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Unveiling the link: anti-protein disulfide isomerase A3 autoantibody expression and polycystic ovary syndrome risk in euthyroid autoimmune thyroiditis women

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Abstract

Background Polycystic ovary syndrome (PCOS) is a common complication of autoimmune thyroiditis (AIT) in women, but the underlying mechanism remains unclear. Protein disulfide isomerase A3 (PDIA3) is a ubiquitous protein. We have reported that PDIA3 autoantibody (PDIA3Ab) production results from autoimmune responses against thyrocytes, resulting in its high expression in euthyroid AIT patients. This study aimed to explore potential correlations between PDIA3Ab expression and concurrent PCOS in euthyroid AIT women.

Methods This is a single-center cross-sectional study. All participants, who visited the First Hospital of China Medical University from April 2023 to May 2024, were assigned to four groups according to AIT and PCOS diagnostic criteria. The PDIA3Ab levels of total IgG and IgG subclasses were detected using ELISA.

Results From highest to lowest, PDIA3Ab total serum IgG levels were categorized as follows: AIT-PCOS group > AIT-non-PCOS group > non-AIT-PCOS group > non-AIT-non-PCOS group Significant differences were observed between each pair of groups, except for the non-AIT-PCOS and non-AIT-non-PCOS groups. Further analysis of the subclasses of PDIA3Ab revealed that serum IgG1 levels in the AIT-PCOS and AIT-non-PCOS groups were significantly higher than those in the non-AIT-PCOS and non-AIT-non-PCOS groups. In addition, the AIT-PCOS group had significantly higher serum IgG3 levels than the other three groups. Binary logistic regression analysis revealed that the PDIA3Ab total IgG level was an independent risk factor for concurrent PCOS in euthyroid AIT women (Q4 vs. Q1: OR, 95%*CI* = 5.082, 1.348–19.16). Furthermore, a trend test demonstrated a titer-dependent increase in PCOS prevalence among AIT women as the PDIA3Ab total IgG level increased.

Conclusions The expression of serum PDIA3Ab may indicate an increased risk of PCOS in euthyroid AIT women and could potentially serve as new targets for markers or immune intervention.

Keywords Anti-PDIA3 autoantibody, Thyroiditis, Autoimmune diseases, Euthyroidism, Polycystic ovary syndrome

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Background

The prevalence of autoimmune thyroiditis (AIT), also known as Hashimoto's thyroiditis, or chronic lymphocytic thyroiditis, in women is as high as 20.35% [1], and it can cause various extrathyroidal lesions, such as spontaneous abortion, adult Hashimoto encephalopathy [2], and fetal neurodevelopmental disorders [3], impair female reproductive function and lead to adverse pregnancy outcomes. In recent years, numerous studies have shown that AIT is closely related to polycystic ovary syndrome (PCOS). A cohort study in Taiwan revealed that the PCOS risk increased by 2.37 times in women with AIT [4]. When AIT patients with PCOS were compared to AIT-only individuals, the proportion of coronary artery disease (CAD) rose by 5.92 times [4]. An Indian prospective case-control study suggested that compared to control subjects, females with euthyroid AIT had a much higher frequency of PCOS (4.3% vs. 46.8%) [5]. Wang et al.'s analysis of infertile women showed a significantly higher prevalence of PCOS in women with AIT (37.1%) compared to those without (19.4%) [6].

Although the mechanism of AIT complicated by PCOS remains unclear, the role of specific autoimmunity has begun to attract attention. 44% of PCOS patients have anti-ovarian antibodies, which mainly target granulosa cells, but the specific target antigen is not yet clear [7]. Moreover, thyroid autoimmunity itself is an independent risk factor for PCOS [8, 9]. Simply correcting thyroid abnormalities could not effectively improve the reproductive function and metabolic disorders of AIT patients with PCOS [10]. Monteleone et al. have proposed that anti-thyroid peroxidase autoantibody (TPOAb) may cause follicle destruction through antibody-dependent cell-mediated cytotoxicity (ADCC) due to the structurally similar antigens between the zona pellucida and the thyroid gland [11]. However, no similar study has provided direct evidence that TPOAb and anti-thyroglobulin autoantibodies (TgAb) promote the occurrence of PCOS, nor confirmed the distribution of thyroid peroxidase (TPO) and thyroglobulin (Tg) in the ovary. Although autoantibodies to the gonadotropin-releasing hormone (GnRH) receptor are expressed in a very small minority of PCOS patients [12], this receptor protein is not found in the thyroid gland or ovary. Edassery et al. found that protein disulfide isomerase A3 (PDIA3) may serve as the target antigen of the antibodies in infertility when analyzing anti-ovarian antibodies in the serum of women with infertility and premature ovarian failure [13]. PDIA3 is mainly expressed in granulosa cells and theca cells [14]. Additionally, it has been observed that the expression level of the classical Vitamin D receptor in the ovary is deficient, and Vitamin D can influence antral follicle development through non-genomic effects mediated by the aforementioned cell surface PDIA3 (also known as 1,25D3-MARRS) [15]. In addition, our previous study has shown that serum anti-PDIA3 autoantibody (PDIA3Ab) levels are significantly increased in both AIT patients and mouse models, potentially contributing to some extrathyroidal lesions, such as spontaneous abortion [16, 17]. Consequently, this research aims to determine the expression levels of PDIA3Ab in euthyroid women with AIT and PCOS.

Methods

Subjects

This single-center cross-sectional study was conducted at the First Hospital of China Medical University between April 2023 and May 2024. All participants provided informed consent, and the protocol was approved by the local ethics committee, specifically the Ethics Committee of the First Affiliated Hospital of China Medical University (Approval Code [2023]2023-92-2). All enrolled women were aged between 18 and 45 years.

AIT was diagnosed when the serum TPOAb was positive (≥5.61 IU/mL) and/or TgAb was positive (≥4.11 IU/mL) along with the hypoechogenicity of the thyroid gland on ultrasound [18, 19]. Postpartum thyroiditis was not included in this study.

The Rotterdam criteria were used to diagnose PCOS when at least two of the following three criteria were present: oligomenorrhea (cycles lasting longer than 35 days) or amenorrhea (<2 menstrual cycles in the past 6 months), clinical manifestations of hyperandrogenism or hyperandrogenemia, and ultrasound showing polycystic ovaries. Other diseases that could cause hyperandrogenic symptoms, including Cushing's syndrome, non-classical congenital adrenal hyperplasia (NCCAH), tumors of the ovary or adrenal gland that secrete androgens, drug-induced hyperandrogenism, and idiopathic hirsutism, are excluded. Additionally, conditions that could cause ovulation disorders, such as functional hypothalamic amenorrhea (FHA), hyperprolactinemia, and premature ovarian insufficiency (POI), are also excluded [20].

