Association of FTO gene variants rs9939609 and rs1421085 with polycystic ovary syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is among the most common complex genetic endocrinopathy, and its etiology and pathophysiology remain controversial. FTO is a large highly polymorphic gene and was coined as the first locus associated with adiposity. The association of the intronic variant FTO rs9939609 or FTO rs1421085 with PCOS has been controversial and unclear, mainly due to ethnic differences among populations. The present study aims to investigate the association of FTO rs9939609 or FTO rs1421085 polymorphisms with PCOS in Saudi Arabian women.

Results: A total of 98 PCOS patients and 99 healthy females were included in this study. PCR and genotyping (TaqMan® SNP Genotyping Assay) were employed. For FTO rs9939609, the genotype TA and the recessive model (TA + AA) in PCOS patients were significantly different compared with control subjects (p = 0.008 and p = 0.007, respectively). The allele frequency of the FTO rs9939609 gene variant was associated significantly (p = 0.027) with PCOS, suggesting that the A allele is a risk factor for PCOS susceptibility. However, for the FTO rs1421085 variant, the genotype and allele distributions did not differ significantly between PCOS patients and controls (p > 0.05).

Conclusions: This is the first report to study the association of FTO rs9939609 and FTO rs1421085 with PCOS in Saudi women. Results suggest that the FTO rs9939609 gene variant could be a genetic predisposing factor for PCOS Saudi women.

Keywords: Polycystic ovary syndrome, Fat mass and obesity-associated gene, PCOS, FTO, Polymorphism, Obesity, Adiposity

Introduction

Polycystic ovary syndrome (PCOS) is among the most common complex genetic endocrinopathy, and its etiology and pathophysiology remain controversial [1]. PCOS is characterised by variable metabolic and reproductive abnormalities [2–4]. Symptoms include menstrual irregularities, infertility, anovulation, elevated serum androgens, sexual dysfunction, and obesity [5, 6]. The prevalence rates of PCOS may vary according to the differences in the attributes of the study population. Worldwide, according to the generally preferred revised Rotterdam diagnostic criteria [7, 8], the prevalence rate of PCOS is between 8 and 13% in women of reproductive age [9] and 6–18% in adolescent girls [10]. To date, there is no specific gene that has been recognised for the onset of PCOS [11, 12].

Obesity is one of the factors that exacerbate PCOS, and there is a general agreement that obesity is associated with the risk of developing the clinical and endocrine features of PCOS [13, 14]. Almost 38%-88% of PCOS women are either overweight or obese [15–17]. The clinical observations have led to believe that the level of BMI can reflect the degree of severity of symptoms in PCOS patients. During adolescence, it appears that the most common precipitant of PCOS symptoms is ordinary obesity [18, 19]. Modest reductions in weight have proven to improve many symptoms in PCOS patients, such as menstrual regularity, fertility, and improving quality of life.
This suggests that obesity is an important contributor to PCOS etiology [13–15, 19–21].

The ubiquity expressed fat mass and obesity-associated (FTO) gene was reported to be associated with obesity genetics [22]. FTO is a large highly polymorphic gene that located in chromosome 16q12.2. FTO was coined as the first locus associated with adiposity [22–25]. The associations of the intronic variant FTO rs9939609 (T>A) or FTO rs1421085 (T>C) with PCOS had been controversial and unclear mainly due to ethnic differences among populations [21, 26–30]. The study aims to investigate the association between two variants of the FTO gene (rs9939609 and rs1421085) with PCOS in Saudi women to estimate the possible role of FTO gene variants in PCOS susceptibility and severity in respect to obesity. To date, there is no information regarding the association of these two variants in the Saudi population.

Materials and methods
Study design and subjects
A retrospective case–control study design was undertaken. Ninety-eight (98) Saudi Arabian women (31.05 ± 0.59 years) who had been diagnosed with PCOS were recruited from the outpatient clinic at King Khalid University Hospital, Riyadh, Saudi Arabia. Diagnosis of PCOS patients was based on the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) diagnostic criteria [31]. Based on the joint ESHRE/ASRM agreement meeting, a refined definition of PCOS was produced that included the presence of two out of the following three criteria: (1) oligo- and/or anovulation, (2) hyperandrogenism, and (3) polycystic ovaries (PCO) morphology detected by ultrasound or pelvic imaging [32, 33]. Additionally, the criteria for patients’ selection were the absence of Cushing’s syndrome, congenital adrenal hyperplasia, androgen-secreting tumor, hyperprolactinemia, diabetes, thyroid dysfunction, and the absence of respiratory infection and cardiovascular diseases. Ninety-nine healthy non-smoker Saudi Arabian women were chosen to serve as controls. All participants were residents of the region of Riyadh Governorate. All participants signed an informed consent that was approved by the Institutional Review Board (IRB) of King Saud University, Riyadh, Saudi Arabia.

