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Original Research

Plasma Circular RNAs as Potential Biomarkers to Assist in the Diagnosis of Postpartum Depression

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Abstract

Background: This research sought to examine alterations in plasma circular RNA (circRNA) expression in women with postpartum depression (PPD), assessing its potential utility as an auxiliary diagnostic biomarker for this condition. **Methods**: Women examined at 42 days postpartum were recruited between June 2024 and December 2024, during which plasma samples and relevant data were collected. A discovery cohort was established, consisting of 3 women with PPD and 3 control participants. This was followed by a validation cohort of 50 women with PPD and 50 controls, identified using the Structured Clinical Interview. The discovery cohort underwent plasma circRNA microarray analysis, whereas the validation cohort used reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to quantify candidate circRNAs and five circRNAs previously associated with major depressive disorder (MDD). **Results**: Plasma levels of circRNA derived from the Interferon Gamma Receptor 2 gene (*circIFNGR2*), circRNA derived from the ATP-Binding Cassette Subfamily C Member 5 gene (*circABCC5*), circRNA derived from the Activating Transcription Factor 7 Interacting Protein gene (*circATF7IP*) were significantly upregulated in the PPD group, whereas circRNA derived from the Dystrophia Myotonica Protein Kinase gene (*circDYM*) was significantly downregulated ($p < 0.05$). The area under the curve (AUC) of the four circRNAs, circIFNGR2, circABCC5, circDYM, and circATF7IP, was 0.62 (sensitivity 28%, specificity 96%), 0.64 (sensitivity 36%, specificity 96%), 0.69 (sensitivity 54%, specificity 84%), and 0.65 (sensitivity 62%, specificity 72%), respectively. The joint AUC of circIFNGR2, circABCC5, circDYM, and circATF7IP was 0.80 (sensitivity 78%, specificity 70%). After adjustment for covariates, circIFNGR2, circABCC5, and circATF7IP remained independent predictive factors. **Conclusions**: Changes in the levels of circIFNGR2, circABCC5 identified by microarray analysis, as well as circDYM, and circATF7IP associated with MDD, were associated with the occurrence of PPD. These findings support the potential utility of circRNAs as adjunct diagnostic biomarkers for PPD and offer valuable insights into the molecular mechanisms underlying the disorder. **Study Registration**: The study has been registered on https://zenodo.org/ (registration number: zenodo. 17906223; registration link: https://zenodo.org/records/17906223).

Keywords: postpartum depression; circular RNA; plasma; biomarker

1. Introduction

Postpartum depression (PPD) encompasses depressive episodes of varying severity occurring within one year after childbirth. Persistent low mood, a marked reduction in interest or pleasure in activities, and feelings of fatigue or diminished energy constitute the three core symptoms [1]. As an obstetric complication, PPD represents a major contributor to maternal mortality worldwide [2,3], with an estimated prevalence of approximately 23% [4]. PPD can severely impair maternal physical and mental health, disrupt family relationships, and exert long-term adverse effects on a child's cognitive, emotional, and behavioral development [5–8].

Recognition and diagnosis of PPD remain challenging. Postpartum changes in sleep, appetite, and energy related to infant care often overlap with depressive symptoms, making differentiation difficult. Additionally, some women are reluctant to report mood changes due to cultural stigma. Although the American College of Obstetricians and Gynecologists (ACOG) recommends routine depression screening at least once during pregnancy and the postpartum period, such screening is effective only when patients who screen positive receive proper diagnosis, treatment, or referral for appropriate medical care [9,10]. Many obstetric clinicians are unable to provide a diagnosis of depression without psychiatric support, and one study reported a referral rate of only 8% among patients who screened positive [11]. Consequently, only a minority of patients access mental health services, and an even smaller proportion receives comprehensive treatment.

With advances in biomedical technology, an increasing number of studies are now exploring biological markers. In mammals, over 98% of gene transcripts are non-protein-coding RNAs (ncRNAs), which perform essential regulatory roles in diverse biological processes [12,

13]. Circular RNAs (circRNAs), a covalently closed-loop subtype of ncRNAs, were long regarded as splicing by-products due to their unique structure, unknown functions, and low expression levels. However, with advances in bioinformatics and high-throughput sequencing technologies, many circRNAs have been found to play roles in the pathogenesis of diverse neuropsychiatric disorders [14–17]. Moreover, owing to their intrinsic resistance to exonucleases such as Ribonuclease R (RNase R), circRNAs exhibit superior stability to their linear counterparts [18], highlighting their potential as diagnostic biomarkers in clinical settings.

Given the diverse pathogenesis of PPD, we also include 5 circRNAs previously validated by our group as differentially expressed in major depressive disorder (MDD): circRNA derived from the Homeodomain Interacting Protein Kinase 2 gene (*circHIPK2*), circRNA derived from the Zinc Finger and BTB Domain Containing 25 gene (*circZBTB25*), circRNA derived from the Activating Transcription Factor 7 Interacting Protein gene (*circATF7IP*), circRNA derived from the Dystrophia Myotonica Protein Kinase gene (*circDYM*), and circRNA derived from the Stromal Antigen 1 gene (*circSTAG1*). Functional studies in animal models indicated that therapeutic intervention targeting these circRNAs effectively mitigated depressive-like behaviors in mice [19–22]. These findings suggest that circRNAs have potential as objective diagnostic biomarkers for depression. To date, no comparable studies have examined this relationship in patients with PPD. This study aims to preliminarily explore and validate the potential association between plasma circRNA levels and PPD by analyzing samples collected from women at 42 days postpartum.