The following were the exclusion criteria for this study: (1) women in pregnancy; (2) patients with previous abnormal thyroid function; (3) patients with chronic systemic ailments such as diabetes and hypertension, and genetic diseases; (4) patients with other autoimmune diseases, pituitary or adrenal diseases and malignant tumors; (5) patients in stress states such as fever, trauma or infection; (6) patients taking Vitamin D supplementation, glucocorticoids, other immunosuppressive or immunomodulatory agents in the past 6 months; (7) patients taking oral contraceptives and other drugs affecting sex hormone levels within the past 3 months.

Finally, all 283 participants in this study were grouped into four separate groups. Group 1: AIT-PCOS group, referred to euthyroid women with AIT with PCOS.

Group 2: AIT-non-PCOS group, referred to euthyroid women with AIT without PCOS. Group 3: non-AIT-PCOS group, referred to euthyroid women without AIT with PCOS. Group 4: non-AIT-non-PCOS group, referred to euthyroid women without AIT without PCOS.

Data collection

Body mass index (BMI, kg/m^2) was calculated for all women after measuring their weight and height. Hirsutism was classified according to the Ferriman-Gallwey score [21].

Laboratory tests

Venous blood samples were taken between 8:00 AM and 9:00 AM during the follicular phase, following an 8–12 h overnight fast. Serum levels of follicular stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estradiol were measured by the chemiluminescence method using the IMMULITE 2000 XPi immunoassay System (Siemens Diagnostics, Los Angeles, USA). The serum levels of free T4 (FT4), free T3 (FT3), thyroid-stimulating hormone (TSH), TPOAb, and TgAb concentrations were measured by chemiluminescent microparticle immunoassay using the ARCHITECT i2000SR immunoassay analyzer (Abbott Diagnostics, Longford, Ireland). Fasting blood glucose (FBG), blood lipids, liver function, and kidney function were measured by the biochemical method using the Cobas C501 automatic biochemical analyzer (Roche Diagnostics, Mannheim Germany). Blood routine parameters were detected by the electrical impedance measurement using the Sysmex XN20 A1 automation blood cell analyzer (Sysmex Diagnostics, Japan).

The serum level of PDIA3Ab was measured using ELISA previously established in our study [17]. Microtiter plates (Nunc, Roskilde, Denmark) were coated with 1 μg/well of recombinant PDIA3 protein (Abcam, Cambridge, UK), blocked with 1% bovine serum albumin (Sigma-Aldrich), and incubated for 2 h at room temperature with participant serum samples diluted 1:50. After 5 washes, the plates were further incubated for 2 h at room temperature with goat anti-human IgG (Bioss, China) labeled with horseradish peroxidase. Following another 5 washes, TMB substrate was added, and the color was allowed to develop at room temperature for ten minutes. The reaction was stopped by adding HCl. Absorbance values were measured at 450 nm using a microplate reader (Bio-Rad 680, Bio-Rad, CA, USA) within 30 min. Each ELISA plate included 4 replicates of positive and negative controls, as well as 3 blank controls during routine runs. For standardization and comparability, we introduced a relative value ratio for PDIA3Ab. These ratios were calculated using the following formula, which allowed us to determine the OD450nm value ratios of PDIA3Ab for each sample on every ELISA plate:

$$\begin{aligned} &Sample\ OD\ value\ ratio \\ &= \frac{Sample\ OD\ value - Average\ OD\ value\ of\ the\ blank\ control\ wells}{Average\ OD\ value\ of\ the\ positive\ control\ wells} \end{aligned}$$

The intra- and inter-assay coefficients of variation of OD values of PDIA3Ab total serum IgG and IgG subclasses were 1.55–9.92% and 13.88–15.46%, respectively.

Statistical analysis

When comparing groups with normally distributed continuous variables, we employed analysis of variance (ANOVA). For continuous variables that did not follow a normal distribution, we utilized the Kruskal-Wallis test for group comparisons, followed by the Bonferroni test for post-hoc analysis. Spearman's correlation test was conducted to investigate the association between PDIA3Ab total serum IgG and FT4, FT3, TSH, TPOAb, TgAb, LH/FSH, estradiol, and testosterone levels. Binary logistic regression was employed to identify factors related to concurrent PCOS. Additionally, receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic utility of PDIA3Ab total serum IgG in AIT women with PCOS. We also evaluated the relationship between the incidence of PCOS in euthyroid AIT women and the serum titers of TPOAb, TgAb, and PDIA3Ab total serum IgG using a trend test. Statistical analysis was performed using SPSS v26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism v9.5.1 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $P<0.05$.

Results

Clinical characteristics of participants

The clinical, biochemical, and hormonal parameters of 4 groups are presented in Tables 1 and 2. No notable differences were observed in age, BMI, serum levels of FT4, FT3, TSH, FSH, and estradiol. However, it was revealed that the serum level of LH was significantly higher in the non-AIT-PCOS group than in the AIT-non-PCOS group and the non-AIT-non-PCOS group. Additionally, LH/FSH was significantly higher in the non-AIT-PCOS group than in the other three groups, and considerably higher in the AIT-PCOS group than in the AIT-non-PCOS group. Furthermore, the serum level of testosterone was lower in the AIT-non-PCOS group than in the AIT-PCOS group and non-AIT-PCOS group, and lower in the non-AIT-non-PCOS group than in the non-AIT-PCOS group (Table 1).

Levels of FBG, cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin

Table 1 The anthropometric parameters, thyroid, and sex hormone indicators of the participants in this study

	euthyroid women with AIT		euthyroid women without AIT	
	AIT-PCOS group (*n* = 55)	**AIT-non-PCOS group (*n* = 43)**	**non-AIT-PCOS group (*n* = 101)**	**non-AIT-non-PCOS group (*n* = 84)**
Age, years	29 (24–34)	30 (27–34)	27 (23–31)	30 (23–38)
BMI, kg/m^2	24.76 ± 5.88	23.50 ± 3.28	25.73 ± 4.44	23.94 ± 4.98
FT4, pmol/L	12.85 (11.78–14.08)	12.95 (11.89–13.72)	12.81 (12.05–13.66)	12.88 (11.78–13.83)
FT3, pmol/L	4.52 ± 0.68	4.39 ± 0.56	4.68 ± 0.50	4.54 ± 0.67
TSH, mIU/L	2.13 (1.10–3.66)	2.10 (1.22–4.28)	1.63 (1.04–2.23)	1.58 (1.09–2.32)
TPOAb, IU/mL	95.51 (3.50-420.94)	32.73 (3.86-448.59)	0.54 (0.19–1.20) $^{\$,\dagger}$	0.57 (0.31–1.57) $^{\$,\dagger}$
TgAb, IU/mL	48.51 (5.20-200.62)	36.06 (7.31-294.71)	1.36 (0.88–1.77) $^{\$,\dagger}$	1.12 (0.82–1.59) $^{\$,\dagger}$
LH, mIU/mL	6.44 (3.85–10.95)	3.7 (2.57–9.13) *	8.60 (6.17–12.25)	4.26 (2.28–10.35) *
FSH, mIU/mL	6.33 (4.17–7.25)	6.58 (4.21–8.74)	5.35 (4.30–6.68)	5.37 (3.26–7.27)
LH/FSH	1.16 (0.66–1.94) *	0.67 (0.46–1.10) $^{*,\$}$	1.63 (1.22–2.17)	0.85 (0.58–1.35) *
Estradiol, pmol/L	188.5 (147.5-290.5)	162 (97.85–307.7)	166 (123.5-291.5)	186 (92.1–309.0)
Testosterone, nmol/L	1.05 (0.69–1.60)	0.69 (0.69–0.99) $^{*,\$}$	1.24 (0.71–1.93)	0.69 (0.69–1.09) *