DNA extraction and genotyping
Three millilitres of peripheral blood were collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Genomic DNA was extracted from patients and controls’ blood [34]. SNP genotyping was performed by the hydrolysis probe method. The TaqMan®SNP Genotyping Assay (Applied Biosystems Inc., USA) was used to analyse the FTO rs9939609 and FTO rs1421085 variants on an ABI 7500 real-time qPCR System (Applied Biosystems Inc., USA) [35]. Briefly, the assay employs the 5’ nuclease activity of Taq polymerase to generate a fluorescent signal during PCR. For each of the FTO rs9939609 and rs1421085 SNPs, two probes were utilised, wild-type and variant allele probes. A 5’ reporter dye (FAM or HEX) and a 3’ quencher dye (Black Hole Quencher (BHQ)) were linked to each probe. The nuclease activity only happens with the perfectly hybridised probes. The ratio of the fluorescent signal for the two reporter dyes is measured and is used as an indication of the sample genotype.

Statistical analysis
Data are reported as mean ± SEM (standard error of the mean). The demographic data of PCOS patients and controls were compared using the t-test. A Pearson Chi-Square (χ²) test and Chi-Square (χ²) test for trend (Armitage trend test) were used to compare the observed and expected numbers of FTO rs9939609 and FTO rs1421085 genotypes for PCOS patients and control for the Hardy–Weinberg equilibrium analysis. A Chi-Square test (χ²) and logistic regression were used to assess the association of the FTO rs9939609 and FTO rs1421085 gene variants with PCOS. The significance level p < 0.05 was considered statistically significant. GraphPad-Prism Software (San Diego, USA) was used for all statistical analyses.

Results
The demographic data of PCOS patients and control subjects are shown in Table 1. No significant differences were observed between PCOS patients and controls in any of the parameters. Hardy–Weinberg equilibrium (HWE) analysis on all subjects revealed that only FTO rs1421085 of the control subjects showed a deviation from Hardy–Weinberg equilibrium (p = 0.012) with a Pearson Chi-Square (χ²) test (Table 2). It was reported that when

| Parameter | Control (n = 99) | PCOS (n = 98) | p value |
|-----------|-----------------|--------------|---------|
| Age (yr)  | 33.10 ± 0.74    | 31.50 ± 0.47 | 0.07    |
| Height (cm)| 158.48 ± 0.55  | 158.41 ± 0.61| 0.94    |
| Weight (kg)| 70.59 ± 1.53   | 72.09 ± 1.37 | 0.47    |
| BMI (kg/m²)| 28.16 ± 0.62   | 28.71 ± 0.51 | 0.49    |

Values are Mean ± Standard Error of Mean (SEM)
BMI: body mass index; PCOS: polycystic ovarian syndrome; p: significance value. p < 0.05 was considered statistically significant
the HWE deviation cannot be attributed to genotyping error or selection or non-random mating and may be due to an unknown factor, the Chi-Square (χ²) test for trend (Armitage trend test) should be used to reduce the chance of false-positive associations [36]. When the Armitage trend test was employed, there was no deviation from Hardy–Weinberg equilibrium (p = 0.998).

The analysis of the genotype and allele distributions of FTO rs9939609 (T>A) and FTO rs1421085 (T>C) variants was performed on PCOS patients and controls (Table 3). For FTO rs9939609, the genotype TA in PCOS patients was significantly different when compared with their respective controls (p = 0.008). Both TT and AA genotypes exhibited no significant difference in distribution (p > 0.05). The recessive model (TA + AA) showed a significant difference (p = 0.007), while the dominant model (TT + TA) did not show any significant difference between PCOS patients and controls (p > 0.05). Moreover, a significant difference of the alleles was evident in PCOS patients compared with healthy controls (p = 0.027), suggesting that the A allele is a risk factor for PCOS susceptibility. However, for the FTO rs1421085

