Influence of KISS1 gene polymorphisms on the risk of polycystic ovary syndrome and its associated variables, in Saudi women

Maha H. Daghestani¹, Mazin H. Daghestani², Mamoon Daghistani³, Khushboo Ambreen⁴, Fadwa S. Albalawi¹, Lina M. AlNeghery⁵ and Arjumand S. Warsy⁶*

Abstract

Background: Polycystic ovary syndrome (PCOS) is a complex multifactorial disorder, affecting millions of women worldwide. The role of genetic polymorphisms of the KISS1 gene on the development of PCOS is still obscure. This study was designed to investigate the probable influence of KISS1 gene polymorphisms on PCOS and its associated variables: BMI, waist-hip ratio, kisspeptin, LH, FSH, and LH-FSH ratio.

Methods: The study comprised 104 PCOS women and 109 controls, with age ranging from 19 to 36 years. BMI, waist-hip ratio, and circulating levels of kisspeptin, LH, and FSH were measured. DNA was extracted, and genotyping of the KISS1 gene was carried out by nucleotide sequencing. The PCOS-associated variables were analyzed in different genotypes of single nucleotide polymorphisms (SNPs) of the KISS1 gene.

Results: The values of waist-hip ratio (WHR), LH, and LH-FSH ratio were significantly higher in PCOS women than controls. BMI, kisspeptin, and FSH levels exhibited no significant difference between the groups. Six novel SNPs of KISS1 gene were identified. Three: rs372790354G > A, rs12998G > A, and rs35431622A > T were investigated. Among these SNPs, the genotype and allele frequencies of rs372790354 showed significant association with PCOS (GA: p = 0.018, AA: p = 0.022, mutant allele-A: p = 0.021) and the G allele was protective. The values of LH, kisspeptin, and WHR of PCOS women were significantly influenced (p < 0.05) by the AA genotype of rs372790354. The other two SNPs rs12998G > A and rs35431622A > T revealed no significant influence on PCOS and associated variables. Haplotypes were constructed, but there was no significant difference between the patients and controls.

Conclusion: In conclusion, this is the first study, which reports a significant influence of KISS1 gene polymorphism (rs372790354G > A) on PCOS and its associated variables. However, more extensive research is necessary to confirm these findings.

Keywords: Polycystic ovary syndrome, KISS1 gene polymorphisms, Kisspeptin, LH, FSH, Waist-hip ratio
Background

Polycystic ovary syndrome (PCOS) is one of the notable common health concerns in the female society of reproductive age ranging from 12 to 45 years with a highly variable prevalence rate of 2.2 to 26% in different ethnic groups [1]. It is a complex endocrine disorder characterized by chronic anovulation, hyperandrogenism, and polycystic ovarian morphology and associated primary clinical manifestations of irregular or missed periods, and excessive hair growth on the face and body [2]. In addition to these endocrinical factors and its related health complications, the complex etiology of PCOS is also influenced by the involvement of genetic, metabolic, and environmental factors [3].

In recent years, considerable research efforts have been made towards understanding the multifactorial etiology of PCOS. In this regard, genetic understanding of this disease in terms of hypothalamic-pituitary-gonadal (HPG) axis associated genetic factors, has received considerable attention by the researchers [4].

Many previous studies have explored mutations in the HPG axis linked various genes such as KISS1, GPR54 receptor, GnRHR, LHR and FSHR, for the development of PCOS in the adult female population [5–7]. Among these genes, KISS1 has emerged as one of the candidate genes contributing to a regulatory role in the female reproductive system with an essential function in gonadotropin secretion of the HPG axis [8]. Some single nucleotide polymorphisms (SNPs) in the KISS1 gene are found to disrupt the healthy functioning of the female reproductive system through disturbing the HPG axis and are postulated to play an essential role in PCOS etiopathogenesis [5, 9].

The effects of KISS1 gene polymorphisms on susceptibility to PCOS have been investigated in two previous studies [9, 10]. However, until now, no adequately powered study is available in the literature, which confidently reveals the role of any relevant polymorphism of the KISS1 gene in PCOS pathogenesis.

