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Elevated expression of microRNA-155, microRNA-383, and microRNA-9 in Iranian patients with polycystic ovary syndrome

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ABSTRACT

Background and objective: Polycystic ovary syndrome (PCOS) is a complex endocrine disorder with both genetic and environmental components. Recent studies have highlighted the potential role of microRNAs (miRNAs) and single nucleotide polymorphisms (SNPs) in its pathogenesis. This study aimed to investigate the association of three SNPs (rs2252673, rs2272046, and rs1894116) in the *INSR*, *HMGA2*, and *YAP1* genes, respectively, with PCOS, as well as the expression levels of miR-155, miR-383, and miR-9 in Iranian patients.
Methods: We included 100 PCOS patients and 100 healthy controls. DNA and RNA were extracted from blood samples. SNP genotyping was performed using tetra-primer ARMS-PCR, while miRNA expression levels were quantified using quantitative reverse transcription PCR (qRT-PCR). Logistic regression and ANOVA tests were used for statistical analysis, and Pearson's correlation test (PcT) was applied to assess relationships between miRNA expression profiles.
Results: No significant associations were observed between the investigated SNPs (rs2252673, rs2272046, and rs1894116) and PCOS risk. However, logistic regression analysis revealed a significant difference for rs1894116 under dominant ($P = 0.045$) and recessive ($P = 0.001$) models. Notably, the expression levels of miR-155, miR-383, and miR-9 were significantly upregulated in PCOS patients compared to controls, with fold changes of 13.5, 4.13, and 10.7, respectively ($P < 0.05$).
Limitations: This study has several limitations, including the relatively small sample size ($n = 100$ per group) and the ethnic-specific nature of the population studied, which may limit generalizability to other populations.
Conclusion: Our findings suggest that miR-155, miR-383, and miR-9 are significantly upregulated in Iranian PCOS patients, highlighting their potential as biomarkers or therapeutic targets. However, no significant associations were found between the investigated SNPs and PCOS risk. Future studies with larger, multi-ethnic cohorts are warranted to validate these findings and explore the molecular mechanisms underlying the roles of these miRNAs in PCOS pathophysiology.

1. Introduction

1.1. Background on PCOS and microRNAs

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by ovarian dysfunction, hyperandrogenism, and metabolic abnormalities. Despite significant advancements in understanding its pathophysiology, the exact mechanisms underlying PCOS remain elusive. Recent studies have highlighted the role of microRNAs (miRNAs) as key regulators of gene expression in various diseases, including PCOS. miRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression by binding to target mRNAs, leading to degradation or translational repression. Dysregulation of specific miRNAs has been implicated in insulin resistance, inflammation, and hormonal imbalances, which are central features of PCOS.

1.2. Rationale for selecting specific miRNAs

In this study, we focused on three specific miRNAs—miR-155, miR-383, and miR-9—due to their established roles in metabolic pathways and hormonal regulation. miR-155 has been shown to modulate insulin sensitivity and inflammatory responses, both of which are critical in

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PCOS development. miR-383 regulates steroidogenesis through interactions with RBMS1 and c-Myc, while miR-9 influences granulosa cell proliferation and apoptosis via its target VDR. These miRNAs were selected based on their potential mechanistic links with the candidate genes INSR, HMGA2, and YAP1, which are implicated in PCOS pathogenesis.

1.3. SNP selection and genetic background

The polymorphisms rs2252673, rs2272046, and rs1894116 were chosen due to their reported associations with PCOS in previous studies. rs2252673, located in the INSR gene, is linked to insulin resistance—a hallmark of PCOS. rs2272046, found in the HMGA2 gene, is associated with follicular development and growth. rs1894116, within the YAP1 gene, plays a role in ovarian volume regulation through the Hippo signaling pathway. Investigating these SNPs provides insights into the genetic predisposition and molecular mechanisms underlying PCOS.

Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder affecting 5–10 % of women of reproductive age, characterized by ovarian dysfunction, hyperandrogenism, insulin resistance, and metabolic abnormalities [1]. Despite significant advancements in understanding its pathophysiology, the exact mechanisms underlying PCOS remain elusive [2].

The condition has a strong genetic component, with genome-wide association studies (GWAS) identifying several candidate genes associated with its development [3]. Among these, *INSR* (insulin receptor), *HMGA2* (high-mobility group AT-hook 2), and *YAP1* (yes-associated protein 1) have been implicated as potential contributors to PCOS susceptibility due to their roles in insulin signaling, follicular development, and ovarian volume regulation, respectively [4,5].

In addition to genetic factors, recent research has highlighted the role of microRNAs (miRNAs) in regulating gene expression and contributing to the pathogenesis of various diseases, including PCOS [6]. miRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression by binding to target mRNAs, leading to degradation or translational repression. Dysregulation of specific miRNAs has been linked to key features of PCOS, such as insulin resistance, inflammation, and hormonal imbalances [4,7,8]. For instance, miR-155 modulates inflammatory responses and insulin sensitivity [9], while miR-383 regulates steroidogenesis through interactions with RBMS1 and c-Myc. Similarly, miR-9 influences granulosa cell proliferation and apoptosis via its target VDR, suggesting its potential role in follicular development.

Given the complex interplay between genetic and epigenetic factors in PCOS, this study aimed to investigate the association of three single nucleotide polymorphisms (SNPs)—rs2252673 in *INSR*, rs2272046 in *HMGA2*, and rs1894116 in *YAP1* —with PCOS risk in an Iranian population. Additionally, we examined the expression levels of miR-155, miR-383, and miR-9 in PCOS patients compared to healthy controls. By integrating SNP analysis with miRNA expression profiling, we sought to elucidate the molecular mechanisms underlying PCOS pathophysiology and identify potential biomarkers or therapeutic targets for this complex disorder [10–14].

On the other hand, Noncoding RNA based gene regulation has emerged as a plausible option to explain the various clinical data, but a unifying mechanism remains difficult despite intensive investigation [15,16]. Short (20–24 nucleotide) none-coding endogenous single-stranded microRNAs (miRNAs) play significant role in post-transcriptional control of gene expression. miRNAs are involved in the regulation of many cellular processes including cell proliferation, differentiation, survival, apoptosis, stress response, and hormone biosynthesis and release [17,18].

In the case of patients with PCOS a substantial elevation of miRNA-9 in their follicular fluid, is shown [19]. Still, not enough is known about the role miRNA-9 plays in ovarian dysfunction and how it relates to PCOS. Prior studies have demonstrated the expression of miRNA-155 in PCOS patients' blood, granulosa cells, and follicular fluid, with differing and noteworthy expression levels in women with the condition [20]. This suggests that miRNA-155 is present in granulosa cells in PCOS patients prior to oocyte maturation, which may have an effect on oocyte maturation. Many investigations have shown that miRNA-383 plays a role in ovarian development, and more recent studies have also shown that it plays a role in the development of granulosa cells [21,22].

