Original
ISSN 2413-0516

Molecular Studies of CAPN-10 Gene (rs2975760) and its Association with Insulin Resistance in Polycystic Ovarian Syndrome of Iraqi Women

Hadbaa H. Al-Murshedi[1]*, Ammar Fadhil Jawad[2], Fadhil J. Al-Tu'ma[1], Eman A. Hadi[3], Maha Mohammed Kadhim Al-Tu'ma[4]

[1]Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq.
[2]Department of Pharmacognosy, College Pharmacy, University of Kerbala, Kerbala, Iraq.
[3]Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.
[4]Department of Anesthesia Techniques, College of Health and Medical Techniques, Al-Zahraa University for Women, Kerbala, Iraq.
*Correspondence to: Hadbaa Helwas Al-Murshedi (Email: hdbaa88@gmail.com)
(Submitted: 10 August 2022 – Revised version received: 22 August 2022 – Accepted: 17 September 2022 – Published online: 26 December 2022)

Abstract

Objectives: To explore an association between CAPN10, SNP-44 (rs2975760) with IR condition in women with PCOS.

Methods: A study included 120 participants of which 68 women have PCOS subdivide according to their body mass index (BMI) into 45 obese (BMI ≥ 30) and 23 non-obese (BMI < 30). The remaining 52 represent the control group who were apparently healthy women with normal weight and normal menstrual cycle. Patients with PCOS were selected from the Infertility Department, Gynecology and Obstetrics Teaching Hospital, Kerbala Health Directorate/Kerbala-Iraq between Nov., 2021 and June, 2022. Diagnosis of PCOS is based on 2 of 3 findings: oligo/anovulation, hyperandrogenism, polycystic ovaries in ultrasound (Rotterdam criteria). Patients were interviewed and examined for weight, height, waist circumference, and hip circumference. Venous blood samples were collected at 9 AM after an overnight fast. IR was assessed by calculating homeostatic model assessment of insulin resistance (HOMA-IR) using the formula (fasting glucose mg/dl x fasting insulin μU/ml)/405, taking normal value <2.7. Genotypes of CAPN10, SNP-44 has been identified using Allele-specific polymerase chain reaction (AS-PCR) technique.

Results: The prevalence of IR based on HOMA-IR was (80%) in obese PCOS and (48%) in non-obese PCOS women. CAPN10, SNP-44 has been reconstructed and analyzed in patients and controls. Genotypes of 45 obese PCOS subjects (TT, $N = 26$; TC, $N = 12$; and CC, $N = 7$), 23 non-obese PCOS subjects (TT, $N = 15$; TC, $N = 6$; and CC, $N = 2$) and control subjects (TT, $N = 39$; TC, $N = 11$; and CC, $N = 2$) were identified. The genotype distribution was statistically different between obese PCOS women and controls (OR = 5.25, $P = 0.048$). The association of SNP-44 allele with IR status was detected. HOMA-IR was greater in CC (10.54 ± 1.29, 9.88 ± 1.41) than in TT (3.30 ± 1.52, $P < 0.001$; 2.82 ± 1.45, $P < 0.001$) and TC (3.76 ± 1.58, $P < 0.001$; 4.10 ± 1.57, $P < 0.05$) in obese PCOS and non-obese PCOS subjects respectively.

Conclusion: In obese PCOS, the C allele was associated with higher insulin secretion and HOMA-IR compared with the T allele. The increased HOMA-IR is an indicator of IR. In this scenario, the C allele might be involved in the pathophysiology of insulin resistance in PCOS.