$^{*}P < 0.05$ as compared with that of the non-AIT-PCOS group

$^{\$}P < 0.05$ as compared with that of the AIT-PCOS group

$^{\dagger}P < 0.001$ as compared with that of the AIT-non-PCOS group

AIT: autoimmune thyroiditis; PCOS: polycystic ovary syndrome; BMI: body mass index; FT4: free T4; FT3: free T3; TSH: thyroid stimulating hormone; TPOAb: anti-thyroid peroxidase autoantibody; TgAb: anti-thyroglobulin autoantibody; LH: luteinizing hormone; FSH: follicular stimulating hormone

Table 2 The metabolic and biochemical indicators of the participants in this study

	Euthyroid women with AIT		Euthyroid women without AIT	
	AIT-PCOS group (*n* = 55)	**AIT-non-PCOS group (*n* = 43)**	**non-AIT-PCOS group (*n* = 101)**	**non-AIT-non-PCOS group (*n* = 84)**
FBG, mmol/L	5.07 ± 0.55	5.18 ± 0.87	5.37 ± 1.29	5.27 ± 1.65
Cholesterol, mmol/L	4.65 (4.06–5.46)	4.29 (3.66–5.32)	4.63 (3.98–5.38)	4.78 (4.18–5.52)
HDL-C, mmol/L	1.32 ± 0.42	1.49 ± 0.35	1.30 ± 0.35	1.46 ± 0.43
LDL-C, mmol/L	2.95 ± 1.02	2.72 ± 0.82	2.87 ± 0.91	2.91 ± 1.02
Triglyceride, mmol/L	1.14 (0.57–1.71)	0.84 (0.62–1.08)	1.17 (0.71–1.89)	1.12 (0.60–1.66)
ALT, IU/L	21 (11–35)	14 (9–20) $^{*,\$}$	20 (13-36.5)	17 (11.25–27.75)
AST, IU/L	20 (16–28)	17 (14–21) $^{*,\$}$	21 (16–28)	19.5 (16–25)
ALP, IU/L	70.29 ± 23.86	60.57 ± 13.07	69.00 ± 18.42	70.52 ± 21.62
GGT, IU/L	18 (14–31)	15 (11.5–22.5)	19 (13–32)	17 (12.25–25.75)
TBIL, umol/L	10.36 ± 4.09	11.94 ± 5.90	11.43 ± 5.17	11.71 ± 4.91
BUN, mmol/L	4.43 ± 1.21	4.32 ± 1.43	4.45 ± 1.13	4.68 ± 1.69
Scr, umol/L	54.11 ± 7.98	54.13 ± 8.98	57.33 ± 9.54	54.91 ± 11.31
eGFR, $ml{\cdot}min^{-1}{\cdot}(1.73m^2)^{-1}$	122.77 ± 9.10	117.50 ± 12.79	122.49 ± 12.33	117.64 ± 15.37
WBC count, $\times 10^9$/L	7.21 ± 1.67	6.23 ± 1.61 *	7.18 ± 1.72	6.31 ± 1.94 *
Neut count, $\times 10^9$/L	4.39 ± 1.23	3.78 ± 1.49	4.32 ± 1.49	3.69 ± 1.74
NLR	1.95 (1.62–2.46)	1.80 (1.41–2.51)	1.82 (1.46–2.22)	1.73 (1.17–2.31)
RBC count, $\times 10^{12}$/L	4.65 ± 0.37	4.45 ± 0.37 *	4.69 ± 0.41	4.50 ± 0.48 *
HB, g/L	132.17 ± 12.74	128.95 ± 14.23 *	136.25 ± 11.69	127.56 ± 15.50 *
PLT count, $\times 10^9$/L	295.68 ± 57.22	260.51 ± 63.71	278.87 ± 57.40	275.67 ± 82.60

$^{*}P < 0.05$ as compared with that of the non-AIT-PCOS group

$^{\$}P < 0.05$ as compared with that of the AIT-PCOS group

AIT: autoimmune thyroiditis; PCOS: polycystic ovary syndrome; FBG: fasting blood glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ALT: alanine transaminase; AST: aspartate transaminase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; TBIL: total bilirubin; BUN: blood urea nitrogen; Scr: serum creatinine; eGFR: estimated glomerular filtration rate; WBC: white blood cell; Neut: neutrophil; NLR: neutrophil to lymphocyte ratio; RBC: red blood cell; HB: hemoglobin; PLT: platelet

(TBIL), blood urea nitrogen (BUN), serum creatinine (Scr), estimated glomerular filtration rate (eGFR), neutrophil (Neut), platelet (PLT) count, and neutrophil to lymphocyte ratio (NLR) were similar among the four groups. However, alanine transaminase (ALT) and aspartate transaminase (AST) levels were significantly lower in the AIT-non-PCOS group compared to the non-AIT-PCOS group and the AIT-PCOS group. Additionally, both red

blood cell (RBC) and white blood cell (WBC) counts, as well as hemoglobin (HB), were significantly higher in the non-AIT-PCOS group compared to the AIT-non-PCOS group and non-AIT-non-PCOS group (Table 2).

PDIA3Ab levels of total IgG and IgG subclasses

Serum PDIA3Ab levels were quantified by the OD450 value ratio of PDIA3Ab in this study. The levels were graded from high to low as follows: AIT-PCOS group > AIT-non-PCOS group > non-AIT-PCOS group > non-AIT-non-PCOS group. Statistical differences were observed between each pair of groups, except for the non-AIT-PCOS group and the non-AIT-non-PCOS group (Fig. 1a). The top 20 cases with the highest PDIA3Ab total serum IgG expression in each group were selected for assessment of IgG subtype levels. PDIA3Ab serum IgG1 levels were significantly higher in the AIT-PCOS and AIT-non-PCOS groups compared to the non-AIT-PCOS and non-AIT-non-PCOS groups. Additionally, the AIT-PCOS group exhibited significantly higher serum IgG3 levels of PDIA3Ab than the other three groups. However, there was no significant difference in the IgG2 and IgG4 serum levels of PDIA3Ab among the four groups, though (Fig. 1b-e).

