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Original Article

A nomogram for predicting insulin resistance in polycystic ovary syndrome based on serum amino acid profiles and anti-Müllerian hormone

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Abstract: Objectives: Insulin resistance (IR) worsens metabolic and reproductive outcomes in polycystic ovary syndrome (PCOS). This study aimed to develop and validate a nomogram incorporating serum amino acid profiles and anti-Müllerian hormone (AMH) for predicting IR in PCOS. Methods: A retrospective observational analysis was performed on 434 PCOS patients treated at The Fourth Hospital of Shijiazhuang between January 2022 and January 2026. Patients were classified as IR+ (n=184) or IR- (n=250) using a homeostasis model assessment of insulin resistance (HOMA-IR) cutoff of 2.69 and were chronologically assigned to training (n=259) and validation (n=175) cohorts. Anthropometric parameters, hormone levels and serum amino acid concentrations were measured. Multivariable logistic regression identified independent predictors for nomogram development. Receiver operating characteristic (ROC) analysis, calibration plot, bootstrap resampling and decision curve analysis (DCA) were applied to evaluate the model's performance. Results: Multivariable analysis identified body mass index (odds ratio [OR]=1.357, 95% confidence interval [CI]: 1.180-1.561), waist circumference (OR=1.096, 95% CI: 1.053-1.141), AMH (OR=0.931, 95% CI: 0.879-0.986), leucine (OR=1.028, 95% CI: 1.012-1.044), isoleucine (OR=1.075, 95% CI: 1.045-1.107), valine (OR=1.015, 95% CI: 1.009-1.022) and tyrosine (OR=1.046, 95% CI: 1.004-1.089) as independent predictors. The nomogram demonstrated excellent discrimination in training (area under the curve [AUC]=0.945) and validation (AUC=0.917) cohorts, with good calibration (Hosmer-Lemeshow P=0.554) and clinical utility on DCA. Conclusion: This nomogram, integrating anthropometric measures, serum AMH and four amino acids, provides an accurate tool for the early identification of IR in PCOS.

Keywords: Polycystic ovary syndrome, insulin resistance, anti-Mullerian hormone, amino acids, nomograms, targeted amino acid profiling

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder among women in their childbearing years, with a global prevalence of approximately 8-13%. The features of the syndrome include continuous anovulation, either clinically or biochemically hyperandrogenism and the presence of polycystic ovary morphology [1]. PCOS also frequently presents with metabolic disorders, with insulin resistance (IR) at its core [2]. It occurs in 50-80% of PCOS patients, depending on the diagnostic criteria and population examined. It is associated with increased hyperandrogenism, impaired ovulation and elevated long-term risks for type 2 diabetes (T2DM), cardiovascular disorders and hepatic steatosis [3]. Thus, given the above problems, early and accurate identification of IR in PCOS patients are required to improve metabolic health and fertility.

Currently, the hyperinsulinemic-euglycemic clamp is the reference standard for quantifying insulin sensitivity, but it is complex and invasive and is rarely used in clinical practice [4]. A very popular index used to assess IR is homeostasis model assessment of insulin resistance (HOMA-IR), derived from fasting glucose and insulin values [5]. However, HOMA-IR has suboptimal sen-

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sitivity and specificity in PCOS populations, partly because of the heterogeneous pathophysiology of the syndrome [6]. Therefore, there is an increasing demand for more accessible and reliable biomarkers that can reflect the all-around features of IR in PCOS [7].

Recent research has identified alterations in serum amino acid levels in IR. Leucine (Leu), isoleucine (Ile) and valine (Val) are branched-chain amino acids (BCAA), while phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) are aromatic amino acids (AAAs); all are generally elevated in IR states such as PCOS [8]. These amino acids are abnormal in the metabolism and may also actively disrupt insulin signaling [9]. Meanwhile, anti-Müllerian hormone (AMH), a marker of ovarian follicular reserve, is frequently elevated in PCOS. Recent studies has reported a negative correlation between AMH and IR, although the underlying mechanisms remain unclear [10]. Amino acid markers and AMH can be combined to improve the predictive ability for IR over traditional clinical and hormonal data; however, an actual tool that integrates these biomarkers has not been developed yet [11].

To address this gap, we developed and validated a nomogram-based predictive model for IR in patients with PCOS by integrating serum amino acid profiles, AMH and anthropometric measures. Such a tool could help doctors find early-stage IR in PCOS women and adjust the treatment plan for them.

Subjects and methods

Research subjects

This retrospective observational study included 434 PCOS patients treated at The Fourth Hospital of Shijiazhuang between January 2022 and January 2026. Based on whether they developed IR, these individuals were assigned to either an IR- group (n=250) or an IR+ group (n=184). According to the time of treatment, the 434 patients were partitioned to a training set (n=259) and a validation set (n=175). The training set consisted of 153 people from the IR- group and 106 people from the IR+ group and the validation set consisted of 97 subjects from the IR- group and 78 subjects from the IR+ group. This research has received ethical clearance from the Medical Ethics Committee of The Fourth Hospital of Shijiazhuang. It is retrospective and the data is anonymous; therefore, written informed consent was not obtained.

