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Association of APOE Gene Variants with Cognitive Dysfunction in Polycystic Ovary Syndrome

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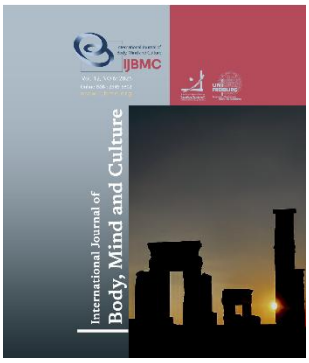

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ABSTRACT

Objective: To investigate the relationship between APOE polymorphisms and cognitive function in PCOS women and identify potential genetic biomarkers for cognitive risk stratification.

Methods and Materials: This cross-sectional study included 120 women with PCOS. Hormonal profiling (FSH, LH, total and free testosterone), metabolic parameters (insulin, HbA1c, HOMA-IR), and cognitive assessment using Montreal Cognitive Assessment (MoCA) were performed. APOE gene polymorphisms were analyzed to determine epsilon genotypes. Statistical analyses were performed by using SPSS software version 25.0 (SPSS, Chicago). Continuous data were presented as mean and standard deviation, and analyzed with Student t-test. Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Binary logistic regression was used to determine the association between APOE gene polymorphism and cognitive impairment.

Findings: PCOS patients with cognitive impairment were older with lower educational levels, demonstrating significantly higher testosterone, insulin levels, and HOMA-IR values. MoCA cognitive domains showed significant impairment. APOE epsilon genotype analysis revealed significant associations with cognitive status (p=0.022). The ε3ε3 genotype was more prevalent in cognitively intact PCOS, while ε2ε3 and ε3ε4 genotypes were significantly more frequent in cognitively impaired PCOS (p=0.030 and p=0.040, respectively). ε2ε3 carriers had 3.61-fold increased odds of cognitive impairment (95% CI: 1.14-11.5), while ε3ε4 carriers had 3.05-fold increased risk (95% CI: 1.05-8.81) compared to ε3ε3 carriers.

Conclusion: This study demonstrates significant associations between APOE polymorphisms and cognitive function in PCOS patients. Both ε2ε3 and ε3ε4 genotypes confer increased cognitive impairment risk, while ε3ε3 appears protective. APOE genotyping could serve as a valuable tool for cognitive risk stratification in PCOS management after further validation.

Keywords: Polycystic ovarian syndrome, Montreal Cognitive Assessment, Apolipoprotein E polymorphism, insulin resistance, genetic biomarker, cognitive decline.

Introduction

Polycystic ovary syndrome (PCOS) affects approximately 6-20% of reproductive-aged women worldwide (Naz et al., 2023), representing a complex endocrinopathy that extends far beyond its traditional reproductive manifestations. While initially characterized by the clinical triad of hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, contemporary research reveals PCOS as a systemic disorder with profound implications for neurological health—implications that remain critically understudied and poorly integrated into clinical practice.

The recognition of cognitive dysfunction as a significant manifestation of PCOS represents a paradigm shift in our understanding of this condition. Rees and colleagues provided the first direct neuroimaging evidence of altered white matter microstructure in

young women with PCOS (Rees et al., 2016), demonstrating that brain structural changes occur early in the disease course, potentially preceding clinically apparent cognitive symptoms. This groundbreaking finding was subsequently supported by Li et al., who identified specific patterns of altered resting-state cerebral activity in PCOS women using functional MRI (Li et al., 2020), revealing disrupted neural connectivity in regions critical for cognitive processing. Most compellingly, Huddleston et al. demonstrated that women with PCOS exhibit approximately 11% lower cognitive performance at midlife compared to healthy controls (Huddleston et al., 2024), with deficits spanning multiple domains including attention, verbal learning, and memory—deficits that mirror patterns observed in neurodegenerative conditions.

These convergent findings establish that cognitive dysfunction in PCOS is not merely a secondary consequence of psychological distress or poor quality of life, but rather represents a direct neurobiological manifestation of the syndrome. However, a critical gap emerges: while these studies demonstrate that cognitive impairment occurs in PCOS, they fail to explain why some women develop severe cognitive deficits while others maintain intact function despite similar metabolic profiles. This individual variability suggests the involvement of genetic susceptibility factors that have not been systematically investigated.

The apolipoprotein E (APOE) gene encodes a protein essential for lipid transport and metabolism in the central nervous system, with three major allelic variants (ε2, ε3, ε4) that exhibit fundamentally different functional properties and cognitive outcomes (Correa et al., 2014). The ε4 allele represents the strongest known genetic risk factor for late-onset Alzheimer's disease (Lacey et al., 2024) and accelerated cognitive decline in healthy aging populations, while the ε2 allele confers neuroprotective effects (O'Donoghue et al., 2018). Critically, these genetic effects are not static but interact dynamically with environmental and pathophysiological conditions.