Keywords: Polycystic ovarian syndrome, insulin resistance, CAPN10 gene

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age and often develops during adolescence.[1] Women with PCOS are at increased risk of infertility.[2] PCOS manifests with the typical clinical features of clinical and/or biochemical hyperandrogenism, ovarian dysfunction (including oligo-amenorrhoea) and polycystic ovaries. The prevalence of PCOS is commonly thought to vary between 5% and 20% depending on diagnostic criteria and sample population.[3] Diagnostic criteria for PCOS mostly use the revised Rotterdam 2003 criteria.[4] Metabolic disturbances often form an important component of the clinical presentation of PCOS. Insulin resistance (IR) is a very common finding in subjects with PCOS which not included among the diagnostic features.[5] IR is usually defined as a pathological condition characterized by a decreased responsiveness or sensitivity to the metabolic actions of insulin. In women with PCOS, IR plays an important role in the development and persistence of this disorder.[6] IR and compensatory hyperinsulinemia stimulate ovarian theca cells to secrete androgens. Insulin also inhibits sex hormone binding globulin (SHBG) production, increasing bioavailability of androgen levels and worsen hyperandrogenism status.[7] Moreover, IR is critically involved in the development of metabolic syndrome, type 2 diabetes (T2D) and cardiovascular disease in PCOS women.[8] The close association between obesity and PCOS is supported by epidemiological data, revealing that between 38% and 88% of women with PCOS are either overweight or obese.[9]

The etiology of PCOS is not completely understood, although it was reported that genetic and lifestyle factors known to influence the etiology of the syndrome. The use of candidate gene analysis has provided several promising genes as genetic modifiers of component phenotypes of PCOS.[10] The results of both linkage and association studies, suggested that some genes involves insulin regulation are important factors in the genetic basis of PCOS.[11] The discovery of single nucleotide polymorphisms (SNPs) linked to a disease can shed light on its origin and help women with a particular phenotype, have a better prognosis. The calpain-10 gene (CAPN-10), plays a role in the secretion and action of insulin by encoding an extremely prevalent member of the calpain-like cysteine protease family. The CAPN-10 gene has been thoroughly examined in PCOS due to the fact that T2D and PCOS share a number of etiologic factors.[12]

The earliest evidence of CAPN10 involvement in PCOS, suggested a statistically significant association between PCOS susceptibility and the SNP-44 (rs2975760).[13] This study was aimed to explore an association between CAPN-10 gene single

nucleotide polymorphism (rs2975760) with insulin resistance in women with PCOS.

Materials and Methods

A case-control study included 120 participants of which 68 women have PCOS subdivided into 45 obese and 23 non-obese subjects. The remaining 52 represent the control group who were apparently healthy women with normal weight and normal menstruation. Patients with PCOS were selected from the Infertility Department, Gynecology and Obstetrics Teaching Hospital, Kerbala Health Directorate/Kerbala-Iraq between November, 2021 and June, 2022. Oral informed consent was obtained from all participants. PCOS was diagnosed in presence of at least two out of the three: oligo/anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries with exclusion of related etiologies. These criteria were defined by the updated 2003 Rotterdam.[14]

Patients were interviewed and examined for weight, height, waist circumference, and hip circumference. Transabdominal pelvic sonography was performed on all of the subjects with PCOS. Five milliliters venous blood samples were collected at 9 AM after an overnight fast. Blood samples were divided into two parts; the first part was collected in gel tube for biochemical analysis. Levels of serum C-peptide, LH and FSH were measured by chemiluminescent automated immunoassay system (Cobas e411). Free testosterone and insulin were measured by enzyme linked immunosorbent assay (ELISA). Glucose was measured with hexokinase method. The second part was collected in EDTA tube for molecular analysis. IR was assessed by calculating homeostatic model assessment of insulin resistance (HOMA-IR) using the formula (fasting glucose mg/dl x fasting insulin µU/ml)/405, taking normal value less than (2.7). Genotypes of CAPN-10, rs2975760 has been identified using Allele-Specific Polymerase Chain Reaction (AS-PCR) technique. Each PCR was carried out in a total volume 25 µl consisting of 3 µl extracted DNA, 1.5 µl each primer, 12 µl master mix and 7 µl nuclease free water. Specific primers were designed with the sequences 5′-GCAGGGAAGCTGGTGAACATG-3′ for the forward primer, 5′-CTCACCTTCAAACGCCTTACTTCA-3′ for the reverse 1 primer and 5′-CTCACCTTCAAACGCCTTACTTCG-3′ for the reverse 2 primer. The PCR products (250 bp) of CAPN-10 gene were separated by electrophoresis in a 5% agarose gel and then visualized by UV Transilluminator.

The data were analyzed using the Statistical Package for Social Sciences (SPSS). Continuous variables were expressed as means ± standard deviation, (SD). Mean comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test.

