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Study of the common variant rs9939609 of *FTO* gene polymorphism in Polycystic Ovary Syndrome

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ABSTRACT

Background: Genes associated with obesity might play a role in pathogenesis of PCOS such as fat mass and obesity associated (*FTO*) gene. *Objective:* To investigate the association between the common variant rs9939609 of *FTO* gene with PCOS in Egyptian women in respect to obesity and insulin resistance. *Methods:* This study was conducted on (50) Egyptian female patients diagnosed as PCOS according to the criteria of Rotterdam Revised (2003) (Group I, patients) and (50) age-matched apparently healthy females (Group II, controls). PCOS patients were classified according to obesity as well as insulin resistance. Detection of FTO rs9939609 polymorphism was done by real time polymerase chain reaction. *Results:* This study revealed a significant difference between group I and group II as regards *FTO* genotypes A/A, A/T and T/T with increase in frequency of A/T and T/T genotypes in PCOS patients (56% A/T and 8% T/T) compared with controls (4% and 0% respectively). *Conclusion: FTO* gene variant rs9939609 is associated with PCOS susceptibility in Egyptian women. The association has been demonstrated in both obese and non-obese patients as well as in PCOS patients with insulin resistance and non-insulin resistance.

Keywords: *FTO*, Single nucleotide polymorphism, rs9939609, PCO

1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the common endocrine diseases in reproductive-age women. PCOS prevalence reaches 8-13% in different ethnic populations (Lim et al., 2019). It is a multifactorial disorder, characterized by menstrual disturbances as well as clinical manifestations of hyper androgenism and polycystic ovaries (Allahbadia and Merchant, 2011 and Mani et al., 2018). The etiologies of PCOS have not been totally elucidated. It frequently coexists with obesity and insulin resistance. It is found that around half of the reported cases of PCOS women are overweight or obese. This implies that obesity is an important role in disease etiology. The evidence from family-based suggests that obesity and PCOS have a significant inherited etiology, due to a shared genetic predisposition contributing to their co-occurrence (Wehr et al., 2010 and Goodman et al., 2015; Reem, 2019). Therefore, improvements in insulin resistance are linked with clinical improvements in patients with PCOS (Morley et al., 2017). Changing lifestyle is recommended to prevent obesity, manage weight and guard against PCOS reproductive and metabolic complications (International PCOS Guideline 2018).

Fat mass and obesity-associated (*FTO*) gene discovery was an important major success in the aera of obesity genetics (Frayling et al., 2007 & Scuteri et al., 2007). The *FTO* gene is located on chromosome 16. It is expressed in a wide range of tissues; the adipose tissue and specific sites in the brain and muscles, suggesting its potential role in fat metabolism and body weight regulation (Wehr et al., 2010 and Cheung et al., 2013 and Daoud et al., 2016). *FTO* gene is reported to be highly polymorphic as several polymorphisms of the gene have been described. *FTO* rs9939609 variant is present in the first intron *of FTO* gene. It has two alleles, A and T alleles (De Luis et al., 2013). Previous studies were conducted to prove the impact *FTO* variants on the risk of PCOS, however controversial results were reported among different ethnic groups.

Aim of the Work

To investigate the association between the *FTO* gene rs9939609 variant with polycystic ovary syndrome in Egyptian women in order to estimate the possible role of *FTO* gene variants in PCOS susceptibility and severity in respect to obesity and insulin resistance.