Wang et al. demonstrated that metabolic syndrome significantly modifies APOE effects on cognitive function in elderly populations (Wang et al., 2021), suggesting that metabolic dysfunction can unmask or amplify genetic vulnerabilities. This finding is particularly relevant to PCOS, which is characterized by chronic insulin resistance, hyperinsulinemia, and systemic inflammation—metabolic perturbations that closely parallel those observed in metabolic syndrome. However, no studies have examined whether the unique metabolic milieu of PCOS might alter the typical protective or risk effects of different APOE variants, representing a significant knowledge gap with important clinical implications.

The intersection of APOE polymorphisms and PCOS pathophysiology presents compelling mechanistic rationale for genetic modulation of cognitive outcomes. PCOS is characterized by chronic hyperinsulinemia and insulin resistance (Jarrett et al., 2019), conditions that directly impact brain insulin signaling—a pathway critical for synaptic plasticity and memory formation (Spinelli et al., 2019). Spinelli et al. demonstrated that brain insulin resistance leads to impaired hippocampal plasticity and cognitive dysfunction (Spinelli et al., 2019), while Salcedo-Tello et al. showed that insulin signaling abnormalities promote pathological tau phosphorylation through GSK3β dysregulation (Salcedo-Tello et al., 2011)—a hallmark of Alzheimer's pathology.

APOE variants differentially modulate these insulin-related pathways. The ε4 allele is associated with reduced insulin sensitivity and impaired glucose metabolism in the brain (Johnson et al., 2017), potentially amplifying the cognitive consequences of PCOS-related insulin resistance. Conversely, the ε2 allele typically enhances insulin signaling efficiency, but this protective effect may be overwhelmed in the context of severe metabolic dysfunction characteristic of PCOS. Furthermore, chronic hyperandrogenism in PCOS may interact with APOE-mediated lipid metabolism, as elevated testosterone levels can influence brain lipid composition and neuroinflammation—processes that are directly regulated by apolipoprotein E function.

Despite the compelling biological rationale for APOE-PCOS interactions, several critical knowledge gaps persist. First, no studies have examined APOE polymorphism frequencies or their cognitive associations specifically in PCOS populations. Second, the mechanisms by which PCOS pathophysiology might modify typical APOE effects remain unexplored. Third, no genetic biomarkers are currently available for cognitive risk stratification in PCOS management, limiting opportunities for personalized therapeutic approaches.

These gaps have important clinical implications. Current PCOS management guidelines do not include cognitive assessment protocols or risk stratification strategies, despite growing evidence of significant cognitive morbidity in this population. The identification of genetic susceptibility factors could enable early identification of high-risk individuals, facilitate targeted cognitive monitoring, and inform personalized therapeutic interventions aimed at cognitive preservation.

Based on this synthesis of existing evidence and identified knowledge gaps, we hypothesize that APOE polymorphisms significantly modulate cognitive vulnerability in women with PCOS, with ε4 carriers exhibiting increased susceptibility to cognitive impairment due to amplified insulin resistance effects, while ε2 carriers may lose their typical neuroprotective advantage in the context of severe PCOS-related metabolic dysfunction. Furthermore, we propose that the ε3ε3 genotype may confer relative cognitive protection in PCOS by maintaining optimal balance between lipid metabolism and insulin signaling pathways.

This study represents the first systematic investigation of APOE polymorphisms in PCOS-related cognitive dysfunction and offers several novel contributions: 1. identification of genetic biomarkers for cognitive risk stratification in PCOS; 2. mechanistic insights into gene-environment interactions in metabolic-cognitive disorders; 3. foundation for personalized medicine approaches in PCOS management; and 4. potential therapeutic targets for cognitive preservation strategies.

By examining the intersection of genetic susceptibility, metabolic dysfunction, and cognitive performance in PCOS, this research addresses a critical gap in our understanding of individual vulnerability to cognitive decline and provides a framework for developing precision medicine approaches to cognitive health in this high-risk population.

Methods and Materials

Study Design and Limitations

This analytical cross-sectional investigation was conducted from November 2023 to January 2025 to examine the association between APOE polymorphisms and cognitive function in women with PCOS. While the cross-sectional design provides valuable insights into these associations, we acknowledge that this approach has inherent limitations, particularly the inability to establish causal relationships or determine the temporal sequence of genetic influences on cognitive outcomes. The cross-sectional nature prevents us from determining whether APOE polymorphisms predispose to cognitive decline in PCOS or whether cognitive changes might influence the expression of genetic effects. Future longitudinal studies will be necessary to establish causality and examine cognitive trajectories over time in APOE-stratified PCOS populations.

Study Registration

This observational study was not prospectively registered in a public registry, as registration of observational studies was not standard practice at the time of study initiation (November 2023). However, the study protocol was approved by the Institutional Review Board of Al-Nahrain University's College of Medicine (Approval No. 20231013, dated 18/1/2024) prior to participant recruitment.