Results

The demographic features of the total study population, as well as baseline values of the various biochemical parameters are presented in Table 1. All fat indices such as waist circumference (WC), waist to hip ratio (WHR) and BMI were significantly higher in PCOS group ($P < 0.001$). This result highlighted the role of obesity in PCOS as reported in previous studies. Gonadal hormones: LH, FSH and LH/FSH ratio were seemingly elevated in PCOS patients. Free testosterone was increased in PCOS group compared to control group ($P < 0.001$). Lipid profile: total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) seem to be normal in women with PCOS of the presented study. However, PCOS subjects still have increased levels of TC, TG and LDL-C; and decreased level of HDL-C in compare to control subjects. Glucose, insulin, C-peptide and HOMA-IR were significantly elevated in women with PCOS compared to control women, pointing to the insulin resistance component in this syndrome.[15] The incidence of IR was 80% in obese women with PCOS and 48% in non-obese women with PCOS, as shown in Figure 1.

Table 1. **Comparison of demographic, hormonal and metabolic features between PCOS patients ($N = 68$) and controls ($N = 52$)**

	PCOS $N = 68$	Control $N = 52$	P-value
Demographic Parameters			
Age (years)	27 ± 6.00	27 ± 5.71	NS
WC (cm)	100 ± 16.71	80 ± 10.94	<0.001
WHR	0.90 ± 0.10	0.82 ± 0.08	<0.001
BMI (kg/m^2)	31.32 ± 5.79	24.09 ± 3.56	<0.001
Hormonal Parameters			
LH* (IU/l)	10.25 ± 1.52	6.67 ± 1.44	<0.001
FSH (IU/l)	6.73 ± 1.69	5.56 ± 1.32	<0.01
LH/FSH	1.69 ± 0.72	1.30 ± 0.60	<0.01
f-testosterone* (pg/ml)	16.65 ± 2.27	4.21 ± 2.50	<0.001
Metabolic Parameters			
Total Cholesterol (mg/dl)	156 ± 31.05	117 ± 20.97	<0.001
Triglycerides* (mg/dl)	95 ± 1.53	70 ± 1.55	<0.001
HDL-C (mg/dl)	40 ± 6.24	43 ± 8.95	<0.01
LDL-C* (mg/dl)	92 ± 1.32	56 ± 1.39	<0.001
Glucose (mg/dl)	92 ± 8.40	84 ± 5.12	<0.001
Insulin* (µU/ml)	18.15 ± 1.76	10.74 ± 1.56	<0.001
C-peptide* (pmol/L)	455.82 ± 1.82	330.64 ± 1.64	<0.01
HOMA-IR*	3.87 ± 1.76	2.22 ± 1.58	<0.001

Data was expressed as mean ± SD; *, refers to logarithmic mean of transformed data; NS, non-significant; WC, waist circumference; WHR, waist to hip ratio; BMI, body mass index; LH, luitinising hormone; FSH, follicle stimulating hormone; LH/FSL, LH to FSH ratio; f-testosterone, free testosterone, HDL, high density lipoprotein-cholesterol; LDL, low density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance.

Extracted DNA samples for study subjects, were found to be pure and the DNA concentrations ranged from (4.64–42.10) ng/u. The mean of DNA concentration and purity were (21.85 ± 8.79), (1.82 ± 0.08) respectively. The results of AS-PCR amplifications were analyzed and three genotypes were obtained for CAPN10, SNP-44, TT (common homozygous-wild type) TC, (heterozygous) and CC, (rare homozygous-mutant type).

Subjects with PCOS were analyzed in two groups, based on BMI: non-obese PCOS (BMI < 30) and obese PCOS (BMI ≥ 30) cases. Chi Square test was carried out to check for the significance of deviation from Hardy-Weinberg equilibrium (HWP) for CAPN10, SNP-44 (rs2975760) in obese PCOS ($P = 0.063$), non-obese PCOS ($P = 0.535$) and control ($P = 0.587$)

women separately. The results indicate that CAPN10 SNP-44 (rs2975760) confirms to the HWE, Table 2. The allele and genotype frequencies were compared between these two groups as well as each of them with controls. The genotype and allele frequency distribution for the CAPN10 SNP-44, (rs2975760) is presented in Figure 2.

