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RECEIVED 10 June 2025

ACCEPTED 22 October 2025

PUBLISHED 04 November 2025

CITATION

Wang Q, Liang J, Liu Q, Liu H, Bai H,
Huang W and Fan P (2025) The promoter T-
413A variant and elevated enzyme levels of
heme oxygenase-1 associated with an
increased risk of polycystic ovarian syndrome.
Front. Endocrinol. 16:1644373.
doi: 10.3389/fendo.2025.1644373

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The promoter T-413A variant and elevated enzyme levels of heme oxygenase-1 associated with an increased risk of polycystic ovarian syndrome

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Background: Oxidative stress and metabolic disorders significantly contribute to the development of polycystic ovarian syndrome (PCOS). Heme oxygenase-1 (HMOX1) plays a key role in the degradation of heme and the regulation of oxidative stress, ferroptosis, and glycolipid metabolism. This study explored the relationship between *HMOX1* promoter T-413A single nucleotide polymorphism (SNP, rs2071746), (GT)_n dinucleotide repeat variant (rs3074372), plasma HMOX1 levels, and the risk of PCOS in Chinese women.

Methods: This case-control study included 1092 women diagnosed with PCOS and 805 controls. The (GT)_n and rs2071746 polymorphisms were identified using polymerase chain reaction amplification, followed by capillary electrophoresis or restriction fragment length polymorphism. HMOX1 levels and clinical, metabolic, hormonal, and oxidative stress indices were analyzed.

Results: The *HOMX1* rs2071746T/A SNP was associated with an increased risk of PCOS based on genotype, recessive, dominant, and allele genetic models ($P < 0.05$). After adjusting for age, body mass index, and recruitment year of participants, the dominant model (odds ratio [OR] = 1.272, 95% confidence interval [CI]: 1.013–1.597, $P = 0.039$) and the TT genotype (OR = 1.395, 95% CI: 1.033–1.883, $P = 0.030$, with the AA genotype as the reference) remained a significant predictor of PCOS in the logistic regression models. No significant differences were observed in the (GT)_n polymorphism of *HMOX1* based on different genetic models. However, the TT/SS combined genotype of *HMOX1* rs2071746T/A and (GT)_n polymorphisms was associated with an increased risk of PCOS (OR = 1.442, 95% CI: 1.021–2.035, $P = 0.037$). Furthermore, elevated HMOX1 levels were related to a slight but significant increase in the risk of PCOS,

and the rs2071746T/A and (GT)_n genetic variants significantly affected obesity, oxidative stress, endocrine abnormalities, and metabolic disorders.

Conclusion: *HMOX1* rs2071746T/A variant and elevated plasma *HMOX1* levels are associated with an increased risk of PCOS.

KEYWORDS

heme oxygenase-1, genetic polymorphism, polycystic ovarian syndrome, oxidative stress, metabolism

1 Introduction

Polycystic ovarian syndrome (PCOS) is the leading endocrine-metabolic disorder among women of reproductive age, with a prevalence of 10–13% (1). Its clinical presentation is diverse and may include reproductive, dermatological, metabolic, and psychological symptoms such as irregular menstrual cycles, infertility, acne, hirsutism, obesity, insulin resistance, dyslipidemia, anxiety, depression, etc. (1, 2). PCOS can affect women throughout their lives and potentially lead to long-term complications, including type 2 diabetes (T2D), cardiocerebrovascular disease, and endometrial cancer. The exact etiology of PCOS remains unknown, making its treatment difficult (1, 3). Increasing evidence indicates that its etiology is complex, involving interactions between multiple predisposing genes, genetic epigenetics, and detrimental environmental factors (2, 4–6).

Heme oxygenase (HMOX) is the rate-limiting enzyme that catalyzes the breakdown of heme, resulting in the production of biliverdin (BV), carbon monoxide (CO), and free iron (Fe²⁺) (7, 8). BV is rapidly turned into bilirubin (BR) by BV reductase (8, 9). Heme and free Fe²⁺ are toxic owing to their oxidative properties; however, in the physiological state, free iron is swiftly sequestered by ferritin, ensuring a harmonious balance within the body (8). CO, BV, and BR possess vasodilator, antioxidant, and anti-inflammatory properties, but excessive accumulation of these products can be toxic (8, 10). The two main isomers of HMOX in humans, inducible

HMOX1 (also known as HO-1) and constitutive HMOX2 (also known as HO-2), have similar structures and catalytic functions but are distributed in different tissues and exhibit distinct features (10). Low HMOX1 expression was observed in most tissues under normal conditions. However, its expression can be markedly increased in response to different pathophysiological stress conditions or stimulation factors (9, 11).

The *HMOX1* is located on chromosome 22q13.1. Two genetic polymorphisms in the promoter of *HMOX1*, the (GT)_n dinucleotide repeat variant (rs3074372) and rs2071746T/A single nucleotide polymorphism (SNP) (rs2071746), can affect the transcriptional activity of *HMOX1* (11). These two polymorphisms are closely linked to certain diseases, including sensitivity to several cancers and coronary heart disease (11), cardiovascular events and mortality in patients undergoing hemodialysis (12), T2D (13), pre-eclampsia (14), chronic obstructive pulmonary disease (15), SARS-CoV-2 viremia in COVID-19 infection (16), and risk of encephalitis in HIV infection (17).