Biochemically, ovarian dysfunction of PCOS is reflected in the excessive production of luteinizing hormone (LH) and a normal or low level of follicle-stimulating hormone (FSH) from the anterior pituitary [11]. Kisspeptins are neuropeptides of different amino acid lengths (Kp-54, Kp-14, Kp-13, and Kp-10), encoded by the KISS1 gene, and unveils a vital regulatory role in the function of the HPG-axis, by utilizing G protein-coupled receptor known as GPR54 [12]. The major pathway of kisspeptin is the direct action on GnRH neurons to release GnRH into the portal circulation, which in turn induces the stimulatory activity of LH and FSH with the release of androgens [13]. Hence, the evaluation of the circulating levels of kisspeptin, LH, and FSH in susceptible women may be of importance in understanding the multifactorial etiology of PCOS.

Likewise, elevated values of body mass index (BMI) and waist-hip ratio (WHR) are considered as conventional anthropometric markers for the feature of obesity. They are proved to be linked with PCOS risk by directly creating disturbances in metabolic as well as endocrine system [14, 15]. Many previous reports also emphasize the significance of BMI and waist-hip ratio as the markers of obesity, having responsibility for the morbidity events of PCOS [16–18].

Hence, KISS1 gene polymorphisms, kisspeptin, LH, FSH, BMI, and waist-hip ratio are the essential interlinking factors that exert their influence on the expression of PCOS. The current study investigates the values of BMI, waist-hip ratio, and the blood levels of kisspeptin, LH, FSH, and the ratio of LH-FSH in PCOS patients and controls. In an attempt to determine the influence of the KISS1 gene polymorphisms on the development of PCOS, the study assesses the genotype and allele frequencies of SNPs of the KISS1 gene for both study groups. Furthermore, we have attempted to demonstrate the possible influence of the KISS1 gene polymorphisms on PCOS associated endocrine and obesity-linked variables (kisspeptin, LH, FSH, LH-FSH ratio BMI, and waist-hip ratio).

Methods

Study subjects

The subjects of this present case-control study consisted of 104 women with well-characterized manifestations of PCOS and 109 control healthy women having no clinical history of PCOS. The study was approved by the Ethical Committee of the Institutional Review Board (IRB), Umm Al-Qura University, Makkah, Saudi Arabia (IRB No. 235). Study participants with an age range of 19–36 years, were recruited from different private hospitals of Makkah, Kingdom of Saudi Arabia.

Enrolled PCOS patients were newly diagnosed, and the criteria for the diagnosis of the PCOS patients was based on two of the three following features, according to Rotterdam 2003 criteria: (I) oligomenorrhea (menstrual period length greater than 35 days) or amenorrhea (menstrual period absent for six months), (II) clinical and/or biochemical signs of hyperandrogenism, (III) polycystic ovaries morphology as seen on ultrasound (at least one ovary contained >12 follicles measuring 2–9 mm in diameter and/or increased ovarian volume of at least 10 ml) [19].

Patients having other reasons, of hyperandrogenism or menstrual irregularity such as Cushing’s syndrome, prolactinoma, and congenital adrenal hyperplasia were excluded. Pregnancy cases and women with the first postpartum year were also not included in our study.
Patients having received any kind of hormonal medicine (such as oral contraceptives, ovulation-inducing agents, and anti-androgens) in the previous 3 months of the research, were excluded.

The selection of the control subjects was based on the enrollment of the healthy women population having no history of PCOS with the regular menstrual cycle (menstrual period up to 7 days and menstrual cycle of 21–35 days), normal androgen levels and no symptoms of hirsutism. The control women were matched with PCOS women for age and BMI.