| Gene polymorphism | Control | PCOS | Control versus PCOS |
|-------------------|---------|------|---------------------|
|                   | O (%)   | E (%)| χ²      | p        | OR (95% CI) | χ² | p     |
| FTO rs9939609 Genotype |         |      |         |         |            |    |       |
| TT                | 29 (29.3) | 47 (48.0) | 2.03 (0.83–4.93) | 2.46 | 0.116       |
| TA                | 55 (55.6) | 39 (39.8) | 0.44 (0.24–0.81) | 6.96 | 0.008*      |
| AA                | 15 (15.2) | 12 (12.2) | 0.50 (0.20–1.20) | 2.46 | 0.116       |
| TT + TA           | 84 (84.8) | 86 (87.8) | 1.30 (0.57–2.90) | 0.35 | 0.553       |
| TA + AA           | 70 (70.7) | 51 (52.0) | 0.45 (0.25–0.81) | 7.24 | 0.007*      |
| Allele            |         |      |         |         |            |    |       |
| T                 | 113 (57.1) | 133 (67.9) | 0.63 (0.42–0.95) | 4.89 | 0.027*      |
| A                 | 85 (42.9) | 63 (32.1) | 1.59 (1.05–2.40) |      |            |    |       |
| FTO rs1421085 Genotype |         |      |         |         |            |    |       |
| TT                | 32 (32.3) | 28 (28.6) | 1.20 (0.57–2.52) | 0.21 | 0.644       |
| TC                | 37 (37.4) | 48 (49.0) | 1.48 (0.76–2.90) | 1.36 | 0.244       |
| CC                | 30 (30.3) | 22 (22.4) | 0.84 (0.40–1.77) | 0.21 | 0.644       |
| TT + TC           | 69 (69.7) | 76 (77.6) | 1.50 (0.80–2.85) | 1.56 | 0.211       |
| TC + CC           | 67 (67.7) | 70 (71.4) | 1.20 (0.65–2.20) | 0.33 | 0.567       |
| Allele            |         |      |         |         |            |    |       |
| T                 | 101 (51.0) | 104 (53.1) | 0.92 (0.62–1.37) | 0.17 | 0.684       |
| C                 | 97 (49.0) | 92 (46.9) | 1.10 (0.73–1.61) |      |            |    |       |

n: Number of individuals; PCOS: polycystic ovarian syndrome; OR: odds ratio; CI: confidence interval; χ²: Chi-Square; p: significance value

*Significant difference. p < 0.05 was considered statistically significant

Table 2 Observed and expected values of FTO rs9939609 (T>A) and FTO rs1421085 (T>C) variants in PCOS patients and controls by Hardy–Weinberg equilibrium

| Gene polymorphism | O | E | χ² | p          | O | E | χ² | p          |
|-------------------|--|--|--|--|---|--|--|--|---|
| FTO rs9939609     |   |   |   |   |   |   |   |   |
| TT                | 29 | 32.2 | 1.77 | 0.00 | 47 | 45.1 | 0.75 | 0.00 |
| TA                | 55 | 48.5 |       | >0.99 | 39 | 42.8 |       | >0.99 |
| AA                | 15 | 18.2 |       | >0.99 | 12 | 10.1 |       | >0.99 |
| FTO rs1421085     |   |   |   |   |   |   |   |   |
| TT                | 32 | 25.8 | 6.30 | 0.00 | 28 | 27.6 | 0.03 | 0.00 |
| TA                | 37 | 49.5 |       | >0.99 | 48 | 48.8 |       | >0.99 |
| CC                | 30 | 23.8 |       | >0.99 | 22 | 21.6 |       | >0.99 |

PCOS: polycystic ovarian syndrome; O: observed data; E: expected data; P: Pearson Chi-Square (χ²) test; A: Chi-Square (χ²) test for trend (Armitage trend test); p: significance value

*Significant difference. p < 0.05 was considered statistically significant

Table 3 Genotype and allele frequencies of FTO rs9939609 (T>A) and FTO rs1421085 (T>C) variants in PCOS patients and controls