Oxidative stress and metabolic disorders significantly contribute to the pathophysiology and progression of PCOS (1, 18–21). Genetic variants of *HMOX2* G554A and A-42G SNPs are associated with endocrine abnormalities and glycolipid metabolic irregularities in patients with PCOS (5). The levels of *HMOX1* mRNA are higher in subcutaneous adipose tissue and granulosa cells (22, 23), but the concentrations of serum HMOX1 are lower in women with PCOS than those among control women (24). However, the association between *HMOX1* polymorphisms and PCOS remains unclear. Therefore, we explored the relationship between *HMOX1* (GT)_n repeats and rs2071746T/A polymorphisms and the risk of PCOS. Additionally, we analyzed how these genetic variants affected plasma HMOX1 levels and various clinical and biochemical parameters in Southwest Chinese women.

2 Materials and methods

2.1 Study participants

This was a case-control study. All participants aged 17–40 years provided written informed consent and were recruited from the Reproductive Endocrinology Outpatient Department of the West

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; BR, bilirubin; BV, biliverdin; CI, confidence interval; CO, carbon monoxide; DBP, diastolic blood pressure; E₂, estradiol; FAI, free androgen index; F-G score, Ferriman–Gallwey score; FSH, follicle-stimulating hormone; Glu, glucose; GSH, glutathione; HA, hyperandrogenism; HDL-C, high-density lipoprotein cholesterol; HMOX1, heme oxygenase-1; HMOX2, heme oxygenase-2; HOMA-IR, the homeostatic model assessment of insulin resistance; Ins, insulin; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; OA, oligo-ovulation or anovulation; OR, odds ratio; OSI, oxidative stress index; PCOs, polycystic ovaries; PCOS, polycystic ovarian syndrome; PCR, polymerase chain reaction; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; T-AOC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides; TOS, total oxidant status; TT, total testosterone; WC, waist circumference.

China Second University Hospital between 2006 and 2024 (Figure 1). This study was approved by the Institutional Review Board of West China Second University Hospital, Sichuan University (2014–014 for P. Fan).

PCOS was diagnosed based on the revised 2003 Rotterdam European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus criteria, which include irregular menstrual cycles, oligo-ovulation, or anovulation (OA), biochemical and/or clinical hyperandrogenism (HA), and polycystic ovaries (PCOs) (25). Detailed definitions of HA, OA, and PCOs have been provided in previous articles (26–28). PCOS was diagnosed if a woman met at least two of the three criteria after ruling out other etiologies such as Cushing syndrome, congenital adrenal hyperplasia, and androgen-secreting tumors (1, 25). Both HA and OA are required in individuals aged < 20 years of age (1). Control women had regular menstrual cycles between 21–35 days, normal ovarian morphology on ultrasonography, and no biochemical or clinical signs of HA.

The participants were excluded if they had infections, cardiovascular diseases, liver or kidney diseases, autoimmune diseases, thyroid disorders, hypogonadism, hyperprolactinemia, premature ovarian insufficiency, endometriosis, or other malignancies. Additionally, the participants were excluded from the analysis when comparing oxidative stress and metabolic and

hormonal parameters between groups/subgroups if they met any of the following criteria: (i) use of hormonal therapy and/or medications that influence glucose and lipid metabolism within 12 weeks before the study; (ii) smoking; (iii) being pregnant or in the luteal phase of their menstrual cycle; and/or (iv) having a fasting glucose (Glu) level of ≥ 7.0 mmol/L and/or a 2-hour plasma glucose after the glucose challenge (2-h Glu) level of ≥ 11.1 mmol/L in the control group.

Clinical indices, including waist circumference (WC), body mass index (BMI), waist-to-hip ratio, diastolic blood pressure (DBP), systolic blood pressure, severity of acne and hirsutism, and ovarian volume were assessed as previously described (27–29).

Blood samples were collected after fasting for 8–12 h. Blood cells were stored at 4°C, and plasma and serum aliquots were preserved at –80°C for later analysis. A 75g oral glucose tolerance test was conducted immediately after fasting blood sampling.

2.2 DNA purification and genotype measurements

Genomic DNA was purified from stored blood cells using a previously described method (30). (GT) $_n$ repeats in *HMOX1* were determined using polymerase chain reaction (PCR) amplification and capillary electrophoresis. The forward primer with 6–