Screening criteria

Inclusion criteria: (1) Female, 18-40 years old; (2) Confirmed diagnosis of PCOS; (3) Full clinical data, including complete anthropometric information, glycemic index, hormone levels and serum amino acid concentrations.

Exclusion criteria: (1) Use of any medication known to affect insulin sensitivity or hormone levels within the past 3 months; (2) Presence of other endocrine or metabolic diseases; (3) Previous treatment for diabetes or dyslipidaemia; (4) Pregnant or breastfeeding women; (5) Other serious systemic diseases.

Diagnostic criteria for PCOS: According to the 2003 Rotterdam criteria and Chinese guidelines for diagnosing polycystic ovary syndrome [12, 13], the identification of PCOS meets the following conditions: (1) Oligomenorrhea/amenorrhea (menstrual cycle >35 days, or fewer than 8 menstrual cycles in the preceding year, or an interval greater than 90 days between two menstrual periods); (2) Polycystic ovarian morphology (transvaginal ultrasound showing more than 12 follicles with a diameter ≤9 mm and/or an ovarian volume exceeding 10 ml in either ovary); (3) Clinical hyperandrogenism (Ferriman-Gallwey hirsutism score ≥5) and/or hyperandrogenemia. The first criterion must be met, along with at least one of the other criteria.

Diagnostic criteria for IR: IR was evaluated using the HOMA-IR. Based on studies in the Chinese PCOS population, IR was defined as HOMA-IR ≥2.69 [14, 15].

Data collection

Data on anthropometric parameters (age, body mass index [BMI], waist circumference [WC], systolic and diastolic blood pressure [SBP, DBP]), fasting plasma glucose (FPG), fasting insulin (FINS), hormone levels and serum amino acid concentrations were retrieved from electronic health records. An AU5800 fully automated biochemical analyzer (Beckman Coulter, USA) was employed to determine FPG and FINS

levels. The HOMA-IR index was derived using the standard formula: FPG × FINS/22.5. Hormone levels (luteinizing hormone [LH], follicle-stimulating hormone [FSH], free testosterone [FT], total testosterone [TT] and AMH) were measured using a fully automated electrochemiluminescence immunoassay system (Cobas e601, Roche Diagnostics, Switzerland). Serum Leu, Ile, Val, Phe, Tyr and Trp were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Triple Quad™ 6500, AB Sciex, USA).

Prior to sample analysis, the assay was rigorously validated for recovery, matrix effects and quality of control (QC). Absolute recovery was evaluated by comparing the peak areas of analytes added to blank serum before extraction (representing total analyte) versus those spiked post-extraction (representing 100% recovery). Mean recoveries ranged from 94.8% to 103.7%. Matrix effects were evaluated using the post-extraction spike method. The Internal Standard Normalized Matrix Factor (IS-normalized MF) was calculated as (peak area in matrix/peak area in solvent) normalized to the internal standard. IS-normalized MF values ranged from 0.96 to 1.04, indicating minimal ion suppression or enhancement. An internal quality control (IQC) sample (Liquichek™ Serum Total Protein Control, Bio-Rad Laboratories, USA) was analyzed after every 10 patient samples. The coefficients of variation (CVs) for all analytes remained <5% over the four-year collection period, confirming assay stability.

Statistical methods

All statistical computations were carried out using R 4.3.3 software. Continuous variables were presented as means with standard deviations and group comparisons were performed via independent samples t-tests. To identify factors independently associated with IR, logistic regression modeling was applied, with IR status as the dependent variable. Multicollinearity was examined using variance inflation factor (VIF), with a threshold of 5 considered indicative of problematic collinearity. Restricted cubic splines (RCS) with 3 knots were fitted for continuous predictors to explore potential nonlinear relationships with IR risk. Receiver operating characteristic (ROC) analysis was used to gauge discriminatory capacity. The final nomogram was constructed using the significant variables in the multivariable logistic model. Its performance was appraised through several metrics: the area under the curve (AUC) from ROC, decision curve analysis (DCA), calibration plots with Hosmer-Lemeshow test, bootstrap resampling (1,000 iterations) for internal validation and validated with a separate dataset. Model performance was compared with the triglyceride-glucose (TyG) index (computed as Ln[fasting triglyceride (mg/dL) × fasting glucose (mg/dL)/2]) and the triglyceride/high density lipoprotein-cholesterol (TG/HDL-C) ratio, using DeLong's test for AUC comparison. All tests were two-tailed and significance was set at $P<0.05$.