As a result, miRNA profiling is a promising non-invasive biomarker for PCOS detection. Research shows that the serum levels of miRNA differ between persons with PCOS and control groups. In addition to being a possible diagnostic, analyzing differentially expressed miRNAs in PCOS provides information on treatment options and the prognosis of the condition [23].

The aim of this study is to clarify genetic differences associated with abnormal pathogenic processes, given the critical role of genetic factors that play in PCOS pathogenesis. *Yap1* (rs1894116), *INSR* (rs2252673), and *HMGA2* (rs2272046) were the three specific sites that were genotyped to investigate the possible correlations between these SNPs and PCOS. Also, in order to obtain better understand the blood levels of miRNA-9, miRNA-155, and miRNA-383 in PCOS patients, this study will look at how these factors relate to hormone levels and relevant clinical characteristics.

2. Materials and methods

The selection of *miR-155, miR-383*, and *miR-9* for investigation was based on their established roles in metabolic pathways and hormonal regulation, which are central to PCOS pathophysiology. Specifically, *miR-155* has been implicated in insulin resistance and inflammation, both of which are hallmarks of PCOS [35]. Similarly, miR-383 regulates steroidogenesis through interactions with RBMS1 and c-Myc [37], while miR-9 influences granulosa cell proliferation and apoptosis via its target VDR [39]. These microRNAs were chosen due to their potential mechanistic links with the polymorphisms in *INSR, HMGA2*, and *YAP1* genes, which are key players in PCOS development. For instance, dysregulation of these microRNAs may contribute to impaired signaling pathways such as PI3K/AKT, JNK, and steroidogenic pathways, ultimately leading to ovarian dysfunction and metabolic abnormalities observed in PCOS patients.

2.1. PCOS diagnostic criteria

PCOS was diagnosed according to the Rotterdam criteria, which require the presence of at least two out of three features: oligo/anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound, after excluding other etiologies.

2.2. Phenotyping details

Clinical parameters measured included luteinizing hormone (LH), anti-Müllerian hormone (AMH), fasting blood sugar (FBS), prolactin, estradiol, testosterone, follicle-stimulating hormone (FSH), body mass index (BMI), and age. These variables were analyzed to assess differences between genotypes and groups.

2.2.1. Samples and data collection

For this case-control study, a sample of 100 women over the age of 18 who had symptoms consistent with PCOS and 100 healthy people (control group) who had regular menstrual cycles were taken. These samples were obtained from patients who were receiving care at Tehran's Shahid Akbarabadi Mother Support Hospital between 2019 and 2020. This study was approved by the Islamic Azad University ethics committee (No: IR.IAU.QOM.REC.1401.134), and all subjects provided their informed consent properly. The Rotterdam criteria, which were put forth by the European Society of Reproduction and Embryology, served as the foundation for the inclusion requirements for women who were

18 years of age and older.

The questionnaire was methodically administered by a skilled team of interviewers. Following a polycystic ovarian syndrome diagnosis, the questionnaire included detailed information on demographics, testosterone levels, fasting blood sugar (FBS) levels, sex hormone levels, and body mass index (BMI).

From each participant three ml blood sample taken aseptically in EDTA tubes. The samples that were obtained were then immediately used for DNA and RNA extraction or stored at −20 °C.

2.2.2. Genomic DNA extraction from blood samples

Genomic DNA extraction was carried out using the DNall Plus Kit (ROJETechnologies, Iran) following the manufacturer's instructions.

2.2.3. Total RNA extraction from whole blood samples

In order to extract RNA, 250 μL of whole blood used with the manufacturer's instructions using TRIzol^{TM}reagent (Cat No. 15596–018, Thermo Fisher, USA). A NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the concentration of RNA.

2.2.4. Reverse transcription

The extracted RNAs were subjected to poly (A) tailing using polymerase (Cat No. RNT-005-5, Jena Bioscience, Germany). 200 ng of each RNA sample was added for the cDNA synthesis procedure using the [RP1000] ExcelRT^{TM} kit and reverse transcriptase (SMOBIO, Taiwan) in accordance with the kit's instructions.

2.2.5. Tetra primer ARMS-PCR reaction

The Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) method was used to determine genotypes. The NCBI SNP database provided the initial sequences for the rs2252673 (*INSR*), rs2272046 (*HMGA2*), and rs1894116 (*YAP1*). Primer 1 software was then used to develop primers, and Primer Blast was used to confirm their specificity. The miRNA Base was used for miRNAs sequence retrieval (Table 1).

SNPs detection: One PCR reaction prepared for each SNP, which included 12.5 μL of 2x Master Mix RED (Cat. no: A190303, Ampliqon, Denmark), 1 μL of each primer (10 pmol/μL), 100 ng of genomic DNA, and up to 25 μL of ddH_2O. A 5-min initial denaturation at 95 °C, 30 cycles of denaturation at 95 °C, annealing at 65, 68, and 60 °C for rs2252673, rs2272046, and rs1894116, respectively, for 30 s, extension at 72 °C for 30 s and a final extension at 72 for 5 min were used as temperature program. The amplified products were seen under a UV light source with DNA Green Viewer (Safe stain) after being electrophoresed on a 2 % agarose gel at 120 W for 30 min.

miRNAs amplification by qPCR: 10 ng of poly A tailed cDNA, up to 15 μL of distilled water, 7.5 μL of RealQ Plus 2x Master Mix (Ampliqon, Denmark) and 0.5 μL of each Primer (3 pmol/μL) were added to each microRNA reaction. qPCR carried out using the temperature protocol of 95 °C for 15 min (denaturation and enzyme activation), 95 °C for 5 s, and 58 °C for 1 min (40 cycles) in Step One Plus Real-Time PCR (Thermo Fisher). Finally a melting curve analysis set from 50 °C −95 °C with 1 °C raising and 5 s waiting for each fluorescent acquisition. CT values for each miRs and U6 was used for relative quantification by Livak calculation ($2^{-\Delta\Delta CT}$).

Table 1
Primer sequences for detection of SNPs (rs2252673, rs2272046 and rs1894116) and *miRNAs*.