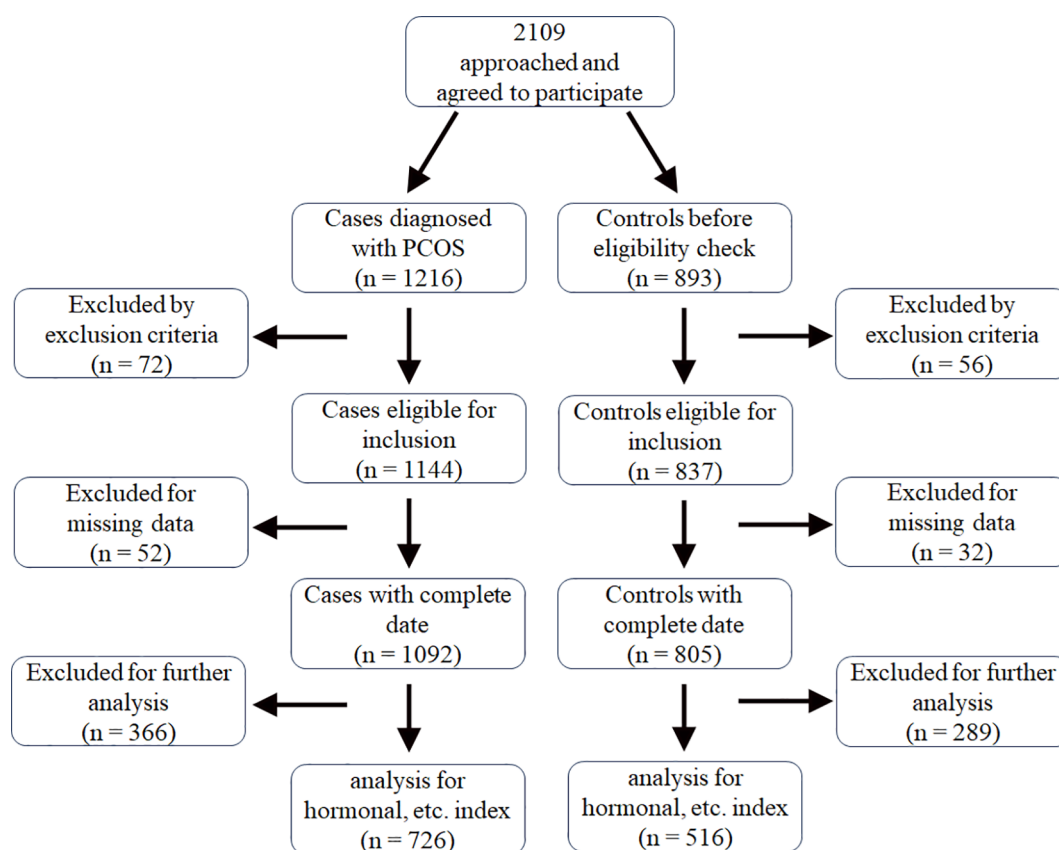


FIGURE 1
The process of recruitment and selection of the case and control group.

carboxyfluorescein (FAM) was 5'-FAM-CCAGCTTTCTGGAACCTTCTG-3', the reverse primer was 5'-GAAACAAAGTCTGGCCATAGGA-3' (31). Samples were amplified using a touchdown PCR protocol (17). The products were then analyzed using a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMapper 4.1 (Applied). Short repeats, with fewer than 27 GT repeats, were classified as S alleles, whereas long repeats, with at least 27 GT repeats, were classified as L alleles (12). For genotyping the rs2071746 SNP, we used PCR and restriction fragment length polymorphism method with a mismatched primer set (the forward primer: 5'-GTTCTGATGTTGCCACCAAGC-3'; the reverse primer: 5'-CTGCAGGCTCTGGGTGTGATTTTG-3'). The PCR products of the rs2071746T/A SNP (151 bp) were then digested with *HindIII* (New England Biolabs, Ipswich, MA, USA), resulting in 20 and 131 bp fragments with the T allele and a whole 151-bp product with the A allele. The results were verified by repeating the genotyping of > 30% of the randomly selected samples, yielding 100% concordance.

2.3 Analysis of HMOX1, oxidative stress, hormonal, and metabolic indices

Plasma HMOX1 concentrations were measured using ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Estradiol, TT, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone binding globulin (SHBG), plasma insulin (Ins) and Glu levels, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein (apo) A1, and apoB concentrations, total antioxidant capacity (T-AOC), glutathione (GSH), total oxidant status (TOS), and oxidative stress index (OSI) were also analyzed as previously described in other studies (20, 21, 28). The homeostatic model assessment of insulin resistance (HOMA-IR) and free androgen index (FAI) were calculated as previously described (18, 28) using the following formulas:

$$\text{HOMA-IR} = \text{fasting Glu (mmol/L)} \times \text{fasting Ins (}\mu\text{U/mL)} / 22.5$$

$$\text{FAI} = \text{TT (nmol/L)} / \text{SHBG (nmol/L)} \times 100$$

2.4 Statistical analysis

Data are presented as mean $\pm$ standard deviation. We used analysis of variance or independent sample t-tests for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Analysis of covariance was used to evaluate differences in clinical and biochemical indices after adjusting for variations in age, BMI, and participant recruitment year. Chi-squared (χ^2) analysis was performed to evaluate deviations in genotypic distribution from Hardy-Weinberg equilibrium and to compare the frequencies of genotypes and alleles between two groups. The Spearman's correlation coefficient was used to assess the relationship between HMOX1 levels and other parameters. Differences were considered statistically significant if the *P* value was <0.05. Data were analyzed using the Statistical Program for Social Sciences (SPSS) 21.0 (IBM SPSS Statistics, IBM Corporation).