Results

Model construction

Anthropometric data in the training set: The baseline anthropometric characteristics of the training set are shown **Table 1**. The IR+ group had markedly higher BMI ($t=7.343$, $P<0.001$), WC ($t=4.842$, $P<0.001$) and SBP ($t=2.577$, $P=0.011$) than the IR- group. No significant differences were observed in age ($P=0.715$) or DBP ($P=0.125$) between the two groups.

Hormone levels in the training set: As shown in **Figure 1**, serum AMH levels were markedly lower in the IR+ group than in the IR- group ($t=2.723$, $P=0.007$). FSH, LH, TT and FT were all not markedly different ($P>0.05$).

Serum amino acid profiles in the training set: The concentrations of BCAA and AAA are compared in **Table 2**. Leu, Ile, Val, Tyr and Phe levels were markedly higher in the IR+ group (all $P<0.001$), whereas Trp levels were comparable between groups ($P=0.948$).

Logistic regression analysis: Univariable logistic regression identified BMI, WC, SBP, AMH, Leu, Ile, Val, Phe and Tyr as associated with IR (**Table 3**). After multivariable adjustment (**Table 4**), BMI (odds ratio [OR]=1.357, 95% confidence interval [CI]: 1.180-1.561), WC (OR=1.096, 95% CI: 1.053-1.141), AMH (OR=0.931, 95% CI: 0.879-0.986), Leu (OR=1.028, 95% CI: 1.012-1.044), Ile (OR=1.075, 95% CI: 1.045-1.107), Val (OR=1.015, 95% CI: 1.009-1.022) and Tyr (OR=1.046, 95% CI: 1.004-1.089) remained independent predictors (all $P<0.05$). SBP ($P=0.156$) and Phe ($P=0.067$) were exclud-

Table 1. Comparison of anthropometric data between patients with and without IR in the training set

Variables	IR- group (n=153)	IR+ group (n=106)	t	P
Age (years)	28.13 ± 4.53	28.34 ± 4.39	0.366	0.715
BMI (kg/m^2)	21.21 ± 2.46	23.68 ± 2.93	7.343	<0.001
WC (cm)	78.45 ± 9.24	84.26 ± 9.85	4.842	<0.001
SBP (mmHg)	109.52 ± 8.67	112.39 ± 9.01	2.577	0.011
DBP (mmHg)	71.65 ± 7.42	73.12 ± 7.68	1.541	0.125

Notes: IR, Insulin Resistance; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; 1 mm Hg=0.133 kPa.

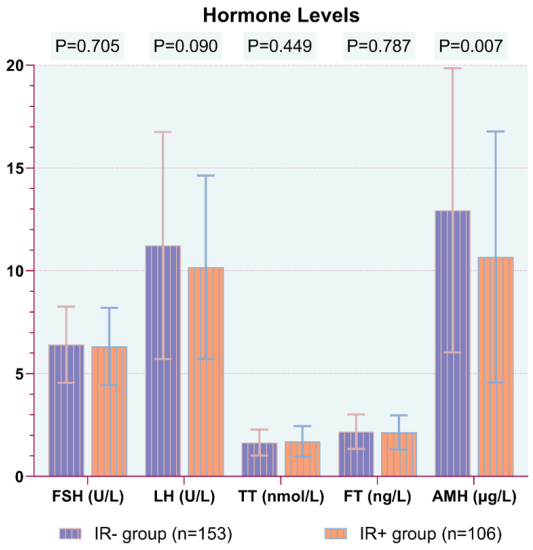

Figure 1. Differences in hormone levels between patients with and without IR in the training set. Notes: IR, Insulin Resistance; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; TT, Total Testosterone; FT, Free Testosterone; AMH, Anti-Müllerian Hormone.

ed from the final model. VIF analysis showed acceptable multicollinearity (BMI=1.82, WC=1.95, AMH=1.21, Leu=3.84, Ile=4.12, Val=3.56, Tyr=1.43). RCS analysis confirmed no significant non-linear deviations (all P for non-linearity >0.05).

ROC analysis: **Table 5** presents the discriminatory performance of the individual risk factors. BMI had the highest AUC (AUC=0.743), followed by Val (AUC=0.701), Leu (AUC=0.684), Ile (AUC=0.682), Tyr (AUC=0.672), WC (AUC=0.670), AMH (AUC=0.595), Phe (AUC=0.593) and SBP (AUC=0.587).