The relationships between the expression of PDIA3Ab and the thyroid and sex hormone parameters

According to a previous study, euthyroid women with AIT with PCOS, regardless of obesity status, demonstrated significantly elevated LH/FSH ratios and

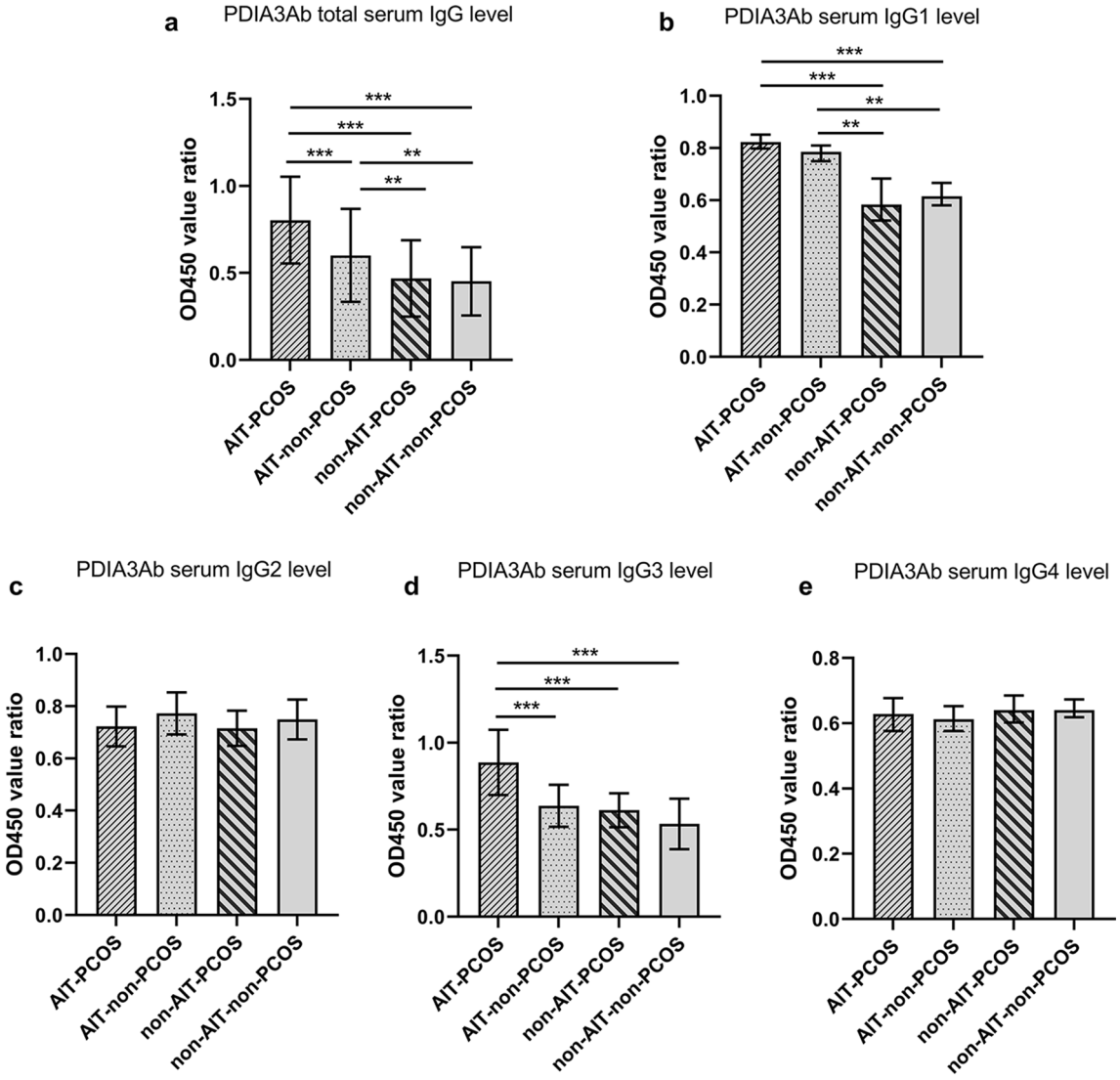

Fig. 1 Analysis of serum PDIA3Ab level. **a.** Comparison of PDIA3Ab total serum IgG levels among 4 groups. One-way ANOVA and Bonferroni's post hoc test were utilized to compare the groups. **b-e.** The top 20 in each group with the highest PDIA3Ab total serum IgG expression were selected to evaluate PDIA3Ab serum IgG1-4 subtype levels. One-way ANOVA with Bonferroni's post hoc test was used to compare IgG1 and 4, and the Kruskal-Wallis test with Bonferroni's post hoc test was used to compare IgG2 and 3. ($^{**}P < 0.01$ and $^{***}P < 0.001$)

Table 3 Spearman correlation analysis of PDIA3Ab total serum IgG level with the levels of thyroid function, thyroid autoantibodies, and sex hormones among the euthyroid women with AIT

PDIA3Ab OD value ratio	FT4	FT3	TSH	TPOAb	TgAb	LH/FSH	Estradiol	Testosterone
AIT-POCS	r_s = -0.079	r_s = 0.122	r_s = 0.233	r_s = 0.133	r_s = 0.147	**r_s= 0.427**	r_s = 0.174	**r_s= 0.297**
	P=0.568	*P*=0.377	*P*=0.087	*P*=0.333	*P*=0.284	***P*=0.005**	*P*=0.296	***P*=0.04**
AIT-non-PCOS	r_s = -0.21	r_s = 0.173	r_s = 0.235	r_s = -0.002	r_s = -0.016	r_s = -0.232	r_s = -0.022	r_s = 0.023
	P=0.177	*P*=0.268	*P*=0.130	*P*=0.992	*P*=0.92	*P*=0.167	*P*=0.911	*P*=0.898

Positive controls serve as the standard OD value ratio of PDIA3Ab total serum IgG

Statistically significant differences are marked **in bold**

PDIA3Ab: protein disulfide isomerase autoantibody; IgG: immunoglobulin G; AIT: autoimmune thyroiditis; PCOS: polycystic ovary syndrome; FT4: free T4; FT3: free T3; TSH: thyroid stimulating hormone; TPOAb: anti-thyroid peroxidase autoantibody; TgAb: anti-thyroglobulin autoantibody; LH: luteinizing hormone; FSH: follicular stimulating hormone

Table 4 Binary logistic regression analysis of the potential risk factors for PCOS without PDIA3Ab included among the euthyroid women with AIT