To collect demographic and clinical data of the patients, we prepared a specially designed questionnaire, and the demographic characteristics (age, weight, height, and hip measurements), history of the menstrual cycle, hirsutism, acne, and previous disease and drug history were recorded. For patients with doubtful records, biochemical laboratory tests and ultrasound investigations were performed. The confirmed cases of PCOS were recruited. Controls were screened for biochemical signs of raised androgens, ultrasonic ovarian morphology, and menstrual health records. Only those with normal results were included in the study.

For each enrolled woman, BMI (weight in kg divided by height in m²) was calculated. Likewise, the calculation of waist-hip ratio was based on measurements of waist circumference by an experienced nurse (the narrowest circumference between the lower costal margins and the iliac crest) and hip circumference (the maximum circumference at the level of the femoral trochanters) with the proper standing position of the study subjects.

Sample collection
In both groups of the study subjects, the blood samples were collected between days 3 and 6 of the menstrual cycle, at about 8:00 am. Each subject was requested to attend the clinic in a fasting state, and 10 ml of the blood sample was drawn from each subject using the venipuncture method. A total of 5 ml of this blood was transferred in an EDTA coated vial, and immediately processed for obtaining buffy coat and plasma, through centrifugation process. Buffy coat was used for DNA extraction, and the plasma was used for the estimation of kisspeptin. Remaining 5 ml of the blood sample was drawn in a red top tube and used to obtain serum by centrifugation process, for the evaluation of FSH and LH. All samples were consequently stored at -80°C until experimental analysis.

Estimation of kisspeptin, FSH and LH levels
The blood plasma level of kisspeptin was measured using an enzyme-linked immunosorbent assay (ELISA) kit from Phoenix Pharmaceuticals Inc., Belmond, CA, following extraction with Phoenix Peptide sep-columns (RK-Sepcol-2). Blood serum levels of FSH and LH were also measured by a specific ELISA kit (Human, Cat. No.65205. GER).

KISS1 genotyping analysis
KISS1 gene is mapped on chromosome 1q32 with its four exons (Fig. 1). Genomic DNA from 104 PCOS patients and 109 controls were obtained, and PCR sequencing was performed for obtaining SNPs of the KISS1 gene. For this purpose, the buffy coat was used to extract the genomic DNA, utilizing a DNA extraction kit from Puregene (Puregene Blood Kit, QIAGEN, Cat. No. 158389, USA). Polymerase chain reaction (PCR) was performed using the following forward and reverse primers: F: 5′-ACCTGCGAAACTACA ACTGG-3′, R: 5′-TGAAGGAACAGGCCG GTTAGT-3′, under the following conditions: initial denaturation step at 95°C for 15 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, with the final extension of 10 min at 72°C. The size of the PCR product was 353 bp. The nucleotide sequencing of the PCR product was carried out using the ABI Big Dye Terminator protocol on ABI 3100 Avant Genetic Analyzer.

Statistical analysis
The statistical analyses were conducted using SPSS for Windows (version 20.0). The descriptive characteristics of the different continuous variables were expressed as mean ± SD. The Student’s t-test was used to obtain the significance of the difference in the mean values of continuous variables between PCOS patients and controls. Genotype and allele frequencies of the KISS1 gene polymorphisms were calculated for both study groups, and the significance of the difference was determined using the Chi-Square test. Odds ratio (OR), its 95% confidence interval (CI), and p-values were obtained. Hardy Weinberg equilibrium was tested.

The levels of PCOS associated variables were analyzed in the different genotypes of each identified SNP of the KISS1 gene. The Pairwise t-test was used to assess the significance of the difference between wild-type homozygous and mutant allele carriers (mutant homozygous and heterozygous). A p-value < 0.05 was considered as statistically significant.

Results
Demographic and endocrine characteristics of study subjects
The demographic characteristics of PCOS patients and controls are presented in Table 1. A significant difference was noted between PCOS patients and controls, only in the value of the waist-hip ratio (p < 0.001) while the age (p = 0.88) and BMI (p = 0.63) exhibited no significant difference between the study groups.
As shown in Table 1, the levels of kisspeptin \((p = 0.415)\) and FSH showed \((p = 0.223)\) no significant difference between PCOS patients and controls. A significant elevation in the level of LH for the PCOS patients \((p = 0.0001)\) was the most noticeable result, as the level was more than three-fold higher in PCOS patients than in the controls. Furthermore, when both groups were examined for the values of the LH-FSH ratio, a significant difference \((p = 0.0001)\) was noted.