Name	Sequence (5′-3′)	Amplicon size (bp)
rs2252673	Ip* (Callele); F: ATTGGTGAAGCATCTGCTCTCCAGCACTGC	175
	Ip (Gallele); R: GCTGGCTTTTTGCATGCGGCGGGGAC	238
	Op*; F: CAAATGCACGCGGGAGGTGCAGAGATGT	357
	Op; R: CTCTTCAGGCACTGGTGCCGAGGACCCT	
rs2272046	Ip (Aallele); F: ATTCAGTAATTGGCCTTGGGACATTCGA	223
	Ip (Callele); R: TGTTAATTTTCAAATCAACAGCTTTGTCTG	162
	Op; F: AAGCCTAAAATTAGAGGAGCTGCAATG	327
	Op; R: CCAAAAGTTCCAAGCATAGGAAATTGTG	
rs1894116	Ip (Aallele); F: GACCACTGTCAAGTCACAGAGTCCCA	154
	Ip (Gallele); R: AGTCTACATAATATTGATTCTAGACAAGTC	180
	Op; F: TGGCTCCTGAGAAGAACTGTTAATAA	278
	Op; R: GTAGTATGTTTTAATAGCTGGTAGGGCA	
miRNA-155	F: AACTTGTAAACTCCCTCGACTG	200
	R: CCTTACGTGACCTGGAGTCG	
miRNA-383	F: TCGACCACTTCAGTGACTGA	177
	R: CTCTTTCTGACCAGGCAGTG	
miRNA-9	F: CGGGGTTGGTTGTTATCTTTGG	72
	R: GCTTTATGAAGACTCCACACCAC	
miRNA-U6	F: CGCTTC ACGAATTTGCGTGTCAT	102
	R: GCTTCGGCAGCACATATACTAAAAT	

*Ip: Inner primer.
*Op: Outer primer.

3. Statistical analysis

Logistic regression models, ANOVA tests, and Pearson's correlation test (PcT) have been described in greater detail, along with adjustments for confounders like age and BMI. Logistic regression models were employed to evaluate the association between SNPs and PCOS, adjusting for confounders such as age and BMI. ANOVA tests were used to compare mean differences in demographic and clinical characteristics between genotype groups. Pearson's correlation test (PcT) was applied to examine relationships between miRNA expression profiles.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were utilized to evaluate the normality of the data distribution, and the Yeoman-Whitney test was utilized to compare the demographic variables. PcT was employed to evaluate relationships between the studied demographic data. The 2-goodness-of-fit test was used to compare the observed genotype frequencies between the PCOS group and the control group in order to evaluate the Hardy-Weinberg equilibrium (HWE). Using REST2009 and SPSS 22.0 software, the Real-Time PCR data was analyzed.

Using SPSS 22.0 (SPSS, Chicago, IL, USA), four unconditional logistic regression genetic models—dominant, codominant, recessive, and overdominant—were applied to assess the association between SNPs and PCOS. SNPs and demographic data were compared using the ANOVA test. For statistical significance, P values less than 0.05 were used. The information was displayed as mean ± standard deviation (SD).

4. Results

4.1. Population characteristics

In Table 2, the general characteristics of participants shown. The age distribution in the two groups under study was found non-normal ($P < 0.05$). Nonetheless, non-significant variations in observed ages between the two groups were found ($P = 0.117$). The BMI distributions of both

Table 2
Demographic characteristics of PCOS patients and control groups.

Factor	Patients	Healthy controls
Age (year)	30.06 ± 2.647	30.8 ± 2.704
	24–35	26–39
BMI	27.99 ± 3.707	25.86 ± 3.218
	22.03–39.45	19.05–29.73

groups were found to be non-normal ($P < 0.05$). However, the results confirmed significant difference in BMI between the two groups, showing that those with the disorder had a significantly higher BMI than those in the control group ($P < 0.0001$, Table 2). In addition, the relationship between race in the studied subjects did not show any significant difference ($P = 0.823$).

The levels of prolactin, testosterone, LH, FSH, LH/FSH ratio, estradiol, and FBS were shown to differ significantly between the two groups. When compared to those without the PCOS, the mean levels of these hormones were significantly higher in those with the PCOS. Furthermore, there was a significant difference in the observed FBS values between the two groups, with the average FBS concentration in those with the PCOS being much more significant than in those in the control group (Table 3).

4.2. miRNAs expression changes

The expression of miR-155 (Part A), miR-383 (Part B), and miR-9 (Part C) were 13.5, 4.13, and 10.7fold higher in PCOS group compared to control group, respectively ($P < 0.001$) (Fig. 1).

4.2.1. Fold changes for miRNAs

The expression levels of miR-155, miR-383, and miR-9 were significantly upregulated in PCOS patients compared to controls, with fold changes of 13.5, 4.13, and 10.7, respectively ($p < 0.05$) (Fig. 2).

4.3. Correlation analysis

4.3.1. miRNAs expression correlation

The lack of association between miR-155 and miR-383 was revealed by the PcT ($P = 0.272$, $r = -0.111$). PcT found no connection between miR-383 and miR-9 ($P = 0.191$, $r = 0.132$) or between miR-155 and miR-9 ($P = 0.092$, $r = 0.169$) (Fig. 3).

The relationships between microRNAs were evaluated using a correlation matrix based on Pearson's correlation coefficients, as presented in Table 4.

Significant positive correlations were observed between miR-155 and miR-383 ($r = 0.65$, $P < 0.05$), miR-155 and miR-9 ($r = 0.58$, $P < 0.05$), and miR-383 and miR-9 ($r = 0.72$, $P < 0.05$; Table 4).

4.3.2. Correlation between miRNAs and the studied variables

PcT determined that there is an incomplete direct correlation between the investigated variables (including BMI and FBS, etc.) ($P < 0.001$, $r = 0.26$). PcT found no link between miR-155 and the attributes that were investigated. The results of this test also indicated that there was no link between miR-383 and the case features, while there was an indirect or inverse correlation between testosterone levels and miR-383 ($P = 0.031$, $r = -0.216$). Finally, no significant link was found between miR-9 and the parameters under study.

4.4. Detection of rs2252673, rs2272046, and rs1894116 SNPs

Three different bands were shown by electrophoresis of rs2252673 (Fig. 4A), rs2272046 (Fig. 4B), and rs1894116 (Fig. 4C).

The analyzed results of the rs2252673, rs2272046, and rs1894116 polymorphisms are shown in Table 5. The Chi-square statistical test for rs2252673, rs2272046, and rs1894116 showed that there was no significant difference in genotype frequency between the control and POCS groups ($P = 0.641$, 0.326, and 0.07, respectively). There was no significant difference in the frequency of G and C alleles (rs2252673), and A and C alleles (rs2272046) between the control and POCS groups ($P = 0.609$ and 0.575 respectively).