Observations of the current study indicated that the homozygotes for the SNP-44, variant CC had significantly higher frequency among obese PCOS cases 15% than the controls 4%, (OR = 5.25, $P = 0.048$). This trend was also seen in the allele distribution pattern, wherein the variant allele C was found in 29% of the obese PCOS cases as compared to 14% of the controls (OR = 2.41, $P = 0.015$), Table 3.

To explore the role of CAPN-10 gene, SNP-44 in the presence of metabolic abnormalities in PCOS. The distribution of genotypes was compared in obese/non-obese PCOS patients. The results of obese PCOS women genotyping in the presented study show that BMI is significantly higher in the rare homozygotes genotype CC compared to heterozygous genotype TC and homozygotes genotype TT. Also, there are no significant difference ($P > 0.05$) when measuring the levels of glucose concentrations. While, insulin, C-peptide and HOMA-IR were significantly higher ($P < 0.001$) in the rare homozygotes genotype CC compared to heterozygous genotype TC and homozygotes genotype TT, Table 4. For non-obese PCOS women, the rare homozygotes genotype CC was had elevated BMI compared to the homozygotes genotype TT. Lipid profile does not correlate with CAPN-10, SNP-44 variant C. Also, there was no significant difference ($P > 0.05$) when measuring the levels of glucose and C-peptide concentrations. While, insulin and HOMA-IR were significantly higher ($P < 0.001$) in the rare homozygotes genotype CC compared to heterozygous genotype TC and homozygotes genotype TT, Table 5.

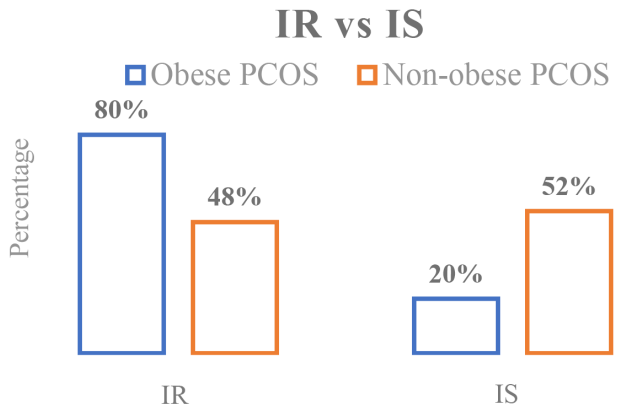

Fig. 1 **The prevalence of insulin resistance (IR) /sensitivity (IS) based on HOMA-IR in both obese and non-obese PCOS women.**

Table 2. **Hardy Weinberg Equilibrium test for study subjects**

	Common Homozygotes	Heterozygotes	Rare Homozygotes
Obese PCOS (*N* = 45)	**TT**	**TC**	**CC**
Frequency	26	12	7
P-value	0.063		
Non-obese PCOS (*N* = 23)			
Frequency	15	6	2
P-value	0.535		
Control (*N* = 52)			
Frequency	39	11	2
P-value	0.587		

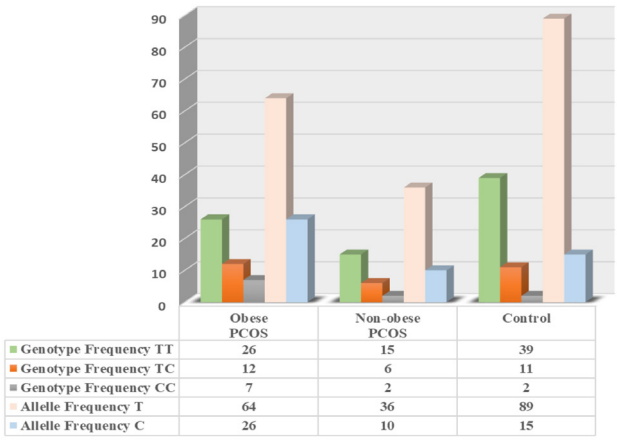

Fig. 2 **Comparison of genotypes frequencies among obese PCOS, non-obese PCOS and control subjects.**