2. Materials and Methods

2.1 Study Population and Methodology

A discovery cohort of three women with PPD and three control participants, along with a validation cohort (n = 50 with PPD, n = 50 controls), was recruited from women who attended a 42-day postpartum follow-up visit at Zhongda Hospital and Nanjing Women and Children's Healthcare Hospital between June 2024 and December 2024. All participants completed the psychological questionnaire, including the Edinburgh Postnatal Depression Scale (EPDS), Social Support Rating Scale (SSRS), Pittsburgh Sleep Quality Index (PSQI), and sociodemographic questionnaires under the guidance and supervision of a trained physician, following predefined inclusion and exclusion criteria. The control group was defined by EPDS scores ($\leq$4), while the PPD group was defined by EPDS scores ($\geq$13) and the clinical structured interview based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), respectively. All participants provided written informed consent prior to the commencement of the study.

Participant selection criteria were as follows: (1) age from 18 years to 45 years, who received routine prenatal care, delivery, and postpartum follow-up at the participating hospital; (2) no serious cardiovascular, neurological, pulmonary, or renal diseases; (3) currently breastfeeding and not taking any medication; and (4) submission of a signed informed consent form. Participant exclusion criteria were: (1) twin pregnancy; (2) pre-existing diabetes before conception; (3) pre-pregnancy hypertensive disorders and preeclampsia; (4) hyperthyroidism; (5) history of miscarriage or stillbirth; (6) clinical diagnosis of schizophrenia and bipolar disorder; (7) family history of mental illness; (8) lack of clinical information; and (9) missing questionnaire information and incomplete clinical information.

2.2 Instruments

2.2.1 Edinburgh Postnatal Depression Scale (EPDS)

The EPDS was used to assess PPD in mothers and consisted of 10 questions with a total score of 30 points. Scores $\geq$13 suggested moderate to severe depression. The internal Cronbach's α = 0.94.

2.2.2 Social Support Rating Scale (SSRS)

The SSRS was used to quantify an individual's access to practical help, emotional support, and active support-seeking from family, friends, and social networks. It included 10 entries divided into three dimensions: objective support (three entries), subjective support (four entries), and utilization of social support (three entries). Higher scores indicated better social support. The internal Cronbach's α = 0.90.

2.2.3 Pittsburgh Sleep Quality Index (PSQI)

The PSQI is a widely employed instrument for assessing sleep disturbances in clinical and research settings, encompassing seven dimensions and scored on a 0–21 scale where elevated scores denote poorer sleep quality. The internal Cronbach's α = 0.89.

2.2.4 Sociodemographic Characteristics and Clinical Baseline Data

Sociodemographic information was collected through a custom-designed questionnaire in Chinese. This covered various domains, including age, marital status, ethnicity, educational status, religious belief, mood disorders, number of pregnancies, number of births, and mode of delivery. Clinical laboratory data covering the antenatal and postpartum periods were retrieved from the institutional electronic medical records. This encompassed assessments of thyroid and liver function from mid to late pregnancy, as well as complete blood count parameters during the postpartum period.

2.3 Sample Collection and Processing

Venous blood samples (2 mL) were collected from the antecubital vein of each participant in the early morning following an overnight fast, using K2EDTA-coated vacuum blood collection tubes (ST750EK, Streck, NE, USA). After gentle inversion, samples underwent centrifugation (1000 $\times$g, 15 min, 4 °C). The resulting plasma supernatant was then aliquoted into nuclease-free vials for cryopreservation at –80 °C until analysis.

2.4 Detection of Plasma circRNA

For circRNA microarray analysis, sample preparation and microarray hybridization were performed following the Arraystar microarray protocols (AS-S-CR-M-1.0 and ASLNC-MV3.0, Arraystar, TX, USA). To enrich circRNAs, total RNA samples were digested with RNase R (RNR07250, Epicentre, WI, USA) to remove linear RNAs. The enriched circRNAs were then amplified and transcribed into fluorescent circRNA utilizing a random priming method (Arraystar Super RNA Labeling Kit; AS-MS-0250, Arraystar, TX, USA). Labeled circRNAs were hybridized onto the Arraystar Human circRNA Array V2 (8x15K, Arraystar, TX, USA). After washing, the slides were scanned using the Agilent Scanner G2505C (G2505C, Agilent Technologies, CA, USA), and array images were analyzed with Agilent Feature Extraction software (version 11.0.1.1, Agilent Technologies, CA, USA).

Validation of differentially expressed circRNAs: 22 circRNAs identified as differentially expressed in the microarray analysis were selected for validation. Among them, 5 circRNAs (*circDYM, circHIPK2, circZBTB25, circSTAG1, circATF7IP*) that had previously been reported to be closely associated with MDD are included for validation using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). In the validation cohort, total RNA was extracted from plasma using the miRNeasy® Serum/Plasma Kit (cat. No. 217184, Qiagen, MD, USA) according to the manufacturer's instructions. RNA concentrations were detected using the One Drop spectrophotometer (Thermo Fisher Scientific, MA, USA). Reverse transcription was performed using the HiScript III All-in-one RT SuperMix Perfect for qPCR Kit (R333-C1, Vazyme, Nanjing, Jiangsu, China). The reaction system was configured, and amplification was carried out using SYBR Green Premix Pro Taq HS qPCR Tracking Kit (AG11733, Accurate Biology, Changsha, Hunan, China) following the manufacturer's protocol, with cDNA as the template. Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. Controls were normalized for each experiment. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. We employed the "reverse primer approach" by designing upstream and downstream primers on either side of the circRNA-specific antisense splicing site, allowing the primers to extend outward across the junction. Simultaneously, we verified the single peak in the melting curve to ensure primer specificity. Primers were synthesized by Geneary (Shanghai, China), and the primer sequences are shown in **Supplementary Table 1**.