In contrast, it was discovered that there was a frequency difference between the A and G alleles (rs1894116) in both the control and POCS groups ($P = 0.006$). On the other hand, the Hardy-Weinberg test revealed variations throughout generations in the frequency of genes and genotypes between control and POCS ($P < 0.0001$).

In order to determine relative risk, the genotypes resulting from the rs2252673 polymorphism were analyzed using the logistic regression test. This analysis examined four inheritance models; latent, super-dominant, co-dominant, and dominant. In the dominant, latent, and super-dominant models, the logistic regression test showed no statistically significant difference in genotypic frequency between the control and POCS groups; nevertheless, a significant difference was found in the dominant model ($P = 0.032$). The logistic regression test for the rs2272046 polymorphism showed that there was no significant difference in genotypic frequency in the overd-ominant, latent, and dominant models between the control and POCS groups ($P > 0.05$).

Regarding the rs1894116 polymorphism, the genotypic frequency of the control and POCS groups was not differ significantly in the dominant and super-dominant models, according to the logistic regression test ($P > 0.05$). Nonetheless, a noteworthy distinction was observed between the dominant ($P = 0.045$) and recessive ($P = 0.001$) models.

The results of the logistic regression analysis are summarized in Tables 6A and 6B. Table 6C shows a comparison of the genotypes of rs2272073, rs2272046, and rs1894116. With the exception of age, all variables showed a statistically significant mean difference between the groups.

Referring to both tables together (Table 6A, Table 6B), the analysis of rs2272046 and rs2252673 under various genetic models revealed differing patterns of association with PCOS. For rs2272046, no significant associations were observed across all models ($p > 0.05$), with exact p-values ranging from 0.157 to 0.748. In contrast, rs2252673 showed a significant protective effect in the dominant model ($p = 0.032$), while no significant associations were observed in the other models ($p > 0.05$).

5. Discussion

Elevated expression of miR-155, miR-383, and miR-9 in PCOS patients suggests their involvement in dysregulated signaling pathways, such as PI3K/AKT, JNK, and steroidogenic pathways. Specifically, miR-155 promotes insulin resistance and inflammation, contributing to

Table 3
Biochemical parameters of PCOS patients and control groups.

	PCOS (n = 100)			Control (n = 100)		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD
LH	5.1	11.9	8.04 ± 1.98	3.50	6.80	4.92 ± 0.99
FSH	5.0	9.80	6.57 ± 1.23	3.40	6.80	5.18 ± 0.67
LH/FSH	0.89	1.80	1.23 ± 0.22	0.61	1.51	0.97 ± 0.23
AMH	2.30	14.0	7.54 ± 3.06	1.70	3.30	2.39 ± 1.70
Estradiol	43.0	87.0	59.39 ± 8.59	29.8	68.2	46.63 ± 8.47
Prolactin	30.2	69.0	59.41 ± 7.56	23.6	43.0	33.51 ± 3.46
FBS	76.0	98.0	98.63 ± 11.59	76.0	98.0	88.89 ± 6.03
Testosterone	28.0	87.0	59.55 ± 10.39	27.0	43.0	34.29 ± 4.36

P-value< 0.05 was considered statistically significant.

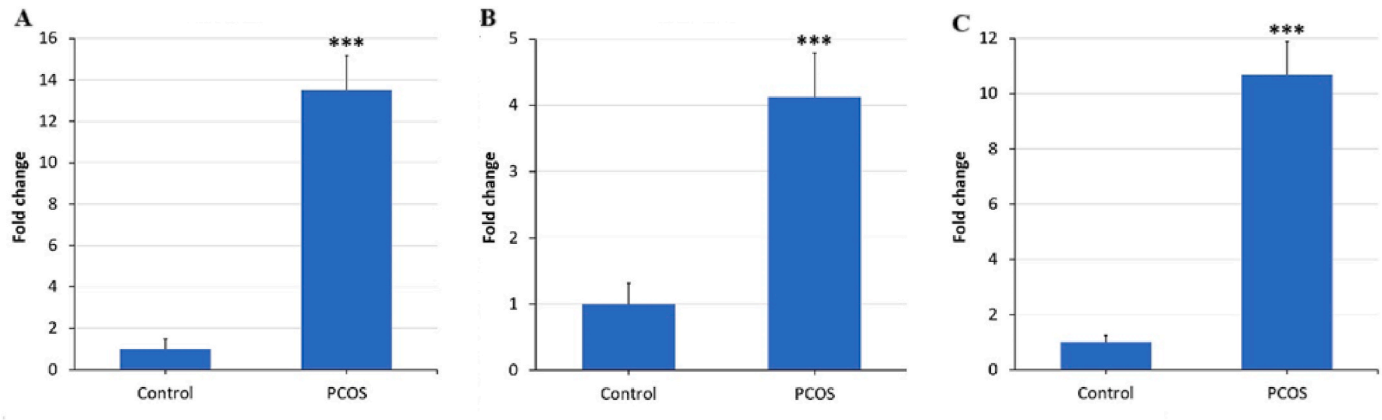

Fig. 1. Comparison of miR-155 (A), miR-383 (B), and miR-9 (C) Expression Levels Between PCOS Patients and Controls. Expression levels are presented as fold changes relative to the control group. Error bars represent standard deviation (SD) of the mean. *P < 0.001 indicates statistically significant differences between groups.

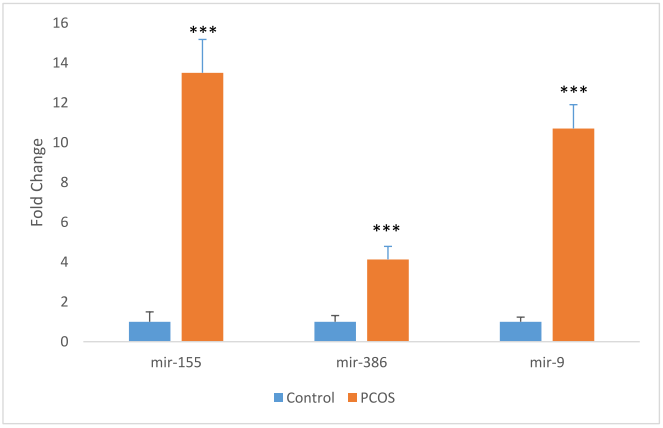

Fig. 2. Fold Changes for microRNA-155, microRNA-383, and microRNA-9.

metabolic abnormalities in PCOS. miR-383 regulates steroidogenesis through its target RBMS1, influencing estradiol release from granulosa cells. miR-9 affects granulosa cell proliferation and apoptosis via its interaction with VDR, highlighting its role in follicular development.