Discussion

Poly cystic ovary syndrome has multiple components- reproductive, metabolic and cardiovascular with long term health implications. None are specific for PCOS, and we speculated that each component might be related with independent genetic risk factors.[16] Obesity in adolescence is associated with greater menstrual cycle irregularity and PCOS.[17]. Three main pathophysiologic components are strongly associated with PCOS: increased LH secretion, higher androgen levels and IR.[18] Hyperandrogenic phenotype of PCOS patients is influenced by both hormonal and metabolic dysfunctions.[19]

Insulin plays an important role in reproduction through its direct effect on ovarian granulosa and theca cells regulating ovulation and steroidogenesis in ovaries. Thus, IR is associated with hyperandrogenism in PCOS.[20] The same finding was recorded previously (Unluer et al., 2013),[21] who reported that IR in PCOS had linked to obesity and obese PCOS have a high probability of IR.[22] Although non-obese women exhibit lower IR is still a common finding in this population. Indeed, several studies have suggested IR as a pathophysiological component independent of weight.[23] The results of lipid profile confirm that PCOS patients might have dyslipidemia as mentioned in previous studies.[24] Rizzo et al. concluded that total cholesterol, triglyceride and LDL concentrations were higher and HDL levels were lower in controls versus PCOS.[25] It was demonstrated that obese women have elevated serum levels of cholesterol and LDL as compared with the corresponding levels in the normal weight group and higher triglycerides and lower HDL than normal or overweight PCOS women.[26] However, many women with PCOS still have a completely normal lipid profile and in larger studies of lipid levels in women with PCOS mostly fall within normal ranges.[27]

The current study supports a role of CAPN-10, SNP-44 in PCOS susceptibility.[28] Variation in the CAPN-10 gene was reported to be linked and associated with T2D susceptibility in

Table 3. **Genotype and allele frequency distribution of SNP-44 T/C (rs2975760) of CAPN10 gene in obese PCOS and control subjects**

Genotype	Obese PCOS		Control		OR	95% CI	*P*-value
TT	26	58%	39	75%		1 reference	
TC	12	27%	11	21%	1.64	0.63–4.26	0.313
CC	7	15%	2	4%	5.25	1.01–27.28	0.048
Total	45	100%	52	100%			
Allele frequency							
T	64	71%	89	86%			
C	26	29%	15	14%	2.41	1.18–4.91	0.015

OR, odd ratio; CI, confidence interval.

Table 4. **The differences in demographic and metabolic parameters in related to TT, TC and CC genotype in obese women with PCOS**

Biochemical parameters	Genotyping frequency			*P*-value
	TT	TC	CC	
BMI (kg/m^2)	33.81 ± 3.05	34.83 ± 2.57	38.75 ± 1.29	$P_a < 0.05$ $P_b < 0.05$
Total Cholesterol (mg/dl)	160 ± 29.32	162 ± 21.09	174 ± 26.27	NS
Triglycerides (mg/dl)	93 ± 1.52	105 ± 1.44	138 ± 1.16	NS
HDL-C (mg/dl)	41 ± 7.68	39 ± 4.81	39 ± 6.59	NS
LDL-C (mg/dl)	95 ± 1.31	98 ± 1.27	105 ± 1.29	NS
Glucose (mg/dl)	92 ± 9.69	92 ± 7.09	91 ± 3.87	NS
Insulin (µU/ml)	14.63 ± 1.49	16.66 ± 1.53	47.08 ± 1.27	$P_a < 0.001$ $P_b < 0.001$
C-peptide (pmol/L)	402.3 ± 1.57	378.2 ± 1.48	1067.9 ± 1.59	$P_a < 0.001$ $P_b < 0.001$
HOMA-IR	3.30 ± 1.52	3.76 ± 1.58	10.54 ± 1.29	$P_a < 0.001$ $P_b < 0.001$

P_a, (TT vs. CC); P_b, (TC vs. CC); P_c, (TT vs. TC).

