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Oviduct Glycoprotein 1 (OVGP1) Diagnoses Polycystic Ovary Syndrome (PCOS) Based on Machine Learning Algorithms

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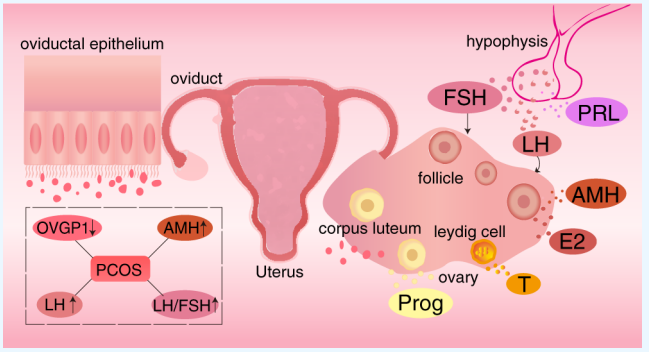
ABSTRACT: *Aims*: To investigate the diagnostic value of oviduct glycoprotein 1 (OVGP1) levels for polycystic ovary syndrome (PCOS). *Materials and Methods*: Serum OVGP1 concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Associations between OVGP1 and endocrine parameters were evaluated by Spearman's correlation analysis. Diagnostic capacity was assessed by utilizing machine learning algorithms and receiver operating characteristic (ROC) curves. *Results*: OVGP1 levels were significantly decreased in PCOS patients and correlated with the serum follicle-stimulating hormone (FSH) concentration and the luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, which are predictors of PCOS occurrence. The diagnostic value of OVGP1 combined with six signatures (LH/FSH, progesterone, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and anti-Müllerian hormone) or three clinical indicators has the potential to significantly improve the accuracy of diagnosing PCOS patients. *Conclusion*: OVGP1 enhances the ability to diagnose when combined with clinical indicators.



1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in patients with ovaries of reproductive age and seriously affects women's reproductive health. The prevalence of PCOS is approximately 6−12% according to the diagnostic criteria.[1,2] PCOS is clinically heterogeneous, there is no single diagnostic test, and the Rotterdam criteria are usually used to diagnose PCOS.[3,4] At present, the biochemical indicators for the diagnosis of PCOS include mainly anti-Müllerian hormone (AMH), the luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio, and progesterone (Prog). Serum levels of AMH are significantly higher in PCOS patients than in controls, reflecting functional ovarian reserve and serving as a biomarker of female reproductive potential.[5] The serum LH level is elevated in PCOS patients, and the LH/FSH ratio reflects ovarian reserve function to a certain extent.[6] In women with PCOS, the endometrium is often characterized by Prog resistance.[7] Therefore, the clinical diagnosis of PCOS relies mainly on these biochemical indicators, and a single diagnostic gold standard is lacking.

The etiology of PCOS is complex, encompassing dysfunctions in the metabolic, reproductive, and psychological domains, and these dysfunctions are frequently accompanied by abdominal obesity, insulin resistance (IR), obesity, metabolic irregularities, inflammation, and cardiovascular risk factors.[8−10] IR can induce dyslipidemia and lipid triads in routine clinical operations for diagnosing IR according to the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) (fasting plasma glucose (FPG, mmol/L) × fasting insulin (FINS, mIU/L)/22.5). OVGP1 is an estrogen-induced secretory protein that plays a key role in sperm capacitation, fertilization, and early embryonic development.[11] Our previous studies confirmed that OVGP1 is a hypertensive factor that directly promotes vascular remodeling and is involved in regulating oxidative stress and metabolism-related pathways.[12] Since PCOS is associated with oxidative stress and some metabolic complications,[13,14] OVGP1 levels may be related to PCOS. However, the OVGP1 expression level and its clinical implications in patients with PCOS have never been reported.

Therefore, in this study, we aimed to explore the value of the serum OVGP1 level in females with PCOS and healthy controls. Spearman's correlation analysis and multivariate linear regression analysis were used to investigate the associations between OVGP1 and endocrine−metabolic parameters, and logistic regression analysis was further employed to identify the risk factors associated with PCOS. In addition, recursive feature elimination based on cross-validation (RFE-CV) algorithm was used to screen existing diagnostic markers for PCOS. Six machine learning algorithms and receiver operating character-

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Table 1. Demographics and Clinical Characteristics of Women with PCOS and Controls[a]

