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Research article

Study the Polymorphism of the Calpain 10 Gene in Polycystic Ovary Syndrome in Iraqi Women

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Abstract: Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder of reproductive-aged women, characterized by oligo-/anovulation, hyperandrogenism, and polycystic ovarian morphology, and frequently accompanied by insulin resistance. Calpain-10 (CAPN10) regulates insulin secretion and glucose homeostasis, making it a plausible candidate gene for PCOS susceptibility. This case–control study examined the CAPN10 rs2975760 (T/C, UCSNP-44) polymorphism and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and insulin in 20 women with PCOS and 20 age-matched controls in Iraq. Genotyping used allele-specific PCR; hormones were measured by electrochemiluminescence immunoassay and ELISA; diagnostic performance was assessed by receiver-operating characteristic (ROC) analysis. The wild-type TT genotype was more frequent in controls (85% vs 60%), the heterozygous TC genotype was more frequent in patients (30% vs 15%; OR = 2.83; P = 0.184), and the CC genotype was observed only in patients (10% vs 0%). The C allele was over-represented in patients (OR = 4.11; 95% CI 1.04–16.29; P = 0.034) by χ^2 but not by Fisher's exact test (P = 0.066). FSH, LH, and insulin were all higher in patients (P < 0.01). Areas under the curve were 0.734 for FSH and 0.751 for LH—both only fair—whereas insulin appeared to separate the groups, most likely reflecting the small sample rather than a genuine diagnostic property. Among patients, TC carriers had higher LH, FSH, and insulin than TT carriers (P < 0.05). These hypothesis-generating findings are consistent with an association between CAPN10 rs2975760 and PCOS in Iraqi women, possibly mediated through insulin resistance, but require confirmation in larger cohorts.

Keywords: CAPN10; polycystic ovary syndrome; insulin resistance; follicle-stimulating hormone; single-nucleotide polymorphism

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder of women of reproductive age, manifesting as irregular menstrual cycles, elevated androgen levels, and the presence of multiple small ovarian follicles. These features frequently impair ovulation and reduce fertility [1, 2]. Although the precise etiology remains undefined, PCOS is understood to arise from the interaction of genetic predisposition with hormonal disturbances, notably insulin resistance, and management relies on lifestyle modification, pharmacological regulation of hormones and, where required, ovulation induction [1]. PCOS is the

most prevalent endocrinopathy among premenopausal women, reported in approximately 6.5–6.7% of this population [3, 4]; application of the 2003 Rotterdam criteria raises the estimated prevalence to as much as 15%, and current estimates suggest that 6–18% of adolescent girls and 8–13% of women of reproductive age are affected [5–7]. Stein and Leventhal first recognized the association between PCOS and obesity in 1935, when 37% of the originally described patients were obese [5]. Metabolic abnormality is a consistent component of the clinical picture of PCOS. The syndrome is heterogeneous, characterised by excessive androgen production, enlarged ovaries and impaired ovulation, and is closely linked to insulin resistance and abdominal obesity [8]. Insulin resistance—a pathological state marked by a diminished metabolic response to insulin—is a prevalent finding in PCOS even though it is not among the diagnostic criteria [9, 10]. Insulin resistance and the resulting compensatory hyperinsulinaemia stimulate androgen secretion by ovarian theca cells and suppress the hepatic production of sex hormone-binding globulin, thereby increasing free androgen availability and aggravating hyperandrogenism [11]. Insulin resistance is also central to the development of metabolic syndrome, type 2 diabetes mellitus (T2DM) and cardiovascular disease in women with PCOS [12], and non-diabetic women with PCOS exhibit both insulin resistance and defects in insulin secretion [13]. A substantial genetic contribution to the etiology of PCOS is well recognized [14].