Table 5. **The differences in demographic and metabolic parameters in related to TT, TC and CC genotype in non-obese women with PCOS**

Biochemical parameters	Genotyping frequency			*P*-value
	TT	TC	CC	
BMI (kg/m^2)	23.43 ± 2.15	25.49 ± 1.99	28.68 ± 0.22	$P_a < 0.01$
Total Cholesterol (mg/dl)	134 ± 23.58	143 ± 16.52	177 ± 29.69	NS
Triglycerides (mg/dl)	72 ± 1.47	91 ± 1.68	148 ± 1.23	NS
HDL-C (mg/dl)	39 ± 4.19	39 ± 6.84	37 ± 9.89	NS
LDL-C (mg/dl)	78 ± 1.27	84 ± 1.12	112 ± 1.43	NS
Glucose (mg/dl)	93 ± 9.93	93 ± 7.89	88 ± 2.82	NS
Insulin (µU/ml)	12.39 ± 1.45	18.06 ± 1.55	45.18 ± 1.45	$P_a < 0.001$ $P_b < 0.05$
C-peptide (pmol/L)	376.0 ± 1.91	461.7 ± 1.77	725.2 ± 1.18	NS
HOMA-IR	2.82 ± 1.45	4.10 ±1.57	9.88 ± 1.41	$P_a < 0.001$ $P_b < 0.05$

P_a, (TT vs. CC); P_b, (TC vs. CC); P_c, (TT vs. TC).

a Mexican American population.[29] In a case-control study involved patients with T2D and normal glucose tolerant subjects, no association of the SNP-44 variant with T2D was found. The frequency of the minor SNP-44 variant C was higher among T2D patients 18% compared with normal glycemic subjects 17%, however, these differences did not reach statistical significance.[30] While SNP-44 has been shown to predict the development of T2D in other study.[31]

Preliminary studies of CAPN-10 gene in PCOS patients provide the first evidence of CAPN-10 involvement in PCOS,

suggesting a statistically significant association between the SNP-44 and PCOS susceptibility.[13] Subsequent studies evaluating the role of CAPN-10 in PCOS have yielded contradictory results. Supporting the CAPN10 gene involvement in PCOS, Gonzalez et al. showed that CAPN-10, SNP-44 allele was associated with PCOS in the Spanish population.[32] Lipid profile does not correlate with CAPN-10 SNP-44 variant C, indicating that this variant does not confer risk to dyslipidemia in PCOS. Although, the associated genetic variation might serve as useful diagnostic tools, it does not necessarily mean that there is an etiological link between a certain allele or genotype and a given trait or disease. In previous studies, CAPN-10 polymorphisms have been found to correlate with several aspects of insulin secretion. However, possible mechanisms whereby calpain-10 modulates insulin secretion was not provided.[33] Although many genes have been researched as potential susceptibility loci, the impact of any one gene may be minimal since PCOS is in fact a complex genetic disorder.

Conclusion

Insulin resistance is commonly associated with PCOS independently of obesity. In obese PCOS, the C allele was associated with higher insulin secretion and HOMA-IR as compared with the T allele. The C allele might be involved in the pathophysiology of insulin resistance in PCOS.

Conflict of Interest

None.