2.5 Statistical Methods

SPSS version 26.0 (International Business Machines Corporation, Armonk, NY, USA) and GraphPad Prism version 8.0.2 (GraphPad Software, Boston, MA, USA) were used for data analysis. The normality of continuous variables was assessed using the Shapiro-Wilk test, while the categorical variables were analyzed using the chi-square test. For continuous variables, an independent samples *t*-test was employed when the data were normally distributed; otherwise, the Mann-Whitney U-test was used. Spearman's rank correlation was utilized to examine linear relationships between levels of circRNAs and severity of PPD, degree of social support, and sleep condition. To adjust for potential confounders, both univariate and multivariate logistic regression models were employed, reporting odds ratios (ORs) with corresponding 95% confidence intervals (CIs). The diagnostic efficacy of candidate circRNAs for PPD was assessed by the area under the curve (AUC) of the receiver operating characteristic (ROC), with the Youden index determining the optimal sensitivity and specificity cutoff. Statistical significance was defined as a two-tailed p-value $<$ 0.05.

3. Results

3.1 Sociodemographic Characteristics and Clinical Data

In the discovery cohort, the mean ages of the PPD and control groups were 30.33 $\pm$ 5.86 and 27.67 $\pm$ 1.16 years, respectively. The average gestational age at delivery was 38.76 $\pm$ 1.00 weeks for the PPD group and 39.62 $\pm$ 0.36 weeks for the control group. The postpartum body mass index (BMI) was 23.77 $\pm$ 2.24 and 21.83 $\pm$ 0.50, respectively. All participants were Han Chinese, married, and had no history of anxiety or depression. Additional sociodemographic data, clinical data, and laboratory findings are summarized in **Supplementary Table 2**. The validation cohort consisted of 50 individuals with PPD and 50 controls. Univariate analysis identified statistically significant differences between the two groups in several factors, including natural conception, spontaneous labor, history of anxiety and depression, exercise during pregnancy, white blood cell (WBC) levels, thyroid-stimulating hormone (TSH) levels, SSRS scores, PSQI scores, and self-injurious ideation ($p <$ 0.05) (Table 1). Univariate logistic analysis identified the following risk factors for PPD: a history of pre-pregnancy anxiety or depression (OR = 14.75, 95% CI: 1.67–1940.94, p = 0.01), assisted reproduction (OR = 3.92, 95% CI: 1.01–15.22, $p < 0.05$), postpartum WBC levels (OR = 1.44, 95% CI: 1.05–1.98, p = 0.03), gestational TSH levels (OR = 1.51, 95% CI: 1.00–2.27, $p < 0.05$), and PSQI scores suggesting sleep disturbance (OR = 2.21, 95% CI: 1.62–3.03, $p <$

Table 1. Sociodemographic characteristics and clinical data of the validation cohort.

Characteristics	N (n = 50)	PPD (n = 50)	t/U/χ^2	p-value
Demographic characteristics				
Age (years), mean (SD)	30.54 (3.62)	31.28 (3.81)	–0.99	0.32
Married, n (%)	48.00 (96.00)	50.00 (100.00)	-	0.50$^{\$}$
Han nationality, n (%)	47.00 (94.00)	48.00 (96.00)	-	1.00$^{\$}$
Undergraduate and junior college, n (%)	32.00 (64.00)	38.00 (76.00)		
Master's degree or above, n (%)	15.00 (30.00)	8.00 (16.00)	2.79	0.25
Junior high school, vocational high school, and below, n (%)	3.00 (6.00)	4.00 (8.00)		
Having religious belief, n (%)	2.00 (4.00)	5.00 (10.00)	-	0.44$^{\$}$
A history of pre-pregnancy anxiety or depression, n (%)	0.00 (0.00)	6.00 (12.00)	-	0.03$^{\$}$
Gravidity, median (interquartile range)	1.00 (1.00, 2.00)	1.00 (1.00, 2.00)	1249.50	1.00
Parity, median (interquartile range)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1225.50	0.79
Planning pregnancy, n (%)	39.00 (78.00)	36.00 (72.00)	0.48	0.49
Natural conception, n (%)	47.00 (94.00)	40.00 (80.00)	4.33	0.04
Exercise during pregnancy, n (%)	46.00 (92.00)	36.00 (72.00)	6.78	0.01
Spontaneous labor, n (%)	20.00 (40.00)	30.00 (60.00)	4.00	$<$0.05
Gestational weeks at delivery, median (interquartile range)	39.79 (38.71, 40.18)	39.14 (38.71, 40.00)	1070.50	0.22
Postpartum BMI (kg/m^2), mean (SD)	23.10 (2.87)	23.37 (3.08)	–0.45	0.66
Newborn is a boy, n (%)	23.00 (46.00)	27.00 (54.00)	0.64	0.42
Laboratory parameters, mean (sd)/median (interquartile range)				
WBC, (10^9/L)	6.26 (5.71, 7.30)	6.62 (6.03, 7.93)	964.50	$<$0.05
RBC, (10^{12}/L)	4.48 (4.33, 4.67)	4.58 (4.42, 4.75)	1040.00	0.15
HGB, (g/L)	132.50 (127.00, 138.00)	134.50 (128.00, 139.00)	1147.50	0.48
Platelets, (10^9/L)	240.50 (207.50, 275.00)	243.00 (205.00, 278.25)	1223.00	0.85
NEUT count, (10^9/L)	3.48 (3.08, 4.23)	3.90 (3.21, 4.97)	991.00	0.07
LYMPH count, (10^9/L)	2.17 (1.89, 2.55)	2.32 (2.08, 2.63)	1064.50	0.20
MONO count, (10^9/L)	0.36 (0.31, 0.42)	0.37 (0.31, 0.45)	1184.00	0.65
ALB, (g/L)	35.65 (34.15, 37.30)	36.20 (34.60, 37.65)	1092.00	0.28
TC, (mmol/L)	6.85 (5.96, 7.56)	7.15 (6.43, 7.96)	959.50	0.06
ALP, (U/L)	148.85 (115.63, 186.80)	139.55 (109.75, 183.33)	1179.00	0.63
LDH-L, (U/L)	179.45 (161.40, 199.43)	181.65 (166.00, 196.03)	1194.50	0.70
TSH, (μIU/mL)	1.77 (1.29, 2.38)	2.25 (1.48, 3.00)	934.00	0.04
FT4, (pmol/L)	13.05 (11.80, 14.40)	12.70 (11.95, 13.60)	1082.00	0.32
Anti-TPO, (IU/mL)	13.25 (10.68, 18.88)	12.90 (9.00, 20.90)	1158.00	0.63
Questionnaire				
PSQI score, median (interquartile range)	4.00 (1.00, 5.25)	9.00 (7.00, 11.00)	216.00	$<$0.001
SSRS score, mean (sd)	42.12 (4.72)	33.78 (5.63)	8.03	$<$0.001
Positive self-harm intention, n (%)	0.00 (0.00)	30.00 (60.00)	-	$<$0.001$^{\$}$