Nomogram model: Using the seven factors independently associated with IR in the multivariable model (BMI, WC, AMH, Leu, Ile, Val, Tyr), we developed a nomogram for estimating the likelihood of IR among PCOS patients (**Figure 2A**). The ROC curve of the nomogram in the training cohort showed good discrimination and had an AUC of 0.945 (**Figure 2B**). DCA demonstrated that the nomogram provided a net benefit across a wide range of threshold probabilities, indicating that model-guided clinical decisions would improve outcomes compared with "treat-all" or "treat-none" strategies (**Figure 2C**). The calibration plot showed good agreement between predicted and observed IR risks (**Figure 2D**). The Hosmer-Lemeshow test indicated adequate fit (χ^2=6.84, P=0.554). Internal validation using 1,000 bootstrap samples yielded an optimism-corrected AUC of 0.925.

Model validation

Anthropometric data in the validation set: The validation set demonstrated similar anthropometric differences to those observed in the training set (**Table 6**). The IR+ group again had a higher BMI (t=6.385, P<0.001), WC (t=4.044, P<0.001) and SBP (t=2.021, P=0.045), but neither age (P=0.594) nor DBP (P=0.172) was markedly different.

Hormone levels in the validation set: Consistent with the training set results, AMH was significantly lower in IR+ patients (t=2.586, P=0.011). All other reproductive hormones did not show notable differences between the groups (**Table 7**, all P>0.05).

Serum amino acid profiles in the validation set: Amino acid alterations were reliably reproduced (**Table 8**). Significantly higher levels of Leu, Ile, Val and Tyr were detected in the IR+ group (all P<0.001) and an increase in Phe (t=2.381, P=0.018) was also observed. Trp levels were not significantly different (P=0.964).

ROC curve: The nomogram was subsequently applied to the validation set and the ROC curve showed good discriminatory ability with an AUC of 0.917; thus, the nomogram had strong predictive power and was generalizable (**Figure 3**).

Sensitivity analysis

To assess the robustness of our model against the inherent limitations of HOMA-IR as a surro-

Table 2. Comparison of amino acid concentrations between patients with and without IR in the training set (nmol/mL)

Variables	IR- group (n=153)	IR+ group (n=106)	t	P
Leu	128.26 ± 20.51	143.17 ± 24.63	5.122	<0.001
Ile	73.92 ± 13.45	85.33 ± 18.08	5.526	<0.001
Val	315.23 ± 57.32	360.19 ± 67.03	5.788	<0.001
Phe	46.19 ± 7.14	48.54 ± 6.71	2.671	0.008
Tyr	59.73 ± 8.86	66.04 ± 9.65	5.429	<0.001
Trp	53.36 ± 11.34	53.45 ± 11.18	0.065	0.948

Notes: IR, Insulin Resistance; Leu, Leucine; Ile, Isoleucine; Val, Valine; Phe, Phenylalanine; Tyr, Tyrosine; Trp, Tryptophan.

Table 3. Univariable logistic regression analysis of factors associated with IR in patients with PCOS

Variables	Coefficient	Std Error	Wald	P	OR	95% CI
BMI	0.337	0.054	6.202	<0.001	1.401	1.264-1.566
WC	0.064	0.014	4.475	<0.001	1.067	1.038-1.098
SBP	0.037	0.015	2.520	0.012	1.038	1.009-1.069
AMH	-0.053	0.020	2.666	0.008	0.949	0.912-0.986
Leu	0.030	0.006	4.811	<0.001	1.031	1.018-1.044
Ile	0.048	0.009	5.128	<0.001	1.049	1.031-1.069
Val	0.012	0.002	5.128	<0.001	1.012	1.008-1.017
Phe	0.049	0.019	2.613	0.009	1.050	1.013-1.090
Tyr	0.075	0.015	4.906	<0.001	1.077	1.047-1.111

Notes: IR, Insulin Resistance; PCOS, Polycystic Ovary Syndrome; OR, Odds Ratio; CI, Confidence Interval; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; AMH, Anti-Müllerian Hormone; Leu, Leucine; Ile, Isoleucine; Val, Valine; Phe, Phenylalanine; Tyr, Tyrosine.

gate marker, we performed a sensitivity analysis using a more liberal HOMA-IR threshold of ≥2.50, prioritizing sensitivity over specificity. For the alternative HOMA-IR threshold, the nomogram maintained excellent performance (training AUC=0.938, validation AUC=0.909), supporting the robustness of the model.

To address potential temporal drift in analyte measurements over the four-year collection period, we included "collection year" as a fixed-effect covariate in the final multivariable model. The inclusion of this variable did not materially alter the effect estimates or statistical significance of the key predictors (BMI, WC, AMH, Leu, Ile, Val and Tyr); all remained robust independent predictors of IR (all P<0.05). The regression coefficients for these predictors varied by less than 3% compared to the original model and the model's AUC remained virtually unchanged (0.941 vs. 0.945 in the training cohort). These findings suggest that any minor inter-batch variability was effectively mitigated by our QC protocols and did not introduce systematic bias into the analysis.