Variable	Control (n = 60)	PCOS (n = 70)	p-value
Age, y	27.783 ± 5.970	26.786 ± 4.259	0.286
BMI, kg/m^2	21.484 (19.628, 23.438)	22.547 (19.721, 27.344)	0.191
FSH, mIU/L	5.750 (4.390, 6.950)	5.530 (4.260, 6.360)	0.552
LH, mIU/L	5.500 (3.660, 9.250)	13.030 (8.060, 20.070)	<0.001
LH/FSH	0.986 (0.612, 1.406)	2.446 (1.708, 3.458)	<0.001
PRL, ng/mL	15.450 (10.510, 19.700)	15.370 (10.990, 21.730)	0.613
E2, pg/mL	49.160 (30.870, 101.400)	47.100 (36.760, 83.360)	0.899
Prog, ng/mL	0.250 (0.140, 1.810)	0.240 (0.120, 0.480)	0.424
T, ng/mL	0.450 (0.220, 19.680)	0.510 (0.350, 0.690)	0.539
DHEAS, mol/L	8.220 ± 2.689	8.377 ± 3.592	0.948
AMH, ng/mL	3.260 (1.620, 4.810)	6.940 (5.050, 9.390)	<0.001
Hcy, mol/L	10.970 (9.300, 12.480)	9.240 (7.150, 9.950)	0.103
HOMA-IR	1.460 (0.936, 2.392)	2.316 (1.582, 3.335)	<0.001
FPG, mmol/L	4.560 (3.790, 4.910)	4.800 (4.410, 5.240)	0.019
1 h PG, mmol/L	6.659 ± 3.169	9.185 ± 2.469	0.014
2 h PG, mmol/L	6.950 (6.760, 9.650)	6.900 (6.200, 8.480)	0.506
3 h PG, mmol/L	5.163 ± 2.294	5.848 ± 1.639	0.315
FINS, mIU/L	7.940 (6.520, 10.600)	11.090 (7.780, 15.260)	<0.001
1 h INS, mIU/L	67.000 (47.900, 116.200)	105.000 (80.520, 181.700)	0.036
2 h INS, mIU/L	57.800 (51.600, 73.140)	101.700 (67.500, 164.200)	0.049
3 h INS, mIU/L	45.250 (24.200, 88.950)	42.440 (25.370, 81.880)	0.822
FCP, ng/mL	2.020 (1.610, 2.600)	2.400 (1.940, 3.310)	0.016
1 h CP, ng/mL	9.943 ± 1.845	12.619 ± 3.854	0.105
2 h CP, ng/mL	9.807 ± 2.750	12.536 ± 3.594	0.083
3 h CP, ng/mL	7.703 ± 2.632	9.010 ± 3.858	0.431
FT3, pmol/L	4.883 ± 0.788	5.056 ± 0.602	0.251
FT4, pmol/L	15.710 (14.270, 17.200)	16.690 (15.200, 18.700)	0.091
T3, nmol/L	1.760 (1.580, 1.990)	1.900 (1.690, 2.040)	0.130
T4, nmol/L	103.000 (89.900, 111.000)	104.000 (91.800, 122.700)	0.396
TSH, μIU/mL	2.190 (1.400, 3.380)	2.400 (1.870, 3.420)	0.512
anti-TPO Ab, IU/mL	10.080 (6.530, 15.280)	10.900 (8.200, 11.900)	0.848
TgAb, IU/mL	16.400 (13.060, 23.200)	17.660 (16.030, 20.410)	0.538
Tg, ng/mL	13.200 (4.650,16.300)	6.980 (3.690, 10.810)	0.035
25(OH)VD, nmol/L	21.316 ± 12.747	23.906 ± 12.431	0.638
TC, mmol/L	4.430 (3.940, 4.990)	4.380 (3.980, 5.070)	0.878
TG, mmol/L	1.085 (0.690, 1.440)	1.080 (0.680, 1.790)	0.599
HDL-C, mmol/L	1.420 (1.180, 1.680)	1.270 (1.050, 1.520)	0.009
LDL-C, mmol/L	2.680 (2.220, 3.260)	2.520 (2.240, 3.300)	0.953
TC/HDL-C	3.036 (2.658, 3.708)	3.414 (2.940, 4.188)	0.012
TG/HDL-C	0.750 (0.493, 1.073)	0.805 (0.482, 1.683)	0.336
LDL-C/HDL-C	1.826 (1.570, 2.489)	2.097 (1.709, 2.625)	0.133

[a]The data are expressed as the means ± standard deviations or medians and quartiles. BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; LH/FSH: luteinizing hormone/follicle-stimulating hormone; PRL: prolactin; E2: estradiol; Prog: progesterone; T: testosterone; DHEAS: dehydroepiandrosterone sulfate; AMH: anti-Müllerian hormone; Hcy: homocysteine; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance index; FPG: fasting plasma glucose; 1 h PG: 1 h plasma glucose; 2 h PG: 2 h plasma glucose; 3 h PG: 3 h plasma glucose; FINS: fasting insulin; 1 h INS: 1 h insulin; 2 h INS: 2 h insulin; 3 h INS: 3 h insulin; FCP: fasting C-peptide; 1 h CP: 1 h postprandial C-peptide; 2 h CP: 2 h postprandial C-peptide; 3 h CP: 3 h postprandial C-peptide; FT3: free triiodothyronine; FT4: free thyroxine; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone; anti-TPO Ab: antithyroperoxidase antibody; TgAb: antithyroglobulin antibody; Tg: thyroglobulin; 25(OH)VD: 25-hydroxyvitamin D; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein; TC/HDL-C: total cholesterol/high-density lipoprotein cholesterol; TG/HDL-C: triglyceride/high-density lipoprotein cholesterol; LDL-C/HDL-C: low-density lipoprotein/high-density lipoprotein cholesterol. The data were compared with normal distribution using Student's t test. The Mann−Whitney U test is used for non-normal distributions. A p-value < 0.05 was considered significant.

istic (ROC) curves were used to evaluate the diagnostic value of OVGP1 for PCOS, and an optimal classifier was ultimately determined for the discovery of novel clinical diagnostic biomarkers.

2. MATERIALS AND METHODS

2.1. Study Design and Participants. In this cross-sectional study, serum was collected from 70 women with PCOS who met the inclusion criteria and 60 age-matched controls at the Xijing Hospital from April 2022 to June 2023. The inclusion criteria were as follows: age 18−40 years[15] and meeting the international evidence-based guideline for the

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assessment and management of PCOS 2023, which revises the 2003 Rotterdam diagnostic criteria.[16] Excluding other causes, a diagnosis of PCOS can be made if one of the following criteria is met: (1) irregular menstrual cycles and clinical manifestations of hyperandrogenism; (2) irregular menstrual cycles and no clinical manifestations of hyperandrogenism or were not obvious, and the biochemical examination showed hyperandrogenemia; (3) only irregular menstrual cycles or hyperandrogenemia, adult women need further ultrasound examination, if the presence of polycystic ovary monography, the diagnosis of PCOS; adolescents were included in the high-risk management of PCOS and were followed up regularly. Other causes to be excluded mainly include thyroid-stimulating hormone, prolactin (PRL), 17α-hydroxyprogesterone, follicle-stimulating hormone, or related causes that need to be excluded if there are clinical characteristics (such as Cushing's syndrome, adrenal tumor, etc.). Hypogonadotropin-induced hypogonadism (usually caused by low fat or prolonged vigorous exercise) can be ruled out in combination with clinical and LH and FSH levels.[17] All participants underwent an analysis of sex hormones (Roche Cobas 6000 CE Line E601 Lab Chemistry Analyzer, Germany), an evaluation of thyroid function (Roche Cobas 6000 CE Line E801 Lab Chemistry Analyzer, Germany), an analysis of a four-item panel of blood lipids (Hitachi 7060 Automatic Biochemical Analyzer, Japan), glucose tolerance tests (BIOELAB ES-480 Automatic Biochemical Analyzer, China), and 25(OH)-vitamin D tests (Roche Cobas 6000 CE Line E601 Lab Chemistry Analyzer, Germany) to establish the baseline characteristics. The study was approved by the ethics committee of the First Affiliated Hospital of the Fourth Military Medical University, and informed consent was obtained from all participants.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA). Serum OVGP1 was measured using an ELISA kit (LifeSpan, Seattle, WA, USA). All procedures were performed in accordance with the manufacturer's instructions. Standard substances of different concentrations were prepared. Then, 100 μL of standard substances and 100 μL of serum samples were added to 96-well plates coated with the OVGP1 protein antibody and incubated at 37 °C for 2 h. The mixture was subsequently discarded, 100 μL of the biotin OVGP1 antibody conjugate was added, the mixture was incubated at 37 °C for 1 h, and the unbound antibodies were removed by washing three times. Next, 100 μL of HRP-conjugated reagent was added, and the mixture was incubated at 37 °C for 0.5 h and washed five times with washing solution. Subsequently, 90 μL of TMB substrate was added, the sample was shielded from light at 37 °C for 10 min, 50 μL of stop solution was added, the absorbance was read at 450 nm with a multifunctional enzyme marker (TECAN Infinite200 PRO), and the OVGP1 concentration was calculated. All of the assays were carried out in duplicate at minimum, and average absorbance data were obtained from three independently repeated experiments.