| Gene polymorphism | Control | PCOS | Control versus PCOS |
|-------------------|---------|------|---------------------|
|                   |         |      | OR (95% CI) | χ² | p     |
| FTO rs9939609 Genotype |         |      |            |    |       |
| TT                | 29 (29.3) | 47 (48.0) | 2.03 (0.83–4.93) | 2.46 | 0.116       |
| TA                | 55 (55.6) | 39 (39.8) | 0.44 (0.24–0.81) | 6.96 | 0.008*      |
| AA                | 15 (15.2) | 12 (12.2) | 0.50 (0.20–1.20) | 2.46 | 0.116       |
| TT + TA           | 84 (84.8) | 86 (87.8) | 1.30 (0.57–2.90) | 0.35 | 0.553       |
| TA + AA           | 70 (70.7) | 51 (52.0) | 0.45 (0.25–0.81) | 7.24 | 0.007*      |
| Allele            |         |      |            |    |       |
| T                 | 113 (57.1) | 133 (67.9) | 0.63 (0.42–0.95) | 4.89 | 0.027*      |
| A                 | 85 (42.9) | 63 (32.1) | 1.59 (1.05–2.40) |      |            |    |       |
| FTO rs1421085 Genotype |         |      |            |    |       |
| TT                | 32 (32.3) | 28 (28.6) | 1.20 (0.57–2.52) | 0.21 | 0.644       |
| TC                | 37 (37.4) | 48 (49.0) | 1.48 (0.76–2.90) | 1.36 | 0.244       |
| CC                | 30 (30.3) | 22 (22.4) | 0.84 (0.40–1.77) | 0.21 | 0.644       |
| TT + TC           | 69 (69.7) | 76 (77.6) | 1.50 (0.80–2.85) | 1.56 | 0.211       |
| TC + CC           | 67 (67.7) | 70 (71.4) | 1.20 (0.65–2.20) | 0.33 | 0.567       |
| Allele            |         |      |            |    |       |
| T                 | 101 (51.0) | 104 (53.1) | 0.92 (0.62–1.37) | 0.17 | 0.684       |
| C                 | 97 (49.0) | 92 (46.9) | 1.10 (0.73–1.61) |      |            |    |       |

n: Number of individuals; PCOS: polycystic ovarian syndrome; OR: odds ratio; CI: confidence interval; χ²: Chi-Square; p: significance value

*Significant difference. p < 0.05 was considered statistically significant
variant, the genotype and allele distributions did not differ significantly between PCOS patients and controls.

Discussion

FTO was identified in 2007 as a sensitive gene for obesity. The intron 1 region of the FTO gene received extensive attention due to the harboring of multiple polymorphisms that were strongly associated with BMI, waist circumference, hip circumference, body fat rate, energy intake, type 2 diabetes mellitus (T2DM), and obesity [22, 24, 37, 38]. FTO is involved in various cellular processes, including fatty acid metabolism, DNA repair, and posttranslational modifications [39]. Although FTO is expressed in the liver, pancreas, muscles, and adipose tissue [40], the highest expression of the FTO gene is in the region that controls energy balance in the hypothalamus implying its crucial role in regulating energy metabolism and appetite [39]. The involvement of FTO polymorphisms with BMI was first reported in diabetic European people. Among the first reported BMI-related FTO variants was the rs9939609 variant [22, 34]. Recent studies have associated the rs9939609 with higher obesity risks in other populations (Chinese, Brazilian, and Iranian populations) [41–43]. It is also associated with increased waist circumference and BMI in Brazilian young individuals [44], increased metabolic syndrome predisposition in Chinese subjects, and distribution of adipose tissue in the Italian subjects [45, 46]. Furthermore, multiple polymorphisms in the first intronic region of FTO have been correlated with the risk of cancers. Again, the classic FTO rs9939609 was associated with breast, lung, prostate, endometrial, renal, and pancreatic cancers. The association between the FTO variants and a wide spectrum of diseases may suggest another role beyond the involvement with BMI [39]. Increasing evidence suggests that FTO can be a main genetic factor in predisposing to PCOS, primarily via its role in BMI and obesity, and secondarily with manipulating hyperandrogenemia and metabolic parameters [6, 39, 47].

PCOS is a metabolic disorder that is closely related to insulin resistance [48], T2DM [49], obesity [14], metabolic syndrome [4], and premature arteriosclerosis [50]. No surprise that genes affecting T2DM and obesity, such as FTO, are regarded as important candidate genes for PCOS. Obesity is found in approximately 50% of PCOS patients. Indeed, as reported previously, FTO is associated and has a profound influence on PCOS [6, 39, 51, 52].