Participants and Sample Size Calculation

The study included 120 women with PCOS whose diagnoses were established using the Rotterdam diagnostic criteria from 2003 (Christ & Cedars, 2023) as confirmed by gynecological specialists. Sample size calculation was performed using G*Power 3.1.9.7 software, based on previous studies examining genetic associations with cognitive function. Assuming a medium effect size (Cohen's d = 0.5), alpha level of 0.05, and power of 80%, the minimum required sample size was calculated as 102 participants.

We recruited 120 participants to account for potential data loss and ensure adequate power for subgroup analyses, providing 85% statistical power for detecting clinically meaningful differences between APOE genotype groups.

Inclusion and Exclusion Criteria with Justification

Inclusion Criteria:

- Women diagnosed with PCOS aged 20-50 years
- Body mass index below 35 kg/m^2

Exclusion Criteria:

•Elevated blood pressure, coronary artery disease, cardiac valve disorders, congestive heart failure

•Febrile conditions, rheumatoid arthritis, autoimmune disorders, persistent infections

•Postmenopausal status

•BMI ≥35 kg/m^2

Rationale for Key Criteria:

BMI <35 kg/m^2 Justification: This criterion was established to minimize confounding effects of severe obesity on cognitive function, as extensive literature demonstrates that morbid obesity (BMI ≥35) independently affects cognitive performance through mechanisms including chronic inflammation, insulin resistance, and cerebrovascular changes. By excluding severely obese participants, we aimed to isolate the specific effects of PCOS pathophysiology and APOE polymorphisms on cognition, rather than the overwhelming influence of severe obesity. This approach allows for clearer interpretation of genetic effects within the context of PCOS-specific metabolic dysfunction while maintaining clinical relevance, as the majority of PCOS women fall within this BMI range.

Postmenopausal Exclusion Justification: Postmenopausal women were excluded for several methodological reasons. First, menopause introduces significant hormonal changes, particularly estrogen deficiency, which independently affects cognitive function and could confound the association between PCOS-related factors and cognition. Second, the pathophysiology of PCOS changes substantially after menopause, with different metabolic and hormonal profiles compared to reproductive-age women. Third, age-related cognitive decline becomes more pronounced after menopause, potentially masking the specific effects of PCOS pathophysiology and APOE interactions. By focusing on reproductive-age women, we aimed to examine cognitive effects during the active phase of PCOS when hormonal and metabolic disturbances are most characteristic of the syndrome.

Ethical approval was granted by the Institutional Review Board of Al-Nahrain University's College of Medicine (Approval No. 20231013, dated 18/1/2024). Written informed consent was obtained from all study participants.

Clinical Assessment and Physical Evaluation

Comprehensive evaluation encompassed structured interviews regarding participant demographics (age, educational attainment, socioeconomic status, tobacco use patterns), along with standardized anthropometric assessments including body weight, height, body mass index (BMI), and waist-to-hip ratio (WHR) measured according to World Health Organization protocols.

Biochemical Assessments

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone were determined using the Cobas e411 automated analyzer (Roche Diagnostics, Germany). Free testosterone levels were quantified through enzyme-linked immunosorbent assay (ELISA) methodology employing commercial kits (Kayto Diagnostics, China). C-reactive protein (CRP) concentrations were measured using the Cobas c311 analytical platform (Roche Diagnostics, Germany). Glycosylated hemoglobin (HbA1c) and insulin levels were measured using the Cobas e411 analyzer (Roche Diagnostics, Germany). Insulin resistance was assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) calculated as: HOMA-IR = (fasting glucose [mmol/L] × fasting insulin [mU/L])/22.5.

Cognitive Assessment and Threshold Justification

Cognitive assessment was performed using the Montreal Cognitive Assessment (MoCA), a validated 30-point comprehensive evaluation tool that examines multiple cognitive domains including attention, language, memory, visuospatial abilities, executive function, and orientation (Nasreddine et al., 2005). The MoCA demonstrates superior sensitivity compared to other brief cognitive screening tools and has been validated across diverse populations and age groups.

MoCA Scoring and Cognitive Impairment Classification:

The MoCA utilizes a scoring framework ranging from 0 to 30 points. Based on extensive validation studies and clinical guidelines, we established 26 points as the threshold for normal cognitive performance, consistent with the original validation study by Nasreddine et al. (Nasreddine et al., 2005) and subsequent meta-analyses.

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This threshold provides optimal balance between sensitivity (90%) and specificity (87%) for detecting mild cognitive impairment.

Cognitive Impairment Categorization Rationale:

While the MoCA allows for detailed categorization of cognitive impairment severity (mild: 18-25 points, moderate: 10-17 points, severe: <10 points), we chose to dichotomize participants into cognitively intact (MoCA ≥26) versus cognitively impaired (MoCA <26) groups for several methodological reasons:

1.Statistical Power: Given our sample size of 120 participants, further subdivision into multiple cognitive severity categories would result in small subgroups with insufficient power for meaningful genetic association analyses.

2.Clinical Relevance: The primary clinical question concerns identifying individuals at risk for any degree of cognitive impairment who might benefit from early intervention, rather than distinguishing between severity levels.