References

1. Azziz, R., Woods, K. S., Reyna, R., Key, T. J., Knochenhauer, E. S. And Yildiz, B. O. 2004. The Prevalence and Features of The Polycystic Ovary Syndrome In An Unselected Population. The Journal of Clinical Endocrinology and Metabolism, 89, 2745–2749.
2. Legro, R. S., Arslanian, S. A., Ehrmann, D. A., Hoeger, K. M., Murad, M. H., Pasquali, R. and Welt, C. K. 2013. Diagnosis And Treatment Of Polycystic Ovary Syndrome: An Endocrine Society Clinical Practice Guideline. The Journal Of Clinical Endocrinology and Metabolism, 98, 4565–4592.
3. March, W. A., Moore, V. M., Willson, K. J., Phillips, D. I., Norman, R. J. and Davies, M. J. 2010. The Prevalence Of Polycystic Ovary Syndrome In A Community Sample Assessed Under Contrasting Diagnostic Criteria. Human Reproduction, 25, 544–551.
4. Fauser, B. C., Tarlatzis, B. C., Rebar, R. W., Legro, R. S., Balen, A. H., Lobo, R., Carmina, E., Chang, J., Yildiz, B. O. and Laven, J. S. 2012. Consensus on Women's Health Aspects of Polycystic Ovary Syndrome (PCOS): The Amsterdam Eshre/Asrm-Sponsored 3rd PCOS Consensus Workshop Group. Fertility and Sterility, 97, 28–38. E25.
5. Mayer, S. B., Evans, W. S. and Nestler, J. E. 2015. Polycystic Ovary Syndrome And Insulin: Our Understanding In The Past, Present And Future. Women's Health, 11, 137–149.
6. Amato, M. C., Vesco, R., Vigneri, E., Ciresi, A. and Giordano, C. 2015. Hyperinsulinism and Polycystic Ovary Syndrome (PCOS): Role of Insulin Clearance. J Endocrinol Invest, 38, 1319–26.
7. Spritzer, P. M. 2014. Polycystic Ovary Syndrome: Reviewing Diagnosis And Management Of Metabolic Disturbances. Arq Bras Endocrinol Metabol, 58, 182–7.
8. Amisi, C. A. 2022. Markers of Insulin Resistance in Polycystic Ovary Syndrome Women: An Update. World Journal of Diabetes, 13, 129.
9. Barber, T. M. and Franks, S. 2021. Obesity and Polycystic Ovary Syndrome. Clinical Endocrinology, 95, 531–541.
10. Jones, M. R. and Goodarzi, M. O. 2016. Genetic Determinants Of Polycystic Ovary Syndrome: Progress And Future Directions. Fertility And Sterility, 106, 25–32.
11. Franks, S., White, D., Gilling-Smith, C., Carey, A., Waterworth, D. and Williamson, R. 1996. Hypersecretion Of Androgens By Polycystic Ovaries: The Role of Genetic Factors in The Regulation of Cytochrome P450c17α. Bailliere's Clinical Endocrinology and Metabolism, 10, 193–203.
12. Haddad, L., Evans, J. C., Gharani, N., Robertson, C., Rush, K., Wiltshire, S., Frayling, T. M., Wilkin, T. J., Demaine, A. and Millward, A. 2002. Variation within The Type 2 Diabetes Susceptibility Gene Calpain-10 and Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology and Metabolism, 87, 2606–2610.
13. Haddad, L., Gharani, N., Evans, J., Cela, E. and Franks, S. M M Linkage And Association of Variants of The Diabetes Susceptibility-Gene Niddm1 CAPN10 with Polycystic Ovarian Syndrome in European Parent-Offspring Trios. 82nd Annual Meeting of The Endocrine Society, Toronto, Ontario, Canada, 2000. 75.
14. Broekmans, F., Knauff, E., Valkenburg, O., Laven, J., Eijkemans, M. and Fauser, B. 2006. PCOS According to The Rotterdam Consensus Criteria: Change in Prevalence Among WHO-II Anovulation and Association with Metabolic Factors. Bjog: An International Journal of Obstetrics and Gynaecology, 113, 1210–1217.
15. Stener-Victorin, E., Zhang, H., Li, R., Friden, C., Li, D., Wang, W., et al. 2019. Acupuncture or metformin to improve insulin resistance in women with polycystic ovary syndrome: study protocol of a combined multinational cross sectional case-control study and a randomised controlled trial. BMJ Open, 9(1), e024733.
16. Franks, S., Gharani, N., Waterworth, D., Batty, S., White, D., Williamson, R. and Mccarthy, M. 1997. The Genetic Basis of Polycystic Ovary Syndrome. Human Reproduction (Oxford, England), 12, 2641–2648.
17. Itriyeva, K. 2022. The Effects Of Obesity On The Menstrual Cycle. Current Problems In Pediatric And Adolescent Health Care, 101241.
18. Crisosto, N., De Guevara, A. L., Echiburú, B., Maliqueo, M., Cavada, G., Codner, E., Paez, F. and Sir-Petermann, T. 2019. Higher Luteinizing Hormone Levels Associated with Antimüllerian Hormone In Postmenarchal Daughters of Women with Polycystic Ovary Syndrome. Fertility and Sterility, 111, 381–388.
19. Hestiantoro, A., Karimah, P. D., Shadrina, A., Wiweko, B., Muharam, R. and Astuti, B. P. K. 2019. Triglycerides, Independent of Ferriman Gallwey Score, Is A Main Determinant of Free Testosterone Index in PCOS. F1000research, 8.
20. Shorakae, S., Ranasinha, S., Abell, S., Lambert, G., Lambert, E., De Courten, B. and Teede, H. 2018. Inter-Related Effects Of Insulin Resistance, Hyperandrogenism, Sympathetic Dysfunction And Chronic Inflammation In Pcos. Clinical Endocrinology, 89, 628–633.
21. Unluer, A., Findik, R., Sevinc, N. and Karakaya, J. 2013. Comparison Of Hba1c Levels In Obese And Non-Obese Polycystic Ovarian Patients. Clinical And Experimental Obstetrics and Gynecology, 40, 148–150.
22. Dahan, M., Abbasi, F. and Reaven, G. 2007. Prevalence of Insulin Resistance among American Women with Polycystic Ovary Syndrome (PCOS) as a Function of Body Mass Index (BMI). Fertility and Sterility, 88, S78–S79.
23. Johnson, P. J., Wiedmeyer, C. E., LaCarrubba, A., Ganjam, V. S., & Messer IV, N. T. 2012. Diabetes, insulin resistance, and metabolic syndrome in horses. Journal of Diabetes Science and Technology, 6(3), 534–540.
24. Liu, Q., Xie, Y.-J., Qu, L.-H., Zhang, M.-X. and Mo, Z.-C. 2019. Dyslipidemia Involvement In The Development Of Polycystic Ovary Syndrome. Taiwanese Journal Of Obstetrics And Gynecology, 58, 447–453.
25. Rizzo, M., Berneis, K., Hersberger, M., Pepe, I., Di Fede, G., Rini, G. B., Spinas, G. A. and Carmina, E. 2009. Milder Forms Of Atherogenic Dyslipidemia In Ovulatory Versus Anovulatory Polycystic Ovary Syndrome Phenotype. Human Reproduction, 24, 2286–2292.
26. Castelo-Branco, C., Steinvarcel, F., Osorio, A., Ros, C. and Balasch, J. 2010. Atherogenic Metabolic Profile in Pcos Patients: Role of Obesity and Hyperandrogenism. Gynecological Endocrinology, 26, 736–742.
27. Legro, R. S., Azziz, R., Ehrmann, D., Fereshetian, A. G., O'keefe, M., Ghazzi, M. N. and Group, P. T. S. 2003. Minimal Response Of Circulating Lipids In Women With Polycystic Ovary Syndrome To Improvement In Insulin Sensitivity With Troglitazone. The Journal Of Clinical Endocrinology and Metabolism, 88, 5137–5144.
28. Simoni, M., Tempfer, C., Destenaves, B. and Fauser, B. 2008. Functional Genetic Polymorphisms And Female Reproductive Disorders: Part I: Polycystic Ovary Syndrome And Ovarian Response. Human Reproduction Update, 14, 459–484.
29. Horikawa, Y., Oda, N., Cox, N. J., Li, X., Orho-Melander, M., Hara, M., Hinokio, Y., Lindner, T. H., Mashima, H. and Schwarz, P. E. 2000. Genetic Variation In The Gene Encoding Calpain-10 Is Associated With Type 2 Diabetes Mellitus. Nature Genetics, 26, 163–175.