2. METHODOLOGY

This study was conducted on 50 Egyptian female with PCOS (Group I, mean age: 26 ± 3.8 years) who were referred to the Gynecology and Obstetrics department & Endocrine clinics of Ain Shams University hospitals, from the first of May 2016 till the end of January 2018. They were diagnosed as PCOS according to the criteria of Rotterdam Revised (2003). This proposes a diagnosis of PCOS when a patient meets two of the following three criteria: 1) Oligomenorrhea or amenorrhea for at least 6 months. Oligomenorrhea was defined as a reduction in the frequency of menses with interval between 40 days and 6 months. 2) Clinical and/or biochemical signs of hyperandrogenism (Increased level of total testosterone and LH/FSH ratio ≥ 2.0). 3) Polycystic ovaries on ultrasound (the presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter) and/or increased ovarian volume (>10 ml). This group was classified according to Body Mass Index (BMI) into two subgroups; subgroup Ia (obese patients, BMI ≥ 30, mean age of 27.5 ± 4.3 years, n= 24) and subgroup Ib (obese patients, BMI < 30, mean age of 25.8 ± 3.3 years, n=26). Moreover,

PCOS patients were also classified according to Homeostasis Model Assessment of Insulin Resistance (HOMA IR) into insulin resistant (HOMA IR ≥ 2.7, n=12) and non-insulin resistant (HOMA IR < 2.7, n=38) patients.

This study was also conducted on 50 age-matched apparently healthy females served as a control group (Group II, mean age of 26.3 ± 2.9 years). They have a normal menstrual cycle and ovarian morphology, and no family history of abnormal menses or hirsutism. None of the control group had evidence of acne, hirsutism, alopecia or any other endocrine dysfunction. Selection Criteria for Subjects: Patients were in their reproductive age, non-pregnant and none of them were on hypoglycemic or hormonal therapy (including oral contraceptives) or undergone ovulation induction for at least 3 months before contributing in the study. Exclusion Criteria: Exclusion criteria for subjects included other causes of hyperandrogenism and ovulatory dysfunction, i.e. Androgen-secreting tumors, thyroid diseases and Cushing's syndrome, hyperprolactinemia.

All individuals included in this study were subjected to: a. Full history taking with special emphasis on menstrual history and medication history; b. Physical examination; c. Anthropometric measures: weight and height were measured. Body Mass Index (BMI) was estimated: the weight (kilograms) divided by the square of height (meters). A cut - off of BMI 30 was chosen to differentiate obese from non-obese (Cynthia et al., 2014). d. Radiological examination: Pelvic ultrasound. e. Laboratory investigations:

a. Hormonal assay of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), prolactin, fasting insulin and serum total testosterone. These hormones were measured on Advia Centaur CP immunoassay system (Siemens Healthcare GmbH Henkestr. 127, 91052, Erlangen, Germany). The ADVIA Centaur assay of FSH, LH, prolactin, insulin is a two-site sandwich immunoassay using direct chemiluminescent technique whereas for total testosterone assay, a competitive immunoassay by direct chemiluminescent technique is used.

b. Fasting glucose and calculation of HOMA IR

HOMA-IR= [Glucose in mg/dl,] X [Insulin in μU/mL] / 405.

c. Detection of *FTO* rs9939609 polymorphism by Real Time Polymerase Chain Reaction (PCR). This process was done through two steps including DNA extraction then amplification and detection.

First step (DNA extraction): Two milliliters of the whole blood were taken in EDTA coated tube for extraction of DNA and performing FTO genotyping. DNA was extracted manually according to manufacturer instructions using QIAamp DNA blood mini kit supplied by QIAGEN (Strasse 1, 40724 Hilden, Germany) as 20 μl QIAGEN protease was pipette first into the bottom of a 1.5 ml microcentrifuge tube and 200 μl of sample was added to it and the extraction steps was followed according to manufacturer instructions. Finally, measuring of DNA concentration and purity using Nano drop spectrophotometer (NanoDrop products 3411 Silverside Rd, Bancroft Building Wilmington, DE 19810, USA) was performed and the eluted purified DNA was stored at -80°C until used.