Comparison with simple indices

We further compared the discriminatory performance of our nomogram against the TyG index and TG/HDL-C ratio. In the training cohort, the AUC for the TyG index was 0.753 and for TG/HDL-C was 0.712. In the validation cohort, the AUCs were 0.741 and 0.705, respectively. Our nomogram demonstrated significantly higher AUCs in both cohorts (Training: 0.945; Validation: 0.917) compared to both the TyG index and TG/HDL-C (DeLong's test, both P<0.001).

Discussion

We developed and validated a nomogram based on BMI, WC, AMH, Leu, Ile, Val and Tyr in this retrospective study of 434 PCOS patients to predict IR. The model showed strong discriminatory performance in both cohorts, outperforming any single biomarker. These findings demonstrate that targeted serum amino acid profiling can effectively assess IR risk in PCOS.

Obesity and central adiposity are well-established risk factors for IR in PCOS [16]. Based on the above analysis, higher BMI and larger WC are independently associated with IR, which is in line with many previous studies showing that excess adipose tissue, especially visceral fat, induces systemic inflammation and lipotoxicity, thereby impairing insulin signaling [17]. PCOS involves a dual elevation of androgenism and obesity that reduces insulin sensitivity in the periphery [18]. The fact that both BMI and WC remained independent predictors after controlling for amino acids and AMH indicates that anthropometric indices capture distinct components in predicting the risk of IR compared with metabolic and endocrine markers [19].

Table 4. Multivariable logistic regression analysis of risk factors for IR in patients with PCOS

Variables	Coefficient	Std Error	Wald Stat	P	OR	OR CI Lower	OR CI Upper
BMI	0.306	0.071	4.285	<0.001	1.357	1.180	1.561
WC	0.092	0.021	4.480	<0.001	1.096	1.053	1.141
SBP	0.033	0.023	1.420	0.156	1.034	0.987	1.082
AMH	-0.071	0.029	-2.422	0.015	0.931	0.879	0.986
Leu	0.028	0.008	3.426	<0.001	1.028	1.012	1.044
Ile	0.072	0.015	4.929	<0.001	1.075	1.045	1.107
Val	0.015	0.003	4.475	<0.001	1.015	1.009	1.022
Phe	0.049	0.027	1.829	0.067	1.050	0.996	1.107
Tyr	0.045	0.021	2.163	0.031	1.046	1.004	1.089

Notes: IR, Insulin Resistance; PCOS, Polycystic Ovary Syndrome; OR, Odds Ratio; CI, Confidence Interval; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; AMH, Anti-Müllerian Hormone; Leu, Leucine; Ile, Isoleucine; Val, Valine; Phe, Phenylalanine; Tyr, Tyrosine.

Table 5. ROC analysis for risk factors in predicting IR in PCOS patients

Variables	Best_threshold	Sensitivities	Specificities	AUC	Youden_index	F1_score
BMI	22.820	0.660	0.758	0.743	0.418	0.657
WC	80.415	0.679	0.601	0.670	0.280	0.603
SBP	109.410	0.642	0.516	0.587	0.158	0.548
AMH	14.830	0.755	0.425	0.595	0.180	0.264
Leu	136.880	0.660	0.699	0.684	0.359	0.631
Ile	75.245	0.726	0.549	0.682	0.275	0.611
Val	338.995	0.660	0.706	0.701	0.366	0.633
Phe	49.780	0.453	0.712	0.593	0.165	0.485
Tyr	67.070	0.453	0.804	0.672	0.257	0.522

Notes: ROC, Receiver Operating Characteristic; IR, Insulin Resistance; PCOS, Polycystic Ovary Syndrome; AUC, Area Under the Curve; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; AMH, Anti-Müllerian Hormone; Leu, Leucine; Ile, Isoleucine; Val, Valine; Phe, Phenylalanine; Tyr, Tyrosine.

AMH showed an inverse association with IR in our cohort; lower AMH levels were associated with increased IR risk [20]. This finding is noteworthy, as PCOS is typically characterized by elevated AMH and higher antral follicle counts. However, emerging evidence suggests that hyperinsulinemia may directly suppress AMH secretion from granulosa cells through insulin receptor signaling [21]. Compensatory hyperinsulinemia in IR may reduce AMH secretion and severe metabolic derangements may impair ovarian follicular development, thereby lowering AMH in PCOS patients with IR [22]. Our results align with several recent studies reporting lower AMH in PCOS patients with IR [23]. Importantly, our cross-sectional data cannot establish causality; the inverse AMH-IR association reflects correlation, not a protective effect of AMH against IR. The directionality of this relationship - whether IR suppresses AMH or whether low AMH predisposes to IR - remains unresolved and warrants longitudinal investigation.