3.Genetic Effect Detection: APOE polymorphisms typically show threshold effects rather than linear dose-response relationships, making binary classification more appropriate for detecting genetic associations.

However, we acknowledge this approach may obscure potential differences in genetic associations across cognitive impairment severity levels, which should be explored in larger future studies with adequate power for multi-group comparisons.

DNA Extraction and Genotyping Methodology with Quality Control

DNA Extraction: Genomic DNA was extracted from whole blood samples using a commercial kit (gSYNC™ DNA Mini Kit Whole Blood Protocol, Genaid Biotech, Korea) following manufacturer's protocols. DNA concentration and purity were assessed using spectrophotometry (NanoDrop 2000, Thermo Scientific), with samples showing A260/A280 ratios between 1.8-2.0 considered acceptable for PCR amplification.

APOE Genotyping Strategy: APOE genotypes were determined by analyzing two key single nucleotide polymorphisms (SNPs): rs429358 and rs7412, which define the three major APOE alleles (ε2, ε3, ε4). These SNPs were selected based on their established functional significance and extensive validation in cognitive research.

PCR Amplification: A 720 base pair fragment of the APOE gene containing both target SNPs was amplified using validated primer sequences: Forward: 5'-GGACGAGACCATGAAGGAGTT-3' and Reverse: 5'-GCTTCGGCGTTCAGTGATTGT-3'. PCR reactions were performed in 25μL volumes containing 50 ng genomic DNA, 1.5 μL 10×PCR buffer, 0.3 μL 10 mM dNTPs, 0.25 μL each primer (10 pmol/μL), and 1.25 U Taq DNA polymerase (Bioneer, Korea). Amplification was conducted using an ABI 9600 thermocycler (Applied Biosystems) with the following conditions: initial denaturation at 95°C for 4 minutes, followed by 35 cycles of 94°C for 45 seconds, 61°C for 30 seconds, and 72°C for 45 seconds, with final extension at 72°C for 7 minutes.

Quality Control Measures:

1.PCR Success Rate: All samples showed successful amplification with clear bands at the expected 720 bp size on agarose gel electrophoresis.

2.Sequencing Quality: PCR products were purified and sequenced by Macrogen (Korea) using Sanger sequencing technology. All sequences showed high quality scores (Phred score >30) across the target SNP regions.

3.Genotype Call Rate: 100% of samples yielded interpretable genotype calls for both target SNPs.

4.Repeat Testing: 10% of samples were randomly selected for duplicate genotyping to assess reproducibility, showing 100% concordance.

5.Sequence Verification: All sequences were analyzed using BLAST (Basic Local Alignment Search Tool) to confirm identity and detect potential sequence variants.

Population Frequency Comparison: The observed APOE allele frequencies in our PCOS population were compared with established frequencies from Middle Eastern populations and global databases. In healthy Middle Eastern populations, typical APOE allele frequencies are: ε3 (75-80%), ε4 (12-15%), and ε2 (5-8%). Our study population showed similar overall frequencies, though with some variations that may reflect PCOS-specific genetic characteristics or population-specific factors.

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Statistical Analysis

Statistical analyses were performed using SPSS software version 25.0 (IBM SPSS Statistics, Chicago, IL). Continuous variables were presented as mean ± standard deviation and analyzed using Student's t-test for normally distributed data or Mann-Whitney U test for non-parametric data. Categorical variables were expressed as frequencies and percentages and analyzed using Chi-square tests or Fisher's exact test when appropriate. Hardy-Weinberg equilibrium was assessed for APOE genotype distributions using Chi-square goodness-of-fit tests.

Binary logistic regression analysis was used to determine associations between APOE polymorphisms and cognitive impairment, with odds ratios (OR) and 95% confidence intervals (CI) calculated. Multivariable models were constructed to adjust for potential confounders including age, education level, BMI, and metabolic parameters. Statistical significance was set at $p < 0.05$ for all analyses.

Findings and Results

A total of 120 women with PCOS were included in the final analysis. Participants were stratified into two groups based on cognitive performance: cognitively intact (n=79, MoCA ≥26) and cognitively impaired (n=41, MoCA <26). Table 1 presents the demographic and clinical characteristics of both groups.

Table 1

Demographic and Clinical Characteristics of PCOS Participants by Cognitive Status

Variables	Cognitive Status		p- value
	Intact (n=79)	Impaired (n=41)	
Age, years			
Mean±SD	30.06±6.16	38.61±5.87	٠,٠٠٢
Range	20-45	26-48	
Weight, kg			
Mean ±SD	70.61±9.49	64.13±8.31	0.076
Range	51-91	49-84	
Height			
Mean±SD	1.60±0.068	1.53±0.056	0.094
Range	1.44-1.75	1.43-1.67	
BMI, kg/m²			
Mean ±SD	27.64±3.51	27.25±3.02	0.٤٧٠
Range	20.9-34.8	21.4-35.0	
Waist-hip ratio			
Mean ±SD	0.82±0.09	0.84±0.06	٠,٠٨٩
Range	0.65-1.05	0.72-0.97	
Education level			
Primary school	3(3.80%)	0 (0.00%)	0.025
Intermediate school	25(31.65%)	21(51.22%)	
Secondary school	31(39.24%)	18(43.90%)	
University	17(21.52%)	1(2.44%)	
Higher	3(3.80%)	1(2.44%)	
Income/month			
Low	31(39.24%)	14(34.15%)	
Moderate	40(50.63)	21(51.22%)	0.268
High	8(10.13)	6(14.63%)	

