Androgen Receptor Coregulator Long Non Coding RNA CTBP1-AS Is Associated With Polycystic Ovary Syndrome in Kashmiri Women

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is one of the most common reproductive, endocrine and metabolic disorders in premenopausal women. Even though the pathophysiology of PCOS is complex and obscure, the disorder is prominently considered as the syndrome of hyperandrogenism. C-Terminal binding protein 1 antisense (CTBP1-AS) acts as a novel Androgen Receptor regulating long noncoding RNA (lncRNA). Therefore, the present study was aimed to establish the possible association of androgen receptor regulating long noncoding RNA CTBP1-AS with PCOS.

Methods: A total of 178 subjects including 105 PCOS cases and 73 age-matched healthy controls were recruited for the study. The anthropometric, hormonal and biochemical parameters of all subjects were analysed. Total RNA was isolated from peripheral venous blood and expression analysis was done by quantitative real time PCR (qRT-PCR). The correlation analysis was performed to evaluate the association between and various clinical parameters and lncRNA CTBP1-AS expression.

Results and conclusion: the mean expression level of CTBP1-AS was found to be significantly higher in the PCOS women than in the healthy controls (-lnCTBP1-AS, 4.23 ± 1.68 versus 1.24 ± 0.29 p<0.001). Further, subjects with higher expression level of CTBP1-AS had significantly higher risk of PCOS compared to subjects with low levels of CTBP1-AS expression (actual OR = 11.36, 95% C.I. = 5.59-23.08, P = < 0.001). The area under ROC curve (AUC) was 0.987 (SE 0.006 and 95% C.I. 0.976−0.99). However, lncRNA CTBP1-AS was found to have no association with different clinical characteristics in PCOS. In conclusion, androgen receptor coregulating lncRNA CTBP1-AS is associated with PCOS women and high expression of CTBP1-AS is a risk factor for PCOS in Kashmiri women

Introduction

Polycystic ovary syndrome (PCOS) is considered one of the most common reproductive, endocrine and metabolic disorders affecting approximately 10% of premenopausal women. PCOS affects approximately 10% of women worldwide[1]. It is a heterogeneous disorder and comes with a wide spectrum of clinical manifestations that include endocrine, metabolic, reproductive and sometimes psychological anomalies. Even though the pathophysiology of PCOS is complex and obscure, the disorder is prominently considered as the syndrome of hyperandrogenism[2]. Hyperandrogenism can either be attributed to excessive serum testosterone levels or hyperactivity of the androgen receptor. The transcriptional activity of AR is regulated by a number of coregulators[3]. Consequently, any abnormal activity of these androgen receptor modulators either due to altered expression or by any mutation may lead to impaired activity of androgen receptor and thereby play a role in the pathogenesis of hyperandrogenism and PCOS.

The recent advancements in genome sequencing technologies revealed that the major portion of the genome is transcriptionally active and encodes for noncoding RNAs [4]. Long Non Coding RNAs (lncRNAs) are the noncoding RNA transcripts of more than 200 nucleotide length, they constitute a significant

proportion of noncoding RNAs[5]. A number of studies have demonstrated their role in a wide variety of biological processes [6,7]. However, the mechanism of action is yet poorly understood. Primarily they act as epigenetic modulators, regulating gene expression at the transcriptional level as well as at post-transcriptional level. Besides dysregulation of lncRNAs has been implicated in various diseases [8]

long non coding RNA C-terminal binding protein 1 antisense (CTBP1-AS) is associated with androgen receptor signalling pathway, acting as AR modulator. CTBP1-AS is located in the antisense region of C-terminal binding protein 1 (CTBP1), which acts by regulating the expression of AR corepressor C-terminal binding protein 1. LncRNA CTBP1-AS acts by repressing the transcription of CTBP1. CTBP1-AS first associates with transcriptional repressor Polypyrimidine Tract Binding Protein associated splicing factor (PSF) and then recruits histone deacetylases-paired amphipathic helix protein Sin3A complexes on to the promoter region of the gene. Thus CTBP1-AS downregulates transcription of CTBP, thereby promoting the transcriptional activity of AR. In addition to this CTBP1-AS itself mimics androgen receptor signalling by inducing upregulation of the androgen receptor regulated genes [9].

The aim of this study was to evaluate the association of androgen receptor coregulator lncRNA CTBP1-AS with the risk of PCOS in ethnic Kashmiri women. We also attempted to find the correlation of lncRNA CTBP1-AS expression with various clinical manifestations of PCOS.