Among the amino acid profiles, the three BCAAs (Leu, Ile, Val) were elevated in insulin-resistant PCOS patients. This finding is consistent with a large body of metabolomic research demonstrating associations between elevated BCAAs and IR in obesity, T2DM and PCOS [24, 25]. Proposed mechanisms include activation of the mammalian target of rapamycin complex 1 (mTORC1), which attenuates insulin receptor substrate 1 (IRS-1) phosphorylation and impairs insulin signaling; generation of propionyl-CoA and succinyl-CoA, which may impair mitochondrial fatty acid oxidation and induction of proinflammatory gene expression [26, 27]. However, we emphasize that these pathways are speculative in the context of our study, as we did not measure any downstream effector molecules. Our data demonstrate associations

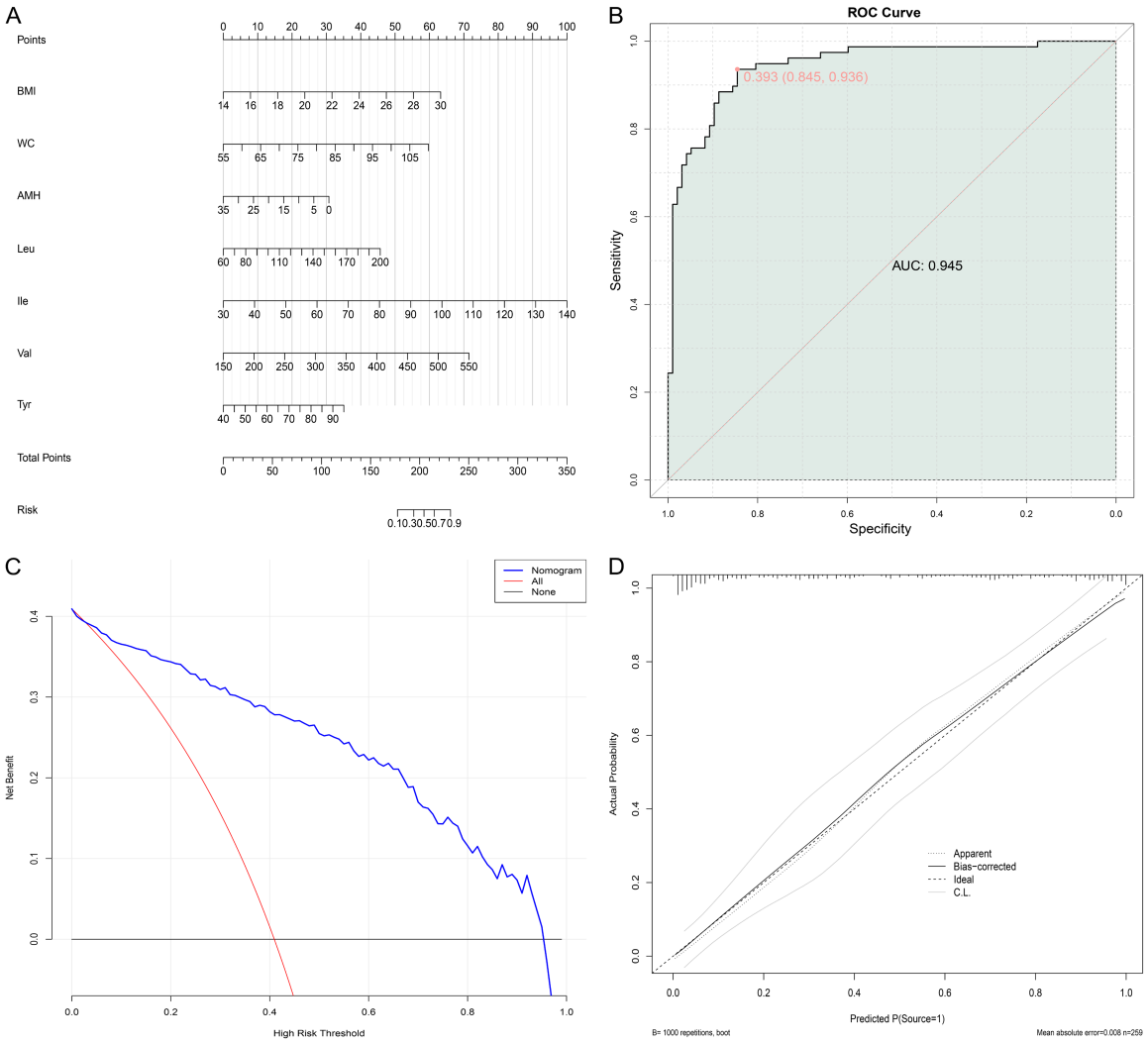

Figure 2. Construction of a nomogram prediction model for IR in patients with PCOS (A) Nomogram; (B) ROC curve; (C) DCA; (D) Calibration curve. Notes: IR, Insulin Resistance; PCOS, Polycystic Ovary Syndrome; ROC, Receiver Operating Characteristic; DCA, Decision Curve Analysis; AUC, Area Under the Curve; BMI, Body Mass Index; WC, Waist Circumference; AMH, Anti-Müllerian Hormone; Leu, Leucine; Ile, Isoleucine; Val, Valine; Tyr, tyrosine.