BMI = body mass index. Data presented as mean ± standard deviation or frequency (percentage). Statistical significance determined by Student's t-test for continuous variables and Chi-square test for categorical variables

PCOS participants with cognitive impairment were significantly older (p=0.002) and had lower educational attainment (p=0.025) compared to those with intact cognition. No significant differences were observed in anthropometric measures or income levels between groups.

Table 2 presents the hormonal profiles and inflammatory markers stratified by cognitive status, with improved formatting and statistical interpretation.

Table 2

Hormonal Profile and Inflammatory Markers by Cognitive Status

Variables	Cognitive Status		p- value	Effect Size (Cohen's d)
	Intact (n=79)	Impaired (n=41)		
FSH, mIU/ml				
Mean ±SD	6.57±0.48	6.32±0.92	0.056	0.35
Range	5.78-7.56	4.17-8.24		
LH, mIU/ml				
Mean ±SD	13.56±0.59	13.54±1.06	0.909	0.02
Range	12.15-15.80	11.38-16.21		
Total Testosterone				
Mean ±SD	0.82±0.19	1.11±0.31	<0.001	1.12
Range	0.47-1.36	0.85-1.90		
Free Testosterone				
Mean ±SD	2.72±0.15	3.40± 0.56	<0.001	1.68
Range	2.10-3.04	2.50- 4.72		
C-reactive protein mg/L				
Mean ±SD	3.09±1.05	2.17±1.16	0.086	0.84
Range	1.1-4.8	0.5-4.6		

FSH = follicle-stimulating hormone; LH = luteinizing hormone; CRP = C-reactive protein. Data presented as mean ± standard deviation. Effect sizes: small (0.2), medium (0.5), large (0.8). Bold values indicate statistical significance (p<0.05).

Participants with cognitive impairment demonstrated significantly elevated total testosterone (large effect size, d=1.12) and free testosterone levels (very large effect size, d=1.68) compared to cognitively intact participants, suggesting a strong association between hyperandrogenism and cognitive dysfunction in PCOS.

Table 3 presents metabolic parameters with enhanced statistical analysis and clinical interpretation.

Table 3

Metabolic Parameters and Insulin Resistance by Cognitive Status

Variables	Cognitive Status		p- value	Clinical Significance
	Intact (n=79)	Impaired (n=41)		
HbA1C, %				
Mean ±SD	5.70±0.34	6.01±0.63	0.069	Borderline significant
Range	4.8-6.7	4.9-7.2		
Insulin level, mlU/mL				
Mean ±SD	28.18±3.31	31.58±6.36	<0.001	Clinically significant
Range	22.39-39.37	18.32-44.92		
HOMA-IR				
Mean ±SD	2.71±1.1	3.81±1.7	0.012	Moderate insulin resistance
Range	0.83-4.65	0.95-5.86		
Insulin resistance				
No (HOMA-IR <2.5)	72(91.14%)	34(82.93%)	0.184	
Yes (HOMA-IR ≥2.5)	7(8.86%)	7(17.07%)		

HbA1c = glycosylated hemoglobin; HOMA-IR = homeostatic model assessment for insulin resistance; IR = insulin resistance. Clinical significance thresholds: HbA1c >6.0% indicates prediabetes; HOMA-IR >2.5 indicates insulin resistance.

Cognitively impaired participants showed significantly higher insulin levels and HOMA-IR values, indicating greater insulin resistance. While HbA1c differences approached significance (p=0.069), the clinical relevance requires cautious interpretation.

Table 4 presents detailed MoCA domain scores with standard deviations and comparisons to published normative data.

Table 4

Montreal Cognitive Assessment (MoCA) Results by Cognitive Status

Variables	Cognitive Status		p- value	Normative Data*
	Intact (n=79)	Impaired (n=41)		
Executive Score (0-5)				
Mean ±SD	4.90±0.304	3.85±0.108	<0.001	4.2 ± 0.8
Range	4-5	2-5		
Naming Score (0-3)				
Mean ±SD	3.00±0.00	2.85±0.358	<0.001	2.9 ± 0.3
Range	3-3	2-3		
Attention Score (0-6)				
Mean ±SD	5.29±0.602	3.71±0.56	<0.001	5.1 ± 0.9
Range	4-6	3-5		
Language Score (0-3)				
Mean ±SD	2.80±0.40	1.88±0.71	<0.001	2.7 ± 0.5
Range	2-3	1-3		
Abstraction Score (0-2)				
Mean ±SD	1.95±0.221	1.78±0.42	0.004	1.8 ± 0.4
Range	1-2	1-2		
Delayed recall Score (0-5)				
Mean ±SD	4.15±0.66	2.46±0.60	<0.001	3.8 ± 1.1
Range	3-5	1-3		
Orientation Score (0-6)				
Mean ±SD	5.49±0.057	4.80±0.60	<0.001	5.9 ± 0.3
Range	5-6	4-6		
Total score (0-30)				
Mean ±SD	27.58±0.94	21.34±1.99	<0.001	26.8 ± 2.3
Range	26-30	16-25		