Note: N, control group; t, Student's t-test; U, Mann-Whitney U test; χ^2, Chi-square Test; $^{\$}$, Fisher's exact test.

Abbreviations: PPD, postpartum depression; SD, standard deviation; BMI, body mass index; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; NEUT, neutrophil; LYMPH, lymphocyte; MONO, monocyte; ALB, albumin; TC, total cholesterol; ALP, alkaline phosphatase; LDH-L, lactate dehydrogenase-L form; TSH, thyroid-stimulating hormone; FT4, free thyroxine; Anti-TPO, anti-thyroid peroxidase antibody; SSRS, Social Support Rating Scale; PSQI, Pittsburgh Sleep Quality Index.

0.001). Protective factors included cesarean section (OR = 0.44, 95% CI: 0.20–0.99, $p < 0.05$), exercise during pregnancy (OR = 0.22, 95% CI: 0.07–0.74, $p = 0.01$), and SSRS score suggestive of good social support (OR = 0.74, 95% CI: 0.66–0.83, $p < 0.001$) (Table 2).

3.2 Differential Expression and Correlation Analysis of circRNAs in Plasma

CircRNA microarrays were used to compare plasma circRNA levels between PPD patients and matched controls in the discovery cohort. Among the 7683 circRNAs detected by microarray profiling (Fig. 1A–C), 22 were found to be differentially expressed between the PPD group and the control group (|Fold Change| >1.20, $p < 0.05$), including 12 upregulated and 10 downregulated circRNAs. Since primers could not be designed for two circRNAs, only 20 differentially expressed circRNAs were validated in the validation cohort (|Fold Change| >1.2 and $p < 0.05$). The complete microarray screening results and the corresponding RT-qPCR findings from the validation cohort are pre-

Table 2. Univariate logistic regression results for PPD.

Variable	B	SE	OR (95% CI)	*p*-value
A history of pre-pregnancy anxiety or depression	2.69	1.48	14.75 (1.67, 1940.94)	0.01[#]
Assisted reproduction	1.37	0.69	3.92 (1.01, 15.22)	<0.05
Cesarean section delivery	–0.81	0.41	0.44 (0.20, 0.99)	<0.05
Exercise during pregnancy	–1.50	0.61	0.22 (0.07, 0.74)	0.01
WBC, (10^9/L)	0.36	0.16	1.44 (1.05, 1.98)	0.03
TSH, (μIU/mL)	0.41	0.21	1.51 (1.00, 2.27)	<0.05
SSRS scores	–3.00	0.06	0.74 (0.66, 0.83)	<0.001
PSQI scores	0.79	0.16	2.21 (1.62, 3.03)	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; [#], perform a firth regression test.

sented in **Supplementary Table 3**. Among the differentially expressed circRNAs, circIFNGR2 ($p < 0.05$) and circABCC5 ($p = 0.01$) exhibited significantly increased expression levels in the PPD group, consistent with the microarray results. CircDYM ($p < 0.01$) had decreased expression levels in the PPD group, and circATF7IP ($p = 0.01$) had elevated expression levels, consistent with previously reported expression trends in MDD-related studies (Fig. 1D–G).

Spearman correlation analysis demonstrated that circIFNGR2 was not correlated with EPDS scores (r = 0.123, $p = 0.224$), SSRS scores (r = –0.158, $p = 0.117$), or PSQI scores (r = 0.152, $p = 0.132$). CircABCC5 was not correlated with EPDS scores (r = 0.192, $p = 0.056$) or SSRS scores (r = –0.095, $p = 0.350$) but was positively associated with PSQI scores (r = 0.199, $p = 0.047$). CircDYM was not significantly correlated with EPDS scores (r = –0.155, $p = 0.123$) but was positively associated with SSRS scores (r = 0.219, $p = 0.029$) and negatively associated with PSQI scores (r = –0.248, $p = 0.013$). CircATF7IP was positively correlated with EPDS scores (r = 0.236, $p = 0.018$) and PSQI scores (r = 0.337, $p = 0.0006$) and negatively associated with SSRS scores (r = –0.306, $p = 0.002$) (Fig. 2). Further studies indicate that only circABCC5 level shows a positive correlation with WBC count (r = 0.205, $p = 0.041$) (**Supplementary Fig. 1**).