2.3. Performance Evaluation of Candidate Signatures via Machine Learning Algorithms. To evaluate the application of the biomarkers, we used RFE-CV algorithms to screen candidate signatures according to clinical indicators in PCOS patients and controls.[18] To accurately predict the performance of the diagnostic model, we used six machine learning classification algorithms in Python, i.e., adaptive boosting (AdaBoost), extreme gradient boosting (XGBoost), decision tree (DT), K-nearest neighbors (KNN), logistic regression (LR), and random forest (RF), to discriminate PCOS patients from controls.[19−21] Furthermore, we used 5-fold cross-validation to ensure the stability and accuracy of the classifiers and calculated five measurements, namely, sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV). The ROC curve was used to evaluate the discriminant ability of the prediction model, and decision curve analysis (DCA) was used to estimate the clinical usefulness and net benefit of the model.[22]

2.4. Statistical Analysis. SPSS 21.0 (IBM Corp., NY, USA) was used for the data analysis. The Kolmogorov−Smirnov test was used to determine whether the data fit a normal distribution. For parametric data, Student's t test was used, and for nonparametric data, the Mann−Whitney U test was used to determine the difference between the two groups. The measurement data are expressed as means ± standard deviations and medians (interquartile distances). The difference in the OVGP1 expression between the two groups is represented by a violin diagram. Spearman's correlation analysis and multiple linear regression models were used to assess the associations between OVGP1 and other diagnostic indicators. Logistic regression analysis was performed to predict the exposure risk in PCOS patients. The net reclassification improvement (NRI)[23] and integrated discrimination improvement (IDI)[24] were used to assess the impact of adding OVGP1 detection on the reclassification of a predictive model. $p < 0.05$ was considered to indicate statistical significance.

3. RESULTS

3.1. Demographics and Clinical Characteristics of the Participants Enrolled in the Study. A total of 130 participants were recruited for this study. The baseline characteristics and clinical outcomes of patients with PCOS and controls are listed in Table 1. There were no significant differences in age or body mass index (BMI) between patients with PCOS and controls. The levels of LH (13.030 (8.060, 20.070) vs 5.500 (3.660, 9.250) mIU/L, $p < 0.001$), LH/FSH (2.446 (1.708, 3.458) vs 0.986 (0.612, 1.406), $p < 0.001$), and AMH (6.940 (5.050, 9.390) vs 3.260 (1.620, 4.810) ng/mL, $p < 0.001$) were significantly higher in the PCOS group than those in the control group, which was consistent with the biochemical performance of PCOS,[25] while the levels of other hormones were not different from those in the control group. IR and metabolic disorders are common symptoms in patients with PCOS.[26] Subsequently, comparisons were made between the two groups regarding endocrine and metabolic parameters. The results showed that the HOMA-IR (2.316 (1.582, 3.335) vs 1.460 (0.936, 2.392), $p < 0.001$), FPG (4.800 (4.410, 5.240) vs 4.560 (3.790, 4.910) mmol/L, $p = 0.019$), the levels of FINS (11.090 (7.780, 15.260) vs 7.940 (6.520, 10.600) mIU/L, $p < 0.001$), and the levels of fasting C-peptide (FCP, 2.400 (1.940, 3.310) vs 2.020 (1.610, 2.600) ng/mL, $p = 0.016$) were significantly increased in PCOS patients compared with the control groups. Likewise, the levels of thyroglobulin (Tg, 6.980 (3.690, 10.810) vs 13.200 (4.650, 16.300) ng/mL, $p = 0.035$), high-density lipoprotein cholesterol (HDL-C, 1.270 (1.050, 1.520) vs 1.420 (1.180, 1.680) mmol/L, $p = 0.009$), and the total cholesterol/HDL-C ratio (TC/HDL-C, 3.414 (2.940, 4.188) vs 3.036 (2.658, 3.708), $p = 0.012$) were also significantly different between the PCOS and control groups. These changes were associated with the clinical phenotypes of PCOS and were consistent with the results of the metabolic parameter analysis.

3.2. The OVGP1 Serum Level Is Significantly Decreased in Patients with PCOS. To evaluate the level of

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OVGP1 in patients with PCOS, we measured differences in the serum OVGP1 concentration between patients with PCOS and controls by using ELISA. As shown in Figure 1a, the serum expression level of OVGP1 was significantly lower in patients with PCOS than in those in the control group (120 ± 39.67 vs 180 ± 93.19 pg/mL, $p < 0.001$), which indicates that OVGP1 may be a diagnostic biomarker for PCOS.

3.3. Correlations between OVGP1 Expression Levels and Clinical Characteristics in PCOS Patients. To explore the correlations between OVGP1 expression and clinical characteristics in patients with PCOS, Pearson and Spearman's correlation analyses were performed to assess the associations between the levels of OVGP1 and clinical indicators of PCOS. The results revealed that the OVGP1 level was positively correlated with the FSH level (rho = 0.39, $p < 0.01$) and negatively correlated with the LH/FSH ratio (rho = −0.25, $p < 0.01$) (Figure 1b,c). However, the OVGP1 level did not significantly correlate with any other diagnostic indicator ($p > 0.05$), as shown in Table S1. Multivariate joint distribution revealed differences in the OVGP1, LH, LH/FSH, AMH, and FSH data distributions between the PCOS and control groups (Figure 2). Furthermore, we performed multivariate linear regression to analyze the associations between OVGP1 and endocrine and metabolic parameters. The results also revealed that the OVGP1 level was significantly positively correlated with the baseline serum FSH concentration ($\beta = 0.444$, $p < 0.001$) (Table 2).