Follicle-stimulating hormone (FSH) and luteinising hormone (LH) are the principal reproductive hormones secreted by the anterior pituitary and are pivotal regulators of ovarian function [15]. Elevated LH and an increased LH/FSH ratio during the follicular phase are reported in 35–77% of women with PCOS [16]. The calpain-10 (*CAPN10*) gene, located on chromosome 2q37.3, spans approximately 31 kb and comprises 15 exons and 14 introns. It was the first gene identified as a susceptibility locus for T2DM through genome-wide scanning and positional cloning [17, 18]. Because insulin resistance provides a shared pathophysiological basis for T2DM and PCOS, *CAPN10* polymorphisms have been proposed to contribute to PCOS risk [19]. Functional studies indicate that calpain-10 regulates glucose homeostasis by facilitating GLUT4 translocation in skeletal muscle and by modulating insulin secretion from β-cells and peripheral glucose-sensing cells, all of which may influence insulin sensitivity [20]. Several single-nucleotide polymorphisms (SNPs) within *CAPN10* have been described, of which UCSNP-44 (rs2975760) has been linked to PCOS and to insulin resistance [21, 22]. Reported associations between CAPN10 variants and PCOS have varied across ethnic groups—positive in some Saudi [21] and Iraqi [24] cohorts but weaker or absent in others—underscoring the value of population-specific data.

Data on *CAPN10* variation in Iraqi women with PCOS remain limited. The present study therefore aimed to determine the distribution of the *CAPN10* rs2975760 (T/C) genotypes and alleles in Iraqi women with PCOS and healthy controls, to compare serum FSH, LH, and insulin between the two groups, to evaluate the diagnostic performance of these hormones by ROC analysis, and to examine whether the rs2975760 genotype is associated with hormone concentrations within the patient group. Although a previous study of Iraqi women (Al-Murshedi et al., 2022 [24]) reported an association between the same CAPN10 variant (rs2975760) and insulin resistance in PCOS, the present work is intended to extend rather than merely confirm that report. First, it examines an independent sample recruited from a different governorate (Al-Qadisiyah). Second, it adds receiver operating characteristic (ROC) analyses of FSH, LH, and insulin, which were not previously undertaken. Third, it explores the relationship between rs2975760 genotype and circulating reproductive and metabolic hormones within the patient group. Given the modest sample size, these analyses are exploratory and hypothesis-generating, and should be interpreted alongside the earlier Iraqi data as part of a cumulative body of evidence.

2. Materials and Methods

2.1 Study design and participants

This case–control study enrolled 40 women: 20 diagnosed with PCOS and 20 age-matched apparently healthy controls. Participants were aged 14–45 years and were recruited from the Women and Children General Hospital and its outpatient clinic in Al-Qadisiyah Governorate between September 2024 and June 2025. The patients' clinical status was confirmed by a physician based on laboratory and ultrasonographic findings. PCOS was diagnosed when at least two of the three Rotterdam criteria were met—oligo-

/anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology—after exclusion of other causes. Informed consent was obtained from all participants before enrolment.

2.2 Sample collection and biochemical analysis

Five milliliters of venous blood was drawn at 09:00 h after an overnight fast. Each sample was divided into two portions. The first was collected into a gel separator tube for biochemical analysis: serum LH and FSH were measured by automated electrochemiluminescence immunoassay (Cobas e411), insulin was quantified by enzyme-linked immunosorbent assay (ELISA), and glucose was determined by the hexokinase method. The second portion was collected into an EDTA tube for molecular analysis. Insulin resistance was estimated using the homeostatic model assessment (HOMA-IR) index (Equation 1), with values below 2.7 regarded as normal.

$$\text{HOMA-IR} = [\text{fasting glucose (mg/dL)} \times \text{fasting insulin (}\mu\text{U/mL)}] \div 405 \quad (1)$$

2.3 Genomic DNA extraction

Genomic DNA was extracted from peripheral blood using the FavorPrep Blood Genomic DNA Extraction Mini Kit (Favorgen, South Korea) according to the manufacturer's instructions. DNA concentration and purity were verified before use; the A260/A280 purity ratio was approximately 1.8, and DNA concentrations ranged from 40 to 100 ng/μL. Extracted DNA was stored at −20 °C until polymerase chain reaction (PCR) analysis.