3.3 The Value of Differentially Expressed circRNA in the Diagnosis of PPD

CircIFNGR2 showed an AUC of 0.62 (95% CI: 0.51–0.73, $p < 0.05$), with a sensitivity of 28% and a specificity of 96%. CircABCC5 exhibited an AUC of 0.64 (95% CI: 0.53–0.75, $p = 0.01$), with a sensitivity of 36% and a specificity of 96%. CircDYM showed an AUC of 0.69 (95% CI: 0.58–0.79, $p = 0.01$), with a sensitivity of 54% and specificity of 84%. Lastly, CircATF7IP showed an AUC of 0.65 (95% CI: 0.54–0.76, $p = 0.01$), with a sensitivity of 62% sensitivity and 72% specificity. The combined model incorporating circIFNGR2, circABCC5, circDYM, and circATF7IP yielded an AUC of 0.80 (95% CI: 0.71–0.88, $p < 0.0001$), with a sensitivity of 78% and a specificity of 70% (Fig. 3A,B, **Supplementary Table 4**). Multivariate logistic regression analysis adjusting for assisted reproduction, cesarean section delivery, history of anxiety and depression, exercise during pregnancy, WBC levels, and TSH levels identified that circIFNGR2 (adjusted OR = 2.26, 95% CI: 1.21–4.23, $p = 0.01$), circABCC5 (adjusted OR = 2.09, 95% CI: 1.35–3.23, $p < 0.01$), and circATF7IP (adjusted OR = 1.74, 95% CI: 1.01–3.02, $p < 0.05$) as independent predictive factors for PPD (Table 3). The combined model incorporating circIFNGR2, circABCC5, and circATF7IP yielded an AUC of 0.75 (95% CI: 0.65–0.85, $p < 0.0001$), with a sensitivity of 66% and a specificity of 80%. The AUC for the combination of circIFNGR2 and circABCC5 was 0.67 (95% CI: 0.58–0.79, $p < 0.01$), with a sensitivity of 54% and a specificity of 82%. The AUC for the combination of circIFNGR2 and circATF7IP was 0.69 (95% CI: 0.59–0.79, $p < 0.01$), with a sensitivity of 52% and a specificity of 80%. The AUC for the combination of circABCC5 and circATF7IP was 0.72 (95% CI: 0.62–0.82, $p < 0.0001$), with a sensitivity of 60% and a specificity of 76% (Fig. 3C–F, **Supplementary Table 4**).

4. Discussion

PPD is widely recognized as a multifactorial disorder involving biological, psychological, and social factors [23,24]; however, its precise pathogenesis remains unclear. Investigating circRNAs as auxiliary diagnostic markers for PPD may help overcome the subjectivity and limitations inherent to screening questionnaires, offering an objective molecular basis for diagnosis. In this study, individual circRNAs showed AUC values ranging from 0.62 to 0.69, with low sensitivity (28%–62%) but high specificity (72%–96%). When the 4 circRNAs were combined into a diagnostic panel, the AUC increased to 0.80, with a sensitivity of 78% and specificity of 70%. Furthermore, comparing diagnostic models with 2 versus 3 circRNAs revealed that increasing the number of circRNAs in the model gradually improved both the AUC and sensitivity. These findings indicate that although individual circRNAs possess diagnostic potential, their discriminative power alone is limited, likely reflecting the complex pathogenic mechanisms underlying PPD. The combined use of 4 circRNAs significantly enhanced the diagnostic performance, as reflected by the increased AUC.