The major finding of the present study is that the first intronic FTO rs9939609 gene variant is associated with PCOS in Saudi Arabian women. However, one must be careful because the presence of an association does not mean causation. The rs9939609 gene variant is strongly conserved across species, the most extensively studied FTO variant, and was previously reported to be associated with PCOS in the Korean [23, 24, 38, 53], Chinese [38, 54–56], UK patients of British/Irish origin [57], Sri.lankan [21, 58], Brazilian [34], Turkish [27], and Tunisian populations [59]. Thus, the finding of the present study, along with the findings of previous studies suggest that the FTO rs9939609 gene variant might have a positive association with the presence of PCOS. Additionally, to the aim to investigate the association of rs9939609 with a larger population of PCOS patients, Liu et al. (2017) performed a meta-analysis that involved 5010 PCOS subjects and 5300 controls. The studies used for the meta-analysis were conducted in Brazil, United Kingdom, China, and Korea and suggested that the FTO rs9939609 gene variant is associated with PCOS risk [60]. An interesting meta-analysis by Cai et al. (2014) revealed that FTO rs9939609 might not be associated with PCOS in Caucasian patients. However, the association was highly significant in PCOS East Asians, which is independent of BMI [61]. On the contrary, rs9939609 was not significantly associated with PCOS itself in Korean women. However, the variant may have a gene dose effect, via an association with obesity, in predisposition to PCOS [62].

Several reports had confirmed that the FTO rs9939609 gene variant impacted body fat contents hence BMI from multiple biological pathways [27, 28, 63]. Furthermore, other studies have reported a positive association between the rs9939609 variant with other PCOS-associated phenotypes [38]. In a cohort of PCOS women from a Polish population, the rs9939609 variant may have a higher impact on obesity and related traits in PCOS [48, 64]. It was reported that there is a correlation between rs9939609 and insulin resistance in PCOS subjects. And the effect size of the rs9939609 variant on BMI in PCOS was greater than that of controls of the same age range in the German population [65]. To the aim to investigate the impact of the FTO rs9939609 on metabolic and endocrine parameters in PCOS patients, Wehr et al., (2010) demonstrated that rs9939609 influenced the anthropometric parameters and hyperandrogenemia and in PCOS women, indicating an important role of this variant not only in T2DM and obesity but also in hyperandrogenism in Austrian women with PCOS [66].

The second finding of the present study was that the FTO rs1421085 gene variant was not associated with PCOS in Saudi Arabian women residing in the Riyadh region. Few studies investigated the association of the rs1421085 variant with PCOS compared to studies on rs9939609. FTO rs1421085 gene variant was not a contributing factor for the development of PCOS in Korean patients [67]. In addition, the findings of this study
are consistent with previously reported data, the *FTO* rs1421085 variant had no association with PCOS in cohorts of 212 Chinese and 207 Romanian women compared with 198 and 100 of matched controls, respectively [68, 69]. On the contrary, in another study in Korea that included 432 patients with PCOS and 927 controls, the *FTO* rs1421085 variant was associated with PCOS in young Korean women [38]. And, in another study that involved 750 PCOS individuals of European origin and 1567 control subjects (BMI-matched), out of the 92 investigated BMI-risk polymorphisms, the association of rs1421085 with BMI was stronger in PCOS women. None of the other investigated variants were associated individually with PCOS [51]. Furthermore, rs1421085 was found to be associated with impaired fasting glucose in PCOS patients in a Central European population [69].

The first intronic *FTO* rs1421085 gene variant was first found to be associated with obesity [24]. However, it was not clear whether the *FTO* rs1421085 variant was associated with overweight or obesity. An overweight reflects an increase in total body mass (calculated BMI), including lean contents and fat contents. Whereas an overweight will reflect elevated fat contents which cannot be accurately estimated using the calculated BMI. It is very important to realise that many SNPs in the *FTO* gene are co-inherited with a wide range of expressing the proportion of fat content to lean content for a given increase in body weight. Additionally, body fat is excessive in PCOS [18]. The results of previous studies regarding the *FTO* rs1421085 gene variant had not been clear nor yielded a repeatable association with obesity rather than BMI alone. 362,129 SNPs of the *FTO* gene were tested in African Americans for association with obesity-related traits. The rs9939609 gene variant was associated with obesity, and this was not the case for the *FTO* rs1421085 gene variant [37]. The *FTO* rs9939609 polymorphism might influence the baseline lipid oxidation in PCOS patients which may explain the impact of the variant on BMI in PCOS patients [64]. However, Li et al. (2013) reported that the variant rs9939609 was associated with PCOS patients in obese, as well as, in non-obese Chinese women [70]. Other studies are needed to investigate the association of the *FTO* rs1421085 variant with PCOS in women of reproductive age in different populations.