Second step (Amplification and detection): Amplification and detection of FTO gene genotyping was performed by Real Time PCR technique using Applied Biosystem 7500 Foster instrument, TaqMan® Universal Master Mix II supplied by Applied Biosysystem (81 Wyman Street Waltham, MA, USA). Reagents supplied include: 1) TaqMan® Universal Master Mix II that is used to amplify complementary DNA (cDNA) and DNA targets for genotyping as it contains AmpliTaq Gold® DNA Polymerase, dNTPs, ROX™ Passive Reference and optimized buffer components. 2) Allelic discrimination Assay: ready to use (specific cat. No. C_30090620_10) that include Sequence-specific forward and reverse primers to amplify the polymorphic of *FTO* rs9939609, in addition to two modified TaqMan probes; one probe matches the A Allele sequence and another probe matches the T Allele sequence. Each TaqMan probe contains a reporter dye at the 5´-end (VIC® dye is linked to the 5´-end of the Allele A probe and FAM™ dye is linked to the 5´-end of the Allele T probe) and a non-fluorescent quencher located at the 3´-end of the probe.

Principle of the assay: During PCR, each probe anneals specifically to complementary sequences between the forward and reverse primer sites. AmpliTaq Gold ® DNA polymerase cleaves only probes which hybridize to the target. Cleavage can separate the reporter dye from the quencher dye with subsequent increase in the fluorescence by the reporter. Thus, the fluorescence signal(s) generated by PCR amplification indicate(s) the sequences that are present in the sample (Livak, 1999).

According to manufacturer instructions, for each sample, PCR reaction mix component was pipetted to a sterile 2mL micro centrifuge tube. PCR reaction mix component total volume per reaction was 20 μL that include; TaqMan® Universal Master Mix II (10.0 μL), TaqMan® Assay, 20X (1.0 μL), DNA template (2.0 μL), RNase-free water (7.0 μL). After mixing and brief centrifugation, 20 μL of PCR reaction mix from each sample was transferred to the associated wells of the 96-well sample block. The plate was centrifuged briefly. The plate was loaded into the real-time PCR system (Applied Biosystem 7500). Thermal cycling parameters for allelic discrimination assays include polymerase activation at 95 °C for 10 min then 40 PCR cycles (denature at 95 °C for 15 sec and anneal/extend at 60°C for 1 min).

Genotype analysis: The system software records the results of the genotyping run on a scatter plot of A Allele (labeled by VIC) versus T Allele (labeled by FAM). Each well of the 96-well reaction plate is illustrated as an individual dot on the plot, as shown in Figure 1.

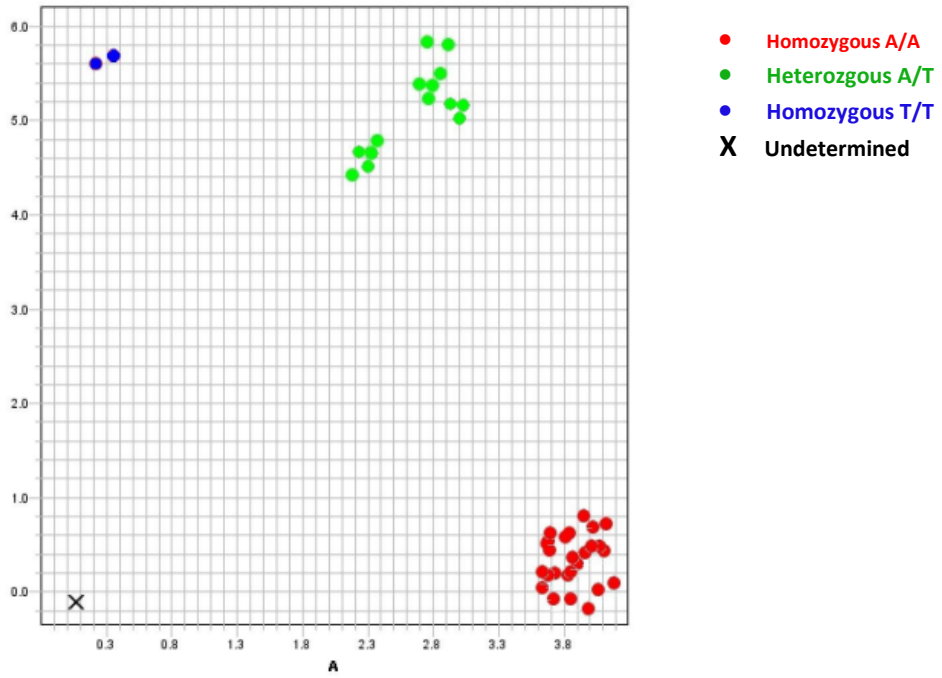

Figure 1 Allelic discrimination plot (*FTO* SNP assay: rs9939609)