3.4. Investigation of OVGP1 and Hormones as Independent Risk Factors for PCOS Occurrence. To investigate whether OVGP1 is an independent risk factor for PCOS occurrence and whether OVGP1 and hormones could be predictors of PCOS occurrence, logistic regression analysis was performed. We found that with a one-unit increase in LH and AMH, the odds ratios (ORs) for PCOS occurrence were 1.228 (95% CI: 1.105−1.366, $p < 0.001$) and 1.276 (95% CI: 1.068−1.526, $p = 0.007$), respectively. Additionally, the ORs for PCOS occurrence were 0.454 (95% CI: 0.263−0.782, $p = 0.004$) and 0.983 (95% CI: 0.972−0.995, $p = 0.006$), with one-unit increases in FSH and OVGP1, respectively (Table 3). These results suggest that an increase in LH and AMH levels or a decrease in FSH and OVGP1 levels could increase the risk of PCOS in females.

3.5. OVGP1 Combined with Six Signatures Improves the Diagnostic Performance of PCOS. To evaluate applicable biomarkers for PCOS, we used the RFE-CV method to screen potential diagnostic markers from all clinical features. Six signatures, including LH/FSH, Prog, TC, TG, HDL-C, and AMH, were constructed (Figure 3a). Next, we used six machine learning algorithms to further evaluate the optimal diagnostic models for these indices. Among these classifiers, the performance of XGBoost was superior to that of each of the other five algorithms (Figure 3b,c); the area under the curve (AUC) for the model was 0.953 (95% CI = 0.916−0.990) in the training set and 0.907 (95% CI = 0.855−0.959) in the testing set (Table 4). When OVGP1 was combined with the six signatures, the AUCs of the XGBoost and LR models increased to 0.963 (95% CI = 0.931−0.995) and 0.833 (95% CI = 0.764−0.902), respectively, indicating that the addition of OVGP1 detection can be used to improve diagnostic performance in patients suspected of having PCOS (Figure 3d). The DCA curve calculates the clinical net benefit of each predictive model within the risk threshold probability range. The results showed that the net benefit of Model 2 (six signatures and OVGP1) was superior to that of

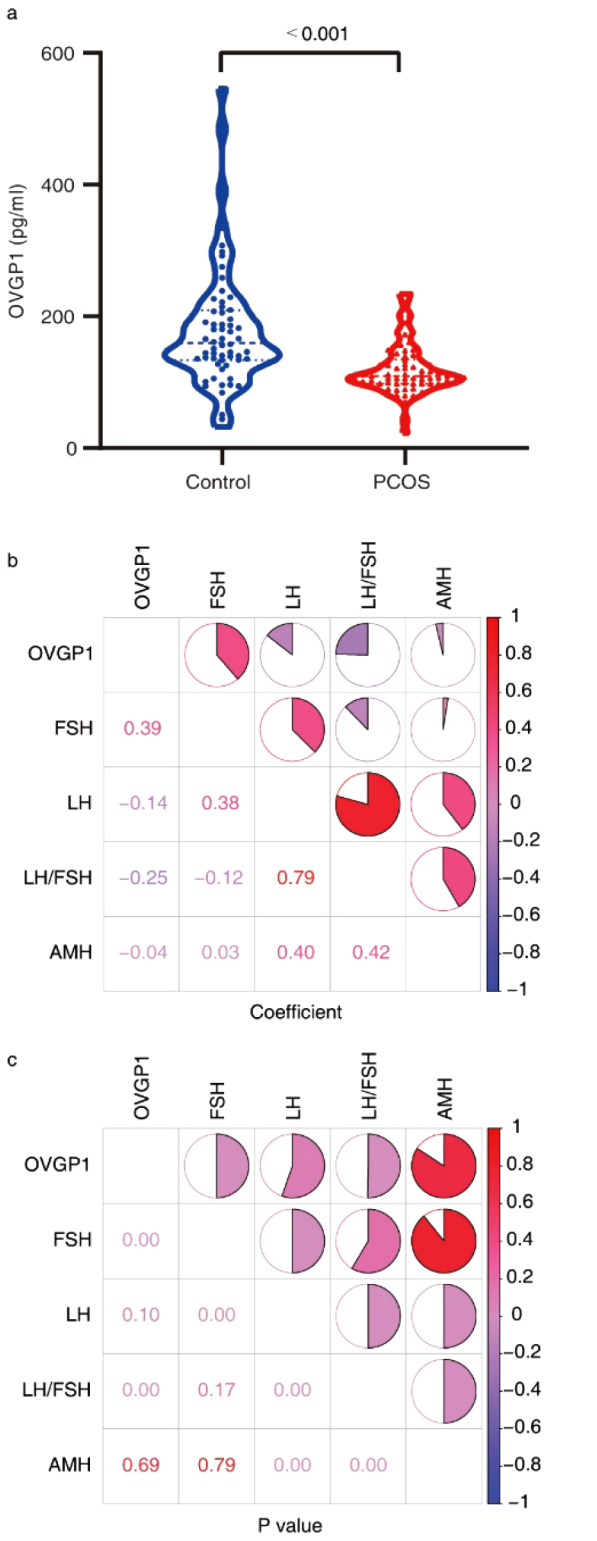

Figure 1. The level of OVGP1 is decreased in patients with polycystic ovary syndrome (PCOS) and is correlated with sex hormones. (a) Violin plot showing the difference in the serum level of OVGP1 between patients with PCOS and controls; one dot represents one sample. (b, c) Representative heatmap showing Spearman's correlation coefficient (b) and p-values (c) between OVGP1 and sex hormones. Spearman's correlation coefficients and p-values are illustrated by the pie area and corresponding number, indicating a positive (red) or negative significant correlation (blue) ($p < 0.05$). The area and color intensity of the round cake indicate the strength of the correlation.