**KISS1 gene polymorphisms**

A total of six SNPs were identified through sequencing of the KISS1 gene. The results of three SNPs (rs372790354 G/A, rs12998 G/A, and rs35431622 A/T)

| Characteristics | PCOS Patients \((n = 104)\) (Mean ± SD) | Controls \((n = 109)\) (Mean ± SD) | \(p\) value |
|-----------------|-----------------------------------------|---------------------------------|------------|
| Age (Years)     | 25.06 ± 3.88                           | 24.96 ± 5.56                   | 0.886      |
| Waist (cm)      | 87.86 ± 13.82                           | 86.15 ± 17.09                  | 0.422      |
| Hip (cm)        | 104.39 ± 11.39                          | 110.48 ± 14.78                 | 0.001*     |
| Waist-Hip ratio | 0.84 ± 0.07                             | 0.77 ± 0.06                    | 0.0001*    |
| BMI (Kg/m²)     | 29.45 ± 5.99                            | 29.89 ± 7.31                   | 0.633      |
| Kisspeptin (ng/ml) | 0.41 ± 0.16                       | 0.39 ± 0.07                    | 0.415      |
| FSH (IU/L)      | 5.09 ± 1.54                             | 4.83 ± 1.55                    | 0.223      |
| LH (IU/L)       | 14.43 ± 7.48                            | 4.56 ± 1.38                    | 0.0001*    |
| LH-FSH ratio    | 2.94 ± 1.07                             | 0.98 ± 0.37                    | 0.0001*    |

*Significant, \(p\) values refer to Student’s t-test; LH luteinizing hormone, FSH follicle stimulating hormone, BMI body mass index
Table 2: Single nucleotide polymorphisms identified in the KISS1 gene by sequencing

| SNP ID    | Chromosome Position | mRNA position | Heterozygosity | MAF     | Allele     | Location | Function         | Amino-acid change |
|-----------|---------------------|---------------|----------------|---------|------------|----------|------------------|-------------------|
| rs372790354 | Chr1:204196427      | –             | –              | –       | G > A      | 5′UTR    | variant          | NA                |
| rs12998   | Chr1:204192819      | 212           | 0.093          | 0.023   | G > A      | Exon 1   | Non-synonymous   | p.Glu20Lys        |
| rs35431622 | Chr1:204190794      | 261           | 0.103          | 0.075   | A > T      | Exon 2   | Non-synonymous   | p.Gln36Leu        |

Table 3: Genotype frequencies of studied SNPs of the KISS1 gene, in PCOS patients and controls

| SNPs          | Genotypes | Frequency | OR (95%CI) | \(\chi^2\) | \(p\)-value |
|--------------|-----------|-----------|------------|-------------|-------------|
|              |           | PCOS patients n (%) | Controls n (%) | Total n (%) |             |
| rs372790354 G/A | GG        | 86 (82.7%) | 92 (84.4%) | 178 (83.6%) | Reference |
|               | GA        | 13 (12.5%) | 17 (15.6%) | 30 (14.1%) | 0.07 (0.01–1.38) | 5.51 | 0.018* |
|               | AA        | 5 (4.8%)   | 0 (0.0%)   | 5 (2.3%)   | 1.17 (0.64–2.15) | 5.20 | 0.022* |
| Total         |           | 104 (100.0%) | 109 (100.0%) | 213 (100.0%) |             |
| rs12998 G/A   | GG        | 87 (83.7%) | 88 (80.7%) | 175 (82.2%) | Reference |
|               | GA        | 12 (11.5%) | 17 (15.6%) | 29 (13.6%) | 0.56 (0.13–2.55) | 0.56 | 0.454 |
|               | AA        | 5 (4.8%)   | 4 (3.7%)   | 9 (4.2%)   | 1.26 (0.33–4.86) | 0.12 | 0.732 |
| Total         |           | 104 (100.0%) | 109 (100.0%) | 213 (100.0%) |             |
| rs35431622 A/T| AA        | 97 (93.3%) | 101 (92.7%) | 198 (92.96%) | Reference |
|               | AT        | 7 (6.7%)   | 8 (7.3%)   | 15 (7.04%) | 0.91 (0.32–2.61) | 0.03 | 0.862 |
|               | TT        | 0 (0.0%)   | 0 (0.0%)   | 0 (0.0%)   | –           | –         | –                |
| Total         |           | 104 (100.0%) | 109 (100.0%) | 213 (100.0%) |             |