Statistical Methods: IBM SPSS statistics (V.21.0, IBM Corp., USA, 2012) was used for data analysis. Descriptive statistics of the different studied groups were done using the mean and standard deviation for parametric data. Comparative statistics between two independent mean groups for parametric data was done using Student t test. One-way analysis of variance (ANOVA) test was used for quantitative data comparison in the different subgroups. For comparing qualitative data in different groups, Chi Squared test/ Fisher exact was used. Measurement of association of data between different groups was assessed by means of Odds ratio test. A probability value (p value) less than 0.05 was considered significant and p value less than 0.01 was considered highly significant.

3. RESULTS

In the present study, A highly significant increase in serum levels of LH, PRL, fasting insulin as well as increased ratio of HOMA IR and BMI was observed in PCOS patient group when compared with the control group ($p<0.01$). In addition, a significant increased in serum fasting glucose level was observed in PCOS patient group versus control group ($p<0.05$). However, no significant difference was observed between the two groups as regards serum FSH, total testosterone and height ($p>0.05$, Table 1).

The *FTO* SNP rs9939609 was analyzed in all individuals of this study using real time PCR. Three genetic variations were detected (A/A, A/T and T/T genotypes). The genotypes distribution between PCOS patients and control group is shown in Figure 2. The genotype analysis showed that AA genotype was significantly less frequent in PCOS patients than control group (36% vs. 96%; respectively; $P<0.01$). While, a significant difference in distribution of both A/T and T/T genotypes was observed between PCOS patient versus control group (A/T= 56% and TT= 8% in PCOS patient versus 4% and 0% in control group; respectively; $p<0.01$).

For the small number of T/T genotype in our study, we combined the T/T and A/T genotypes as a dominant model to analyze the association of *FTO* rs939609 genotypes with PCOS patients. The (A/T+T/T) genotypes frequencies were significantly greater in PCOS patients compared to the controls (64% vs 4%; (A/T+T/T) vs. A/A genotype, OR = 42.7 [95% CI 4.9-370.2], $p < 0.001$; Table 2).

Moreover, a highly significant increase in frequency of T allele was detected in PCOS patient group compared with control group (36% in patient vs. 2% in control, $X^2=18.8$, p-value <0.001) with OR = 27.6, [95% CI 3.5-216.8], $P<0.001$; Table 2). In comparing subgroup Ia versus Ib, the genotype analysis showed that A/A genotype was more frequent in non-obese subgroup than obese PCOS patients (46.2% vs. 25%, respectively). On the contrary, A/T genotype was more frequent in obese versus non-obese PCOS patient (75% vs. 38.5%, respectively); however; these observed discrepancies in *FTO* genotype distribution did not reach a significant

difference (X^2= 4.9, P >0. 05). In addition, no significant difference was detected in comparing FTO genotypes A/A and both genotypes (A/T+T/T) distribution between obese subgroup versus non-obese subgroup (X2 =1.2, p-value > 0.05). Moreover, the T allele carrier genotype (A/T+T/T genotypes) was more frequent in obese PCOS in respect to non-obese with no significant association (75% versus 54%; (A/T+T/T) vs. A/A genotype, OR = 2.6 [95% CI: 0.47-14.1], p>0.05; Table 3).