2.4 Genotyping of CAPN10 rs2975760

The *CAPN10* rs2975760 (T/C) polymorphism was genotyped by allele-specific PCR using a LABNET thermal cycler and the primer set listed in Table 1, which was designed from the NCBI GenBank reference sequence. Amplification comprised an initial denaturation at 94 °C for 4 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 50 s, and extension at 72 °C for 2 min, with a final extension at 72 °C for 7 min. PCR products were resolved on a 5% agarose gel stained with SafeRed and visualized under a UV transilluminator, with a 100 bp DNA ladder (Bioneer, South Korea) as the molecular size marker.

Table 1. Allele-specific PCR primers for the *CAPN10* rs2975760 (T/C) polymorphism, with sequences and amplicon sizes.

Primer	Sequence (5′→3′)	Product size
Forward inner (T allele)	GACTGCAGGGCGCTCACGCTTGCGGT	200 bp
Reverse inner (C allele)	TTAGCCTCACCTTCAAACGCCTTACTGCG	220 bp
Forward outer	CTGGTGACATCAGTGCCCAGTGAGCCCT	365 bp (control)
Reverse outer	TCACCATGGGAGTGAGCCTCTGGCATTG	—

The outer primer pair generates the internal control fragment; the inner primers are allele-specific.

2.5 Statistical analysis

Data were analyzed with SPSS v22.0. Continuous variables are expressed as mean ± standard error of the mean and were compared between the two groups using the independent-samples t-test. Genotype and allele distributions were compared using the chi-square (χ^2) test, and associations were expressed as odds ratios (OR) with 95% confidence intervals (CI). Because several genotype cells were sparse, the 2 × 3 genotype table and the CC-versus-TT comparison were additionally evaluated with Fisher's exact test, and a Haldane–Anscombe (+0.5) correction was applied to any odds ratio involving a zero cell. Conformity of the control-group genotype frequencies to Hardy–Weinberg equilibrium was tested by a χ^2 goodness-of-fit test. Continuous variables are additionally summarised as standard deviations (SD) alongside the standard error of the mean so that the spread of values can be judged. Differences in hormone concentrations across genotypes were assessed using analysis of variance with least significant difference (LSD) post hoc comparisons. Because the LSD procedure does not correct for multiple comparisons and the genotype subgroups were small, these post-hoc results are treated as exploratory. Receiver-operating-characteristic (ROC) curves were constructed to evaluate the diagnostic performance of FSH, LH, and insulin, and the area

under the curve (AUC), sensitivity, specificity, and optimal cut-off value were determined [23]. Sensitivity and specificity were calculated as shown in Equations 2 and 3, where TP, TN, FP, and FN denote true-positive, true-negative, false-positive, and false-negative cases, respectively. A P value below 0.05 was considered statistically significant. Exact P values generated by SPSS are reported to three decimal places.

$$\text{Sensitivity (\%)} = [TP \div (TP + FN)] \times 100 \quad (2)$$

$$\text{Specificity (\%)} = [TN \div (TN + FP)] \times 100 \quad (3)$$

3. Results and Discussion

3.1 Genotype and allele distribution of CAPN10 rs2975760

The distribution of the *CAPN10* rs2975760 genotypes and alleles in the two groups is presented in Table 2 and Figure 1. The wild-type TT genotype was more frequent among controls (17/20; 85%) than among patients (12/20; 60%), consistent with a protective role. The heterozygous TC genotype was more frequent in patients (6/20; 30%) than in controls (3/20; 15%); although this difference did not reach statistical significance ($\chi^2 = 1.76$; P = 0.184), by χ^2 (Fisher's exact P = 0.260), the odds ratio (OR = 2.83; 95% CI 0.59–13.63) indicates a trend towards approximately two- to three-fold increased risk. The homozygous mutant CC genotype was detected exclusively in the patient group (2/20; 10%) and was absent from controls. For the overall 2 × 3 genotype distribution, Fisher's exact test was not significant (P = 0.191), and the control genotype frequencies did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 0.13$, P = 0.72; exact P = 1.00). Because the CC genotype was absent from controls, the CC-versus-TT odds ratio is not directly estimable; a Haldane–Anscombe correction gives OR = 7.0 (95% CI 0.31–158.8), a very wide interval that includes the null and shows this estimate to be uninformative in the present sample.