Power values were calculated according to the disease allele frequency of the rs2071746T/A SNP in *HMOX1* and the sample size (prevalence = 0.12, significance level = 0.05) using the Genetic Association Study Power Calculator (http://csg.sph.umich.edu/abecasis/gas_power_calculator/index.html).

Using the online SNPStats (<https://www.snpsstats.net/start.htm>), we assessed the linkage disequilibrium between two genetic polymorphic loci in view of the *D'* parameter.

3 Results

3.1 Clinical and biochemical characteristics of the participants

Owing to the significant discrepancies in BMI and age between the PCOS and control groups (Table 1), and the relatively long recruitment period of participants between 2006 and 2024 in this study, we adjusted for these three confounding factors in our subsequent analyses.

Table 1 shows that the PCOS group had significantly higher acne grade scores, F-G scores, average ovarian volumes, DBP, WC, waist-to-hip ratio, TT, LH, LH/FSH ratio, FAI, fasting Ins, HOMA-IR, 2-h Glu and 2-hour insulin after the glucose challenge (2-h Ins),

TABLE 1 Clinical and biochemical parameters in women with PCOS and controls.

	Controls (n = 805)	PCOS (n = 1092)	<i>P</i>	<i>P</i> ^a
Age (years)	28.25 $\pm$ 4.10	25.10 $\pm$ 4.15	<0.001	
BMI (kg/m ²)	21.15 $\pm$ 2.81	23.04 $\pm$ 4.14	<0.001	
WC (cm)	73.64 $\pm$ 8.08	79.15 $\pm$ 11.07	<0.001	<0.001
Waist-to-hip ratio	0.81 $\pm$ 0.60	0.85 $\pm$ 0.07	<0.001	<0.001
F-G score	0.24 $\pm$ 0.72	1.74 $\pm$ 2.03	<0.001	<0.001
Acne grade score	0.13 $\pm$ 0.34	0.67 $\pm$ 0.90	<0.001	<0.001

(Continued)

TABLE 1 Continued

	Controls (n = 805)	PCOS (n = 1092)	<i>P</i>	<i>P</i> ^a
SBP (mmHg)	112.38 ± 11.15	114.16 ± 10.61	0.001	0.315
DBP (mmHg)	73.33 ± 8.58	75.43 ± 8.75	<0.001	0.003
Mean ovarian volume (mL)	7.36 ± 2.90	9.92 ± 4.04	<0.001	<0.001
Hormonal levels*				
E ₂ (pmol/L)	313.87 ± 324.44	275.17 ± 273.34	0.035	0.657
TT (nmol/L)	1.45 ± 0.52	2.26 ± 0.80	<0.001	<0.001
SHBG (nmol/L)	55.32 ± 27.19	32.63 ± 19.03	<0.001	<0.001
FAI	3.14 ± 1.89	9.53 ± 6.87	<0.001	<0.001
LH (IU/L)	7.05 ± 6.24	13.15 ± 8.05	<0.001	<0.001
FSH (IU/L)	6.55 ± 2.63	6.05 ± 2.10	0.001	0.015
LH/FSH	1.16 ± 1.13	2.24 ± 1.23	<0.001	<0.001
Metabolic profile*				
Fasting Ins (pmol/L)	60.37 ± 35.46	98.46 ± 72.40	<0.001	<0.001
2-h Ins (pmol/L)	365.80 ± 270.73	715.01 ± 566.96	<0.001	<0.001
Fasting Glu (mmol/L)	5.23 ± 0.47	5.34 ± 0.84	0.003	0.861
2-h Glu (mmol/L)	5.98 ± 1.27	7.21 ± 2.46	<0.001	<0.001
HOMA-IR	2.21 ± 1.30	3.79 ± 3.01	<0.001	<0.001
TG (mmol/L)	1.00 ± 0.54	1.41 ± 1.14	<0.001	<0.001
TC (mmol/L)	4.25 ± 0.72	4.43 ± 0.80	<0.001	<0.001
HDL-C (mmol/L)	1.51 ± 0.33	1.37 ± 0.34	<0.001	0.002
LDL-C (mmol/L)	2.35 ± 0.64	2.60 ± 0.77	<0.001	<0.001
TG/HDL-C	0.73 ± 0.61	1.19 ± 1.37	<0.001	<0.001
ApoA1 (g/L)	1.46 ± 0.21	1.42 ± 0.21	0.001	0.752
ApoB (g/L)	0.75 ± 0.17	0.83 ± 0.20	<0.001	<0.001
Oxidative stress parameters*				
TOS (nmol H ₂ O ₂ Equiv./mL)	11.41 ± 5.34	15.25 ± 10.31	<0.001	<0.001
T-AOC (U/mL/min)	14.51 ± 2.60	15.92 ± 3.51	<0.001	<0.001
OSI	0.79 ± 0.41	0.99 ± 0.76	<0.001	<0.001
GSH (nmol/mL)	1.11 ± 0.25	1.18 ± 0.25	<0.001	0.005
TOS/GSH	10.50 ± 5.76	12.71 ± 9.33	<0.001	<0.001
HMOX1 (μg/L)	4.51 ± 2.41	5.02 ± 4.61	0.018	0.011