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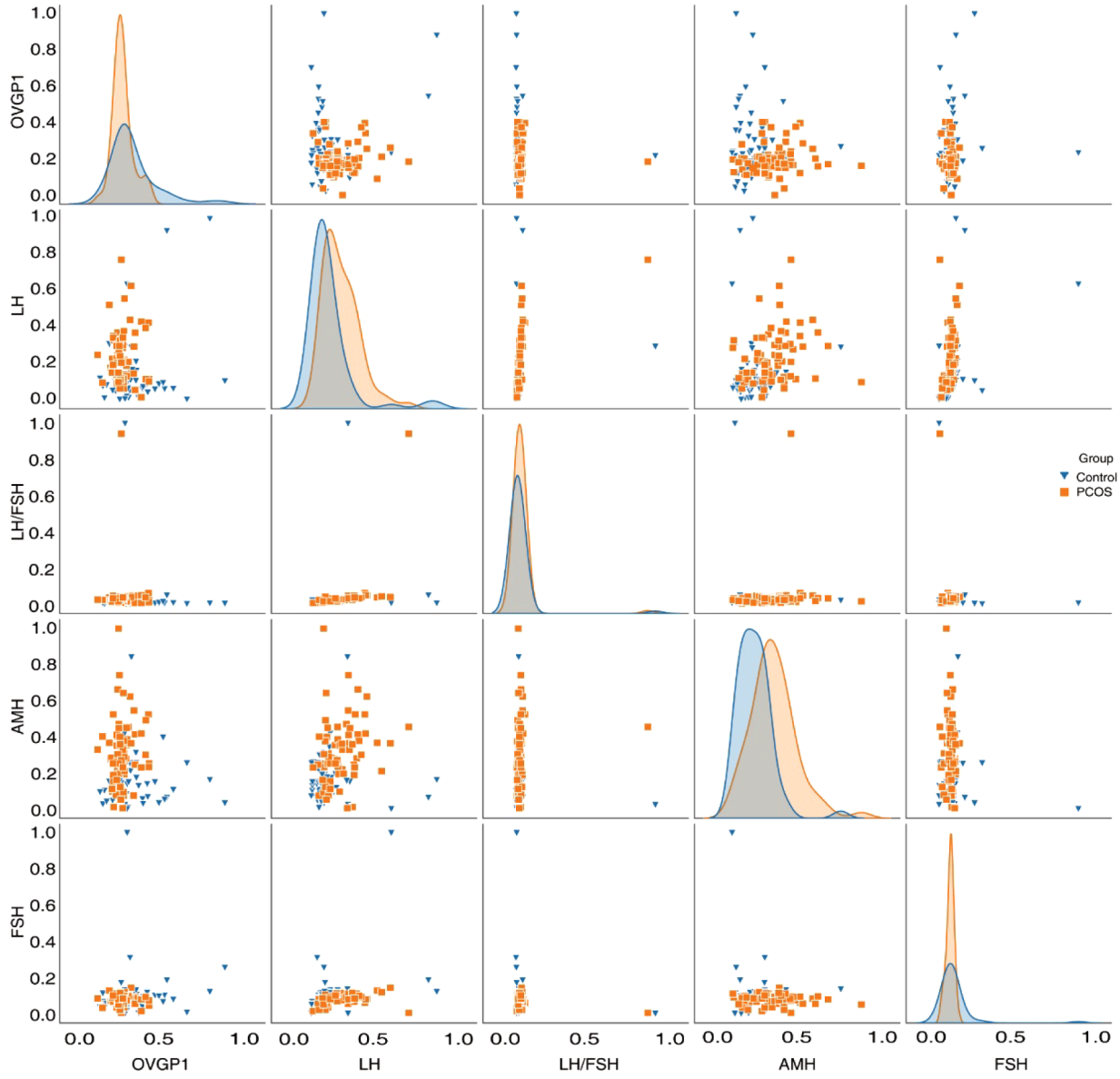

Figure 2. Joint distribution plots of OVGP1 and representative sex hormones for each sample in patients with polycystic ovary syndrome (PCOS) and control individuals. The orange square represents the PCOS group, and the blue triangle represents the control group. The diagonal represents the density estimate of the univariate distribution. The density plot shows the probability density of each variable in the dataset. The off-diagonal points are the scatter plots between the pairwise variables.

Table 2. Multivariate Linear Regression Analysis between OVGP1 and Endocrine−Metabolic Parameters[a]

Variable	*B*	SE	*β*	*t*	*p*-value	95% CI
FSH, mIU/L	2.999	0.774	0.444	2.999	0.000	1.464−4.534
LH, mIU/L	−0.715	0.894	−0.098	−0.715	0.426	−2.489−1.058
LH/FSH	0.059	0.650	0.009	0.059	0.927	−1.230−1.349
Prog, ng/mL	−0.066	0.469	−0.013	−0.142	0.888	−0.996−0.864
AMH, ng/mL	−1.041	1.790	−0.055	−0.582	0.562	−4.591−2.509
HOMA-IR	−3.536	11.602	−0.138	−0.305	0.761	−26.541−19.469
FPG, mmol/L	1.652	4.251	0.055	0.389	0.698	−6.778−10.082
FINS, mIU/L	3.234	3.646	0.455	0.887	0.377	−3.996−10.464
FCP, ng/mL	−20.217	12.129	−0.355	−1.667	0.099	−44.266−3.832

[a]Dependent variable: OVGP1; $R = 0.189$; adjusted $R = 0.119$; $F = 2.718$; $p<0.007$, CI: confidence interval.

Model 1 (six signatures), which met the practical requirements of clinical diagnosis (Figure 3e). In addition, when the two models were compared, as shown in Figure 3f, the NRI was 3.81% and the IDI was 7.4% (1.1%−13.7%, $p = 0.021$), which suggested that the combination of OVGP1 with the other six signatures improved the diagnostic performance of PCOS.