Table 6. Comparison of anthropometric data between patients with and without IR in the validation set

Variables	IR- group (n=97)	IR+ group (n=78)	t	P
Age (years)	28.05 ± 4.48	28.41 ± 4.42	0.534	0.594
BMI (kg/m^2)	21.13 ± 2.46	23.74 ± 2.95	6.385	<0.001
WC (cm)	78.51 ± 9.17	84.33 ± 9.79	4.044	<0.001
SBP (mmHg)	109.74 ± 8.67	112.46 ± 9.04	2.021	0.045
DBP (mmHg)	71.61 ± 7.39	73.18 ± 7.72	1.371	0.172

Notes: IR, Insulin Resistance; BMI, Body Mass Index; WC, Waist Circumference; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; 1 mm Hg=0.133 kPa.

only, not causal mechanisms. All three BCAAs remained independent predictors in the multivariable model. Despite moderate correlations among BCAAs, each retained independent predictive value, suggesting they may capture complementary aspects of amino acid metabolic dysregulation. Phe was not retained in the final model after adjustment, possibly due to metabolic interconversion with Tyr, which was an independent predictor [28, 29].

Tyr, an AAA, was also independently associated with IR in our cohort. Elevated Tyr in IR has been linked to increased protein turnover and defective insulin-mediated suppression of proteolysis [30, 31]. Tyr is a precursor for catecholamine synthesis and sympathetic nervous sys-

Table 7. Comparison of hormone levels between patients with and without IR in the validation set

Variables	IR- group (n=97)	IR+ group (n=78)	t	P
FSH (U/L)	6.38 ± 1.83	6.31 ± 1.87	0.246	0.806
LH (U/L)	11.46 ± 5.49	10.15 ± 5.42	1.581	0.116
TT (nmol/L)	1.65 ± 0.62	1.72 ± 0.76	0.677	0.499
FT (ng/L)	2.17 ± 0.84	2.14 ± 0.82	0.216	0.829
AMH (μg/L)	12.98 ± 6.76	10.46 ± 5.97	2.586	0.011

Notes: IR, Insulin Resistance; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; TT, Total Testosterone; FT, Free Testosterone; AMH, Anti-Müllerian Hormone.

Table 8. Comparison of amino acid concentrations between patients with and without IR in the validation set (nmol/mL)

Variables	IR- group (n=97)	IR+ group (n=78)	t	P
Leu	128.21 ± 20.44	143.14 ± 24.52	4.393	<0.001
Ile	73.85 ± 13.36	85.26 ± 17.98	4.662	<0.001
Val	315.07 ± 57.14	360.03 ± 66.84	4.796	<0.001
Phe	46.04 ± 7.18	48.54 ± 6.56	2.381	0.018
Tyr	59.73 ± 8.75	66.08 ± 9.49	4.594	<0.001
Trp	53.38 ± 11.21	53.45 ± 10.93	0.046	0.964

Notes: IR, Insulin Resistance; Leu, Leucine; Ile, Isoleucine; Val, Valine; Phe, Phenylalanine; Tyr, Tyrosine; Trp, Tryptophan.