At the allelic level, the mutant C allele was over-represented among patients (10/40 alleles; 25.0%) relative to controls (3/40 alleles; 7.5%), yielding an odds ratio of 4.11 (95% CI 1.04–16.29; P = 0.034). However, this allelic association was not significant by Fisher's exact test (P = 0.066); because each participant contributes two alleles, the allele-based test treats 40 alleles per group as independent observations and is therefore reported as secondary to the genotype comparison. Conversely, the wild-type T allele predominated in controls (37/40; 92.5%) compared with patients (30/40; 75.0%), with a reciprocal odds ratio of 0.24 (95% CI 0.06–0.96). These results are consistent with the C allele acting as a putative genetic risk factor for PCOS and the T allele as protective, although the loss of significance under exact testing indicates that the association is preliminary, and are consistent with previous reports associating *CAPN10* rs2975760 with PCOS in other populations [24, 25]. Taken together with the genotype data, the findings suggest a gene-dosage pattern in which the TC genotype represents an intermediate state of genetic susceptibility and the CC genotype confers the greatest risk, particularly in the context of obesity and insulin resistance. This gene-dosage interpretation is offered only as inference: with just two CC carriers, and none among controls, the data cannot establish a dose-response relationship, and the pattern requires testing in a substantially larger sample.

Table 2. Genotype and allele distribution of the *CAPN10* rs2975760 (T/C) polymorphism in women with PCOS and healthy controls.

Genotype / Allele	Patients n (%)	Controls n (%)	χ^2	P value	OR	95% CI
TT (wild-type)	12 (60.0)	17 (85.0)	—	Reference	1.00	—
TC (heterozygous)	6 (30.0)	3 (15.0)	1.76	0.184	2.83	0.59–13.63
CC (homozygous mutant)	2 (10.0)	0 (0.0)	—	—	n.e.	—
Total (genotypes)	**20 (100)**	**20 (100)**				
T allele	30 (75.0)	37 (92.5)	4.50	0.034	0.24	0.06–0.96
C allele	10 (25.0)	3 (7.5)	4.50	0.034	4.11	1.04–16.29
Total (alleles)	**40 (100)**	**40 (100)**				

OR, odds ratio; CI, confidence interval; n.e., not estimable (zero cell in the control group). Given the sparse cells, genotype comparisons were confirmed with Fisher's exact test: the overall 2 × 3 genotype distribution was not significant (P = 0.191) and the TC-versus-TT comparison gave P = 0.260. The allelic comparison, significant by χ^2 (P = 0.034), was not significant by Fisher's exact test (P = 0.066). Because the CC genotype was absent from controls its odds ratio is not directly estimable; a Haldane–Anscombe (+0.5) correction gives OR = 7.0 (95% CI 0.31–158.8). Control genotype frequencies conformed to Hardy–Weinberg equilibrium (χ^2 = 0.13, P = 0.72; exact P = 1.00). Allele percentages are calculated from 2n alleles per group (n = 20; 40 alleles).