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Table 3. Logistic Regression Analysis to Investigate OVGP1 and Hormones as Independent Predictive Factors for PCOS Occurrence

Biomarkers	*B*	SE	Wals	*p*-value	OR (95% CI)
FSH, mIU/L	−0.791	0.278	8.104	0.004	0.454 (0.263−0.782)
LH, mIU/L	0.206	0.054	14.519	0.000	1.228 (1.105−1.366)
LH/FSH	−0.098	0.078	1.575	0.210	0.907 (0.778−1.057)
AMH, ng/mL	0.244	0.091	7.182	0.007	1.276 (1.068−1.526)
HOMA-IR	3.096	1.810	2.925	0.087	22.112 (0.636−768.600)
FPG, mmol/L	−0.974	0.818	1.417	0.234	0.378 (0.076−1.876)
FINS, mIU/L	−0.631	0.382	2.722	0.099	0.532 (0.252−1.126)
FCP, ng/mL	0.768	0.632	1.477	0.224	2.156 (0.625−7.446)
TC, mmol/L	0.209	0.361	0.336	0.562	1.233(0.608−2.501)
TG, mmol/L	−0.184	0.322	0.328	0.567	0.832 (0.443−1.563)
Prog, ng/mL	−0.083	0.071	1.364	0.243	0.921 (0.801−1.058)
OVGP1, pg/mL	−0.017	0.006	7.469	0.006	0.983 (0.972−0.995)

3.6. Diagnostic Value of the Combination of OVGP1 and Other Clinical Indicators in Patients with PCOS.

Considering the practical clinical application of these methods, the cost of seven detection indicators is too high and sex hormones are commonly used in the clinical diagnosis of PCOS. LH, LH/FSH, and AMH serum levels are the most frequently used criteria for assessing ovarian reserve.[27] Consequently, we compared the diagnostic efficacy of individual hormones, such as LH, LH/FSH, and AMH, as well as their combination. The AUCs of the ROC curves were 0.789 for LH, 0.846 for LH/FSH, and 0.778 for AMH. We found that all the ROC curves had high accuracy, with an AUC > 0.7, which reveals the predictive efficacy of all four indicators (Figure 4a). The combined diagnosis had an AUC of 0.930 (95% CI: 0.885−0.975), with a sensitivity of 90.00%, a specificity of 81.67%, and an accuracy of 86.15%, surpassing that of a single diagnostic index. Moreover, when OVGP1 was included, the AUC increased to 0.932 (95% CI: 0.888−0.976), the specificity increased to 86.67%, and the accuracy increased to 88.46% for differentiating between patients with PCOS and controls (Figure 4b,d). The DCA showed that the two diagnostic models are suitable for clinical application (Figure 4c). Similarly, the IDI and NRI were also used to compare the advantages and disadvantages of the two prediction models, and the results showed an NRI of 5% and an IDI of 8.5% (95% CI: 2.2%−14.8%, $p = 0.008$). These results indicate that the reclassification ability of OVGP1 was significantly improved after its inclusion.

4. DISCUSSION

In this study, we are the first to report the level of OVGP1 in patients with PCOS and report that OVGP1 is significantly decreased in patients with PCOS, is positively associated with FSH, and is negatively associated with the LH/FSH ratio. We also screened the ability of six signatures (LH/FSH, Prog, TC, TG, HDL-C, and AMH) to discriminate PCOS patients from controls via machine learning algorithms and found that the diagnostic value of OVGP1 alone and in combination with the six signatures or clinically applied markers for distinguishing PCOS patients from controls is high. Moreover, our study shows that a decrease in the level of OVGP1 expression can increase the risk of PCOS.

Anovulation is a major cause of infertility in 90% of women with PCOS, and OVGP1 is a major tubal glycoprotein in many species that is essential for sperm motility, fertilization, and embryonic development, and it may also be involved in female ovulation function.[11,28,29] Genetic mechanisms play an important role in the etiology of PCOS. The ERK-1 and ERK-2 pathways are among the most important of these mechanisms. In some studies, it has been stated that OVGP1 levels may be related to ERK gene pathways.[30−33] The MEK/ERK pathway is closely related to androgen secretion;[14] therefore, OVGP1 may also be involved in androgen secretion. Hyperlipidemia, insulin resistance, and chronic inflammation are associated with PCOS.[34] In this study, we also found that HDL-C levels were decreased and HOMA-IR was increased in women with PCOS. Our previous study revealed that OVGP1 induced vascular oxidative stress and inflammation,[12] which suggested that OVGP1 is associated with the pathophysiology of PCOS. The exact mechanism of OVGP1 in PCOS patients should be explored in future studies.

In addition, reproductive function is closely related to the endocrine system, and PCOS is characterized by a series of interrelated changes in reproductive hormones,[35] and the relationship between OVGP1 and PCOS has not been evaluated at either the clinical or basic level. Therefore, we conducted this first clinical study to analyze the correlation between OVGP1 and diagnostic indicators of PCOS.

Reproduction occurs through the hypothalamus−pituitary−ovary axis, which coordinates reproductive behavior with ovulation. Gonadotropin-releasing hormone (GnRH) release from the hypothalamus regulates the secretion of FSH and LH, and E2 levels increase as follicles develop.[36,37] An increase in E2 in turn stimulates the release of GnRH, and the release of OVGP1 is dependent on estrogen.[38,39] In our study, a significant positive correlation between OVGP1 and FSH was evident, which suggested that OVGP1 may play an important role in promoting follicle development and ovulation. Studies have reported that in women, LH and FSH are positively correlated from the beginning of the reproductive stage up to menopause,[40] which is consistent with our findings. We next used multiple linear regression to determine how OVGP1 and FSH are correlated, and the results confirmed our findings and highlighted how complex and close the regulatory relationships between sex hormones are. Subsequent logistic regression analysis revealed that a decrease in the level of OVGP1 was an independent risk factor for PCOS and may indirectly influence the development of PCOS by regulating hormone levels.

We also evaluated biochemical indicators as predictors of PCOS via machine learning algorithms. In particular, we observed that the XGBoost model had the best predictive performance for PCOS diagnosis and that the analysis of the six signatures through the XGBoost model was able to predict