Values are presented as average ± standard deviation.

apoA1, apolipoprotein A1; apoB, apolipoprotein B; BMI, body mass index; DBP, diastolic blood pressure; E₂, estradiol; FAI, free androgen index; F-G score, Ferriman–Gallwey score; FSH, follicle-stimulating hormone; Glu, glucose; GSH, glutathione; HDL-C, high-density lipoprotein cholesterol; HMOX1, heme oxygenase-1; HOMA-IR, the homeostatic model assessment of insulin resistance; Ins, insulin; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; OSI, oxidative stress index; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; T-AOC, total antioxidant capacity; TC, total cholesterol; TG, triglycerides; TOS, total oxidant status; TT, total testosterone; WC, waist circumference. 2-h Ins and 2-h Glu, 2-hour plasma insulin and glucose after the glucose challenge.

P Continuous variables were compared between the two groups using the independent samples t-test (normally distributed) or the Mann-Whitney U test (non-normally distributed).

P^a Comparisons of the parameters were corrected for differences in age, BMI, and recruitment year of participants between the two groups using analysis of covariance.

*Controls (n = 516), PCOS (n = 726).

LDL-C, TC, TG, TG/HDL-C ratio, apoB, TOS, T-AOC, GSH, OSI, TOS/GSH ratio, and plasma HMOX1 levels, but lower serum FSH, SHBG, and HDL-C concentrations than the control group (*P* < 0.05).

We further compared the plasma HMOX1 levels in lean (BMI < 23 kg/m²) and overweight/obese (BMI ≥ 23 kg/m² and/or waist circumference > 80 cm) subgroups after adjusting for age, BMI, and recruitment period of participants. The results showed that the

HMOX1 levels were higher in the lean PCOS subgroup ($n = 339$) than in the lean control subgroup ($n = 382$) (5.00 ± 5.00 vs. 4.48 ± 2.36 $\mu\text{g/L}$, $P = 0.034$), but no statistical significance in the overweight/obese PCOS subgroup ($n = 387$) than in the overweight/obese control subgroup ($n = 134$) (5.04 ± 4.29 vs. 4.48 ± 2.15 $\mu\text{g/L}$, $P = 0.115$).

3.2 Correlation of HMOX1 levels with clinical and biochemical indicators and risk of PCOS

The Spearman's correlation analysis showed that plasma HMOX1 levels were positively correlated with 2-h Glu, WC, fasting Ins, HOMA-IR, TG/HDL-C ratio, BMI, 2-h Ins, TC, fasting Glu, FAI, T-AOC, apoB, and WHR in patients with PCOS ($r = 0.138, 0.132, 0.132, 0.129, 0.127, 0.118, 0.105, 0.093, 0.092, 0.087, 0.085, 0.080$, and 0.079 , respectively; $P < 0.05$). Although statistically significant, the correlations between HMOX1 and PCOS traits were quantitatively modest.

Binary logistic regression analysis demonstrated that elevated HMOX1 levels were associated with an increased risk of PCOS after correcting for differences in participant recruitment year, age, and BMI (odds ratio [OR] = 1.053, 95% confidence interval [CI]: 1.008–1.100, $P = 0.019$).

3.3 Distributions of *HOMX1* rs2071746T/A and (GT)n genotypes and alleles

Table 2 summarizes the genetic models for the rs2071746T/A and (GT)n repeat polymorphisms in *HOMX1*. The distribution of genotypes for both polymorphisms was consistent with Hardy–Weinberg equilibrium in women with and without PCOS ($P > 0.05$).

The frequencies of the TT genotype and T allele in the *HOMX1* rs2071746T/A SNP were significantly higher in the PCOS group than those in the control group. The OR indicated that this difference was statistically significant for the dominant model, the recessive model, and the TT vs. AA genotype model, and the allele model (all $P < 0.05$). After adjusting for age, BMI, and recruitment year of participants, the dominant genetic model remained statistically significant in the binary logistic regression model (OR = 1.272, 95% CI: 1.013–1.597, $P = 0.039$) and the TT genotype remained a significant predictor for PCOS in a multinomial logistic regression model, with the AA genotype as the reference (OR = 1.395, 95% CI: 1.033–1.883, $P = 0.030$). The genetic association power is 0.984 for rs2071746T/A SNP. No statistically significant differences were observed between the two groups for the (GT)n repeat polymorphism of *HMOX1* when analyzed using different genetic models ($P > 0.05$; Table 2).

The combined genotypes of *HMOX1* rs2071746T/A and (GT)n polymorphisms exhibited a significant difference in frequency between patients with PCOS and controls ($P = 0.031$; Supplementary Table 1). The TT/SS was a risk factor for PCOS (OR = 1.442, 95% CI: 1.021–2.035, $P = 0.037$) in a multinomial logistic regression model using the AA/LL combined genotype as the reference, with participant

recruitment year, age, and BMI as covariates. Moderate linkage disequilibrium was observed between the rs2071746T/A and (GT)n polymorphisms ($D' = 0.8517$, $r^2 = 0.5539$).