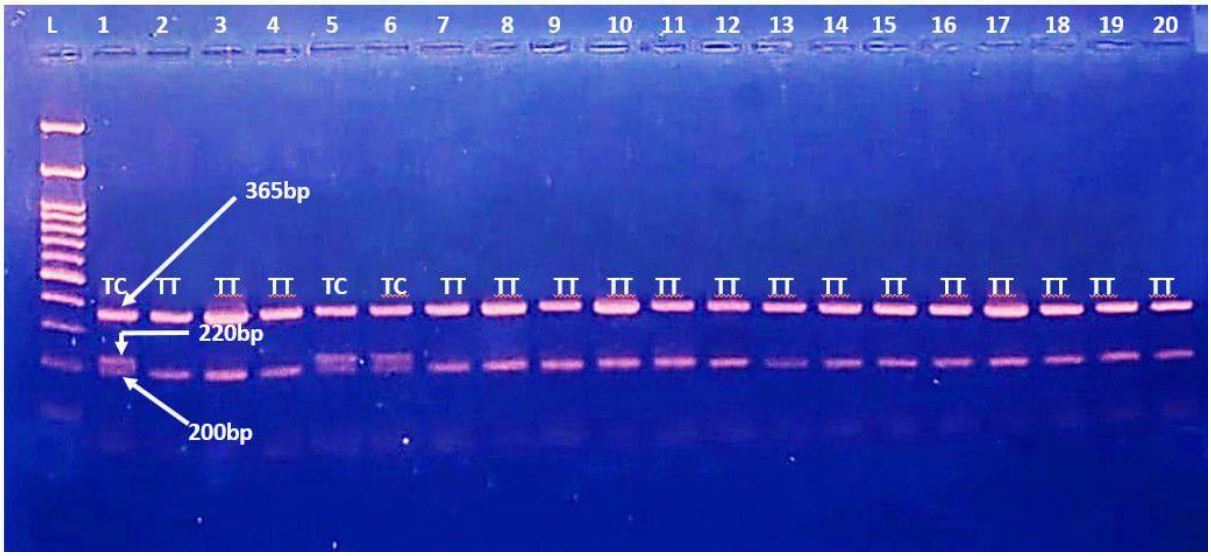


Figure 1. Genotype distribution and allele frequency of the *CAPN10* rs2975760 (T/C) polymorphism in women with PCOS and healthy controls.

3.2 Serum FSH concentrations

Mean serum FSH was significantly higher in women with PCOS than in controls (6.984 ± 0.356 vs 5.512 ± 0.392 mIU/mL; P = 0.008; Table 3). The corresponding standard deviations were 1.59 and 1.75 mIU/mL. This finding accords with several previous reports [24, 26–29], although it contrasts with the results of Ibrahim and Alobaidi [30]. PCOS is hormonally heterogeneous, and a relative elevation of FSH may be observed in particular subtypes—especially where ovarian reserve is diminished or premature ovarian insufficiency is emerging. Insulin resistance and related metabolic disturbances can further perturb the ovarian hormonal milieu and thereby raise FSH. In physiological terms, LH and FSH are released in response to hypothalamic gonadotropin-releasing hormone (GnRH), and certain disorders of GnRH pulsatility may overstimulate FSH secretion [31]. This elevation nonetheless warrants critical appraisal. The classic gonadotropin signature of PCOS is a raised LH with normal or low FSH and an elevated LH/FSH ratio, so a higher FSH in patients is atypical. Both group means (6.98 and 5.51 mIU/mL) lie within the normal early-follicular range, so the clinical meaning of a statistically significant difference of about 1.5 mIU/mL is uncertain and may partly reflect the small sample. The possibility that some participants had diminished ovarian reserve or emerging ovarian insufficiency rather than classic Rotterdam-defined PCOS cannot be excluded and should be weighed when interpreting this result. Notably, the ratio of the mean LH to the mean FSH was similar in the two groups (approximately 1.2 in both), so the increase in the LH/FSH ratio that is characteristic of PCOS was not evident here.

Table 3. Serum follicle-stimulating hormone (FSH) concentrations in women with PCOS and healthy controls.

Trait	Group	n	Mean	Std. error	P value
FSH (mIU/mL)	Patients	20	6.984	0.356	0.008
	Controls	20	5.512	0.392	

Values are mean ± standard error of the mean; independent-samples t-test.

3.3 Diagnostic performance of FSH

ROC analysis of FSH (Figure 2, Table 4) returned an AUC of 0.734 (95% CI 0.596–0.871), corresponding to only fair discrimination. At the optimal cut-off of > 6.77 mIU/mL, sensitivity was 65% and specificity 85%. FSH therefore performs modestly and is not a sufficiently reliable stand-alone marker for identifying women with PCOS.

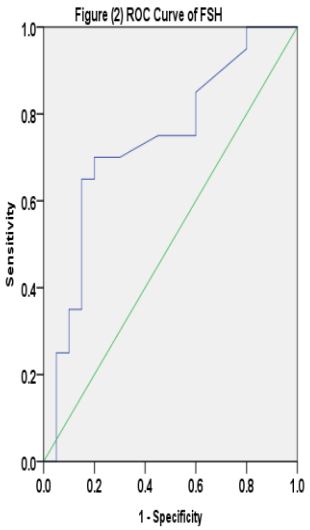


Figure 2. Receiver-operating-characteristic (ROC) curve for serum FSH.