Materials And Methods

Recruitment of Subjects

In this case–control study, a total of 178 subjects were recruited of which 105 were drug naïve PCOS women and 73 were age-matched healthy controls. PCOS cases were recruited from the patients attending OPD of Department of Endocrinology, Sher-e-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, Jammu and Kashmir, India and Department of Obstetrics and Gynaecology, Govt. Medical College, Srinagar, Jammu and Kashmir, India for PCOS related complications from October 2017 to March 2020. The inclusion criteria for PCOS patients was based on the Rotterdam criteria [10]. All those subjects who presented with PCOS mimicking disorders including thyroid dysfunction , hyperprolactinemia , congenital adrenal hyperplasia, androgen secreting tumours and Cushing's syndrome were excluded from the study. Controls recruited for the study were age matched healthy women with regular cycles, no signs of hyperandrogenism clinical/ biochemical, with no history of autoimmune or endocrine disorders. All of the subjects were ethnic Kashmiris living in Kashmir province

Ethics statement

This study was approved by the ethical committee Government Medical College Srinagar under ethical approval no. 94/ETH/GMC/ICMR. All subjects were recruited after written informed consent was obtained from them.

Anthropometric and clinical evaluation

All the subjects underwent general anthropometric measurements that include height, weight, waist, hip, waist-hip ratio (WHR), body mass index (BMI) and FG score. A detailed history of clinical symptoms like menstrual cycles, hirsutism, acne, alopecia, and acanthosis nigricans was also taken from all study subjects. Height was measured using a height measuring scale in standing position without shoes and weight was measured using a weighing scale with light clothing. For measurement of waist circumference, minimum value between the iliac crest and the lateral costal margin was determined in a standing position, and hip circumference was measured as the maximum value over the buttocks. WHR was calculated by dividing waist circumference by the hip circumference. BMI was calculated as weight(kg)/Height(m²). Hirsutism was measured by Ferriman-Gallwey scoring system and a score of greater than 8 was taken as significant. All the PCOS women underwent transabdominal ultrasonography (USG) to measure the number of small peripheral cysts and/or increased ovarian volume.

Hormonal and Biochemical assessment

For hormonal and biochemical assessment, blood sample was collected in clot activator vials from all the participants after an overnight fast on 2nd – 3rd day of their menstrual cycle. The hormonal profile included Testosterone, luteinizing hormone, Follicle stimulating hormone. Thyroid stimulating hormone to exclude thyroid disorders, Prolactin to exclude hyperprolactinemia were estimated by Radioimmunoassay (RIA) using RIA kits (Immunotech S.R.O, Prague, Czech Republic) on Beckman coulter UniCelDxl 800 (Access Immunoassay system). Enzyme-linked immunosorbent assays were used to measure Sex Hormone-Binding Globulin (SHBG), androstenedione, dehydroepiandrosterone sulfate (DHEAS), and fasting insulin using Calbiotech, CA USA and DGR Instruments GmbH Marburg ELISA kits using SkanIt RE 4.0 software on Thermo Scientific Multiskan FC ELISA reader. The biochemical parameters done included oral glucose tolerance test, cholesterol, triglyceride, High Density Lipoprotein cholesterol levels, Low-Density Lipoprotein cholesterol levels, Urea, Creatinine, SGOT, SGPT. All the biochemical parameters were measured on semi-automatic analyzer (ERBA Chemtouch 7, Biochemistry Analyzer, Wiesbaden, Germany) using ERBA bioassay diagnostic kits.

The free androgen index (FAI) was derived using the formula:

$$FAI = \frac{Total\ Testosterone}{SHBG} \times 100.$$

Insulin resistance was calculated

$$\text{"HOMA IR} = fasting\ glucose\ (mg/dL) \times Fasting\ Insulin\ (IU/L)/405\text{"}.$$

Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated by formula:

$$QUICKI = 1/\log\ (Fasting\ Insulin\ u/mL + \log\ (fasting\ glucose\ mg/dL)$$

Peripheral blood mononuclear cell isolation

For isolation of peripheral blood mononuclear cell 2ml blood sample in Na-EDTA vials was obtained from all the subjects. Blood samples were diluted by addition of equal volume of phosphate buffer saline (PBS) followed by density gradient centrifugation using Ficoll-Paque plus (GE Healthcare Bio-Sciences Sweden). After density gradient centrifugation Peripheral blood mononuclear cell (PBMNCs) were isolated and washed twice with PBS to remove any contamination of Ficoll and plasma. The cells thus obtained were stored at -80°C for further processing.