In a meta-analysis study that included twelve studies published from 2008 to 2015, and included a total of 6287 PCOS patients and 6667 controls for assessing the association with rs9939609, and 1549 PCOS patients and 1611 controls for assessing the association with rs1421085, the *FTO* rs9939609 gene variant was significantly associated with PCOS. However, the association between rs1421085 and PCOS needed further confirmation as reported by the authors. These studies were performed in USA, Brazil, UK, France, Romania, China, and Korea [71]. In another systematic review and meta-analysis that included seven studies describing eight distinct PCOS cohorts in which seven of the cohorts were genotyped for rs9939609 and one for rs1421085, and included 2548 PCOS women (sample size ranged from 136 to 469), *FTO* variants showed a statistically larger effect in PCOS cohorts than in the reference groups [22, 72]. The studies were conducted in USA, Austria, Czech Republic, Germany, Poland, France, Romania, and UK [55]. A recent meta-analysis on the associations of *FTO* variants with PCOS, which included forty-six studies, revealed that rs9939609 is significantly associated with PCOS (7629 PCOS vs 10511 controls) which was not the case for the rs1421085 variant (1256 PCOS vs 1638 controls). The meta-analysis proved that the rs9939609 variant may serve as a predisposing factor for PCOS, especially for Asians [1]. Very recently, it was reported that the *FTO* rs9939609 variant may increase susceptibility to PCOS development independent from serum adipocytokine levels [47].

Although the diagnostic criteria for PCOS do not include obesity [14, 73], the Northern Finland Birth Cohort (NFBC) reported a significant association between BMI and PCOS at all ages. The outcome of weight loss has shown that it can lead to clinically meaningful improvements of PCOS manifestations [17, 74, 75]. Weight gain and obesity often result in clinical and biochemical manifestation in women who are genetically susceptible to the development of PCOS [17]. On the contrary, the increased prevalence of PCOS in obese and overweight women was independent of the presence or absence of the metabolic syndrome of PCOS [76, 77]. The co-occurrence of PCOS and obesity may be attributed to a contributing genetic predisposition and a significant inherited etiology as suggested by evidence from family-based studies [38, 66, 78]. Obesity may be an important contributor to PCOS but is not one of the main etiologic defects leading to this disease [77, 79].

**Conclusion**

This is the first report to study the association of *FTO* rs9939609 and *FTO* rs1421085 with PCOS in Saudi women. *FTO* rs9939609 gene variant is significantly associated with PCOS patients, while *FTO* rs1421085 exhibited no significant difference. Results suggest that the *FTO* rs9939609 gene variant could be a genetic predisposing factor for PCOS Saudi women. A discrepancy between populations is evident, and further studies on other and large populations to evaluate the association between these two SNPs and the risk of PCOS are highly required.
Limitations
(1) The study employed only two genetic variants of the FTO gene. Other variants of the FTO gene should be considered in future studies. (2) The study was restricted to Saudi Arabian women. Further studies are required in other populations. (3) Biochemical and clinical parameters including hyperandrogenism or serum testosterone levels, and modified Ferriman-Gallwey (mFG) score have been assessed for the study population, for the purpose of complete PCOS diagnosis, before the recruitment of PCOS patients for this study. And these data are not available with the authors. (4) The results need to be confirmed in larger samples.

Abbreviations
ASRM: American Society for Reproductive Medicine; BMI: Body mass index; CI: Confidence interval; EDTA: Ethylenediaminetetraacetic acid; ESHRE: European Society for Human Reproduction and Embryology; FTO: Fat mass and obesity-associated; HWE: Hardy–Weinberg equilibrium; IRB: Institutional Review Board; KKUH: King Khalid University Hospital; NFBC: Northern Finland Birth Cohort; PCO: Polycystic ovaries; PCOS: Polycystic ovary syndrome; PCR: Polymerase chain reaction; SEM: Standard error of the mean; SNP: Single nucleotide polymorphism; T2DM: Type 2 Diabetes mellitus.

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Authors’ contributions
All authors listed have made a substantial and intellectual contribution to the work and approved it for publication. AAA and HMA were involved in data acquisition and methodology. HMA was involved in formal analysis and literature search. AFA and ZAB contributed to the supervision and project administration. AFA, ZAB, and MFK have made substantial contributions to the conception, design of the work, analysis, and interpretation of data. AFA and MFK contributed to the manuscript preparation, editing, and review. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
All participants signed an informed consent that was approved by the Medical Ethics Committee of King Khalid University Hospital (KKUH). The study protocol was approved by the Institutional Review Board (IRB) of King Saud University, Riyadh, Saudi Arabia.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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