Table 4. Parameters of the ROC curve for serum FSH.

Parameter	Patients (n = 20)	Controls (n = 20)
Area under the curve (AUC)	0.734	
Standard error	0.070	
95% CI	0.596–0.871	
Sensitivity	65%	
Specificity	85%	
Cut-off point	> 6.77 mIU/mL	
True positive	13	
False negative	7	
True negative		17
False positive		3

3.4 Serum LH concentrations

Mean serum LH was significantly higher in the PCOS group than in controls (8.517 ± 0.477 vs 6.860 ± 0.388 mIU/mL; $P < 0.001$; Table 5). The corresponding standard deviations were 2.13 and 1.74 mIU/mL. Elevated LH is a characteristic hormonal signature of PCOS and is attributed principally to disturbance of hypothalamic–pituitary–ovarian regulation. Under normal conditions, GnRH is released in a balanced pulsatile rhythm that elicits proportionate pituitary secretion of LH and FSH; in PCOS, GnRH pulse frequency is abnormally accelerated, preferentially stimulating LH over FSH. The resulting increase in the LH/FSH ratio impairs follicular maturation and contributes to chronic anovulation [32]. Ovarian theca cells additionally show an exaggerated response to LH, increasing androgen synthesis and producing the clinical features of hirsutism, acne and menstrual irregularity, while the excess androgen further aggravates insulin resistance [33]. These findings are in agreement with previous reports that women with the reproductive phenotype of PCOS exhibit relatively elevated LH [34, 35].

Table 5. Serum luteinising hormone (LH) concentrations in women with PCOS and healthy controls.

Trait	Group	n	Mean	Std. error	P value
LH (mIU/mL)	Patients	20	8.517	0.477	< 0.001
	Controls	20	6.860	0.388	

Values are mean ± standard error of the mean; independent-samples t-test.

3.5 Diagnostic performance of LH

ROC analysis of LH (Figure 3, Table 6) yielded an AUC of 0.751 (95% CI 0.601–0.910), again indicating fair discrimination. At the optimal cut-off of > 7.9 mIU/mL, sensitivity was 75% and specificity 80%, so LH performs somewhat better than FSH but remains, on its own, a fair rather than a strong diagnostic indicator.

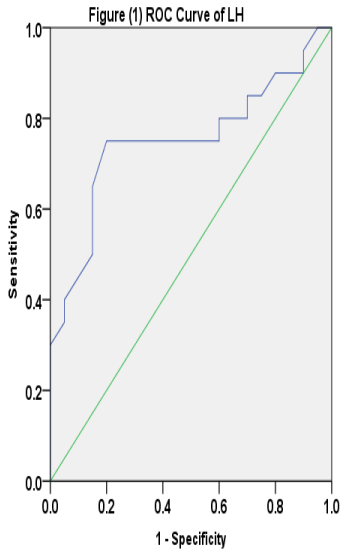


Figure 3. Receiver-operating-characteristic (ROC) curve for serum LH.

Table 6. Parameters of the ROC curve for serum LH.

Parameter	Patients (n = 20)	Controls (n = 20)
Area under the curve (AUC)	0.751	
Standard error	0.076	
95% CI	0.601–0.910	
Sensitivity	75%	
Specificity	80%	
Cut-off point	> 7.9 mIU/mL	
True positive	15	
False negative	5	
True negative		16
False positive		4