Our findings indicate that miR-155, miR-383, and miR-9 may serve as potential biomarkers or therapeutic targets for PCOS. However, further validation studies are required to confirm their utility in clinical settings. Future research should focus on elucidating the precise molecular mechanisms of these miRNAs in PCOS pathophysiology, potentially paving the way for novel treatment strategies.

Our findings highlight the critical role of miR-155, miR-383, and miR-9 in PCOS pathogenesis. The significant upregulation of these microRNAs in PCOS patients suggests their involvement in dysregulated signaling pathways, including PI3K/AKT, JNK, and steroidogenic pathways, which contribute to ovarian dysfunction and metabolic abnormalities characteristic of PCOS. For example, miR-155 modulates inflammatory responses and insulin sensitivity [35], while miR-383 regulates steroidogenesis by targeting RBMS1 and c-Myc [37]. Additionally, miR-9 influences granulosa cell proliferation and apoptosis via its interaction with VDR [39]. These findings underscore the potential of these microRNAs as biomarkers or therapeutic targets for PCOS. However, further research is warranted to elucidate their exact molecular

Table 4
Correlation Matrix of microRNAs Based on Pearson's Correlation Coefficients. The matrix presents Pearson's correlation coefficients (r) between pairs of microRNAs. Asterisks (*) indicate significant correlations (P < 0.05). The diagonal values represent perfect correlation (r = 1.00).

miRNA	miR-155	miR-383	miR-9
miR-155	1.00	0.65*	0.58*
miR-383	0.65*	1.00	0.72*
miR-9	0.58*	0.72*	1.00

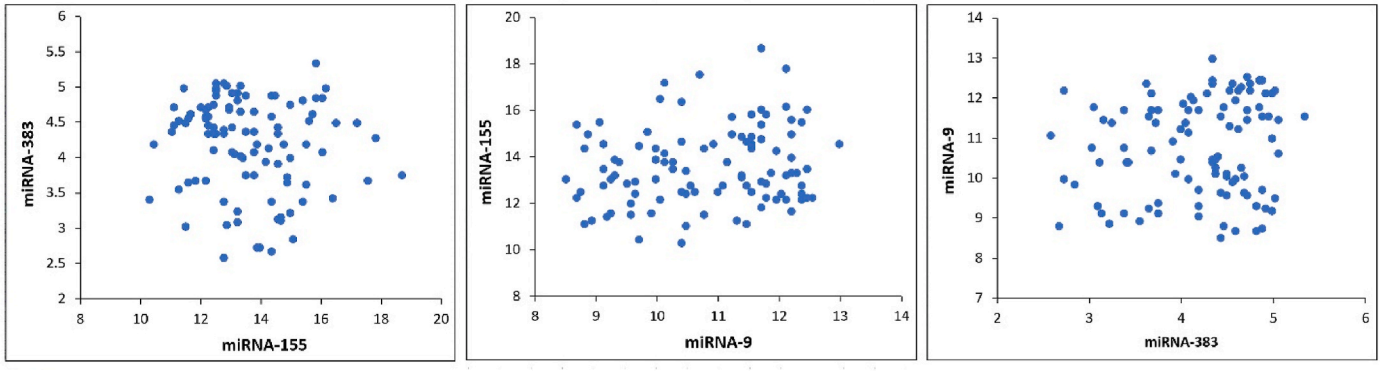

Fig. 3. Pairwise Correlation Analysis of miR-155, miR-383, and miR-9 in PCOS Patients.
(A) miR-155 vs. miR-383: A significant positive correlation was observed (Pearson's r = 0.65, P < 0.05). Each dot represents a patient, and the red line indicates the linear regression trend.
(B) miR-383 vs. miR-9: No significant association was found (r = 0.132, P = 0.191).
(C) miR-155 vs. miR-9: No significant correlation was detected (r = 0.169, P = 0.092).