	Model 1 OR (95%CI)	*P*	Model 2 OR (95%CI)	*P*	Model 3 OR (95%CI)	*P*
Age, years	0.958(0.89–1.022)	0.190	0.955(0.895–1.020)	0.169	0.943(0.879–1.012)	0.105
BMI, kg/m^2	ND	ND	1.062(0.971–1.161)	0.189	1.061 (0.962–1.171)	0.236
FT4, pmol/L	ND	ND	ND	ND	0.867(0.665–1.132)	0.294
FT3, pmol/L	ND	ND	ND	ND	1.362(0.640–2.899)	0.423
TSH, mIU/L	ND	ND	ND	ND	0.910(0.725–1.120)	0.349
TPOAb, IU/mL	ND	ND	ND	ND	1.001(0.999–1.002)	0.393
TgAb, IU/mL	ND	ND	ND	ND	0.999(0.998–1.001)	0.482

In model 1, only age was included for analysis

In model 2, age, and BMI were included for analysis

In model 3, all those potential risk factors were included, which consisted of age, BMI as well as serum levels of FT4, FT3, TSH, TPOAb, and TgAb

ND, not done in this model

PDIA3Ab: protein disulfide isomerase autoantibody; AIT: autoimmune thyroiditis; PCOS: polycystic ovary syndrome; BMI: body mass index; FT4: free T4; FT3: free T3; TSH: thyroid stimulating hormone; TPOAb: anti-thyroid peroxidase autoantibody; TgAb: anti-thyroglobulin autoantibody

testosterone levels [9]. Thus, using Spearman correlation analysis, the associations between serum FT4, FT3, TSH, TPOAb, TgAb, LH/FSH, estradiol, and testosterone levels and PDIA3Ab total serum IgG expression were investigated in euthyroid AIT women. The analysis revealed two positive correlations within the AIT-PCOS group: between PDIA3Ab total serum IgG and LH/FSH ratio (r_s = 0.427, *P*=0.005), and PDIA3Ab total serum IgG and testosterone levels (r_s = 0.297, *P*=0.04) (Table 3). The results suggest that the increased LH/FSH ratio and hyperandrogenism may play a pathogenic role in the development of PCOS in AIT patients, as reported by previous research. This may be attributed to the simultaneous expression of PDIA3Ab, which could be a significant factor contributing to PCOS in AIT patients.

Risk factors for concurrent PCOS in euthyroid women with AIT

Previous studies have suggested a close relationship between age, BMI, and PCOS [22, 23]. Recent research has also indicated that thyroid autoimmunity and inflammation could be significant risk factors for the development of PCOS, potentially playing a crucial role in its pathogenesis [8, 9]. Binary logistic regression analysis was conducted to assess the independent association of various factors with concurrent PCOS in euthyroid women. The results from the binary logistic regression analysis models, which did not include PDIA3Ab, indicated that age, serum levels of FT4, FT3, and TSH within normal reference ranges, as well as serum TPOAb and TgAb were not independently associated with concurrent PCOS among euthyroid women with AIT (Table 4). However, in euthyroid women without AIT, age [OR (95% *CI*) in Model 1: 0.955 (0.916–0.995); Model 2: 0.954 (0.915–0.995); Model 3: 0.958 (0.916–1.002)] and BMI [OR (95% *CI*) in Model 2: 1.090 (1.019–1.165); Model 3: 1.095 (1.022–1.174)] were the independent risk factors of concurrent PCOS (Table 5).

To further explore its potential independent relationship with the prevalence of concurrent PCOS in euthyroid women, the OD value ratio of PDIA3Ab total serum IgG was divided into four grades based on quartiles or the upper limit of reference ranges. This was done for binary logistic regression analysis after adjusting for potential confounding factors as mentioned in Model 1–3 above. The elevated level of PDIA3Ab total IgG in the serum was observed to be independently correlated with an increased risk of concurrent PCOS in euthyroid AIT women [Q4 vs. Q1, OR (95% *CI*) in Model 1: 5.715 (1.618–20.184); Model 2: 3.677 (1.116–12.116); Model 3:

Table 5 Binary logistic regression analysis of the potential risk factors for PCOS without PDIA3Ab included among the euthyroid women without AIT

	Model 1 OR (95%CI)	*P*	Model 2 OR (95%CI)	*P*	Model 3 OR (95%CI)	*P*
Age, years	0.955(0.916–0.995)	0.028	0.954(0.915–0.995)	0.027	0.958(0.916–1.002)	0.06
BMI, kg/m^2	ND	ND	1.090(1.019–1.165)	0.012	1.095(1.022–1.174)	0.01
FT4, pmol/L	ND	ND	ND	ND	1.010(0.848–1.202)	0.914
FT3, pmol/L	ND	ND	ND	ND	1.229(0.698–2.164)	0.475
TSH, mIU/L	ND	ND	ND	ND	0.858(0.651–1.130)	0.275

In model 1, only age was included for analysis

In model 2, age, and BMI were included for analysis

In model 3, all those potential risk factors were included, which consisted of age, BMI as well as serum levels of FT4, FT3, and TSH

ND, not done in this model

PDIA3Ab: protein disulfide isomerase autoantibody; AIT: autoimmune thyroiditis; PCOS: polycystic ovary syndrome; BMI: body mass index; FT4: free T4; FT3: free T3; TSH: thyroid stimulating hormone