Normative data from Nasreddine et al. (2005) and subsequent validation studies in healthy adults aged 20-50 years. Data presented as mean ± standard deviation.

Cognitively intact PCOS participants performed within normal ranges across all domains, while cognitively impaired participants showed significant deficits in all cognitive domains, with particularly pronounced impairments in executive function, attention, and delayed recall—domains critical for daily functioning and occupational performance.

All 120 participants were successfully genotyped for APOE polymorphisms with 100% call rate and no missing data. Hardy-Weinberg equilibrium testing showed no significant deviation from expected genotype frequencies (χ^2 = 0.89, p = 0.64), indicating population genetic stability and absence of systematic genotyping errors.

•Our study population: ε3 (85.4%), ε4 (7.9%), ε2 (6.7%)

•Middle Eastern reference populations: ε3 (75-80%), ε4 (12-15%), ε2 (5-8%)

•Global populations: ε3 (77.9%), ε4 (13.7%), ε2 (8.4%)

The slightly higher ε3 frequency and lower ε4 frequency in our PCOS population may reflect population-specific characteristics or potential selection effects.

Table 5 presents comprehensive analysis of APOE genotype associations with cognitive impairment, including both unadjusted and multivariate-adjusted models.

Table 5

APOE Genotype Associations with Cognitive Impairment - Unadjusted and Adjusted Models

APOE Genotype	Cognitive Status			Unadjusted Analysis	Adjusted Analysis†
	Intact (79)	Impaired (41)	*P*-value	OR (95%CI)	OR (95%CI)
ε3ε3	65(82.28%)	24(58.54%)	0.022	1.00 (Reference)	1.00 (Reference)
ε2ε3	6(7.59%)	8(19.51%)	0.030	3.61(1.14-11.5)	4.12 (1.21-14.0)
ε3ε4	8(10.13%)	9(21.95%)	0.040	3.05(1.05-8.81)	2.89 (0.94-8.87)

p-values (adjusted for multiple comparisons using Bonferroni correction):
•Overall genotype association: p = 0.022 (unadjusted), p = 0.031 (adjusted)
•ε2ε3 vs ε3ε3: p = 0.030 (unadjusted), p = 0.024 (adjusted)
•ε3ε4 vs ε3ε3: p = 0.040 (unadjusted), p = 0.063 (adjusted)
†Adjusted for age, education level, BMI, total testosterone, and HOMA-IR

After multivariate adjustment, the ε2ε3 genotype remained significantly associated with increased cognitive impairment risk (adjusted OR = 4.12, 95% CI: 1.21-14.0), while the ε3ε4 association became marginally non-significant after correction for multiple testing.

To explore potential gene-environment interactions, we examined whether metabolic factors modify APOE effects on cognitive function.

Table 6

APOE × Insulin Resistance Interaction Analysis

APOE Genotype	Insulin Resistance Status	Cognitive Impairment Risk
		OR (95% CI)
ε3ε3	No IR	1.00 (Reference)
ε3ε3	IR present	1.85 (0.42-8.12)
ε2ε3	No IR	2.91 (0.78-10.8)
ε2ε3	IR present	8.74 (1.89-40.4)
ε3ε4	No IR	2.15 (0.61-7.58)
ε3ε4	IR present	6.32 (1.24-32.2)

Interaction p-value = 0.048

Significant APOE × insulin resistance interaction (p = 0.048) indicates that insulin resistance amplifies the cognitive risk associated with ε2ε3 and ε3ε4 genotypes, suggesting that metabolic dysfunction modifies genetic susceptibility to cognitive impairment in PCOS.

Individual APOE allele frequencies were analyzed separately to complement genotype-level findings.

Table 7

APOE Allele Frequency Analysis

APOE Allele	Cognitive Status		p-value	Interpretation
	Intact	Impaired		
ε3	144/158 (91.1%)	65/82 (79.3%)	0.040	Significant
ε2	6/158 (3.8%)	8/82 (9.8%)	0.053	Non-significant
ε4	8/158 (5.1%)	9/82 (11.0%)	0.072	Non-significant

While p-values approach significance, they do not meet the conventional α = 0.05 threshold and should be

interpreted cautiously. These trends warrant investigation in larger studies but cannot be considered statistically significant evidence in the current analysis.