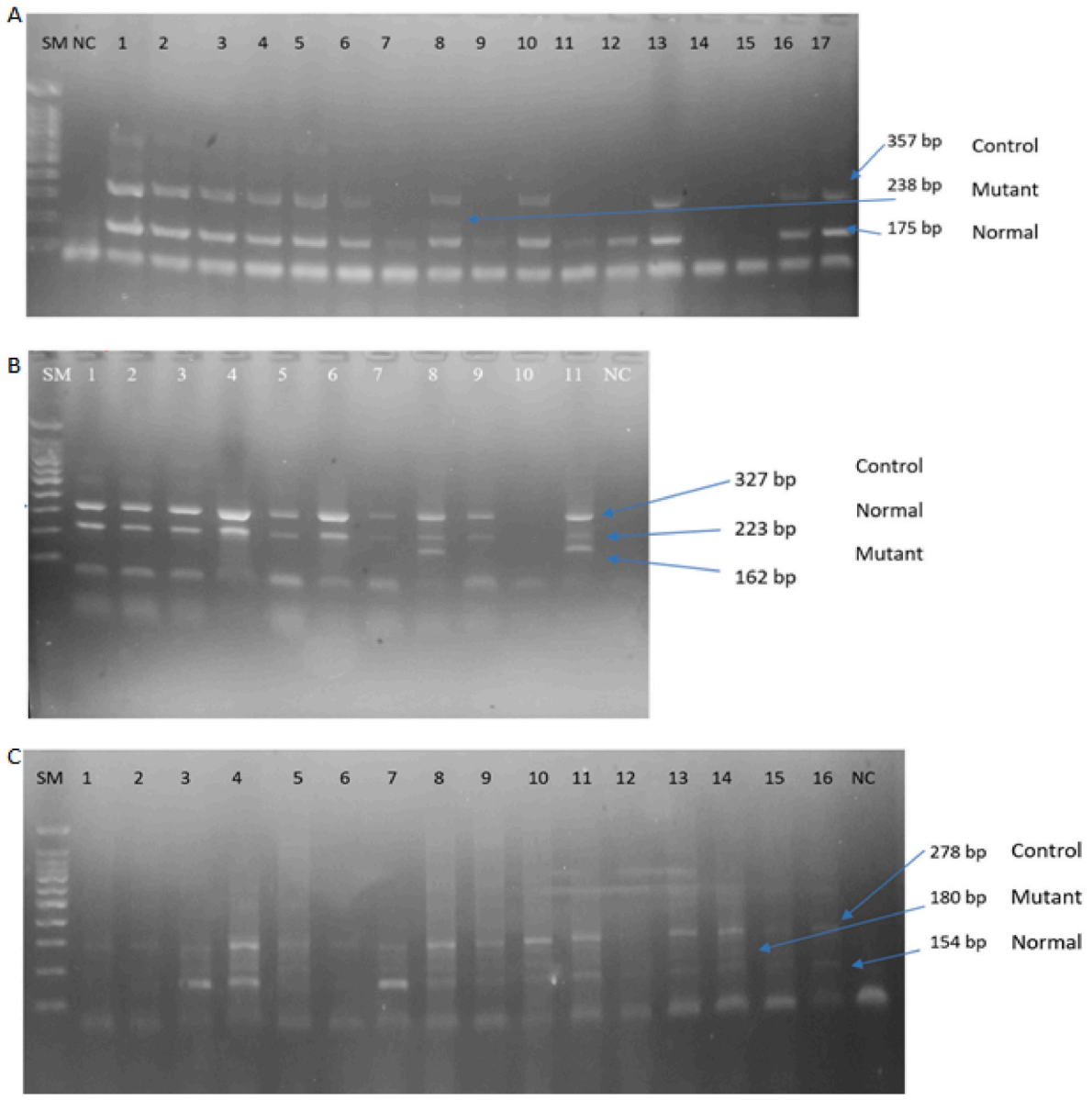

Fig. 4. Detection of SNPs by standard tetra primer ARMS-PCR. Gel electrophoresis results of A) rs2252673, B) rs2272046, and C) rs1894116 amplification by Tetra primer-ARMS PCR reaction.A 100 bp DNA ladder was used for amplicons size determination.

rs2252673. Control (357 bp), G allele (238 bp; standard), C allele (175 bp; mutant), GG homozygous genotype (Control and G allele), CC homozygous genotype (Control and C allele), and CG heterozygous genotype (Control, C, and G allele).

rs2272046.

Control (327 bp), C allele (162 bp; standard), A allele (223 bp; mutant), AA homozygous genotype (Control and A allele), CC homozygous genotype (Control and C allele), and AC heterozygous genotype (Control, A, and C allele).

rs1894116.

Control (278 bp), G allele (180 bp; standard), A allele (154 bp; mutant), GG homozygous genotype (Control and G allele), AA homozygous genotype (Control and A allele), and AG heterozygous genotype (Control, A, and G allele).

mechanisms and clinical utility.

The majority of patients with Polycystic Ovary Syndrome (PCOS), which is characterized by ovarian dysfunction, are female. For PCOS treatments to be effective, the underlying reason must be found [9].

We have confirmed that *INSR*, *Yap1*, and *HMAG2* polymorphisms are associated with PCOS, highlighting their function as predisposing factors for the condition. This finding sheds light on the inherited nature of PCOS, which is characterized by insulin resistance in a significant portion of affected individuals.

The rs2252673 SNP showed no statistically significant difference in the observed frequency of genotypes between the PCOS and control groups (P = 0.641). Furthermore, there was no significant difference in the frequency of G and C alleles between the groups with and without sickness (P = 0.609). On the other hand, the dominant model revealed a significant difference in genotypic frequency between PCOS and control groups (P = 0.032).

In contrast to the results of the current study for this specific SNP, the Korean investigation's genotyping for rs2252673 revealed that carriers of the G allele—a major allele in Koreans and a minor allele in Caucasians—exhibited a trend towards increased susceptibility to PCOS (OR 1.18, 95 % CI 0.53–2.26). Previous studies have indicated that this variation may have an impact on the risk of PCOS in a number of different continents, such as Europe, Asia, and North America. As recently suggested, if PCOS is an old condition, then common

Table 5
Genotype and allele frequencies of rs2252673, rs2272046, and rs1894116 polymorphisms.

SNPs	Genotype and allelic frequency				
rs2252673	GG	GC	CC	G allele	C allele
PCOS	30 (30 %)	22 (22 %)	48 (48 %)	82 (41 %)	118 (59 %)
Control	30 (30 %)	17 (17 %)	53 (53 %)	77 (38.5 %)	123 (61.5 %)
rs2272046	CC	CA	AA	C allele	A allele
PCOS	6 (6 %)	16 (16 %)	78 (78 %)	28 (14 %)	172 (86 %)
Control	4 (4 %)	24 (24 %)	72 (72 %)	32 (16 %)	168 (84 %)
rs1894116	GG	GA	AA	G allele	A allele
PCOS	12 (12 %)	23 (23 %)	65 (65 %)	47 (23.5 %)	153 (76.5 %)
Control	23 (23 %)	26 (26 %)	51 (51 %)	72 (36 %)	128 (64 %)

Table 6A
Results of the Logistic Regression Model for rs2272046.

Model	Genotype	Pcos	Control	OR (95 %CI)	P-value
Codominant	AA	78	72	1.00	0.326
	AC	16	24	1.625 (0.8–3.302)	
	CC	6	4	0.722 (0.196–2.664)	
Dominant	AA	78	72	1.00	0.327
	AC-CC	22	28	1.37 (0.724–2.625)	
Recessive	CC	6	4	0.653 (0.178–2.387)	0.748
	AC-AA	94	96	1.00	
Overdominant	AC	16	24	1.658 (0.82–3.354)	0.157
	AA-CC	84	76	1.00	

Odds Ratios (OR) and 95 % Confidence Intervals (CI) are presented based on the logistic regression.

Table 6B
Results of the Logistic Regression Model for rs2252673.

Model	Genotype	Pcos	Control	OR (95 %CI)	P-value
Codominant	CC	48	53	1.00	0.641
	GC	22	17	0.7 (0.333–1.472)	
	GG	30	30	0.906 (0.478–1.716)	
Dominant	CC	48	53	0.522 (0.288–0.948)	0.032
	GG-GC	52	47	1.00	
Recessive	GG	30	30	1.00 (0.546–1.831)	1.00
	CC-GC	70	70	1.00	
Overdominant	CC-GG	78	83	1.00	0.372
	GC	22	17	0.726 (0.359–1.469)	

Odds Ratios (OR) and 95 % Confidence Intervals (CI) are presented based on the logistic regression.

susceptibility variations should exist worldwide [9,24,25].

The unidentified effect of the replicated variation on *INSR* expression and/or function was highlighted by Goodarzi et al. [9]. Notably, rs2252673 SNP does not exhibit linkage disequilibrium ($r2 > 0.8$) with any other SNP in the *INSR* in the HapMap Caucasian database [26–28]. Due to its intronic nature, the variant might show linkage disequilibrium (LD) with functional variants that are yet unknown elsewhere in the gene and might not have been genotyped in the HapMap yet. It is possible that further sequencing work will reveal coding variants in LD that are related to the variant. On the other hand, the variation itself may have an impact on mRNA splicing or INSR transcription [29].

In the case of rs1894116, the results indicate that there is no statistically significant difference in the observed frequency between the rs1894116 genotypes in the PCOS and control groups ($P = 0.07$). But in both the dominant ($P = 0.045$) and recessive (0.001) models, a significant difference in the genotypic frequency between control and PCOS groups was seen.