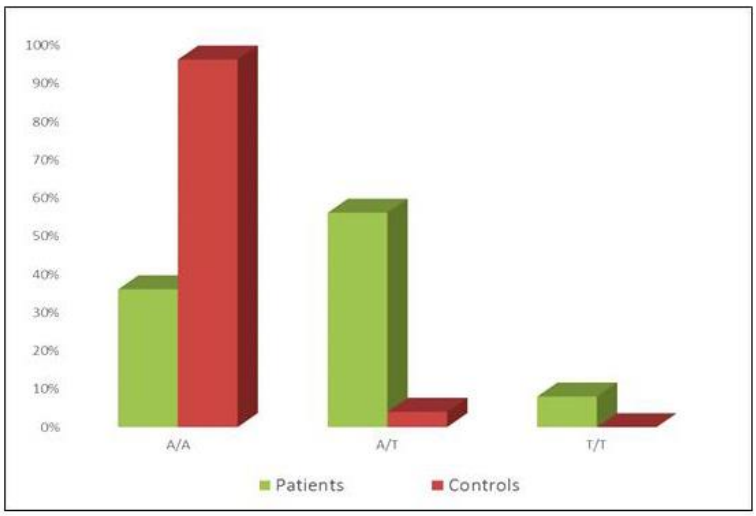

Figure 2 *FTO* genotypes in PCOS patients and controls

Table 1 Descriptive and comparative analysis of different studied parameters between PCOS patients (Group I) versus controls (Group II)

Parameters	**Group I (n=50)**	**Group II (n=50)**	**Student t-test**	
	Mean ± SD	**Mean ± SD**	**t**	**P-value**
FSH (mIU/mL)	5.8±1.3	7.5±4.3	-1.8	0.067
LH (mIU/ mL)	11.1±2.7	5.1±3.2	7.13	0.0**
Prolactin (µg/L)	13.5±6.3	7.8±2.9	4.04	0.0**
Total Testosterone (mmol/L)	2.7±1.0	2.5±0.26	1.0	0.32
Fasting Glucose (mg/dL)	123±46.6	100.7±19.4	2.2	0.032*
Fasting Insulin (µU/mL)	9.1±3.8	6.3±2.1	3.2	0.003*
HOMA- IR	3.0±2.4	1.6±0.6	2.9	0.006*
Weight (Kg)	86.8±10.0	79.0±7.4	3.1	0.003*
Height (Meter)	1.67±0.6	1.673±0.1	-0.16	0.867
BMI	31.1±2.8	28.2±2.1	4.0	0.0**

t: Student t Test for parametric data. p >0.05: non-significant difference (NS). *p <0.05: significant difference (S). **p<0.01: highly significant difference (HS).

Table 2 Genotype and Allele Frequencies of rs9939609 Polymorphism in FTO Gene in PCOS Patients (group I) versus Controls (group II)

	Group I (n=50)		**Group I (n=50)**		**Total**		**X^2**	**P-value**	**OR (CI 95%)**
	N	**%**	**N**	**%**	**N**	**%**			
FTO genotypes									
A/A	18	36	48	96	66	66	20.1	< 0.0*	42.7 (4.9-370.2)*
A/T+T/T	32	64	2	4	34	34			
FTO alleles									
A allele	64	64	98	98	162	81	18.8	< 0.01*	27.6 (3.5-216.8)**
T allele	36	36	2	2	38	19			

X^2: Chi square test / Fisher Exact, *p<0.001: highly significant difference (HS), CI: Confidence Interval

OR*: Odds Ratio revealing the frequency of association of genotype (A/T + T/T) in PCOS patient as that of control, OR**: Odds Ratio revealing the frequency of association of T allele in PCOS patients as that of controls

Table 3 Comparative analysis of *FTO* genotypes and alleles between obese (Subgroup Ia) versus non-obese (Subgroup Ib) PCOS patients

	Subgroup Ia (n=24)		Subgroup Ib (n=26)		Total		X^2	P-value	OR (CI 95%)
	N	%	N	%	N	%			
FTO genotypes									
A/A	6	25	12	46	18	36	1.2	0.277	2.6 (0.47 to 14.1)*
A/T+T/T	18	75	14	54	32	64			
FTO alleles									
A allele	30	62.5	34	65.38	64	64	0.01	0.934	1.13 (0.35 to 3.6)**
T allele	18	37.5	18	34.62	36	36			