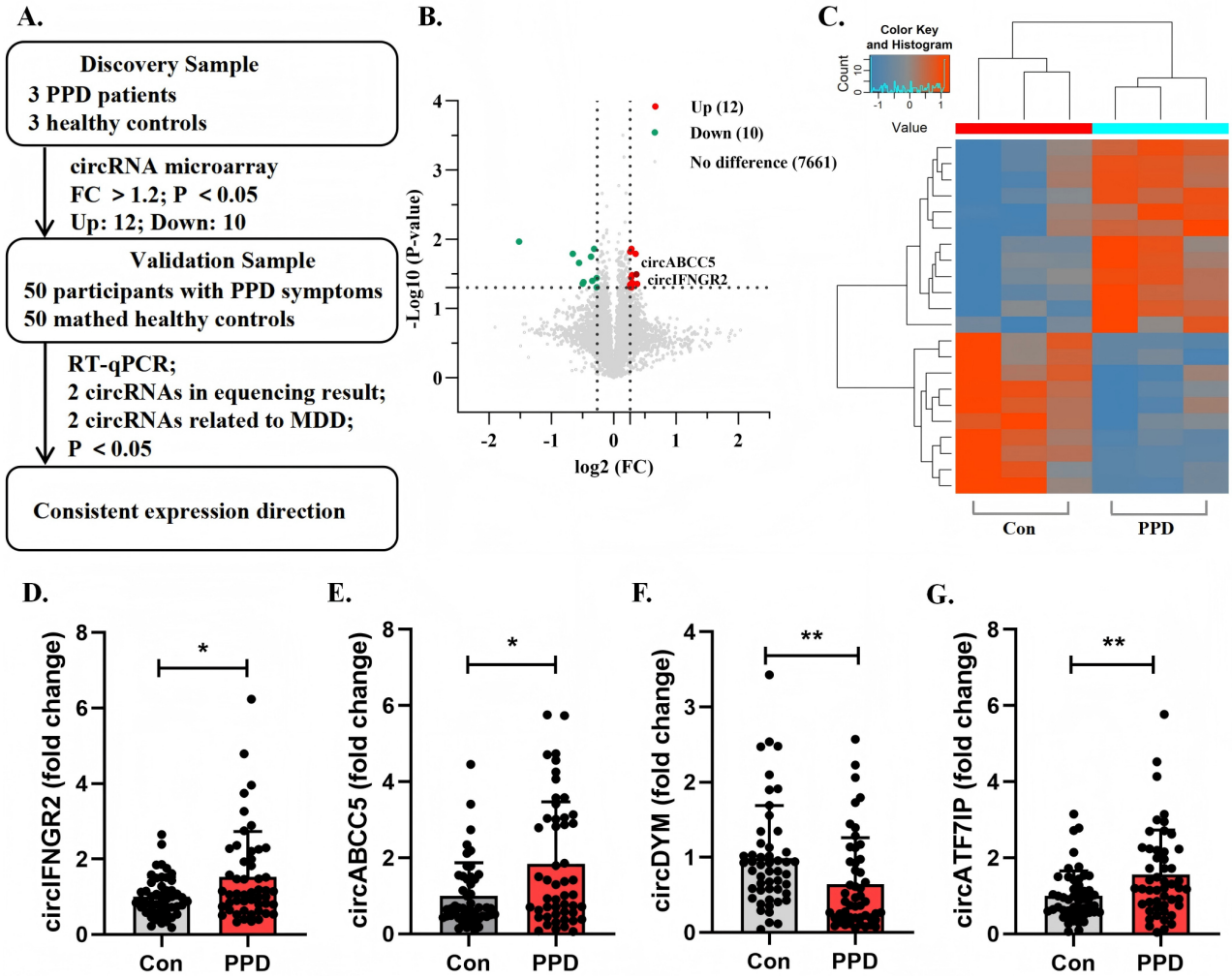

Fig. 1. Screening and validation of differentially expressed circular RNAs. (A) Experimental flowchart. (B,C) Volcano plot and heatmap of microarray analysis results. (D–G) Differentially expressed circRNAs verified by RT-qPCR in the validation cohort. (D) Relative expression levels of circIFNGR2. (E) Relative expression levels of circABCC5. (F) Relative expression levels of circDYM. (G) Relative expression levels of circATF7IP. Note: * $p < 0.05$, ** $p < 0.01$. Abbreviations: Con, control group; FC, fold change; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; MDD, major depressive disorder; circRNA, circular RNA; *circIFNGR2*, circRNA derived from the Interferon Gamma Receptor 2 gene; *circABCC5*, circRNA derived from the ATP-Binding Cassette Subfamily C Member 5 gene; *circDYM*, circRNA derived from the Dystrophia Myotonica Protein Kinase gene; *circATF7IP*, circRNA derived from the Activating Transcription Factor 7 Interacting Protein gene.

Table 3. Evaluation of differentially expressed circRNAs using univariate and multivariate logistic regression.

Variables	OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
circIFNGR2	2.10 (1.17, 3.76)	0.01	2.26 (1.21, 4.23)	0.01
circABCC5	1.71 (1.19, 2.45)	0.01	2.09 (1.35, 3.23)	<0.01
circDYM	0.43 (0.22, 0.84)	0.01	0.50 (0.23, 1.10)	0.84
circATF7IP	2.05 (1.21, 3.46)	0.01	1.74 (1.01, 3.02)	<0.05

The EPDS is the most widely employed tool for PPD screening in both clinical and research settings. Although previous study has reported that an EPDS cutoff score of 10 can achieve sensitivity and specificity exceeding 80% [25], it remains a symptom-based self-report measure. Cultural stigma may lead some individuals to underreport or conceal their symptoms, resulting in the underestimation of true prevalence. In contrast, the predictive model developed in this study, based on multiple circRNAs, allows more objective identification of individuals at risk for PPD symptoms.

Beyond improving diagnostic objectivity, understanding the molecular basis of PPD is essential for elucidating its underlying mechanisms. The neuroimmune-inflammatory hypothesis has garnered increasing attention in depression