30. Jensen, D. P., Urhammer, S. A., Eiberg, H., Borch-Johnsen, K., Jørgensen, T., Hansen, T. and Pedersen, O. 2006. Variation In Capn10 In Relation To Type 2 Diabetes, Obesity And Quantitative Metabolic Traits: Studies In 6018 Whites. Molecular Genetics And Metabolism, 89, 360–367.
31. Lyssenko, V., Almgren, P., Anevski, D., Orho-Melander, M., Sjögren, M., Saloranta, C., Tuomi, T., Groop, L. and Group, B. S. 2005. Genetic Prediction Of Future Type 2 Diabetes. Plos Medicine, 2, E345.
32. Gonzalez, A., Abril, E., Roca, A., Aragón, M. J., Figueroa, M. J., Velarde, P., Ruiz, R. O., Fayez, O., Galán, J. J. and Herreros, J. A. 2003. Specific Capn10 Gene Haplotypes Influence The Clinical Profile of Polycystic Ovary Patients. The Journal of Clinical Endocrinology and Metabolism, 88, 5529–5536.
33. Neumeyer, S., Hemani, G. and Zeggini, E. 2020. Strengthening Causal Inference For Complex Disease Using Molecular Quantitative Trait Loci. Trends In Molecular Medicine, 26, 232–241.