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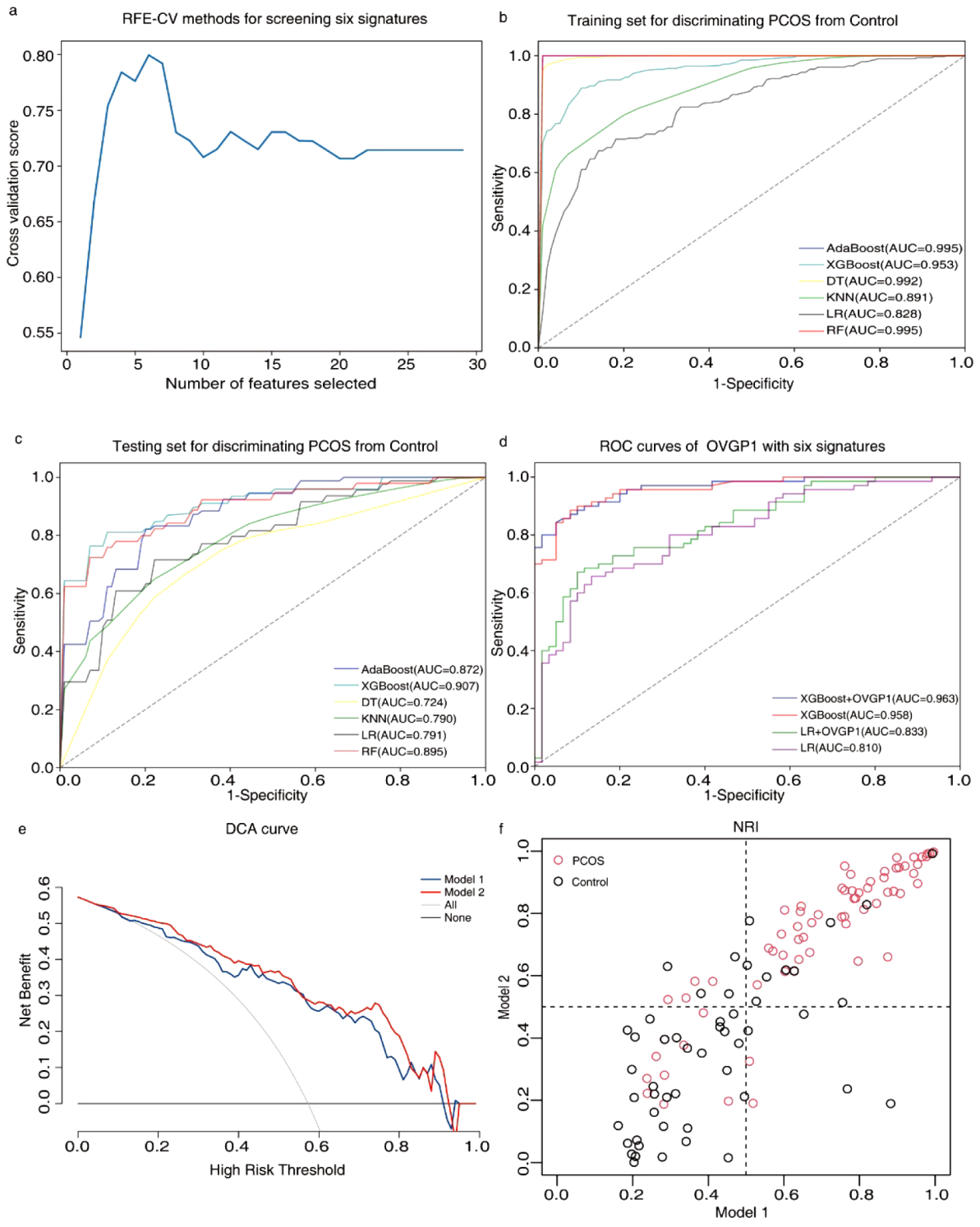

Figure 3. Identification of six signatures by the RFE-CV algorithm and evaluation of their diagnostic values in patients with PCOS. (a) Six diagnostic indicators were screened from among all the clinical features based on the RFE-CV algorithm. (b, c) ROC plot of the performance of the six classification algorithms based on the AUC for discriminating PCOS patients from controls in the training (b) and test (c) sets. (d) ROC curves for six candidate diagnostic indicators with or without OVGP1 based on the XGBoost and LR models. (e) Decision curve and (f) the net reclassification index for diagnostic efficacy verification. Model 1 represents the combination of six candidate signatures, and Model 2 represents the combination of six candidate signatures with OVGP1. The black circles represent controls, and the red circles represent PCOS patients.

PCOS with an accuracy of 88.46%; however, combining OVGP1 with the six signatures resulted in an increased accuracy of 89.23%. In the context of routine PCOS diagnosis, the primary focus is on sex hormone levels. We developed a diagnostic model encompassing sex hormones, and the AUC was 0.932 (95% CI = 0.888−0.976), with an accuracy of 88.46%. Silva used a machine learning algorithm to incorporate 14 variables with an accuracy of 86% and an AUC of 97%.[41] These results indicate that our model (four indicators) is well suited for clinical implementation, while reducing the cost of examination.

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Table 4. Classification Performance for the Six Signatures between PCOS Patients and Controls

		Sensitivity (%)	Specificity (%)	ACC (%)	PPV (%)	NPV (%)	AUC (95% CI)
AdaBoost	Training set	100.00	100.00	100.00	100.00	100.00	0.995 (0.983−1.000)
	Test set	80.72	74.67	79.23	77.85	78.11	0.872 (0.811−0.933)
XGBoost	Training set	89.24	89.15	89.23	90.51	87.70	0.953 (0.916−0.990)
	Test set	83.54	83.08	83.85	84.88	83.22	0.907 (0.855−0.959)
DT	Training set	96.09	99.21	97.50	99.23	95.55	0.992 (0.977−1.000)
	Test set	66.34	75.11	71.54	74.06	66.42	0.724 (0.638−0.81)
KNN	Training set	83.18	74.19	79.23	79.44	78.98	0.891 (0.835−0.947)
	Test set	72.76	68.67	72.31	72.66	68.95	0.790 (0.713−0.867)
LR	Training set	78.26	70.21	74.81	75.47	74.06	0.828 (0.758−0.898)
	Test set	72.09	70.86	71.54	71.39	71.37	0.791 (0.714−0.868)
RF	Training set	100.00	100.00	100.00	100.00	100.00	0.995 (0.983−1.000)
	Test set	81.12	77.33	79.23	79.42	78.18	0.895 (0.840−0.950)