3.4 Effects of genotypes on clinical and biochemical indicators

We analyzed the effect of *HMOX1* rs2071746T/A and (GT)n genetic variants on plasma HMOX1 levels and clinical and biochemical parameters in women with and without PCOS.

Supplementary Table 2 shows that patients with the AT genotype of *HMOX1* rs2071746T/A SNP had a greater TOS/GSH ratio than those with the TT genotype ($P = 0.028$). The controls with the TT genotype exhibited a lower acne grade score than those with the AA genotype ($P = 0.043$) and lower HDL-C levels ($P = 0.038$) than those with the AT genotype; whereas the controls with the AT genotype exhibited lower GSH levels ($P = 0.022$) than those with the AA genotype.

The same parameters were analyzed for different genotypes of *HMOX1* (GT)n repeat polymorphism (Supplementary Table 3). Patients with the LL genotype displayed a lower waist-to-hip ratio and TT levels ($P < 0.05$) than those with the SS genotype. The controls with the LL genotype exhibited a lower BMI than those with the SS and SL genotypes ($P < 0.05$). The controls with the SL genotype showed higher FAI ($P = 0.019$) than those with the SS genotype.

No statistically significant differences in plasma HMOX1 levels were observed between the different genotypes of *HMOX1* rs2071746T/A and (GT)n genetic variants in the control and PCOS groups ($P > 0.05$; Supplementary Tables 2, 3).

4 Discussion

For the first time, we demonstrated that the TT genotype and T allele of the rs2071746T/A SNP are associated with an increased risk of PCOS in Chinese women. We also proved that the TT/SS combined genotype of the rs2071746T/A and (GT)n repeat variants is a risk factor for PCOS. Furthermore, we found that plasma HMOX1 levels were significantly higher in patients with PCOS than those in the control women, and elevated HMOX1 levels were related to a slight but significant increase in the risk of PCOS, suggesting that patients with PCOS have a compensatory increase in HMOX1 levels. *HMOX1* rs2071746T/A and (GT)n repeat polymorphisms significantly affected BMI, waist-to-hip ratio, TT, FAI, acne grade score, HDL-C, GSH, and TOS/GSH ratio, but not plasma HMOX1 levels among the PCOS and/or control participants, supporting that the two variants may be involved in obesity, endocrine abnormalities, oxidative stress, and metabolic disorders.

Oxidative stress, metabolic disorders, and iron homeostasis imbalance play significant roles in the occurrence and progression of PCOS (18–21, 32). HMOX catalyzes the degradation of heme and is crucial for controlling the dynamic equilibrium of heme and its products (BV, BR, CO, and Fe^{2+}) (7, 8). In addition to the recovery of Fe^{2+} from heme, HMOX participates in the regulation of multiple

TABLE 2 Association of *HMOX1* T-413A (rs2071746) and (GT)*n* repeat polymorphisms with the risk of PCOS using different genetic models.

	Controls (n = 805)	PCOS (n = 1092)	Unadjusted		Adjusted	
			OR (95% CI)	P	OR (95% CI)	P
T-413A (rs2071746)						
Genotype						
AA	173 (21.5%)	193 (17.7%)				
AT	426 (52.9%)	556 (50.9%)	1.170 (0.919–1.489)	0.201	1.140 (0.872-1.493)	0.342
TT	206 (25.6%)	343 (31.4%)	1.493 (1.141–1.952)	0.003	1.395 (1.033-1.883)	0.030
P _{HWE}	0.233	0.456				
Recessive						
TT + AT	632 (78.5%)	899 (82.3%)				
AA	173 (21.5%)	193 (17.7%)	1.257 (1.014–1.603)	0.037	1.212 (0.936-1.570)	0.144
Dominant						
TT	206 (25.6%)	343 (31.4%)				
AA + AT	599 (74.4%)	749 (68.6%)	1.332 (1.086–1.632)	0.006	1.272 (1.013-1.597)	0.039
Allele						
A	772 (48.0%)	942 (43.1%)				
T	838 (52.0%)	1242 (56.9%)	1.215 (1.067–1.382)	0.003	/	/
(GT)n repeat						
Genotype						
LL	224 (27.8%)	298 (27.3%)				
SL	422 (52.4%)	542 (49.6%)	0.965 (0.779–1.197)	0.748	0.935 (0.736-1.189)	0.584
SS	159 (19.8%)	252 (23.1%)	1.191 (0.915–1.551)	0.193	1.134 (0.844-1.522)	0.404
P _{HWE}	0.292	0.983				
Recessive						
LL + SL	646 (80.2%)	840 (76.9%)				
SS	159 (19.8%)	252 (23.1%)	1.219 (0.975-1.524)	0.082	0.842 (0.655-1.081)	0.178
Dominant						
LL	224 (27.8%)	298 (27.3%)				
SS + SL	581 (72.2%)	794 (72.7%)	0.973 (0.794-1.193)	0.796	1.041 (0.827-1.309)	0.733
Allele						
L	870 (54.0%)	1138 (52.1%)				
S	740 (46.0%)	1046 (47.9%)	1.081 (0.950-0.239)	0.239	/	/