3.6 Serum insulin concentrations

Mean serum insulin was markedly higher in women with PCOS than in controls (18.257 ± 0.305 vs 10.886 ± 0.324 µIU/mL; P < 0.001; Table 7). The corresponding standard deviations were 1.36 and 1.45 µIU/mL. This elevation reflects compensatory hyperinsulinaemia arising from insulin resistance, a hallmark of the syndrome that is present even in lean patients. When peripheral tissues—muscle, liver and adipose tissue—lose sensitivity to insulin, secretion increases to offset the impaired cellular response; up to 70% of women with PCOS are reported to be insulin-resistant [24]. Insulin acts directly on ovarian granulosa and theca cells to modulate steroidogenesis and ovulation, and hyperinsulinaemia is therefore closely linked to the hyperandrogenism of PCOS [33]. The present result is consistent with earlier studies [24, 36, 37] and reinforces

the value of early assessment of insulin status and of therapeutic strategies directed at improving insulin sensitivity as a core element of PCOS management. Because insulin is not part of the Rotterdam diagnostic criteria, the very large between-group difference should be interpreted cautiously, since any role of hyperinsulinaemia or insulin resistance in the selection of cases would render the separation partly circular.

Table 7. Serum insulin concentrations in women with PCOS and healthy controls.

Trait	Group	n	Mean	Std. error	P value
Insulin (μIU/mL)	Patients	20	18.257	0.305	< 0.001
	Controls	20	10.886	0.324	

Values are mean ± standard error of the mean; independent-samples t-test.

3.7 Diagnostic performance of insulin

ROC analysis of insulin (Figure 4, Table 8) produced an AUC of 1.000, with sensitivity and specificity of 100% at a cut-off of > 15.275 μIU/mL—complete separation of patients and controls in this cohort. Insulin thus emerged as by far the strongest of the three markers evaluated. This result should nevertheless be interpreted with caution: perfect discrimination in a sample of this size is more likely to reflect the small number of participants and the absence of overlap in the observed distributions than a genuinely infallible test, and it requires confirmation in a larger and more heterogeneous cohort before any diagnostic cut-off is adopted in practice. Moreover, insulin is not a diagnostic criterion for PCOS; the perfect discrimination observed here is best regarded as a statistical artefact of the small sample and the non-overlapping distributions rather than evidence of a genuine diagnostic marker, and the phrase "complete separation" is used only descriptively.

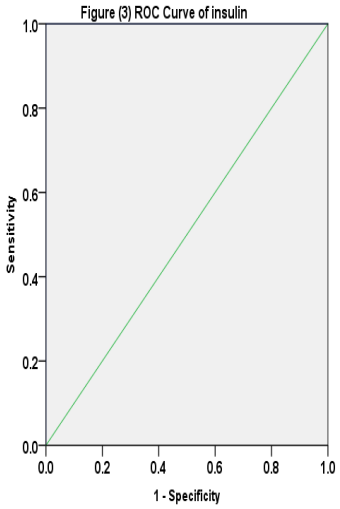


Figure 4. Receiver-operating-characteristic (ROC) curve for serum insulin.

Table 8. Parameters of the ROC curve for serum insulin.

Parameter	Patients (n = 20)	Controls (n = 20)
Area under the curve (AUC)	1.000	
Standard error	0.000	
95% CI	1.000–1.000	
Sensitivity	100%	
Specificity	100%	
Cut-off point	> 15.275 μIU/mL	
True positive	20	
False negative	0	
True negative		20
False positive		0

3.8 Association between CAPN10 genotype and hormone concentrations

Within the PCOS group, *CAPN10* rs2975760 genotype was significantly associated with hormone concentrations (Table 9). Carriers of the heterozygous TC genotype had significantly higher LH (10.483 ± 0.772 mIU/mL) and FSH (9.067 ± 0.529 mIU/mL) than carriers of the wild-type TT genotype (8.157 ± 0.546 and 6.287 ± 0.374 mIU/mL, respectively; $P < 0.05$), whereas the CC and TT genotypes did not differ significantly for these two hormones. TC carriers likewise showed significantly higher insulin (20.333 ± 0.452 μIU/mL) than either TT (18.446 ± 0.320) or CC (18.850 ± 0.784) carriers, indicating an association between this genotype and greater insulin resistance. These observations are consistent with the established function of calpain-10 in insulin signalling and glucose metabolism. Variants of *CAPN10* have been reported to impair pancreatic β-cell activity and to increase insulin resistance; the resulting hyperinsulinaemia disrupts the hypothalamic–pituitary–ovarian axis, altering gonadotropin secretion and exacerbating hyperandrogenism and the clinical features of PCOS [18, 38]. It should be noted, however, that only two patients carried the CC genotype, so estimates for that group are imprecise and the apparent absence of a CC–TT difference cannot be interpreted as evidence of no effect. Accordingly, the CC genotype is presented for descriptive purposes only and excluded from formal inference. Because the LSD procedure does not adjust for multiple comparisons, the higher hormone levels in TC carriers should be regarded as exploratory; a conservative post-hoc approach and, ultimately, a larger sample are required before any genotype-hormone relationship can be considered established.