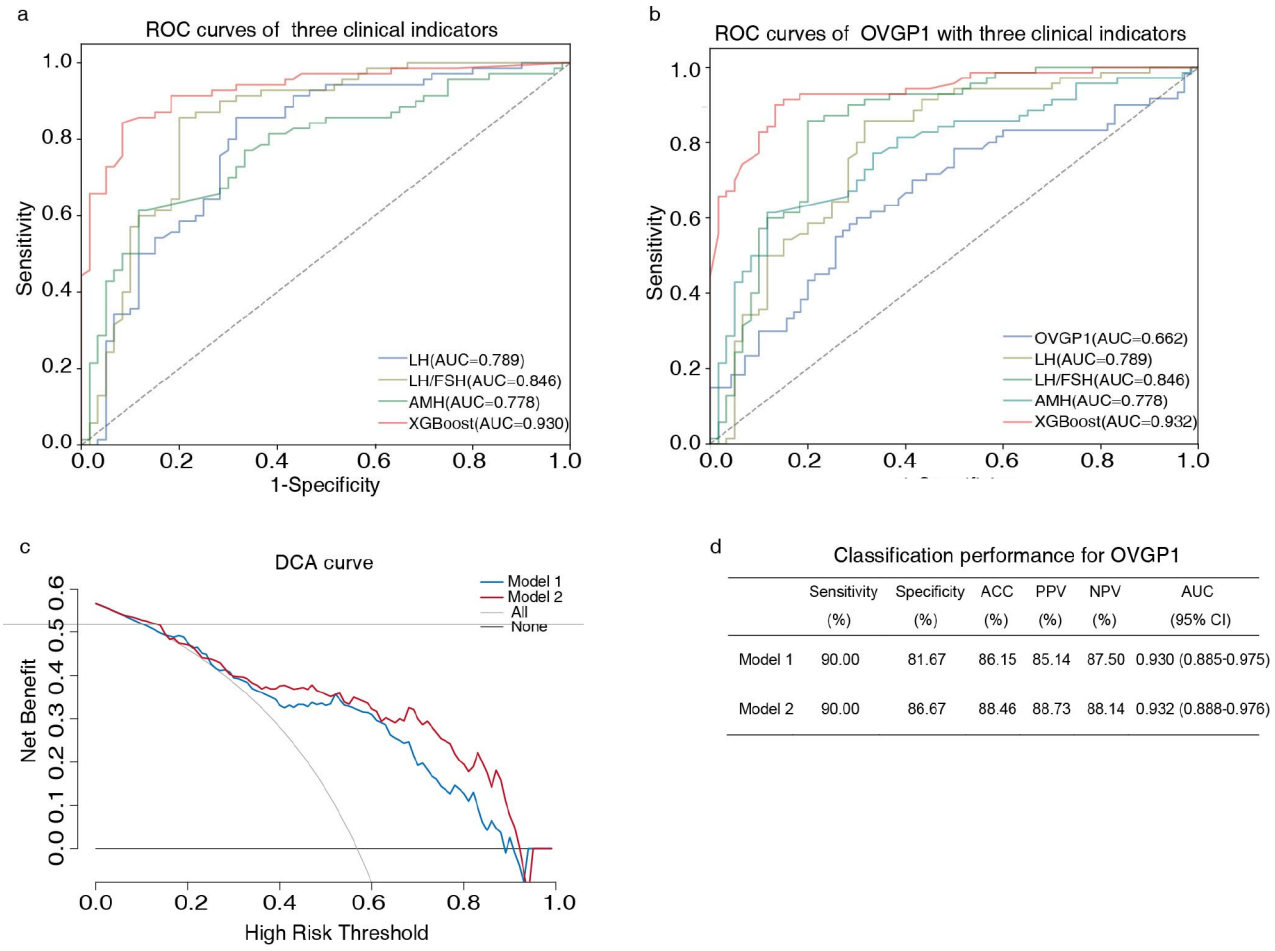

Figure 4. Evaluation of the diagnostic value of combining OVGP1 with clinical applicative indicators in patients with PCOS. (a) ROC plot of LH, LH/FSH ratio, and AMH levels individually or in combination for differentiating patients with PCOS from controls. (b) ROC plot of LH, the LH/FSH ratio, and AMH levels combined with OVGP1 for differentiating patients with PCOS from controls. (c) DCA curves for diagnostic efficacy verification. (d) Classification performance for OVGP1 combined with clinical indicators between PCOS patients and controls. Model 1 represents the combination of LH, the LH/FSH ratio, and AMH; Model 2 represents the combination of LH, the LH/FSH ratio, AMH, and OVGP1.

This finding implies that well-designed machine learning has the potential to significantly enhance our ability to diagnose PCOS early, with associated cost savings and a reduced burden of PCOS on patients and the health system.[42,43]

The main objective of our study was to provide a new diagnostic index for PCOS. Feature selection methods were applied to select the optimal subset of features to combine with OVGP1, and the results showed that XGBoost with RFE feature selection achieved the highest performance compared to that of a single index. The application of a machine learning algorithm can be useful for guiding more personalized and effective approaches for diagnosing PCOS and preventing its comorbidities. However, this study has several limitations. First, the sample in this study was limited, and a larger multicenter study with more participants is needed to externally validate the diagnostic value of OVGP1. Second, the lack of multilayer distribution analysis and the presence of some patients with irregular menstruation in the control group may lead to the

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underestimation of the diagnostic efficacy of OVGP1 for PCOS; in the future, additional clinical samples need to be collected for clinical verification. Finally, the potential functions of OVGP1 in patients with PCOS should be explored via cell and animal experiments.

5. CONCLUSION

In this study, we report OVGP1 levels in PCOS patients for the first time. The level of OVGP1 is significantly decreased in PCOS patients and is associated with FSH and the LH/FSH ratio, and the OVGP1 level may be an independent predictor of PCOS occurrence. Furthermore, we identified six signatures (LH/FSH, Prog, TC, TG, HDL-C, and AMH) by machine learning that perform well in the diagnosis of PCOS patients with a diagnostic accuracy of 88.46%. Considering the cost and benefit, the accuracy of the combination of three clinical indicators (LH, LH/FSH, and AMH) was 86.15%. With the addition of the OVGP1 index, the accuracy rate increased to 89.23% and 88.46%, respectively, which improved the diagnostic ability and emphasized that OVGP1 helps to diagnose PCOS.

■ ASSOCIATED CONTENT

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article (and/or its Supporting Information).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03111.

Table S1: Correlation between OVGP1 levels and baseline clinical characteristics in patients with polycystic ovary syndrome (PCOS) and controls (XLSX)

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Author Contributions

#F.W. and X.L. contributed equally to this work. C.B. designed the study. F.W. and X.L. collected the clinical samples. F.W. and J.W. performed the experiments on OVGP1 and laboratory indicator expression levels. X.H. and C.B. analyzed and interpreted the laboratory data. F.W. and C.B. wrote the manuscript. J.L. revised the manuscript. All authors approved the final version of the manuscript for submission.

Notes

The study was approved by the ethics committee of the First Affiliated Hospital of the Fourth Military Medical University and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The authors declare no competing financial interest.

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