RNA isolation and cDNA Synthesis

Total RNA was isolated from blood leukocytes by TRIzol method [11]. All of the RNA samples were subjected to DNase treatment using Sigma Aldrich DNase treatment kit according to manufacturer's protocol in order to eliminate any traces of genomic DNA. The RNA thus obtained was checked for integrity on 2% agarose gel. Qualitative and quantitative analysis of all the RNA samples was done on NanoDrop (Thermo Scientific). All RNA samples with an absorbance $A_{260/280}$ ratio of 1.9–2.0 were subsequently used for cDNA synthesis by RT-PCR. 1.5mg of total RNA was reverse transcribed into cDNA using Thermo Scientific RevertAid First Strand cDNA Synthesis Kit as per manufacturer's protocol in applied biosystems thermal cycler.

Quantitative real-time polymerase chain reaction

Expression levels of lncRNA CTBP1-AS were measured by Quantitative real-time polymerase chain reaction (qRT-PCR) using 10ml KAPA SYBR® FAST SYBR green , 0.3ml of forward and 0.3ml of reverse primers and cDNA less than 100ng (1ml) in a 20ml reaction following manufacturer's protocol. qRT-PCR was performed in Roche LightCycler® 480 Instrument II 96 well plate having following reaction protocol pre-incubation at 95°C for 5mins, amplification for 40 cycles at 95°C for 20 sec, 62°C for 15 sec and 72°C for 15 sec. Amplification was followed by melting curve analysis with following conditions 95°C for 5sec, 60°C for 1min and 95°C continuous. The amplified product was subsequently run on 2% agarose gel to confirm product size. The relative expression of CTBP1-AS was estimated by Livak method and Beta-Actin was used as reference gene [12]. Each reaction of qRT-PCR was performed in triplicates.

The following primers were used to quantify the lncRNA CTBP1-AS levels: 5´- TTGATGAGGTGGTGGTTGTG -3´ (forward) and 5´ - GACCCTTACTTGTCGGATGG -3´ (reverse), β-Actin RNA was quantified as a control to normalize differences in total RNA levels using the following primers 5´ - ATCGGAACGGTGAAGGTGACA -3´ (forward) and 5´- ATGGCAAGGGACTTCCTGTAAC -3´ (reverse).

Statistical Analysis

All baseline quantitative variables were expressed as mean ± SD. The anthropometric, hormonal and biochemical parametric variables between PCOS and controls and were compared by unpaired student t-test. Chi square test was used to study association of CTBP1-AS expression and various non-parametric variables. The relation between different metabolic and endocrine markers with CTBP1-AS was evaluated by Pearson or Spearman rank correlation coefficient. The expression of CTBP1-AS was divided into

binary groups in both PCOS cases and in healthy controls based on limits derived from the healthy control group as follows <1.81 for the low expression and >1.81 for high expression. Receiver operator characteristic (ROC) and area under curve (AUC) was calculated using Sigma Plot 10. The statistical analysis was done using statistical computing tool vassarstats (http://vassarstats.net/). P- value of <0.05 was considered as statistically significant.

Results

Clinical Characteristics of subjects

The Clinical, hormonal and metabolic characteristics PCOS patients ($n = 105$) and controls ($n = 73$) are summarized in Table 1. The baseline characteristics of controls and PCOS patients like age, menarche, and height were comparable with no statistically significant difference. However, weight, BMI, Waist-Hip ratio (WHR), FG score, was significantly higher in PCOS patients compared to controls.

Various hormones like total Testosterone (59.25 ± 21.40 vs 25.99 ± 8.78, $P < 0.001$), androstenedione (3.40 ± 0.83 vs 2.71 ± 0.64, < 0.001) DHEA-S (3.76 ± 1.17 vs 3.16 ± 1.23, P 0.001), fasting insulin (17.31 ± 5.37 vs 6.24 ± 3.66 < 0.001), LH (9.76 ± 4.89 Vs 6.18 ± 1.64 < 0.001) were significantly higher in PCOS women than controls. While the levels of FSH (6.21 ± 1.55 vs 7.103 ± 1.61, 0.002) and SHBG (44.59 ± 13.66 vs 61.39 ± 20.87 < 0.001) where significantly lower in PCOS cases compared to controls. Details are summarized in table 1.

Expression of lncRNA CTBP1-AS in PCOS women and controls

Shown in Fig. 1, the mean expression level of CTBP1-AS in peripheral blood leukocytes was significantly higher in the PCOS women than that in the healthy controls (-lnCTBP1-AS, 4.23 ± 1.68 versus 1.24 ± 0.29 p < 0.001).