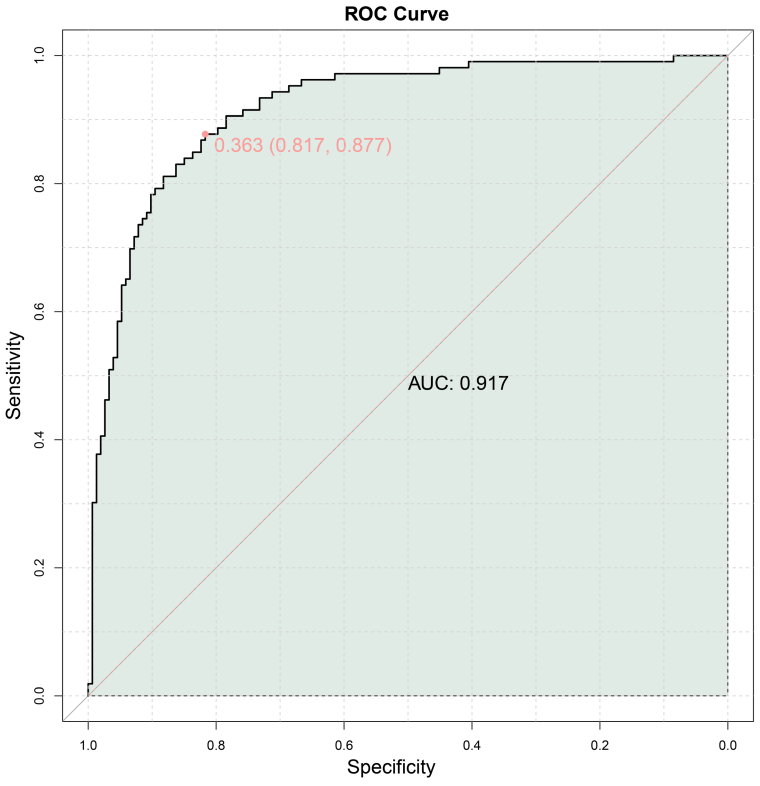

Figure 3. Validation of the predictive model for IR in patients with PCOS. Notes: IR, Insulin Resistance; PCOS, Polycystic Ovary Syndrome; ROC, Receiver Operating Characteristic; AUC, Area Under the Curve.

tem overactivity has been implicated in IR [32]. Additionally, Tyr and its metabolites may modulate insulin receptor function [33]. A recent systematic review confirmed dysregulation of AAA metabolism in PCOS, with elevated serum levels driven by inflammation and oxidative stress [29]. The absence of Trp differences between groups indicates that not all amino acids are affected by IR in PCOS.

Our nomogram, based on seven predictors, demonstrated strong discriminatory power (AUC 0.945 in training, 0.917 in validation), outperforming previous IR prediction models for PCOS. Guo et al. constructed a nomogram based on BMI, TG, alanine aminotransferase and FPG with AUCs of 0.911 and 0.901 in internal and external validation sets [3]. The significant improvement in AUC confirms that adding AMH and targeted amino acid profiling to basic anthropometric measures provides meaningful incremental value over the TyG index or TG/HDL-C ratio. While these simple indices remain valuable first-line screening tools due to their low cost and accessibility, our nomogram offers enhanced precision for risk stratification. This is particularly relevant for subgroups such as lean PCOS patients or those with normal fasting glucose, in whom simple indices may lack sensitivity for detecting subtle metabolic dysregulation.

Several limitations to this study should be noted. First, the retrospective, single-center design may have introduced selection bias and limits the generalizability of our findings. Multi-center prospective

validation is needed before clinical implementation. Second, HOMA-IR was used to define IR rather than the clamp technique, which is considered the gold standard. Although HOMA-IR is widely used in large-scale clinical studies and has been validated in Chinese PCOS populations, its suboptimal sensitivity and specificity may have led to outcome misclassification. Our sensitivity analysis using an alternative HOMA-IR threshold supported the robustness of our findings, but clamp-based validation would be ideal. Third, because the data are cross-sectional, causality cannot be inferred and the direction of relationships among amino acids, AMH and IR cannot be determined. Longitudinal studies are needed to clarify temporal relationships. Fourth, we did not assess dietary intake or physical activity, both of which may influence amino acid metabolism and insulin sensitivity. Fifth, although LC-MS/MS is a high-precision analytical technique, it is not widely available in routine clinical settings and inter-laboratory variability in amino acid reference ranges remains a challenge. Future work should focus on developing simplified, cost-effective assays for the key amino acids. Sixth, our cross-sectional design did not allow direct investigation of mechanistic pathways such as gut microbiota-BCAA interactions, fatty acid oxidation disorders, or mitochondrial dysfunction, which are well-established in IR pathophysiology. Future studies incorporating metagenomic, lipidomic and functional assays are needed to explore these mechanisms and their potential contribution to the associations observed in our study. Additionally, formal pathway enrichment analysis was not feasible in our study because our targeted amino acid profiling measured only six analytes, rather than a comprehensive metabolomic panel. Future studies employing untargeted metabolomics or multi-panel approaches should perform formal Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment and network analysis to determine mechanistic connections in PCOS-associated IR. Finally, our study included only Chinese women; thus, the results may not be directly generalizable to other ethnic groups. Despite these limitations, our nomogram provides a practical tool to identify PCOS patients at high risk of IR, enabling timely lifestyle or therapeutic interventions.

Conclusion

In summary, we developed and validated a nomogram for predicting IR in PCOS patients based on BMI, WC, serum AMH and four amino acids (Leu, Ile, Val, Tyr). The model demonstrated good discriminatory power in both the training and validation cohorts, with AUC values of 0.945 and 0.917, outperforming any individual clinical or biochemical marker. These findings suggest that combining anthropometry, hormone data and targeted amino acid profiling can improve IR prediction in PCOS. The nomogram provides a practical, non-invasive tool for the early detection of high-risk patients, facilitating tailored management strategies. Future prospective studies with larger, multi-center designs are warranted to be carried out to externally validate the model in diverse populations and to explore its application in guiding treatment decisions, such as initiating lifestyle modifications or insulin-sensitizing drugs. Furthermore, longitudinal research is essential to clarify the causal pathways linking altered amino acid profiles, AMH and the development of IR in PCOS.

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Disclosure of conflict of interest

None.

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