Subgroup Ia (obese) BMI ≥ 30 and subgroup Ib (non-obese) BMI < 30, $X^{2:}$ Chi Square test/ Fisher Exact. P <0.05: non-significant difference (NS), OR*: Odds Ratio revealing the frequency of association of genotype (A/T+ T/T) in obese subgroup as that of non-obese, OR **: Odds Ratio revealing the frequency of association of T allele in obese subgroup as that in non-obese
CI: Confidence Interval

Furthermore, no significant difference was detected in A allele and T allele frequencies between both subgroups (X^2=0.01, p-value > 0.05) and no significant association of T allele was detected in obese PCOS patients (OR: 1.13, [95% CI 0.35-3.6], P > 0.05; Table 3). The *FTO* genotypes distribution in insulin resistant and non-insulin resistant PCOS patients was also assessed in our studied population. The AA genotype in insulin resistant versus non-insulin resistant was 33% versus 37%; respectively, with no significant difference (X^2 = 0.024, P > 0.05). Moreover, there was no significant association of genotypes (A/T+T/T) with insulin resistant PCOS patients (OR = 1.17, [95% CI 0.12-8.1], P > 0.05, Table 4).

Table 4 Comparative analysis of *FTO* genotypes and Alleles between insulin resistant versus non- insulin resistant PCOS patients

	Insulin resistant (n=12)		Non-insulin resistant (n=38)		Total		X^2	P-value	OR (CI 95%)
	N	%	N	%	N	%			
FTO genotypes									
A/A	4	33	14	37	18	36	0.024	0.876	1.17 (0.12 to 8.1)*
A/T+T/T	8	67	24	63	32	64			
FTO alleles									
A allele	16	66.7	48	63.2	64	64	0.02	0.902	0.86 (0.22 to 3.37)**
T allele	8	33.3	28	36.8	36	36			

Non-insulin resistant: HOMA IR < 2.7 and Insulin resistant ≥ 2.7, OR*: Odds Ratio revealing the frequency of association of genotype (A/T + T/T), with insulin resistant as that of non-insulin resistant PCOS patients, OR**: Odds Ratio revealing the frequency of association of T allele in insulin resistant as that in non-insulin resistant group in PCOS patients, CI: Confidence Interval, $X^{2:}$ Chi Square test/ Fisher Exact, P> 0.05: non-significant difference (NS)

Similarly, no significant difference was detected in allelic distribution between insulin resistant and non-insulin resistant PCOS patients (X^2 = 0.02, p > 0.05) with no significant association of T allele in insulin resistant PCOS patients (OR= 0.86, [95% CI 0.22-3.37], P > 0.05; Table 4). In our study, there was no differences existing in the parameters of endocrine and metabolic characteristics among *FTO* gene genotypes (A/A, A/T and T/T) (P > 0.05, Table 5).

4. DISCUSSION

Fat mass and obesity-associated gene (*FTO*) is linked to obesity, especially the common variant rs9939609. Polycystic ovary syndrome (PCOS) is a complex endocrine-metabolic disorder that is associated with obesity. Therefore, *FTO* is considered a potential candidate gene for PCOS; however its association with PCOS is still confusing and needs to be clarified in different ethnic population (Chen and Fang, 2018). In the current study, *FTO* rs9939609 polymorphism is associated with PCOS in Egyptian women, not only in obese, but also in non-obese. This association was detected also in the presence or absence of insulin resistance in such patients. A

significant difference was observed among *FTO* gene genotypes in PCOS patients vs. controls, as genotypes (A/T+T/T) were more frequently associated with PCOS patients.