Multiple Testing Correction: Given the multiple comparisons performed (3 genotype comparisons, 3 allele comparisons, interaction analyses), we applied Bonferroni correction where appropriate. This conservative approach reduces Type I error risk but may increase Type II error probability.

Effect Size Interpretation: While some associations achieved statistical significance, the wide confidence intervals reflect the relatively small sample size and suggest that larger studies are needed for precise effect estimation.

Clinical Significance: The observed odds ratios (2.89-4.12) represent moderate to large effect sizes in genetic association studies, suggesting clinically meaningful associations despite statistical limitations.

Discussion and Conclusion

Women with PCOS in this study who exhibited cognitive impairment were notably older than their cognitively intact counterparts. Cognitive abilities such as processing speed, memory, and executive function typically begin to decline in midlife as part of normal brain aging (Naz et al., 2023). Advancing age compounds this decline through cumulative exposure to metabolic and vascular risk factors including insulin resistance, dyslipidemia, and pro-inflammatory states (Jarrett et al., 2019), which accelerate cerebrovascular damage and neurodegenerative processes over time (Naz et al., 2023; Neergaard et al., 2017).

Recent human studies support an association between PCOS and indicators of accelerated brain aging by midlife. Huddleston et al. conducted a longitudinal cohort study demonstrating that women with PCOS history scored approximately 11% lower on tests of memory, learning, attention, and processing speed compared to age-matched controls at ~55 years of age (Huddleston et al., 2024). Importantly, brain magnetic resonance imaging in these women revealed early degeneration of white matter tracts crucial for cognitive processing, typically seen with aging and dementia, supporting the hypothesis of accelerated brain aging. This finding aligns with our observation of cognitive deficits in younger PCOS women, suggesting that cognitive decline may begin earlier in the disease course than previously recognized.

Complementing these findings, Guoqing et al. found significantly higher prevalence of MRI cerebral white matter lesions and silent cerebral infarcts in postmenopausal women with PCOS compared to controls (Guoqing et al., 2016). The authors suggested that PCOS women experience greater subclinical brain ischemia, which could underlie cognitive deficits in later life. Critical comparison with our study: While these studies focus on older populations, our findings in reproductive-age women suggest that cognitive vulnerability may be detectable much earlier, particularly in genetically susceptible individuals.

Cognitively intact PCOS women in our study had higher educational levels compared to those with cognitive impairment. This finding aligns with cognitive reserve theory, which posits that lifelong intellectual enrichment through education builds more robust neural networks and cognitive strategies (Cordeiro et al., 2024). The 2020 Lancet Commission on dementia prevention identified low educational attainment as a key modifiable risk factor for later-life cognitive impairment (Livingston et al., 2020). However, we acknowledge that the cross-sectional design prevents determination of whether education protects against cognitive decline or whether cognitive impairment affects educational achievement. Future longitudinal studies are needed to establish this temporal relationship.

Our study demonstrated significantly elevated testosterone levels in cognitively impaired participants. Sukhapure et al. similarly found that higher free testosterone levels in women correlated with poorer visuospatial learning and psychomotor speed (Sukhapure et al., 2022). However, Jarrett et al. emphasized that glycemic control, rather than hormone levels, was the primary driver of cognitive differences in PCOS (Jarrett et al., 2019). Our data suggest both mechanisms may be operative, as we observed both elevated testosterone and insulin resistance in cognitively impaired participants.

Limitation acknowledgment: We cannot definitively separate the independent effects of hyperandrogenism from metabolic dysfunction, as these factors are closely interrelated in PCOS pathophysiology. The significant correlation between testosterone levels and insulin

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resistance in our population (data not shown) suggests potential confounding that requires careful interpretation.

PCOS women with cognitive impairment exhibited significantly higher insulin levels and HOMA-IR values. This finding extends previous research by Castellano et al., who observed 9-14% lower cortical glucose uptake in normal-weight PCOS women compared to controls, with regional hypometabolism correlating inversely with HOMA-IR (Castellano et al., 2015). Critical comparison: While Castellano's study used neuroimaging to demonstrate metabolic brain changes, our study provides the first evidence linking insulin resistance to cognitive performance in PCOS through genetic susceptibility factors.

The mechanisms underlying insulin resistance-related cognitive impairment involve multiple pathways. The hippocampus and cortex express insulin receptors, with insulin acting as a neuromodulator of synaptic plasticity and memory (Spinelli et al., 2019). Chronic hyperinsulinemia may downregulate insulin transport across the blood-brain barrier, leading to brain insulin resistance and impaired cognitive processes. Additionally, insulin signaling regulates GSK3β, and when insulin signaling is impaired, GSK3β becomes overactive, promoting abnormal tau phosphorylation—a hallmark of Alzheimer's pathology (Salcedo-Tello et al., 2011).