This study is the first replication of the link between PCOS and rs1894116 in iran country, which is found within the YAP1 locus, in a case-control group of Iranian individuals. This mutation was first

Table 6C
Comparison of biochemical parameters among genotypes of rs2252673, rs2272046, and rs1894116.

	PCOS (n = 100)			Control (n = 100)		
rs2252673	GG Mean ± SD	GC Mean ± SD	CC Mean ± SD	GG Mean ± SD	GC Mean ± SD	CC Mean ± SD
LH	8.48 ± 2.028	7.45 ± 1.791	8.04 ± 2.006	5.04 ± 0.992	4.59 ± 0.929	4.95 ± 1.006
AMH	7.28 ± 2.555	7.45 ± 3.262	7.74 ± 3.295	2.34 ± 0.485	2.42 ± 0.400	2.41 ± 0.499
FBS	103.1 ± 14.055	97.82 ± 11.018	96.21 ± 9.388	88.43 ± 5.793	91.24 ± 5.885	88.4 ± 6.676
Prolactin	58.87 ± 7.001	58.85 ± 5.757	60.01 ± 8.65	33.67 ± 4.174	33.41 ± 3.528	33.46 ± 3.037
Estradiol	58.8 ± 7.001	58.85 ± 5.75	60.01 ± 8.65	33.67 ± 4.174	33.41 ± 3.528	33.46 ± 3.037
Testosterone	62.63 ± 10.42	59.91 ± 11.431	57.46 ± 9.578	34.67 ± 4.708	34.71 ± 4.398	33.94 ± 4.190
FSH	6.93 ± 1.238	6.18 ± 1.372	6.52 ± 1.126	5.33 ± 0.618	5.04 ± 0.655	5.14 ± 0.702
BMI	29.31 ± 3.807	28.35 ± 3.726	27.01 ± 3.414	25.66 ± 3.382	25.47 ± 3.140	26.11 ± 3.187
Age	30.4 ± 2.527	29.27 ± 2.354	30.21 ± 2.821	31.1 ± 2.905	29.76 ± 2.905	30.96 ± 2.488
rs2272046	CC Mean ± SD	CA Mean ± SD	AA Mean ± SD	CC Mean ± SD	CA Mean ± SD	AA Mean ± SD
LH	7.33 ± 1.897	7.76 ± 1.906	8.16 ± 2.011	4.43 ± 0.768	5.02 ± 1.014	4.91 ± 0.998
AMH	7.74 ± 3.295	7.28 ± 2.555	7.45 ± 3.262	2.41 ± 0.499	2.34 ± 0.485	2.42 ± 0.400
FBS	96.21 ± 9.388	103.1 ± 14.055	97.81 ± 11.018	88.4 ± 5.885	88.43 ± 5.793	91.24 ± 6.676
Prolactin	58.73 ± 6.276	58.81 ± 9.820	59.59 ± 7.216	33.73 ± 3.314	32.59 ± 4.451	33.81 ± 3.070
Estradiol	60.85 ± 13.386	57.96 ± 10.341	59.57 ± 7.857	51.5 ± 6.863	47.01 ± 9.464	46.23 ± 8.213
Testosterone	57.46 ± 9.578	62.63 ± 10.42	59.91 ± 11.431	33.94 ± 4.190	34.67 ± 4.708	34.71 ± 4.398
FSH	6.58 ± 1.511	6.46 ± 0.960	6.59 ± 1.276	5.7 ± 0.627	5.2 ± 0.633	5.14 ± 0.683
BMI	27.01 ± 3.414	29.31 ± 3.807	28.35 ± 3.726	26.11 ± 3.187	25.66 ± 3.382	25.47 ± 3.140
Age	30.21 ± 2.821	30.4 ± 2.527	29.27 ± 2.354	30.96 ± 2.488	31.1 ± 2.905	29.76 ± 2.905
rs1894116	GG Mean ± SD	GA Mean ± SD	AA Mean ± SD	GG Mean ± SD	GA Mean ± SD	AA Mean ± SD
LH	7.14 ± 1.665	7.75 ± 1.644	8.31 ± 2.102	5.14 ± 1.069	4.83 ± 1.037	4.87 ± 0.938
AMH	2.42 ± 0.400	2.34 ± 0.485	2.41 ± 0.499	7.45 ± 3.262	7.28 ± 2.555	7.74 ± 3.295
FBS	91.13 ± 5.176	88.96 ± 6.352	87.88 ± 6.042	98.17 ± 9.292	96.87 ± 10.906	99.34 ± 12.275
Prolactin	33.42 ± 4.360	32.31 ± 2.884	34.19 ± 3.183	58.85 ± 7.375	61.36 ± 6.651	58.82 ± 7.884
Estradiol	46.23 ± 8.213	47.01 ± 9.464	51.5 ± 6.863	59.57 ± 7.857	57.96 ± 10.341	60.85 ± 13.386

(*continued on next page*)

Table 6C (*continued*)

Testosterone	62.63 ± 10.42	59.91 ± 11.431	57.46 ± 9.578	34.67 ± 4.708	34.71 ± 4.398	33.94 ± 4.190
FSH	6.08 ± 1.115	6.73 ± 1.400	6.6 ± 1.190	5.14 ± 0.900	5.27 ± 0.636	5.15 ± 0.581
BMI	29.31 ± 3.807	28.35 ± 3.726	27.01 ± 3.414	25.66 ± 3.382	25.47 ± 3.140	26.11 ± 3.187
Age	30.17 ± 2.758	29.96 ± 2.654	30.08 ± 2.665	30.68 ± 2.297	30.67 ± 2.542	30.92 ± 2.979

P-value <0.05 was considered statistically significant.

discovered by Shi et al. and has since been independently verified in a different group of Han-Chinese PCOS patients [30]. Consistent with our results, Bakhashab's investigation in the Saudi population found no evidence of a significant correlation between PCOS or its manifestations and YAP1 rs1894116; this finding may have been due to the small sample size [31]. YAP1, one of the transcriptional targets of the highly conserved Hippo pathway, is encoded by the *YAP1* gene. It makes sense that the Hippo signaling system would regulate increased ovarian volume, given that this is a prominent clinical characteristic of PCOS. To support this theory, however, additional translational research is necessary [30,32].

Moreover, the current study's findings show that there is no statistically significant difference (P = 0.326) in the frequency difference between the rs2272046 genotypes in the PCOS patient and control groups. The observed frequency difference between the A and C alleles in both the control and PCOS groups is also not significant (P = 0.575).