*Significant, \(p\) values refer to Chi-Square \(\chi^2\) test, OR odds ratio, CI confidence interval
performed between wild type homozygous and mutant allele carriers (mutant homozygous and heterozygous). The results are presented in Tables 5 and 6. The SNP rs372790354 was revealed to exhibit a significant influence (\( P < 0.05 \)) on the endocrine (LH and kisspeptin) and obesity-linked (waist-hip ratio) parameters of PCOS patients. While other parameters (FSH, LH-FSH ratio, and BMI) of PCOS patients were not influenced by rs372790354. When the influence of this SNP on the studied parameters of control subjects was investigated, no significant difference was obtained (Table 5).

The other two SNPs: rs12998 G/A, and rs35431622 A/T, exhibited no significant influence on the values of endocrine as well as obesity like parameters in the PCOS patients. Likewise, in the control group, no significant differences were observed for the levels of the studied parameters between the genotypes of these two SNPs (Table 6).

Haplotype analysis was carried out using SNPstat, and the results are presented in Table 7. Five haplotypes exhibited polymorphism in the PCOS and control group. The most frequently identified haplotype in both groups was GGA (rs12998, rs372790354, and rs35431622), but the results in the two groups were not significantly different. None of the other haplotypes showed any significant difference between the patients and controls.

**Discussion**

The present study describes the probable influence of three identified SNPs (rs372790354 G/A, rs12998 G/A, and rs35431622 A/T) in the KISS1 gene in PCOS pathogenesis. Importantly, for the first time, we have attempted to demonstrate the influence of these three novel SNPs on the endocrine (kisspeptin, LH, FSH, LH-FSH ratio) and obesity-linked parameters (BMI and waist-hip ratio), in women with and without PCOS.

The genotype and allele frequencies were calculated and compared between PCOS patients and controls to elucidate the role of these studied SNPs in PCOS. For the SNP rs372790354, the genotypes AA and GA exhibited significant association with the risk of PCOS. The mutant AA genotype was found only in PCOS patients, and the frequency of the GA genotype was significantly higher in control subjects. The allele G showed a protective effect.

In recent years, the KISS1 gene has been addressed as one of the crucial regulators in controlling the function and maturation of the reproductive system [20]. This gene consists of four exons, of which only third and fourth exons are finally translated into 145 amino acids and is cleaved into shorter four forms of kisspeptin containing amino acids of 54, 14, 13, and 11 and exhibit the same affinity for their receptor-GPR54 [8]. The role of kisspeptin (a major product of KISS1 gene) has been well identified in puberty, ovulation, brain sex differentiation, and fertility, with an essential regulatory function in the normal release of hypothalamic GnRH and consequently in LH secretion [21, 22]. The pathophysiological mechanism of PCOS is also reflected in the inappropriate GnRH/LH secretion [23, 24]. However, clear evidence for the role of KISS1/kisspeptin expression in the mechanism of PCOS pathogenesis is still not obtained.