Table 9. Effect of *CAPN10* rs2975760 genotype on serum hormone concentrations within the PCOS group.

Hormone	Genotype	Mean	Std. error	LSD ($\alpha = 0.05$)
LH (mIU/mL)	TT	8.157 b	0.546	2.324
	TC	10.483 a	0.772	
	CC	8.600 ab	1.337	
FSH (mIU/mL)	TT	6.287 b	0.374	1.592
	TC	9.067 a	0.529	
	CC	7.700 ab	0.916	
Insulin (μIU/mL)	TT	18.446 b	0.320	1.361
	TC	20.333 a	0.452	
	CC	18.850 b	0.784	

Within each hormone, means not sharing a common letter differ significantly at $P < 0.05$ (LSD post-hoc test). LSD, least significant difference.

4. Conclusions

This study provides preliminary evidence of an association between the *CAPN10* rs2975760 (T/C) polymorphism and polycystic ovary syndrome in Iraqi women from Al-Qadisiyah Governorate. The mutant C allele was significantly over-represented among patients (OR = 4.11; $P = 0.034$) by χ^2, although not by Fisher's exact test ($P = 0.066$), and is therefore identified as a putative risk allele, while the wild-type T allele and the TT genotype appear protective. The CC genotype was confined to the patient group, and the TC genotype showed a non-significant trend towards increased risk, together suggesting a gene-dosage effect that requires confirmation in a larger sample. Serum FSH, LH, and insulin were all significantly elevated in women with PCOS relative to controls. In ROC analysis, FSH (AUC 0.734) and LH (AUC 0.751) showed only fair discrimination and are not adequate as stand-alone diagnostic markers. In contrast, insulin showed apparently complete separation of the groups in this cohort; because insulin is not a PCOS diagnostic criterion and the sample was small, this most likely reflects sample size rather than genuine diagnostic accuracy—a result that warrants validation in a larger, more heterogeneous population. Within the patient group, TC carriers exhibited significantly higher LH, FSH, and insulin levels than TT carriers, consistent with a mechanism in which CAPN10 variation contributes to PCOS through impaired insulin signaling and consequent disruption of the hypothalamic–pituitary–ovarian axis. Overall, given the small sample and the sensitivity of the key allelic result to the statistical test used, these findings should be regarded as preliminary and hypothesis-generating rather than definitive.

Study limitations

The principal limitation of this work is the small sample size (20 patients and 20 controls), which reduces statistical power, produces wide confidence intervals—particularly for the rare CC genotype, observed in only two patients—and precludes reliable adjustment for potential confounders such as body mass index and age. A single SNP was examined, and although fasting glucose and HOMA-IR were measured, these were not analyzed in relation to genotype. Anthropometric measures—body mass index, waist circumference, and obesity status—were not available, and because insulin resistance is strongly influenced by adiposity, the observed CAPN10-insulin association may be confounded; multivariable adjustment (for example, logistic regression incorporating age, BMI, and insulin resistance) was therefore not possible and is recommended for future work. Genotyping relied on allele-specific PCR read from agarose gels without orthogonal confirmation (for example, Sanger sequencing), and independent validation of a subset of samples would strengthen the genotype calls. The participants spanned a wide age range (14–45 years) that included adolescents, who were not analysed separately. The allelic association was significant by the χ^2 test but not by Fisher's exact test, underscoring its fragility, and no a priori power calculation was performed. Larger, multi-center studies incorporating additional *CAPN10* variants, anthropometric data, and formal indices of insulin resistance are needed to confirm and extend these findings.

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