Data are presented as number (%). Genetic models (genotype, recessive, dominant, and allele models). OR, odds ratio; CI, confidence interval; *P*_{HWE}, *P* value of Hardy–Weinberg equilibrium. The assessment of Hardy–Weinberg equilibrium, the comparisons of the frequencies between two groups, and the unadjusted OR (95% CI) using Chi-squared (χ^2) analysis. Adjusted OR (95% CI) using the multivariable (for genotype model with the AA or LL genotype as the reference) or binary logistic regression analysis including age, BMI, and recruitment year of participants as covariates.

signaling pathways via its products, BV, CO, and Fe²⁺, as well as its substrate, heme (8). Under physiological conditions, low HMOX1 expression is found in most tissues, except in cells of the reticuloendothelial system (8, 10). Unlike the constitutive isoform HMOX2, which is barely regulated at the transcriptional and translational levels (5, 8), HMOX1 can be rapidly induced under various stress conditions (7, 8, 10, 11). BV and BR are important endogenous antioxidants and cellular signaling molecules that play significant roles in regulating immunity and glycolipid metabolism, and CO is a gaseous mediator with vasodilatory, anti-inflammatory, anti-proliferative, and anti-apoptotic properties, while heme and free Fe²⁺ can facilitate the production of reactive oxygen species

(7, 8, 10). Therefore, besides its cytoprotective effects, HMOX1 induction may also be involved in the development of certain diseases. Several studies have indicated that genetic overexpression or chemical induction of HMOX1 can protect against hypertension, cardiovascular diseases, metabolic conditions, and kidney diseases (33–35). Increased HMOX1 activity may promote oxidative stress by increasing free intracellular iron and accelerating the consumption of cytosolic NADPH, thereby contributing to chronic inflammation, ferroptosis, and cell injury (8). It has been reported that elevated plasma HMOX1 levels in individuals with T2D are associated with a higher disease risk (36). However, another study showed that low serum HMOX1 levels in non-obese women are an independent risk factor for PCOS (24). In this study, we found that plasma HMOX1 levels were significantly higher in the PCOS group compared to the control group, and the lean PCOS subgroup compared to the lean control subgroup. Furthermore, elevated HMOX1 levels were related to an increased risk of PCOS. The possible reasons for the inconsistent results of HMOX1 levels in PCOS may be discrepancies in the sample size and study population. Contrary to the report of lower HMOX1 concentrations in PCOS (24), our finding of elevated HMOX1 levels could represent a protective compensatory response to various chronic unfavorable stimuli in PCOS, aligning with the canonical role of HMOX1 as an oxidative stress sensor.

Increased oxidative stress in PCOS, as shown in this study, can enhance *HMOX1* transcription by activating nuclear factor erythroid 2-related factor 2 transcription factor (11, 37). Patients with PCOS have iron overload, abnormal heme metabolism, and chronic inflammation due to chronic oligomenorrhea, excessive androgen, and compensatory hyperinsulinemia (32, 38). High levels of heme and activation of the inflammatory factor nuclear factor kappa B can promote the expression of *HMOX1* (11, 33). The HMOX1 and its downstream metabolites, including CO, BV, and BR, may play a protective role through their antioxidant, anti-inflammatory, and vasodilator functions (8, 10). However, the sustained induction of *HMOX1* and the iron overload may paradoxically dysregulate ferroptosis through iron-mediated production of peroxidized lipids, potentially contributing to ovarian dysfunction. In addition to transcriptional regulation, HMOX1 activity is regulated by critical protein-protein interactions (PPIs) and post-translational modifications (39). PPIs affect the stability, oligomerization, subcellular localization, and function of HMOX1 (39–42). The post-translational modifications, such as phosphorylation, acetylation, and ubiquitination, also play an important role in regulating the level and activity of HMOX1 (43–45). In a word, the possible reasons and mechanisms for elevated HMOX1 levels are complex in PCOS. Further studies are needed to explain this phenomenon and its exact pathophysiological mechanisms.

Genetic variants in the promoter of *HMOX1* may affect the expression of *HMOX1* (11), thereby affecting the incidence and progression of diseases. The A allele of *HMOX1* rs2071746T/A SNP is associated with a higher transcription activity of *HMOX1* (11, 46). A meta-analysis revealed a lower susceptibility to coronary heart disease in individuals carrying the A allele (11). The AA genotype

increases the occurrence of hypertension in the Japanese women (47), but decreases the risk of ischemic heart disease in the Japanese population (48). The T allele of the rs2071746T/A variant is a risk factor for the development of esophageal varices in patients with cirrhosis (46), and the TT genotype is more likely to cause proteinuria in Korean patients with T2D (49) and SARS-CoV-2 viremia in COVID-19 infection (16). Our findings indicated that women with the TT genotype and T allele have a higher risk of developing PCOS. Moreover, we found that this genetic polymorphism may contribute to oxidative stress, hyperandrogenism status, and metabolic disorders through influencing TOS/GSH ratio, GSH and HDL-C levels, and acne grade score in the study population. However, we did not observe significant differences in plasma HMOX1 levels according to different genotypes of the rs2071746T/A SNP, suggesting that this genetic variant may not be a key factor affecting HMOX1 expression in the study population.