In this study, the results showed that the SNP rs372790354, which is significantly different in PCOS patients, is located in the 5′UTR (untranslated region) of the KISS1 gene. The 5′UTR has been recognized as playing an important role in the regulation of gene expression through regulating the process of RNA transcription, mRNA stability and its localization, and translational efficiency [25, 26]. Moreover, 5′UTR efforts great contribution in the initiation step of translation. The multi-step process of gene expression is primarily depended on this initiation translation step [27].

Keeping in view the role of 5′UTR in gene expression regulation, our study has hypothesized that the rs372790354 polymorphism of the KISS1 gene, can
dysregulate the multi-step process of gene expression. This step may indirectly promote alteration in the functional activity of the KISS1 gene product- kisspeptin and its receptor GPR54. The clear evidence for the considerable influence of the SNP rs372790354 on kisspeptin level of PCOS women is illustrated in Table 4, which highlights a significant difference in kisspeptin level, between homozygous wild-type (GG) and mutant (AA) genotypes of this SNP. While in the control group, the level of kisspeptin was not influenced by any of the genotypes of rs372790354.

Moreover, when both study groups were compared for the level of kisspeptin (Table 1), no significant difference was obtained between PCOS patients and controls. No significant elevation of the kisspeptin level in PCOS patients in our study may be due to the small sample size. One of our previous studies, reported by Albalawi et al. [9], also highlights no significant difference in the level of kisspeptin between PCOS women and controls. It showed that the SNP rs4889 exhibited a significant influence on the kisspeptin level. Several previous studies have also shown a non-significant difference in the level of kisspeptin in women with PCOS as compared to non-PCOS women [28, 29]. Thus, these observations of our study suggest that the kisspeptin level of PCOS women was not significantly higher as compared to controls, but is significantly influenced by the SNP rs372790354 of KISS1 gene.

It is well established that PCOS has a complex multifactorial etiology and is associated with increased

| Parameters | Genotypes of rs372790354 | P-value (Pair wise Comparison) |
|------------|--------------------------|-------------------------------|
|            | GG       | GA      | AA      |                      |
| BMI (Kg/m²) | C        | 29.88 ± 7.21 | 29.89 ± 8.06 | –                     |
|            | P        | 29.43 ± 5.90 | 29.62 ± 5.01 | 29.40 ± 10.4          |
| Waist-hip ratio | C      | 0.77 ± 0.06 | 0.79 ± 0.07 | –                     |
|            | P        | 0.83 ± 0.07 | 0.84 ± 0.08 | 0.88 ± 0.16           |
| FSH (IU/L) | C        | 4.76 ± 1.60 | 5.17 ± 1.22 | –                     |
|            | P        | 5.16 ± 1.53 | 4.41 ± 1.08 | 5.52 ± 2.39           |
| LH (IU/L) | C        | 4.53 ± 1.45 | 4.68 ± 1.02 | –                     |
|            | P        | 12.54 ± 2.55 | 11.85 ± 3.43 | 14.93 ± 8.02          |
| LH-FSH ratio | C      | 0.99 ± 0.39 | 0.94 ± 0.26 | –                     |
|            | P        | 2.96 ± 1.08 | 2.91 ± 1.12 | 2.45 ± 0.62           |
| Kisspeptin (ng/ml) C | 0.42 ± 0.15 | 0.37 ± 0.06 | –                     |
|            | P        | 0.41 ± 0.16 | 0.42 ± 0.15 | 0.28 ± 0.19           |

*Significant, C control, P PCOS patients, BMI Body mass index, LH luteinizing hormone, FSH follicle stimulating hormone, p values indicate comparison of features between wild-type homozygous and mutant allele carriers (mutant homozygous and heterozygous)
| Parameters | Genotypes of rs12998 | P-value (Pair wise Comparison) | Genotypes of rs35431622 | P-value (Pair wise Comparison) |
|------------|----------------------|-------------------------------|--------------------------|-------------------------------|
|            | GG  | GA  | AA  | No. C  | n = 88 | n = 17 | n = 4 | n = 101 | n = 8 | n = 7 |
| No. P      | n = 87 | n = 12 | n = 5 |  |