Association Between Expression of lncRNA CTBP1-AS and Presence of PCOS

Further, we sought to evaluate the relationship between different expression levels of CTBP1-AS and risk of PCOS. The subjects were divided into binary groups of high and low expression based on restrictions set by control group. As shown in Table 2, subjects with higher expression level of CTBP1-AS had significantly higher risk of PCOS compared to subjects with low levels of CTBP1-AS expression.

Receiver operator characteristic of lncRNA CTBP1-AS Expression between PCOS cases and healthy controls

Receiver operator characteristic (ROC) curve analysis was performed with the healthy controls as a reference, the area under the curve (AUC) was 0.987, with a standard error of 0.006 and 95% confidence interval of 0.976−0.99 (Fig. 2).

Correlation Between lncRNA CTBP1-AS Level and Clinical Parameters in PCOS

Correlation between CTBP1-AS expression level and various clinical parameters in PCOS cases and in controls was done by calculating Pearson or Spearman rank correlation. The expression of lncRNA CTBP1-AS was used as dependent variable, and clinical parameters both in PCOS cases and in controls as independent variables. As shown in Table 3, no association was found between expression of lncRNA CTBP1-AS and any of the biochemical characteristics .

Association between lncRNA CTBP1-AS expression and Hirsutism

We sought to evaluate the association between expression levels of CTBP1-AS and clinical hyperandrogenic trait, hirsutism in PCOS women. The PCOS cases were divided into binary groups based on presence and absence of hirsutism and expression levels were compared by T-test. As shown in Table 4 no significant association was found between expression of CTBP1-AS and hirsutism (P = 0.64)

Association of CTBP1-AS Expression with total testosterone

To explore the association of lncRNA CTBP1-AS expression with total testosterone (TT). The PCOS subjects were grouped into high TT (> 50ng/dl) and low TT (< 50ng/dl) group and T-test was used to compare the expression levels. No significant association was found between the expression of CTBP1-AS and total testosterone (P = 0.93) (Table 5).

Association between lncRNA CTBP1-AS expression obesity

Association between expression of lncRNA CTBP1-AS and obesity was evaluated by T-test. The subjects were categorised into two groups those having BMI < 25 kg/m^2 and those having BMI > 25 kg/m^2. As shown in table 6, no significant difference in the expression of CTBP1-AS was found between two groups in both cases (P = 0.897) and controls (P = 0.154).

Discussion

This study is first of its kind that investigated the association of lncRNAs CTBP1-AS with the risk of PCOS and its clinical manifestation in North Indian PCOS women. Androgen receptor (AR) regulating lncRNA CTBP1-AS was significantly overexpressed in PCOS women than in healthy controls. We also found that high expression CTBP1-AS as a risk factor for PCOS but has not a significant role in driving various clinical manifestations associated with PCOS.

PCOS is a multisystemic disorder associated with varied immediate complications like hirsutism, acne, alopecia, menstrual irregularities and a range of long term metabolic, cardiovascular, and psychological complications [13,14]. Previous studies have shown that PCOS women are predisposed to obesity [15–18]. Our study showed similar results, BMI (P< .001), WHR (P< .001) were significantly higher in women with PCOS than in healthy women. Studies have shown PCOS women have abnormal metabolic, hormonal parameters [15–20]. Consistently, in our study various metabolic parameters were found deranged in PCOS women. Insulin resistance marker HOMA IR (P < .001) was significantly higher in PCOS women compared

to healthy females and insulin sensitivity was also found significantly lower in PCOS than in controls; QUICKI (P < .001). Women with PCOS are predisposed to high risk liver disease and have elevated levels of liver enzymes [21,22]. Similar results were demonstrated in our study, liver enzymes AST (*P* < .001), ALT (*P* < .001) were significantly higher in PCOS women than in controls.

The etiology of PCOS is still obscure nevertheless, hyperandrogenism is the cardinal feature of PCOS which plays a central development of PCOS [23]. The primary complaints of PCOS like hirsutism, alopecia, Acne, polycystic ovaries, menstrual irregularities are the outcomes of hyperandrogenism [23–25]. Hyperandrogenic effect is either due to high levels of serum testosterone or hyperactivity of androgen receptor. The activity of the androgen receptor is regulated by different coactivators and corepressors [3,26]. Studies have shown hyperactive AR at ovarian and extraovarian sites including hypothalamus, skeletal muscles, and adipocytes play a crucial role in PCOS pathophysiology. The amplified androgenic response via hyperactivity of androgen receptor especially at neuroendocrine site promotes abnormal GnRH pulsation, early follicular development, subsequently follicular arrest, ovulatory dysfunction and other hyperandrogenic manifestations associated with PCOS [23,27,28]. Thus, androgen receptor, its downstream signalling molecules together with coregulators are critical elements for understanding the mechanism of progression of PCOS, its clinical manifestations and development of novel AR based therapeutic treatments.