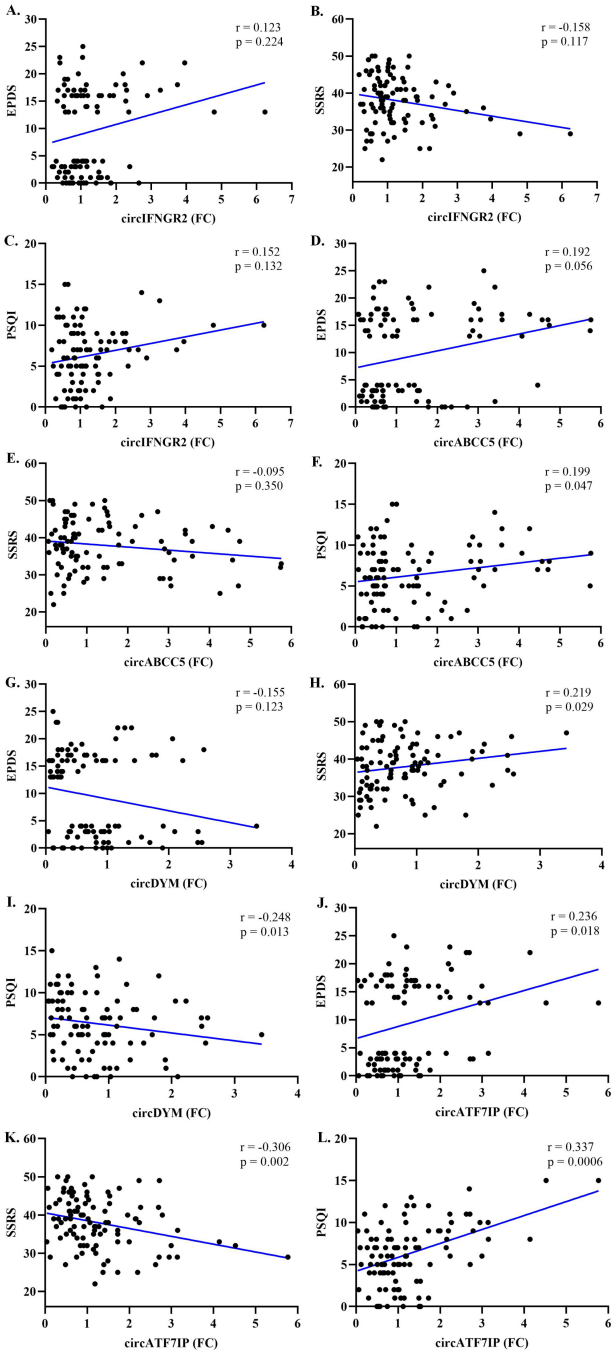

Fig. 2. Correlation between plasma circIFNGR2, circABCC5, circDYM and circATF7IP levels and questionnaire assessments in participants. (A,D,G,J) Correlation between four differentially expressed circular RNAs and EPDS scores. (B,E,H,K) Correlation between four differentially expressed circular RNAs and SSRS scores. (C,F,I,L) Correlation between four differentially expressed circular RNAs and PSQI scores. Abbreviations: EPDS, Edinburgh Postnatal Depression Scale.

research. As the resident immune cells of the brain, microglia play a pivotal role in driving neuroinflammation through phenotypic shifts and the release of inflammatory factors. Observations of activated microglia in specific brain regions, ranging from animal models of depression [26] to human clinical studies [27,28], support the notion that microglia-mediated neuroinflammation is a key mechanism in MDD [29]. Importantly, circRNAs have emerged as key regulators within this pathway. For instance, circDYM acts as a miR-9 sponge, suppressing microglial activation through heat shock protein 90 (HSP90) ubiquitination, ultimately ameliorating depression-like behaviors [30]. Similarly, downregulation of circATF7IP has been shown to attenuate microglia-induced neuroinflammation and alleviate depression-like behavior in mice [20].

In our study, correlation analysis revealed that circABCC5 levels positively correlated with WBC count ($p < 0.05$), further suggesting that circRNA may participate in PPD development by regulating immunomodulatory processes. Originating from the *ABCC5* gene, circABCC5 functions as a pivotal transporter with specificity for nucleotide second messengers, notably cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [31]. Simultaneously, these cyclic nucleotides can modulate inflammatory signaling. As a central regulator, nuclear factor-κB (NF-κB) controls the expression of numerous proinflammatory and immunoregulatory factors. Studies have shown that the nitric oxide (NO)-cGMP pathway can negatively regulate vascular NF-κB signaling, resulting in the suppression of local inflammation. Furthermore, both cAMP and cGMP are recognized as key mediators in neuroimmunity, influencing immune regulation, synaptic transmission, neural plasticity, neuronal survival, and memory consolidation [32]. In addition, we found that the levels of circABCC5, circDYM, and circATF7IP correlated with PSQI scores. A high PSQI score indicates a sleep disorder. Research has shown that complex interactions exist between sleep, the immune system, and the central nervous system. Sleep disturbances trigger sustained activation of the inflammatory system and the hypothalamic-pituitary-adrenal (HPA) axis, while prolonged HPA axis activation further stimulates and exacerbates inflammatory responses [33]. Some studies have found significant changes in the levels of inflammatory factors such as tumor necrosis factor α (TNF-α), C-reactive protein (CRP), interleukin (IL)-1β, IL-6, IL-8, and IL-10 during the development of PPD [34–36]. A proposed mechanism for increased PPD vulnerability involves the dysregulation of bidirectional communication between the HPA axis and the immune system [37]. These findings collectively suggest that circRNAs may participate in PPD pathogenesis by modulating neuroimmune pathways linked to sleep disorders. Furthermore, it provides a new perspective for understanding the mechanism of PPD.