Table 5 Comparative analysis of different studied parameters in relation to FTO genotypes

Parameters	A/A	A/T	T/T	ANOVA	
				F	p-value
Age	26.222±4.116	26.643±3.629	28.500±6.364	0.269	0.767
FSH (mIU/mL)	6.111±1.504	5.519±1.212	6.300±1.414	0.696	0.509
LH (mIU/ mL)	12.189±2.408	10.100±2.666	12.150±2.475	2.027	0.156
Prolactin (µg/L)	17.167±8.097	11.150±4.156	13.200±1.273	2.909	0.076
Total Testosterone (mmol/L)	3.000±1.057	2.614±1.071	2.150±0.919	0.673	0.520
Fasting Glucose (mg/dL)	124.333±40.293	125.571±53.970	99.500±0.707	0.262	0.772
Fasting Insulin (µU/mL)	8.576±3.906	9.896±3.808	5.600±2.121	1.251	0.306
HOMA- IR	2.911±2.381	3.371±2.630	1.375±0.516	0.587	0.565
Weight (Kg)	86.778±8.927	87.571±11.291	81.500±7.778	0.301	0.743
Height (Meter)	1.681±0.057	1.660±0.063	1.695±0.078	0.500	0.614
BMI	30.667±2.343	31.721±3.176	28.350±0.071	1.395	0.269

F: ANOVA test for parametric test, *P* <0.05: non-significant difference (NS)

These results were in agreement with previous Chinese studies of Li et al. (2013) and Tao et al. (2013). They found an association between FTO rs9939609 polymorphism and PCOS susceptibility with no role of obesity in this association. This indicated that *FTO* might interact with PCOS directly. On the other side, a previous study by Yan et al. (2009) conducted on Chinese women as well as a study by Barber et al. (2008) on UK population detected a significant association between *FTO* rs9939609 polymorphism and PCOS, especially in obese patients. However, no association of *FTO* rs9939609 polymorphism with PCOS susceptibility has been observed in Caucasian and Korean population (Saxena et al., 2013, and Kim et al., 2014). It was reported that *FTO* rs9939609 are not a major determinant of PCOS, however, *FTO* rs9939609 variant allele was associated with increased BMI, suggesting that *FTO* polymorphism is linked to PCOS risk indirectly by affecting BMI (Kim et al., 2014). A previous meta-analysis reported that *FTO* rs9939609 polymorphism might not be related to PCOS risk in all population. However, in East Asians, a direct association between *FTO* gene polymorphism and PCOS susceptibility could exist, independent of BMI (Chen and Fang, 2018). Therefore, the relation among PCOS, BMI / obesity and *FTO* rs9939609 A/T polymorphism is still unclear.

Numerous case-control studies have reported the associations between *FTO* gene rs9939609 A/T polymorphism and PCOS, however, without a distinct result. *FTO* might contribute to PCOS directly or through the combined direct and indirect ways. More studies are needed to clarify this association (Liu et al., 2017). Moreover, the discrepancies detected among studies revealed that *FTO* gene association with obesity in PCOS patients could be affected by the genetic load among different ethnic population (Yan et al., 2009 and Claussnitzer et al., 2015 and Bego et al., 2019). Obesity is associated with hyperandrogenism and menstrual disturbance of PCOS and it can worsen PCOS complications as Type 2 DM (Li et al., 2013). In addition, insulin resistance may also contribute to endocrine dysfunction and elevated androgen level which, in turn, may increase visceral obesity which may have synergetic effect on PCOS by *FTO* function. Therefore, *FTO* may be one of the molecular determinants connecting obesity to PCOS (Bego et al., 2019).

FTO gene, as confirmed by our study, confers risk to the pathogenesis of PCOS. It acts as a transcriptional co-activator in epigenetic regulatory mechanism and nucleic acid repair or modification processes, which is important in modulating the hyperandrogenism status and is involved in the ovarian dysfunction of PCOS (Gerkin et al., 2007). Therefore, the effect of *FTO* at the molecular aspect and epigenetic regulation suggests that *FTO* could be a pleiotropic factor involved in various diseases such as PCOS, obesity and T2DM. However, the mechanism and biological pathways of *FTO* protein in PCOS has not been fully elucidated (Zhang et al., 2018 and Lim et al., 2019).