LncRNAs have a tremendous role in normal cellular processes and development [29–31]. LncRNAs act as regulators of gene expression, post-transcriptionally they can act as regulators by regulating splicing, translation, stability of proteins and mRNA decay etc [32]. Consequently, they assume important role in disease pathogenesis and progression. Studies have shown that dysregulation of lncRNAs are involved in various disorders like cancer, metabolic diseases and other endocrine-related disorders such as diabetes mellitus. Studies have revealed that lncRNAs have been involved in the development and regulation of the female reproductive system moreover, they play a crucial role in the pathogenesis of various gynaecological-related diseases like endometriosis and various gynaecological cancers such as cervical, endometrial, and ovarian cancers [33].

C-Terminal binding protein 1 (CTBP1) is one of the androgen receptor coregulator which plays an important role in androgen signalling by acting as a corepressor of AR thereby decreasing the androgenic effect. The CTBP1 corepressor is in turn regulated by its antisense lncRNA CTBP1-AS. This lncRNA is extensively studied in AR signalling cascade. The role of CTBP1-AS as novel positive regulator of AR transcriptional activity was confirmed by Takayama and co-workers. They showed that CTBP1-AS acts by promoting the repression of CTBP1 protein and it also the releases repressors of AR-regulated genes from their regulatory regions. Thus CTBP1-AS lncRNA promotes hyperactive androgen response and subsequent transcriptional activation of genes regulated by the androgen [9]. In present study, we showed CTBP1-AS is significantly overexpressed (*P* < .001) in PCOS women compared to controls. Further, we also found women with higher expression of CTBP1-AS are at greater risk of developing PCOS than women with lower CTBP1-AS expression. Similar results were reported by Liu et.al, who investigated 23

PCOS women and 17 controls of Chinese ethnicity and found significantly higher CTBP1-AS expression in PCOS women [34]. Taken together these studies suggest CTBP1-AS as an important factor for hyperactivity of AR and development of androgenic disorders like PCOS.

The clinical manifestations of PCOS are varied and complex. Primary clinical manifestations like hirsutism acne alopecia menstrual irregularity are outcomes of multiple biological interactions which may require simultaneous presence of various genetic, epigenetic and environmental factors [35–40]. Although, in present study we showed CTBP1-AS is associated with PCOS. However, we didn't find any correlation between CTBP1-AS expression and various clinical manifestations of PCOS. Hirsutism ($P = 0.74207$) Acne ($P = 0.74207$) Alopecia ($P = 0.689985$) were not significantly associated with expression of CTBP1-AS. Our study is consistent with previous study by carried out by Liu et.al, on Chinese women with PCOS [34]. This may be attributed to the fact that in this complex syndrome, multiple factors come into the play and act together so as to bring about a phenotypic change or a specific clinical manifestation. We assume that CTBP1-AS may be one of these contributing factors that in association with other factors is responsible for development of various clinical manifestations in PCOS. However, further mechanistic insights are needed so as to understand how and to what extent CTBP1-AS influences the different phenotypic features in PCOS.

We further evaluated the correlation of CTBP1-AS expression with different hormonal and biochemical parameters. We didn't find any significant correlation between CTBP1-AS expression and various hormonal or biochemical parameters. Testosterone ($P = 0.99$), Insulin ($P = 0.53$), SHBG ($P = 0.67$), LH ($P = 0.51$), FSH ($P = 0.72$), Androandrostenrdione ($P = 0.93$) didn't show significant correlation with CTBP1-AS expression. However, contrary to our results, Lui and co-workers found a positive correlation between expression of CTBP1-AS and serum total testosterone ($P = 0.027$) in a small case control study on Chinese women (cases/controls 23/17) [34]. The possible reason for such correlation may be because of small sample size in their study. Furthermore, it is believed that CTBP1-AS affects downstream signalling of androgen by enhancing the transcriptional activity of AR and may not have direct effect on biosynthesis of androgens. However, more elaborated studies need to be carried out to so as to understand the effect of CTBP1-AS on biosynthesis of testosterone.

Conclusion

In conclusion, our study showed that AR coregulating lncRNA CTBP1-AS is associated with PCOS women and high expression of CTBP1-AS is a risk factor for PCOS in Kashmiri women. Higher expression levels of CTBP1-AS may act as an important factor for hyperactivity of AR and may contribute towards the pathophysiology of PCOS. However, further investigations are needed to understand the mechanism of action of CTBP1-AS and identify signalling pathway. This will help to further understand the role played by lncRNA CTBP1-AS in pathophysiology of PCOS, its clinical manifestations and in identifying therapeutic targets.

