-AS* level in PCOS. Further study is needed to explore the mechanism by which *CTBP1-AS* influences phenotype of PCOS.

As far as we know, data on the functional importance of the lncRNA *CTBP1-AS* on AR activity are sparse and mainly focus on prostate cancer. Nevertheless, it has been identified as critical to the androgen-mediated transcriptional regulation.[27] Abnormal AR activity regulated by coregulators may predispose an individual to the development of PCOS. Association of AR activity with other coregulators in some diseases that

influence target organ response to androgens further supports this hypothesis.[23] Additionally, in a very recent study, Yang et al[47] found lncRNA-dependent mechanisms of AR-regulated transcriptional activity in prostate cancer cells, revealing that lncRNAs may be part of AR transcription regulatory network. Our present findings will also have an impact on the research field of steroid hormone receptors, as we still have limited knowledge with regard to those receptors' lncRNA coregulators.

One limit of the current research is the relatively small sample size, but our cohort with PCOS appeared to be well suited for the investigations performed, since the cohort was rather homogenous. Another limitation is the use of peripheral blood leukocytes. Gene analysis in peripheral blood leukocytes does not necessarily reflect *CTBP1-AS* expression status in specific target tissues such as the ovary, and further work would be required to validate the use of peripheral blood leukocytes for understanding the gene expression status in a hormonally regulated tissue. We adjusted for age and BMI when assessing association with PCOS. Obesity is thought to promote the development of PCOS.[48] There is a normal age-related decline in androgen levels.[49] Insulin promotes the synthesis of androgen in thecal cells directly.[50] This is the reason why BMI, HOMA-IR, and age should be adjusted when establishing the presence of hyperandrogenemia in women.

Conclusion

In conclusion, in a perspective of AR coregulators, our study differs from previous genetic investigations about the association between CAG repeat polymorphism and PCOS. Our current study established the possibility that abnormal *CTBP1-AS* expression is a risk factor for PCOS in the Chinese population, and it is a predictor of serum TT level variability in Chinese women with PCOS. Accordingly, although there is as yet no evidence for a causal link between *CTBP1-AS* and PCOS, it is conceivable that *CTBP1-AS* might contribute, at least to some extent, to the etiology of PCOS. In light of these findings, our next focus will be to investigate the biological functions and mechanisms of *CTBP1-AS* in vivo and whether there is a causal link between lncRNA *CTBP1-AS* and PCOS in other cell lines and large other ethnic groups.

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Authors' Note

Zhenteng Liu and Cuifang Hao contributed equally to this work. This work was performed at the Central Laboratory of Yuhuangding Hospital of Yantai and Medicine and Pharmacy Research center of Binzhou Medical University, Yantai, Shandong, China.

Declaration of Conflicting Interests

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