Because the polymorphism rs2272046 is located in an HMGA2 intron, there is conjecture that it may affect the risk of PCOS by modifying the translation or degradation of HMGA2 mRNA. This SNP, rs2272046, and rs74980477 ($D' = 1$, $r2 = 1$) show perfect linkage disequilibrium, with the latter being anticipated to modify the function of the *myc* gene. The regular expression of the myc oncoprotein plays a pivotal role in initial oocyte growth and the autonomous growth of granulosa cells, suggesting that dysregulation may influence follicular development [33,34]. Our results are consistent with the research by Bakhashab on PCOS, which found no significant correlation between the HMGA2 rs2272046 variation and PCOS [31]. Nonetheless, HMGA2 rs2272046 is, in fact, associated with the risk of PCOS, according to Jiao's investigation on PCOS patients in a Chinese Han community [6].

Our study's results on miR expression showed a considerable upregulation, with increases of 13.5, 4.13, and 10.7fold for miR-155, miR-383, and miR-9, respectively. In as study, Xia et al. showed the increase of miR-155 in PCOS patient tissues and suggested its role in PCOS pathogenesis, is in line with our findings. These findings highlight the critical regulatory role of miR-155 in the pathophysiology of PCOS. Disease incidence is closely associated with the control of signaling pathways (JNK and PI3K/AKT pathway). Xia et al. elucidated that miR-155 mimic stimulation augmented the activation of PI3K/AKT and JNK pathways, thereby promoting PCOS growth [35]. Ysrafil et al. showed the downregulation of miR-155–5p, approximately by 892.15-fold in ovarian cancer cell line [33,36], which is consistent with our current study.

According to Yin et al. study, miR-383 was primarily expressed in mouse ovarian follicle granulosa cells (GC) and oocytes. Targeting the RNA binding motif, single-stranded interacting protein 1 (RBMS1), overexpression of miR-383 significantly increased estradiol release from GC. Notably, both primary and mature miR-383 in GC were considerably reduced in expression upon suppression of the transcription factor steroidogenic factor-1 (SF-1). This finding implies that miR-383 is transcriptionally regulated by SF-1. Moreover, SF-1 has been linked to the control of estradiol release from granulosa cells mediated by miR-383 and RBMS1/c-Myc. All of these results point to the possibility that miR-383 targets RBMS1 in order to partially inactivate c-Myc and thereby promote steroidogenesis [37].

A comprehensive study comprising the extraction of ovarian granulosa cells and whole blood from PCOS patients was carried out by Kong et al. In line with earlier studies showing increased miR-9 levels in PCOS women's follicular fluid, their results showed a substantial elevation of miR-9 expression in PCOS women compared to controls [19]. Folic developmental anomalies have been linked to aberrations in GC proliferation and apoptosis; women with anovulatory PCOS showed significantly greater rates of proliferation and significantly lower rates of apoptosis in their GCs compared to both regular and ovulatory PCOS [38]. Kong's study also examined the impact of miR-9 mimic and inhibitor on GC proliferation and apoptosis in vitro, revealing that its target, VDR, influenced these effects. Notably, it was discovered that GCs' expression of miR-9 was upregulated by high insulin concentrations [39].

Our study provided several key findings, but it also had limitations. One of the main limitations of this study was the relatively small sample size (100 patients with PCOS and 100 controls), which may not be fully representative of the broader population. Using larger sample sizes in future research could help to increase the validity and precision of the results. Furthermore, all participants in this study were selected from a specific geographic population (Iranian patients), which may have had unique genetic and environmental influences on the results, thus limiting the generalizability of the results to other populations.

6. Limitations

While this study provides valuable insights into the roles of microRNAs and genetic polymorphisms in polycystic ovary syndrome (PCOS), it is important to acknowledge several limitations that may influence the interpretation of our findings.

6.1. Small sample size

The study included a relatively small sample size (100 PCOS patients and 100 controls), which may limit the generalizability of the results. Larger sample sizes in future studies could enhance the statistical power and provide more robust evidence regarding the associations between the investigated SNPs and miRNAs with PCOS.

6.2. Ethnic-specific population

All participants were selected from a specific geographic population (Iranian patients), which may introduce ethnic-specific biases. Genetic variations can differ significantly across populations, and thus, the findings may not be fully applicable to other ethnic groups. Multi-ethnic studies are essential to validate these results and ensure their broader applicability.

6.3. Lack of functional studies

This study focused on correlational analyses and did not include functional experiments to investigate the direct effects of the identified microRNAs and SNPs on PCOS-related pathways. Future research should incorporate functional assays, such as cell culture experiments or animal models, to elucidate the precise molecular mechanisms underlying their roles in PCOS pathophysiology.

6.4. Use of blood samples instead of ovarian tissue

Blood samples were used for RNA extraction, which may not fully represent tissue-specific changes occurring in ovarian tissues, such as granulosa or follicular cells. These tissues are directly involved in the pathogenesis of PCOS, and their analysis could provide more accurate insights into the disease mechanisms. Future studies utilizing ovarian tissue samples would help bridge this gap.

6.5. Potential confounding factors

Although efforts were made to control for confounding variables such as age and BMI, other environmental or lifestyle factors (e.g., diet, physical activity) that may influence gene expression and SNP associations were not comprehensively assessed. Incorporating these variables in future studies could lead to a more nuanced understanding of PCOS etiology.

In summary, while our findings contribute to the growing body of knowledge on the genetic and epigenetic factors associated with PCOS, addressing these limitations in future research will be crucial to advancing our understanding of the disease and identifying potential biomarkers or therapeutic targets.

7. Conclusion

In conclusion, our study highlights the elevated expression of *miR-155, miR-383*, and *miR-9* in PCOS patients, suggesting their potential as non-invasive biomarkers for early diagnosis or as therapeutic targets to modulate insulin resistance, inflammation, and steroidogenesis in PCOS. These findings are consistent with recent studies demonstrating the critical role of miRNAs in PCOS pathophysiology, particularly in regulating pathways such as PI3K/AKT, JNK, and steroidogenic signaling. However, further research is warranted to validate these findings and explore the exact molecular mechanisms underlying their roles in PCOS development. Larger, multi-ethnic studies incorporating functional analyses, including in vitro and in vivo models, are recommended to address current limitations and advance our understanding of this complex disorder. Elucidating the precise roles of these miRNAs in PCOS pathophysiology could pave the way for novel diagnostic and therapeutic strategies, underscoring the importance of further research in this area.

Consent for publication

All authors have agreed to publish this manuscript.

Availability of data and material

Data will be available by corresponding author with request.

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Declaration of competing interest

The authors declare that they have no conflict of interest with respect to the author or publication of this article.

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Data availability

Data will be made available on request.

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