Limitations

Several limitations should be considered when interpreting our findings. First, our study included a small sample size. Due to the low incidence of PPD and challenges

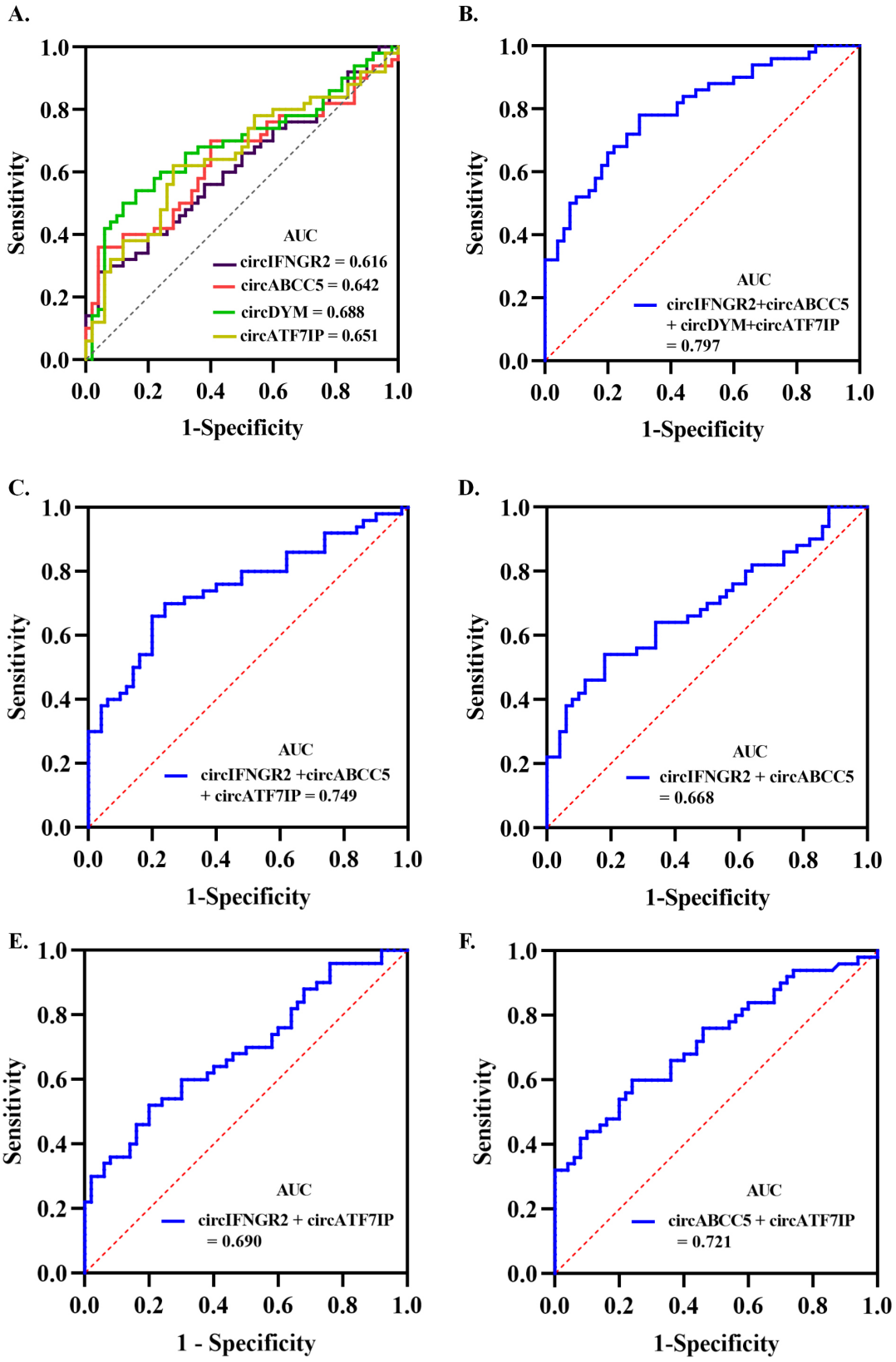

Fig. 3. AUC for ROC analysis of circRNAs that distinguish individuals with PPD symptoms from a normal control population. (A) AUC for the independent prediction of PPD by 4 circRNAs. (B) AUC for the combined prediction of PPD by 4 circRNAs. (C) AUC for the combined prediction of PPD by 3 circRNAs identified as relatively independent predictive factors. (D–F) AUC for the combined prediction of PPD by 2 circRNAs in each combination. Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic.

in sample collection, a 3-to-3 sample ratio was adopted for microarray analysis in the discovery cohort. Although the included samples were sufficiently representative, including individuals with severe depressive symptoms and self-harm tendencies who subsequently required hospitalization, the small sample size still poses a risk of false positives. Given the exploratory nature of this study, we validated as many relevant circRNAs as possible in the validation cohort, including those associated with MDD. The generalizability and reliability of these findings still require validation in larger, multicenter, multiethnic cohorts. Second, the dynamic changes of circRNA levels during the course of the disease cannot be fully captured by the single-timepoint sampling approach used in this study. Future research should adopt longitudinal sampling strategies to monitor circRNA expression profiles at multiple stages of pregnancy and the postpartum period. Lastly, the specific pathophysiologic mechanisms through which different circRNAs contribute to PPD require additional exploration.

5. Conclusions

In this study, we identified significant associations between circulating circRNA levels and PPD. Overall, our findings support the potential use of circRNAs as adjunct diagnostic biomarkers for PPD and offer valuable insights into the molecular mechanisms underlying the disorder.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request and have been publicly deposited in Zenodo (https://zenodo.org/records/17906223).

Author Contributions

FZ was responsible for the experimental operations, data organization and article writing. YZ was responsible for the experimental design and guidance. DQ was responsible for the collection and processing of clinical samples. HY was responsible for organizing the experimental results, making tables and pictures. XW was responsibe for data collection and article revision. HY was responsible for research design, revision, paper review and guidance. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study performed in accordance with the "Declaration of Helsinki" and approved by the Ethics Committee of Zhongda Hospital (2023ZDSYLL438-p01) and the Ethics Committee of Nanjing Women and Children's Healthcare Hospital (2024KY-080-01). Informed consent was obtained from all participants prior to data collection.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG47150.

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