Our observation that ε3ε4 genotype is over-represented among PCOS women with cognitive impairment aligns with established literature showing that ε4 carriers perform worse cognitively with aging (Gharbi-Meliani et al., 2021; Johnson et al., 2017). Large cohort studies demonstrate that both ε4 heterozygotes and homozygotes have poorer cognition and higher dementia risk in older populations (Saddiki et al., 2020). However, the association became marginally non-significant after multiple testing correction (p=0.063), indicating the need for cautious interpretation.

ε2ε3 Genotype: Unexpected Findings Requiring Careful Interpretation The prominent role of ε2ε3 as a risk genotype in our study is unusual and contradicts established literature. In general populations, ε2 is typically protective, associated with lower Alzheimer's disease risk and better cognitive outcomes relative to ε3 (Berlau et al., 2009; Berlau et al., 2007). We acknowledge this contradiction and emphasize several important limitations:

1. Small sample size: Only 14 participants carried the ε2ε3 genotype, resulting in wide confidence intervals (1.21-14.0) that reflect statistical uncertainty.

2. Population-specific effects: Our Middle Eastern population may have different genetic backgrounds that modify typical APOE effects.

3. Metabolic context: The severe metabolic dysfunction in PCOS may override usual ε2 protective effects, though this remains speculative.

These findings require replication in independent PCOS populations before drawing definitive conclusions about ε2ε3 risk associations.

Our most robust finding was the significant APOE × insulin resistance interaction (p=0.048), suggesting that metabolic dysfunction amplifies genetic susceptibility to cognitive impairment. This aligns with Johnson et al.'s finding that ApoE4 mice on high-fat diets showed exaggerated hippocampal memory impairment relative to ApoE3 mice (30), indicating that ε4 carriers are especially susceptible to metabolic stress effects on cognition.

However, we acknowledge important limitations: The interaction analysis involved small subgroups (some cells n<10), requiring cautious interpretation. Additionally, we cannot exclude the possibility that other unmeasured genetic or environmental factors contribute to these interactions.

Amyloid and Tau Pathology: Butler et al. demonstrated that Aβ clearance is positively regulated by ApoE2/E3 and negatively by ApoE4 (31), with ε4 carriers tending to accumulate more amyloid plaque and tau pathology (Wadhwani et al., 2019; Yi et al., 2018). In PCOS women who develop cognitive impairment, ε4 may exacerbate Alzheimer-like pathology. However, we emphasize that our cross-sectional study cannot determine whether such pathological processes are occurring in our population.

Vascular and Inflammatory Mechanisms: APOE isoforms affect vascular integrity and inflammation. The ε2 allele is associated with increased risk of cerebral amyloid angiopathy and hemorrhagic stroke (Nelson et al., 2013; Yu et al., 2015), while ε4 is linked to blood-brain barrier breakdown and neuroinflammation. PCOS involves endothelial dysfunction and dyslipidemia, potentially exacerbating these vascular effects. However,

these mechanistic explanations remain speculative without direct evidence from our study.

Population Stratification and Genetic Confounding: Our ethnically homogeneous Middle Eastern population reduces population stratification concerns but limits generalizability. We acknowledge that other genetic variants affecting cognitive function were not assessed, and polygenic risk scores for cognitive decline could provide important context for interpreting APOE effects.

Confounding Factors: Despite multivariate adjustment for age, education, BMI, testosterone, and HOMA-IR, residual confounding likely remains. The strong associations between age and education with cognitive status suggest these factors may partially mediate apparent APOE effects.

Sample Size and Statistical Power: While meeting calculated sample size requirements, the study remains underpowered for detecting modest genetic effects, particularly in interaction analyses. The wide confidence intervals reflect this limitation.

This cross-sectional study provides preliminary evidence for associations between APOE polymorphisms and cognitive function in women with PCOS. The ε2ε3 genotype showed increased cognitive impairment risk (adjusted OR = 4.12), while a significant gene-environment interaction indicated that insulin resistance amplifies genetic susceptibility to cognitive dysfunction.

However, important limitations require cautious interpretation. The modest sample size, cross-sectional design, and contradictory ε2ε3 findings compared to established literature necessitate replication in independent populations. These preliminary results do not support clinical implementation of APOE genotyping but contribute to understanding genetic factors in PCOS-related cognitive dysfunction.

Future longitudinal studies with larger samples are needed to validate these associations and explore their clinical relevance. This research represents an initial step toward elucidating the complex interplay between genetic susceptibility and metabolic dysfunction in cognitive outcomes among women with PCOS.

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Declaration of Interest

The authors of this article declared no conflict of interest.

Ethical Considerations

The study protocol adhered to the principles outlined in the Helsinki Declaration, which provides guidelines for ethical research involving human participants. Ethical considerations in this study were that participation was entirely optional. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board/Ethics Committee of Al-Nahrain University's College of Medicine (Approval No. 20231013, dated 18/1/2024). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

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Authors' Contributions

M.M.D., study concept and design, data collection, laboratory analysis, manuscript original drafting and writing; F.B.H., study supervision, methodology guidance, manuscript review and editing; Q.S.A., statistical analysis, data interpretation, practical assistance in data collection. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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