In our study, significant differences in allelic and genotypic distributions of *FTO* gene were detected. The T carrier allele (A/T+T/T) genotypes was significantly more frequently associated with PCOS patients than that of controls (64%, OR = 42.7 [95% CI 4.9-370.2], P < 0.001) with no significant difference in its frequency of association between patient subgroup; obese vs. non-obese or insulin resistant vs. non-insulin resistant. On the contrary to our findings, A carrier allele of *FTO* gene was more frequently associated with PCOS susceptibility compared to T allele in previous studies done by Yan et al. (2009) conducted on 215 Chinese women (OR = 1.62 [95% CI 1.1-2.43], P = 0.019) and Barber et al. (2008) conducted on 463 UK women (OR = 1.30 [95% CI 1.12-1.51],

p < 0.01). Moreover, meta-analysis studies reported that A allele of rs9939609 A/T polymorphism of *FTO* gene may be associated with PCOS risk (Cai et al., 2014). A more recent study confers A allele to be linked with PCO patients and T allele was significantly higher in controls with significant correlation between *FTO* gene and BMI in Sri Lankan women, probably due to its effect on body mass index (BMI) (Umayal et al., 2017), whereas, Zhang et al. (2018) detected increased frequency of TT genotype and T allele in obese group than control group in ethnic Mongolians.

The differences in allelic frequency among studies may be due to the different genetic background between ethnicities and the power of the study (sample size). In addition, the potential sources of heterogeneity in the studies should include other determinants such as BMI, waist-to-hip ratio (WHR) as well as insulin resistance. As in our study, most PCOS patients are non-insulin resistance; this may explain the difference in allelic distribution between our study and another population studied. However, because of limited data, these factors cannot be explored in previous studies due to insufficient information. Finally, it is well known that the etiology of PCOS involving in genetic and environmental interactions that needs more investigations (Liu et al., 2017).

Although Wehr et al. (2010) showed increased hyperandrogenemia indices in PCOS along with rs9939609 variant, it was not observed in our study and a previous study done by Li et al. (2013). The reason may confer to the differences in endocrine and metabolic profiles among different ethnic population. In addition, subjects enrolled in our study were reproductive-aged young females with less frequent metabolic disorders compared with old aged females. Further studies on a larger population and different ethnicities are recommended in order to increase the statistical power and clarify the relationship between *FTO* gene polymorphism and PCOS across different population groups.

To our knowledge, this study is the first one to investigate *FTO* gene variants in Egyptian women with PCOS, however, several limitations of this study should be acknowledged. First, the number of patients included was relatively small, thus might weaken statistical power. Second, the gene to gene and gene to environment interactions could not be assessed in our study due to insufficient information.

5. CONCLUSION

FTO gene variant rs9939609 is associated with PCOS susceptibility in Egyptian women in the population studied. The association has been demonstrated in both obese and non-obese patients as well as in PCOS patients with insulin resistance and non-insulin resistance.

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Author contributions

Wessam El Sayed Saad, @, MD - Manuscript writing and preparation for publication, Statistical analysis and scientific review, Sharing in analytical part

Aziza Ahmed ELSebai, MD - Statistical analysis and scientific review

Maram Mohamed Maher, MD - Idea of the paper, Data collection

Alaa Mahmoud Heikal, MS.Ce - Data collection, Analytical part

Informed consent

Written & oral informed consent was obtained from all participants before enrollment in the study.

Ethical approval

The study protocol and methodology was approved by Ethical Committee of Faculty of Medicine, Ain Shams University, Ethical code: FMASU MS 168/2016.

Funding statement

This study has not received any external funding.

Conflict of interest

The authors declare that there are no conflicts of interest.

Data and materials availability

All data associated with this study are present in the paper.

Peer-review

External peer-review was done through double-blind method.

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