Declarations

Funding

Conflict of interest

On behalf of all the authors, the corresponding author declares that there is no conflict of interest.

Availability of data and materials

The data and materials will be provided by authors on reasonable request.

Authors' contributions

M.N.: Major work done, Manuscript Writing, Project Development, Recruitment of Participants, Sample collection, Data Collection,

S.M.A: Primer designing and qPCR validation.

S.U.A: Data Collection, Manuscript Revision.

S.A. : Recruitment of Participants, Manuscript revision, data collection

I.M.: Diagnosis and recruitment of Cases

S.A.: Conception and design, Manuscript writing, Project development and management.

All authors read and approved the final draft.

Ethics approval

This study was approved by the ethical committee Government Medical College Srinagar under ethical approval no. 94/ETH/GMC/ICMR

Consent of participation

All participants were recruited after written informed consent was obtained from them.

Consent for publication

All authors have approved the manuscript for submission.

Acknowledgements

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Tables

Table 1
Clinical Characteristic of Controls and Patients With PCOS.

Variables	PCOS cases (n = 105)	Controls (n = 73)	P-Value
Age (years)	23.24 ± 3.87	24.08 ± 2.20	0.15
Weight(kg)	63.91 ± 9.07	55.06 ± 5.25	< 0.001*
Height (cm)	157.86 ± 5.42	156.65 ± 5.36	0.13
BMI(kg/m2)	25.63 ± 3.40	22.43 ± 1.84	< 0.001*
Waist (cm)	87.05 ± 10.22	78.04 ± 6.62	< 0.001*
Hip (cm)	94.44 ± 7.47	90.50 ± 6.19	0.002*
WHR	0.94 ± 0.076	0.86 ± 0.059	< 0.001*
Menarche (years)	13.02 ± 1.26	13.36 ± 0.82	0.122
FG Score	16.34 ± 5.78	4.23 ± 2.10	< 0.001*
LH (IU/L)	9.76 ± 4.89	6.18 ± 1.64	< 0.001*
FSH (IU/L)	6.21 ± 1.55	7.10 ± 1.61	0.002*
LH: FSH	1.60 ± 0.77	0.88 ± 0.20	< 0.001*
TT (ng/dL)	59.25 ± 21.40	25.99 ± 8.78	< 0.001*
SHBG (nmol/L)	44.59 ± 13.66	61.39 ± 20.87	< 0.001*
Andro (ng/mL)	3.40 ± 0.83	2.71 ± 0.64	< 0.001*
DHEAS (ng/mL)	3.76 ± 1.17	3.16 ± 1.23	0.001*
Insulin F (μIU/ml)	17.31 ± 5.37	6.24 ± 3.66	< 0.001*
Glu F (mg/dL)	89.12 ± 8.50	84.57 ± 8.57	0.001*
Glu 2h (mg/dL)	125.64 ± 21.82	105.68 ± 15.21	< 0.001*
Chol (mg/dL)	169.72 ± 29.77	148.13 ± 20.69	< 0.001*
TG (mg/dL)	128.12 ± 39.43	104.33 ± 13.10	< 0.001*
HDL (mg/dL)	43.48 ± 5.82	47.24 ± 7.50	< 0.001*
LDL (mg/dL)	104.17 ± 22.57	95.75 ± 12.20	0.003*
Urea (mg/dL)	24.48 ± 9.43	21.53 ± 4.16	0.02*
Creatinine (mg/dL)	0.88 ± 0.35	0.76 ± 0.11	0.006*
FAI	4.92 ± 2.04	1.66 ± 0.75	< 0.001*

Variables	PCOS cases (n = 105)	Controls (n = 73)	P-Value
HOMA-IR	3.81 ± 1.27	1.30 ± 0.78	< 0.001*
QUICKI	0.31 ± 0.02	0.39 ± 0.03	< 0.001*
AST/SGOT U/L	29.98 ± 13.88	20.02 ± 6.30	< 0.001*
ALT/SGPT U/L	31.23 ± 17.38	23.03 ± 5.16	< 0.001*

Data presented as Mean ± SD. *P-value <0.05 significant. P values calculated by Independent Student's t-test.