Of the polymorphisms observed in the *HMOX1* promoter region, the (GT)_n repeat variant has been extensively studied (11, 13). The range of (GT)_n repeat numbers is 10–50 (17), and the number of repeat lengths shows a bimodal distribution, with peaks at (GT)₂₃ and (GT)₃₀ repeats in East Asian and Caucasian populations, and a trimodal form, with crest values at (GT)₂₃, (GT)₃₀, and (GT)₃₉ among African-Americans (11). Generally, the short (S) allele is defined as the number of (GT)_n repeats < 25 or 27, and the long (L) allele is defined as the number of (GT)_n repeats ≥ 25 or 27 in different reports (11–13, 49). The S alleles are linked to increased transcriptional activity compared with the L alleles (11, 13), and individuals with the SS genotype of the (GT)_n variant have higher levels of *HMOX1* mRNA than those with the LL genotype (50). Individuals with the S allele or SS genotype have a reduced risk of coronary heart disease (11), T2D (13), rheumatoid arthritis (50), and encephalitis in HIV infection (17), but an increased risk of melanoma (51). Whereas individuals with the L allele or LL genotype have an increased risk of hypertension (52), chronic obstructive pulmonary disease (15), and preeclampsia (late-onset and non-severe form) (14). Our study revealed no significant differences were observed between the PCOS and control groups based on different genetic models. However, this polymorphism may be involved in obesity and endocrine disorders, probably by affecting waist-to-hip ratio, BMI, TT levels, and FAI in the study population. Moreover, we did not observe significant differences in plasma HMOX1 levels according to different genotypes of the (GT)_n repeat variant.

Additionally, in this study, a moderate linkage disequilibrium was observed between the rs2071746T/A and (GT)_n repeat polymorphisms. The TT/SS combined genotype of the two genetic polymorphisms was a risk factor for PCOS. However, we did not observe significant differences in the HMOX1 levels according to the different combined genotypes in the control and PCOS groups ($P > 0.05$, data not indicated). Several studies have shown that *HMOX1* expression is also controlled by genetic polymorphisms (11). The (GT)_n and rs2071746T/A polymorphisms may be involved in 5'-UTR alternative splicing of the *HMOX1* primary transcript, which may affect translational efficiency and mRNA stability, and thus regulate the translational process of *HMOX1* (53). Therefore, a

more detailed and systematic investigation of the correlation between genotype and gene expression, along with *in vitro* studies, is required to clarify the potential mechanism.

The present study has some limitations. First, we did not measure the levels of BV, BR, and iron, which are critical downstream products of HMOX1 enzymatic activity; this could provide further evidence to reveal the relationships between *HMOX1* genetic variants, HMOX1 levels, and PCOS and the underlying mechanism. Second, we did not evaluate oxidative stress and hormonal and metabolic indices because of confounding factors in some participants, which could have had an effect on the statistical effectiveness of these parameters. Third, lifestyle factors (e.g., dietary patterns, physical activity levels) and longitudinal treatment histories were not collected during enrollment, preventing us from adjusting for differences in these potential confounders in our analyses.

In conclusion, this study indicates that the *HMOX1* rs2071746T/A SNP is associated with the risk of PCOS, and that the T allele, TT genotype, and its coexistence with the SS genotype of the (GT)_n repeat variant are genetic risk factors for PCOS among Chinese women. We further demonstrated that patients with PCOS have higher plasma HMOX1 concentrations and that elevated HMOX1 levels are associated with an increased risk of PCOS. We found that the *HMOX1* rs2071746T/A and (GT)_n repeat polymorphisms may contribute to obesity, oxidative stress, endocrine abnormalities, and metabolic disorders. Our findings suggest that induction of the heme-degrading enzyme HMOX1 and its genetic polymorphisms in the promoter may be involved in the pathophysiology of PCOS.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Institutional Review Board of West China Second University Hospital, Sichuan University (2014-014 to PF). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

QW: Formal Analysis, Methodology, Writing – original draft, Investigation. JL: Resources, Data curation, Writing – original draft, Investigation. QL: Investigation, Writing – original draft, Project administration, Validation. HL: Writing – review & editing, Resources, Supervision, Data curation. HB: Writing – review &

editing, Supervision. WH: Writing – review & editing, Supervision, Resources, Conceptualization. PF: Formal Analysis, Methodology, Conceptualization, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was supported by the National Natural Science Foundation of China (81370681) and the Program for Chang Jiang Scholars and Innovative Research Team in the University, Ministry of Education (IRT0935).

Acknowledgments

The authors thank all participants who donated blood samples for this study. We also thank You Li, Qi Song, Dehua Wan, Ying Wang, Feng Zhang, Jinxia Zhang, Yan Gong, Renjiao Zhang, Jun Tan, and Yujin Zhang for their support with sample and data collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2025.1644373/full#supplementary-material>

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