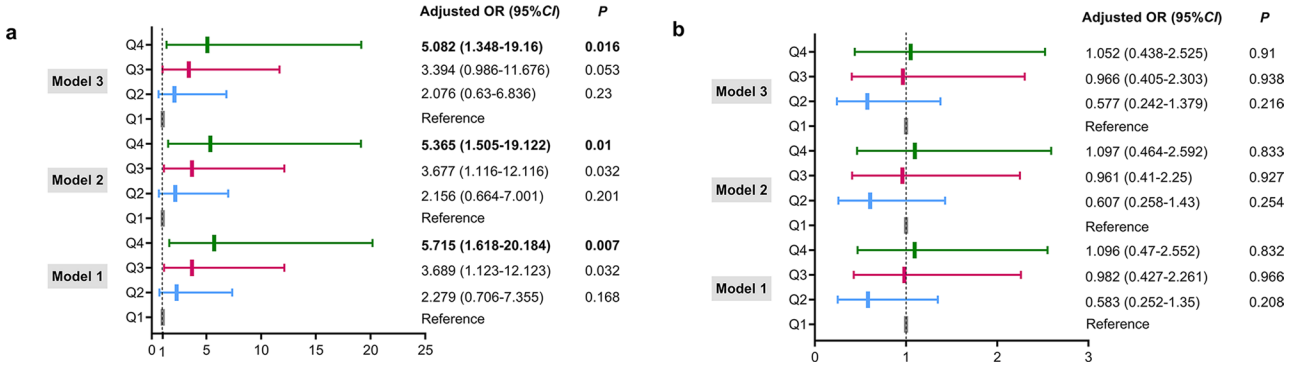

Fig. 2 The odds ratio of PDIA3Ab total serum IgG expression related to PCOS in the euthyroid women with AIT ($n=98$, Fig. 2a) and those without AIT ($n=185$, Fig. 2b). Adjusted OR were shown according to quartile of OD value ratio of PDIA3Ab total serum IgG (Fig. 2a: Q1, < 0.5273; Q2, 0.5273 to 0.6977; Q3, 0.6977 to 0.9415; Q4, > 0.9415. Figure 2b: Q1, < 0.344; Q2, 0.344 to 0.4369; Q3, 0.4369 to 0.5494; Q4, > 0.5494). Q1 was the reference group in all models. Binary logistic regression analysis was performed in models 1–3 after adjusting potential confounding factors. Model (1) Adjusted for age. Model (2) Adjusted for age and BMI. Model (3) Adjusted for age, BMI, serum levels of FT4, FT3, TSH, TPOAb (only in euthyroid AIT women), and TgAb (only in euthyroid AIT women)

5.082 (1.348–19.16)]. However, non-AIT women did not observe this association (Fig. 2).

Assessment of the relationship between PDIA3Ab, TPOAb, and TgAb and the occurrence of concurrent PCOS in euthyroid AIT women

Next, we conducted a logistic regression plot analysis to estimate the probability of concurrent PCOS in AIT women. As serum PDIA3Ab total serum IgG expression increased, the predicted probabilities of PCOS in AIT women gradually increased (Fig. 3a). Further investigation was performed to assess the predictive role of PDIA3Ab total serum IgG in the development of concurrent PCOS in euthyroid AIT women, utilizing the ROC curve and titer-dependent trend analysis. According to the ROC curve for the OD value ratio of PDIA3Ab total serum IgG in euthyroid AIT women, the area under the curve (AUC) was 0.700±0.053. The largest Youden index (0.299) corresponds to a cut-off value of 0.5920. This value can predict a significant increase in the risk of concurrent PCOS in euthyroid AIT women, with a sensitivity of 76.4% and a specificity of 53.5% (Fig. 3b).

Additionally, based on the quartiles or the upper limit of reference ranges, the levels of each autoantibody in the serum were categorized into four grades for titer-dependent trend analysis (Figs. 3c and 4). The data showed a significant increase in the prevalence of concurrent PCOS with the rising PDIA3Ab total serum IgG level in euthyroid AIT women (Fig. 3c). However, there was no significant change in the prevalence of concurrent PCOS with the increase in serum TPOAb or TgAb levels (Fig. 4). These outcomes support the conclusions of the logistic regression analysis and provided additional evidence for the critical predictive role of elevated PDIA3Ab total serum IgG expression in the concurrent occurrence of PCOS in euthyroid AIT women.

Discussion

AIT is the primary autoimmune illness in women of reproductive age [24]. Elevated levels of TPOAb, TgAb, and other non-thyroid tissue-specific autoantibodies are the primary indicators for autoantibody detection. More and more scholars are realizing that this autoimmune response may involve systemic tissues, rather than

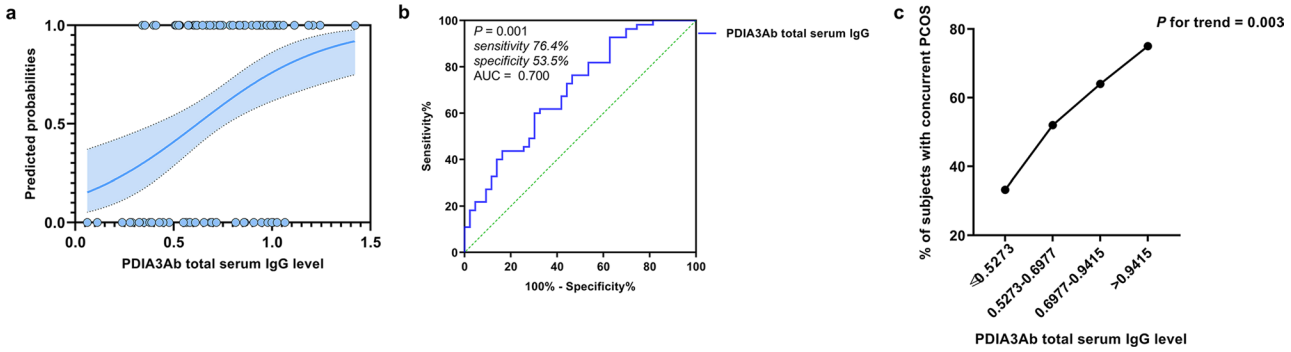

Fig. 3 Analysis of the relationship between PDIA3Ab total serum IgG expression and the occurrence of PCOS among euthyroid AIT women. **(a)** A logistic plot showing the probability of concurrent polycystic ovary syndrome relative to PDIA3Ab total serum IgG expression. **(b)** The ROC curve of PDIA3Ab total serum IgG expression for prediction of PCOS. The area under the curve was 0.700 ± 0.053 ($P = 0.001$). (c) The titer-dependent relationship between PDIA3Ab total serum IgG expression and the prevalence of PCOS. Four grades were divided based on the quartile of OD value ratio of PDIA3Ab total serum IgG in the whole euthyroid AIT women

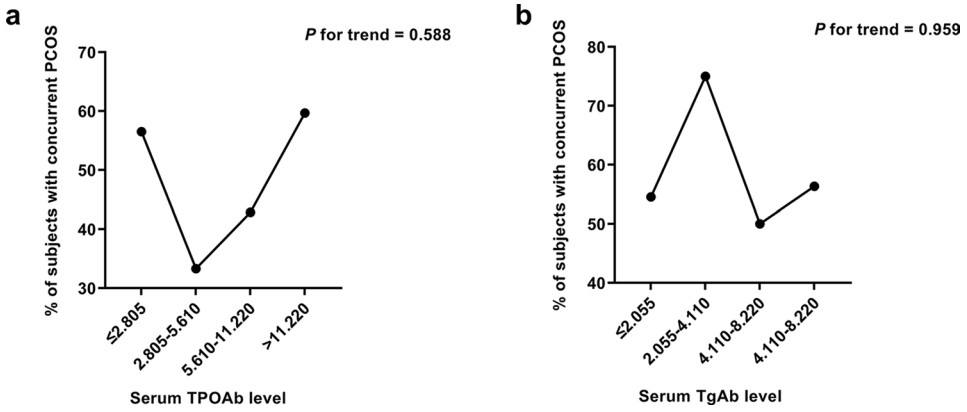

Fig. 4 The titer-dependent relationship between serum TPOAb and TgAb and the concurrent occurrence of PCOS in euthyroid AIT women. The reference ranges for serum TPOAb (≤ 5.61 IU/mL) and TgAb (≤ 4.11 IU/mL) were provided by the manufacturer (Abbott Diagnostics, Longford, Ireland)

organ-specific damage. For example, real-world studies have found that women with AIT have a significantly increased risk of developing PCOS, a significant complication that affects reproductive function in women with AIT [4, 6, 10, 25]. PCOS is an endocrine disorder characterized by high levels of androgens, irregular or absent ovulation, and cyst-like formations on the ovaries. It can lead to infertility, miscarriage, and metabolic disorders. At present, it is unclear how AIT patients are prone to developing PCOS, and there are no simple and feasible predictive factors or effective prevention and treatment strategies.