PCOS; polycystic ovary syndrome, BMI; body mass index, WHR; Waist to hip ratio, FG; Score ferrimangallwey score, LH; luteinizing hormone, FSH; follicle stimulating hormone, TT; total testosterone, SHBG; sex hormone binding globin, Andro; Androandrostenrdione, DHEAS; dihydroepian-drostenedionesulphate, Glu F; glucose fasting, CHOL; cholesterol, TG; triglycerides, HOMA IR; homeostasis model assessment-estimated insulin resistance, QUICKI; quantitative insulin sensitivity check index, FAI; free androgen index, AST aspartate aminotransferase, ALT alanine aminotransferase

Table 2
Odds Ratio of PCOS Events by Expression of CTBP1-AS.

CTBP1-AS Expression	Cases	Controls	Odds ratio	95% CI	P
Low < 1.81	21 (20.0%)	54 (73.97%)	11.36	5.59−23.08	< 0.001*
High > 1.81	84 (80.0%)	19 (26.02%)			

Abbreviations: CTBP1-AS; C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome; OR, odds ratio; 95% CI, confidence intervals. CTBP1-AS expression for binary groups cut offs were <1.81 for the low expression, >1.81 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 X^2 Test. P values < 0.05 is in bold

Table 3
Partial Pearson or Spearman Rank Correlation Coefficients of the
Expression of CTBP1-AS and Patients' Characteristics

Parameter	CASES		CONTROLS	
	r	p-value	r	p-value
BMI	-0.0517	0.597253	0.0814	0.531127
WHR	0.077	0.437180	-0.2291	0.075390
FG score	-0.111	0.261101	0.1873	0.149595
DHEAS	0.0487	0.625176	-0.1397	0.284537
Andro	-0.0079	0.936392	-0.1051	0.421195
SHBG	0.0416	0.675360	0.1478	0.254784
Testo	-0.0006	0.992041	-0.1039	0.426920
FAI	0.006	0.952272	-0.1989	0.124108
LH	0.0645	0.510727	-0.0605	0.640088
FSH	0.0343	0.727053	0.0582	0.654360
LH:FSH	0.0379	0.697342	-0.089	0.492899
INSULIN	-0.0622	0.530097	-0.0665	0.611954
FASTING GLU	0.1104	0.261101	-0.0237	0.857769
2HR	0.0858	0.386323	0.0797	0.544204
CHOL	0.0607	0.536627	-0.0424	0.742569
TG	0.007	0.944329	0.0113	0.928592
LDL	-0.0579	0.556483	-0.1085	0.404300
HDL	0.087	0.375540	0.1123	0.387826
HOMA	-0.0194	0.841875	-0.0591	0.654360
HIRSUTISM	0.0326	0.74207	0.0591	0.65436
CYCLES	-0.0643	0.517139	-0.0102	0.432692
ACNE	-0.065	0.510727	-0.0043	0.976168
ALOPECIA	-0.039	0.689985	-0.0884	0.499164

BMI; body mass index, WHR; Waist to hip ratio, FG; Score Ferriman Gallwey score, LH; luteinizing
hormone, FSH; follicle stimulating hormone, TT; total testosterone, SHBG; sex hormone binding globin,

Andro; Androandrostenrdione, DHEAS; dihydroepian-drostenedionesulphate, Glu F; glucose fasting, CHOL; cholesterol, TG; triglycerides, HOMA IR; homeostasis model assessment-estimated insulin resistance, FAI; free androgen index. The correlation coefficient (r).

Table 4

Association between expression of lncRNA CTBP1-AS and Hirsutism

Parameter	PCOS		
Hirsutism	Present (n = 93)	Absent (n = 12)	p-value
CTBP1-AS Expression (Fold change)	4.20 ± 1.65	4.44 ± 2.03	0.64

Abbreviations: CTBP1-AS; C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome

Table 5

Association between expression of lncRNA CTBP1-AS and TT

Parameter	PCOS		
Total Testosterone	> 50ng/dl (n = 69)	< 50ng/dl (n = 36)	P-value
CTBP1-AS Expression (Fold change)	4.22 ± 1.67	4.26 ± 1.76	0.93

Abbreviations: CTBP1-AS; C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome
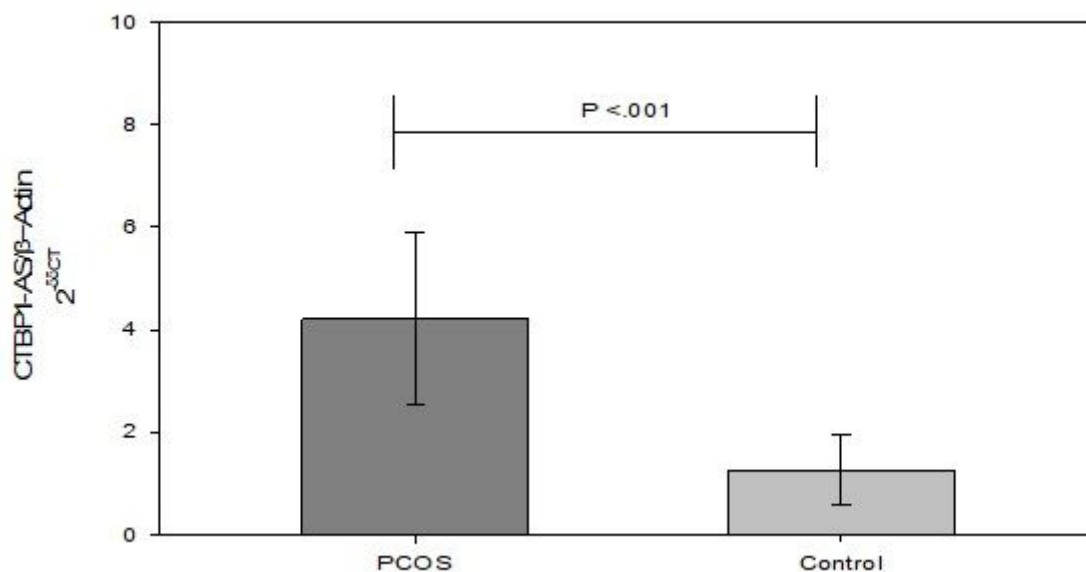
Table 6

Association between expression of lncRNA CTBP1-AS and obesity

Parameter	PCOS			controls		
BMI	< 25 kg/m^2) (n = 52)	> 25 kg/m^2) (n = 53)	P-value	< 25 kg/m^2) (n = 60)	> 25 kg/m^2) (n = 13)	P-value
CTBP1-AS Expression (Fold change)	4.18 ± 1.81	4.23 ± 1.56	0.897	1.2 ± 0.67	1.66 ± 0.80	0.154

Abbreviations: CTBP1-AS; C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome BMI; body mass index
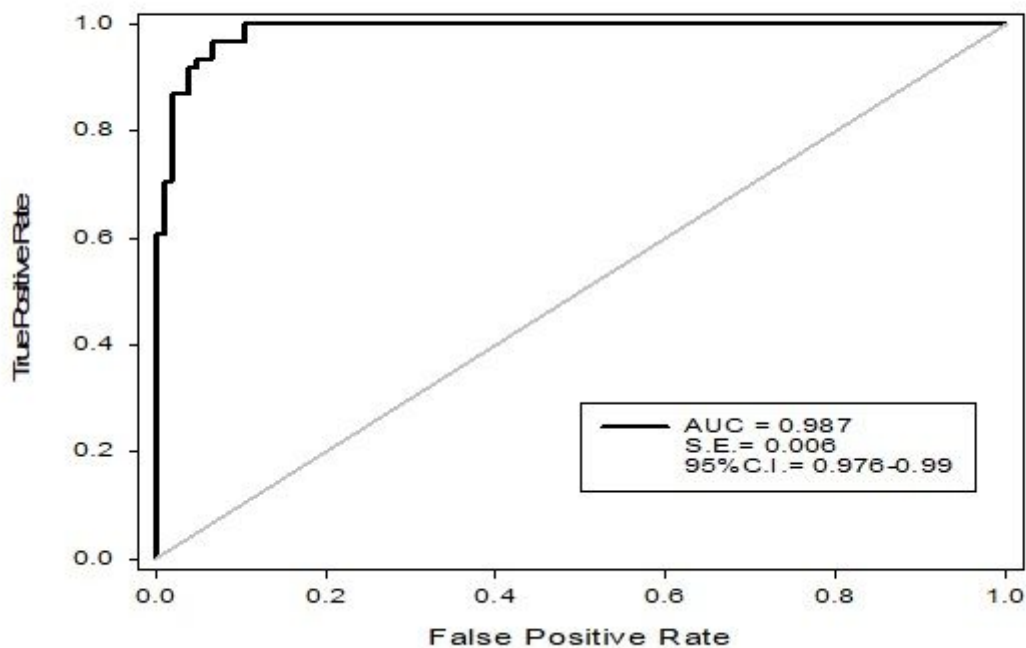
Figures

Figure 1

The expression profile of CTBP1-AS in peripheral blood leukocytes in PCOS cases and healthy controls measured by quantitative real- time PCR. Data are expressed as mean ± SD *P < 0.05. CTBP1-AS indicates C-terminal binding protein 1 antisense; PCOS, polycystic ovary syndrome

Figure 2

ROC curve analysis of lncRNA CTBP1-AS in PCOS cases and healthy controls.