We thoroughly examined the possible risk factors for concurrent PCOS incidence in euthyroid AIT and non-AIT women in this study. Age, BMI, and serum levels of FT4, FT3, TSH, TPOAb, and TgAb are not independent risk factors for concurrent PCOS in the AIT group; instead, age and BMI are the only independent risk factors for the occurrence of PCOS in the non-AIT group. This suggests that other factors may have contributed to the development of PCOS in these AIT women.

Edassery et al. discovered that PDIA3 could be a target antigen in infertile women by analyzing serum anti-ovarian antibodies in women with infertility and premature ovarian failure [13]. PDIA3 is expressed in the cell membrane, cytoplasm, and nucleus, and it has multiple functions [26–28]. When expressed on the membrane, it is an important antigen associated with immunogenic cell death (ICD) [26]. Stress can also promote the translocation of PDIA3 to the membrane and increase its autoantibody (PDIA3Ab) production [29]. PDIA3 also serves as a membrane receptor for active Vitamin D, mediating its non-genomic effects [28]. Interestingly, the level of the classic Vitamin D receptor in the ovaries is very low. Vitamin D mainly promotes antral follicle development and estrogen and progesterone synthesis through PDIA3-mediated non-genomic effects [15].

Many scholars have suggested the potential involvement of autoimmune mechanisms in PCOS [8, 9, 30], such as the presence of autoantibodies. Regrettably, only a limited number of studies have verified that PCOS patients exhibit significantly elevated levels of

non-organ-specific antibodies. Previous small pilot studies have indicated a higher occurrence of *anti-nuclear antibody (ANA)* positivity in women with PCOS [31, 32]. However, a study by Petrikova et al. found no significant increases in ANA, *anti-Sjögren's syndrome A (SSA)*, *anti-Sjögren's syndrome B (SSB)*, anti-dsDNA, anti-ribonucleoprotein (RNP), antineutrophil cytoplasmic (ANCA)/*myeloperoxidase (MPO)*, or ANCA/*proteinase 3 (PR3)* in 152 PCOS patients and 76 healthy controls who were tested for these antibodies [30]. Although anti-GnRH antibodies are produced in a few PCOS patients [12], the receptor is not distributed in the thyroid and ovaries. In other words, previous studies had not fully elucidated the relevant target antigens and autoantibodies causing PCOS. We reported high expression of PDIA3Ab in AIT women with PCOS, which is innovative. Other studies have focused on major anti-thyroid antibodies, such as TPOAb and TgAb. TPOAb may destroy follicles through ADCC because of the similarity in structure between antigens in the zona pellucida and the thyroid gland [11]. However, further research has not provided direct evidence that TPOAb and TgAb contribute to the development of PCOS. Consistently, we did not discover a clear and independent association between TPOAb and TgAb titers and an increased risk of concurrent PCOS in AIT.

PDIA3Ab is highly expressed in the serum of patients with rheumatic heart disease, autoimmune hepatitis, and type 2 diabetes [29]. In our earlier investigation, PDIA3Ab levels were higher in the AIT animal model caused solely by mouse Tg immunization of CBA/J mice [16]. PDIA3Ab may result from immune damage to thyroid tissue in AIT individuals, possibly due to the diffusion of the intermolecular epitope, and is not caused by other concurrent autoimmune diseases [17]. In our current study, euthyroid AIT women had a significantly greater level of PDIA3Ab than non-AIT controls [17]. Specifically, the serum PDIA3Ab level was notably elevated in euthyroid AIT women with PCOS compared to those without PCOS. Additionally, the logistic regression analysis results demonstrated that PDIA3Ab total serum IgG expression was an independent risk factor for concurrent PCOS in the euthyroid AIT group but not in the non-AIT group. Trend analysis also showed a positive linear correlation between serum PDIA3Ab total serum IgG level and the concurrent prevalence of PCOS in euthyroid AIT women. All of these findings indicate that PDIA3Ab may contribute to the development of concurrent PCOS in euthyroid AIT women. Furthermore, as an important risk factor, it could also serve as a predictive marker for the disorder.

It is worth noting that, Spearman correlation analysis showed that PDIA3Ab total serum IgG levels were positively associated with LH/FSH ratio and testosterone levels in the AIT-PCOS group. Previous studies revealed that the binding of the active Vitamin D to PDIA3 stimulates the interaction between phospholipase A2 (PLA2) activating protein (PLAA) and PDIA3. Subsequently, PLA2 is activated, triggering the rapid release of arachidonic acid (AA) [33]. In PCOS patients, the activation of PLA2 is reduced, while cyclooxygenase-2 (COX-2) gene expression is significantly up-regulated, which can promote the metabolism of AA to prostaglandin (PG) E2, which can stimulate the production of testosterone [34–36]. On the other hand, PDIA3 is present in lipid rafts and cytoplasmic complexes in the membrane of signal transducer and activator of transcription 3 (STAT3)-expressing cells and inhibits the translocation of STAT3 activated by cytokines such as *interleukin 6* (IL-6) to the nucleus, making activated STAT3 unable to activate gene transcription [27]. We speculate that PDIA3Ab can prevent the above process, allowing activated STAT3 to enter the nucleus, of course, this needs to be verified by subsequent experiments. Activation of STAT3 can enter the nucleus and also raise COX-2 gene expression eventually leading to an increase in testosterone [34, 36]. Therefore, we speculate that the elevated PDIA3Ab expression in AIT women may contribute to the development of hyperandrogenism through the above mechanisms, which requires further research through in vivo and in vitro experiments.

The association between PDIA3Ab expression and the occurrence of concurrent PCOS in euthyroid AIT and non-AIT women has been systematically assessed in this study. Our findings revealed that the grades of the PDIA3Ab total serum IgG levels (from high to low) were classified as follows: AIT-PCOS group>AIT-non-PCOS group>non-AIT-PCOS group>non-AIT-non-PCOS group. The levels of IgG subclasses in the human body are as follows: IgG1>IgG2>IgG3>IgG4. Among them, IgG1 and IgG3 exhibit the strongest complement activation and ADCC, while IgG2 primarily responds to polysaccharide antigens, and IgG4 demonstrates the lowest biological activity [37, 38]. PDIA3Ab serum IgG3 levels were significantly higher in the AIT-PCOS group compared to the other three groups, while the levels of PDIA3Ab serum IgG2 and IgG4 were not significantly different among the four groups. On one hand, the increased titer of PDIA3Ab may counteract the activation of T cells [16]. On the other hand, PDIA3 is involved in the assembly of major histocompatibility complex (MHC) class I molecules and the antigen presentation process, which helps activate $CD8^+$T cells to exert a cytotoxic effect [39]. PDIA3Ab and its subtypes can damage target cells expressing PDIA3 through complement and humoral immune pathways, but this antibody is not the primary antibody that destroys the thyroid. It is speculated that PDIA3Ab may mainly act on extrathyroidal lesions of AIT such as

the ovary. We acknowledge that we have not completed the investigation of the mechanisms of PDIA3Ab in the development of PCOS in euthyroid AIT women. Therefore, their cause-effect relationship awaits further study.

Our study possesses several strengths. It has come to our knowledge that this is the inaugural investigation into the correlation between PDIA3Ab expression and concurrent PCOS in both AIT and non-AIT women. The study has revealed that PDIA3Ab is a significant independent risk factor for concurrent PCOS in euthyroid AIT women. The ROC curve of PDIA3Ab total serum IgG level alone for predicting the occurrence of concurrent PCOS in euthyroid AIT women was 0.700 (95%*CI*: 0.596–0.804). The corresponding cut-off OD450 value ratio of PDIA3Ab total serum IgG for the maximum Youden index was 0.592, indicating a significant increase in the risk of concurrent PCOS in euthyroid AIT women with a sensitivity of 76.4% and a specificity of 53.5%. Furthermore, and this has never been documented before, the titer-dependent associations between those autoantibody levels and the prevalence of PCOS have been thoroughly evaluated in those euthyroid AIT women alone. PDIA3Ab total serum IgG showed a titer-dependent connection with the prevalence of PCOS, although neither serum TPOAb nor TgAb did. Finally, LH/FSH ratio and serum testosterone were positively related to the serum PDIA3Ab expression in AIT women with PCOS, but not in AIT women without PCOS. All of these results suggest that serum PDIA3Ab expression may be directly connected to a greater risk of concurrent PCOS in euthyroid AIT women, but not serum TPOAb or TgAb expression, and that an elevated level of PDIA3Ab could be a significant factor in AIT-related PCOS. Serum PDIA3Ab level measurement not only provides an independent predictor of concurrent PCOS risk but also helps to explain the mechanism of spontaneous abortion caused by PCOS from a new perspective.

Our study still has limitations: (1) The limited sample size may introduce bias into the findings. To further assess the predictive efficacy of PDIA3Ab on the concurrent PCOS risk among euthyroid AIT women, a bigger sample investigation is required. (2) The predictive value of PDIA3Ab total serum IgG for diagnosing PCOS in AIT patients is limited (AUC=0.700, 95%*CI*: 0.596–0.804). Our future work will concentrate on investigating the epitope targeted by PDIA3Ab. Specific antibodies against the antigen epitope may not only enhance the predictive value of PDIA3Ab diagnosis but also potentially intervene to prevent the occurrence and development of PCOS through the induction of immune tolerance. (3) The potential impact of PDIA3Ab on the female reproductive system is complex. Previous studies have demonstrated that PDIA3 affects ovarian and placental function. Our prior research found that PDIA3Ab was linked to spontaneous abortion in euthyroid AIT women during pregnancy. The study focused on the connection between PDIA3Ab and PCOS. It's important to note that having PCOS does not necessarily conflict with having had a spontaneous abortion. However, it's worth pointing out that our findings do not rule out patients with a history of spontaneous abortion. This is a limitation of our study. In future research, we aim to explore this topic further.

Conclusions

When considered collectively, the results of this research show that serum PDIA3Ab expression is a significant independent risk factor for concurrent PCOS in euthyroid AIT women, with a titer-dependent relationship. It might be employed as a novel biomarker to predict the probability of developing PCOS in euthyroid AIT women, as well as a suitable indicator for monitoring the tracking the effectiveness of associated immunotherapy. Therefore, further exploration of the mechanism of PDIA3Ab causing AIT women to be more susceptible to PCOS may be beneficial to clinical inhibition of the progress of PCOS and grasp the opportunity of treatment.

Abbreviations

AIT	Autoimmune thyroiditis
PCOS	Polycystic ovary syndrome
PDIA3	Protein disulfide isomerase A3
PDIA3Ab	Protein disulfide isomerase autoantibody
NCCAH	Non-classical congenital adrenal hyperplasia
FHA	Functional hypothalamic amenorrhea
POI	Premature ovarian insufficiency
LH	Luteinizing hormone
FSH	Follicular stimulating hormone
CAD	Coronary artery disease
TPO	Thyroid peroxidase
Tg	Thyroglobulin
TPOAb	Anti-thyroid peroxidase autoantibody
TgAb	Anti-thyroglobulin autoantibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
GnRH	Gonadotropin-releasing hormone
TSH	Thyroid-stimulating hormone
FT4	Free T4
FT3	Free T3
BMI	Body mass index
ANOVA	Analysis of variance
FBG	Fasting blood glucose
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
ALT	Alanine transaminase
AST	Aspartate transaminase
ALP	Alkaline phosphatase
GGT	Gamma-glutamyl transferase
TBIL	Total bilirubin
BUN	Blood urea nitrogen
Scr	Serum creatinine
eGFR	Estimated glomerular filtration rate
Neut	Neutrophil
PLT	Platelet
NLR	Neutrophil to lymphocyte ratio
RBC	Red blood cell
WBC	White blood cell
HB	Hemoglobin
ROC	Receiver operating characteristics

AUC	Area under the curve
ICD	Immunogenic cell death
ANA	Anti-nuclear antibody
SSA	Sjögren's syndrome A
SSB	Sjögren's syndrome B
RNP	Ribonucleoprotein
ANCA	Antineutrophil cytoplasmic
MPO	Myeloperoxidase
PR3	Proteinase 3
PLA2	Phospholipase A2
PLAA	PLA2 activating protein
AA	Arachidonic acid
COX-2	Cyclooxygenase-2
PG	Prostaglandin
STAT3	Signal transducer and activator of transcription 3
IL-6	Interleukin 6
MHC	Major histocompatibility complex

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Author contributions
The study was designed by ZYC and JL. Data collection was conducted by ZYC, CXZ, CFM, YDH, YZN, BRG, JSW, and LL. Experiments and data analysis were performed by ZYC and KC. The draft of the manuscript was written by ZYC and reviewed by WPT, ZYS, and JL. All authors read and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was conducted under the approval of the Ethics Committee of the First Affiliated Hospital of China Medical University (Approval Code [2023]2023-92-2). The participants had signed written informed consent in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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