**Obesity linked Parameters**

| Parameters | C | P |  |
|------------|---|---|---|
| BMI (Kg/m²) | 29.82 ± 7.04 | 30.45 ± 9.33 | 29.00 ± 4.02 |
|            | 29.44 ± 5.86 | 28.88 ± 5.48 | 31.04 ± 9.96 |
|            | 0.77 ± 0.07 | 0.79 ± 0.07 | 0.79 ± 0.06 |
|            | 0.84 ± 0.07 | 0.81 ± 0.07 | 0.89 ± 0.15 |

**Endocrine Parameters**

| Parameters | C | P |  |
|------------|---|---|---|
| FSH (IU/L)  | 4.73 ± 1.54 | 5.04 ± 1.48 | 6.10 ± 1.83 |
|            | 5.11 ± 1.53 | 4.59 ± 1.26 | 5.80 ± 2.18 |
|            | 0.77 ± 0.07 | 0.79 ± 0.07 | 0.79 ± 0.06 |
|            | 0.84 ± 0.07 | 0.81 ± 0.07 | 0.89 ± 0.15 |

*Significant, C control, P PCOS patients, BMI Body mass index, LH luteinizing hormone, FSH follicle stimulating hormone, p values indicate comparison of features between wild-type homozygous and mutant allele carriers (mutant homozygous and heterozygous). For SNP rs35431622: TT = 0, in both PCOS patients and controls.

**Table 7** Construction of haplotypes of the three studied SNP in the PCOS patients and control group

| Serial No. | SNP | Haplotype Freq. | Freq. | OR (95% CI) | P-value |
|------------|-----|-----------------|-------|-------------|---------|
| rs12998    | rs372790354 | rs35431622 | Control | Case |       |
| 1 | G | G | A | 0.8413 | 0.8302 | 1.00 | – |
| 2 | A | A | A | 0.0508 | 0.0665 | 1.22 (0.56–2.63) | 0.62 |
| 3 | A | G | A | 0.0516 | 0.0393 | 0.82 (0.37–1.78) | 0.61 |
| 4 | G | A | A | 0.0196 | 0.0304 | 1.43 (0.47–4.33) | 0.53 |
| 5 | G | G | G | 0.0244 | 0.02 | 0.74 (0.18–3.05) | 0.68 |
secretion of LH, normal or low level of FSH, and an increased ratio of LH-FSH [30, 31]. In our study, PCOS patients showed significantly higher values of LH and LH-FSH ratio as compared to controls. While the level of FSH was almost the same between the two study groups. Likewise, many previous reports highlighted a significantly elevated level of LH and increased LH-FSH ratio in PCOS subjects with no significant alteration in the FSH level, compared to controls [32, 33]. Importantly, when the influence of the SNP rs372790354 on endocrine parameters (LH, FSH, and LH-FSH ratio) was investigated, it was seen that the level of LH in the PCOS group was influenced by this SNP and was significantly higher in mutant AA genotype. While, in the control group, no significant influence of SNP rs372790354 was observed. Likewise, the level of FSH and LH-FSH ratio showed no significant difference in the genotypes of this SNP in both study groups. These results suggest a significant influence of the AA genotype of rs372790354 on increased LH levels of PCOS women.

Results of several previous studies show that the pathology of PCOS is influenced by the obesity-linked parameters, including BMI and WHR [34, 35]. Hence, we also determined the values of BMI and WHR in both PCOS and control subjects and then observed any possible effects of rs372790354 on these obesity-linked parameters. Our results showed that there was no difference in BMI in the two groups, but WHR was significantly different. These findings confirm the results of other recent studies [17, 36]. Further, when the influence of rs372790354 was investigated on BMI and WHR, only WHR in PCOS patients showed a significant difference between the genotypes (GG and AA).

Based on these findings, we suggest that the rs372790354 polymorphism of the KISS1 gene may have a direct effect on the functional activity of kisspeptin in terms of its altered behavior towards receptor GPR54. Consequently, the disturbed kisspeptin-GPR54 pathway encourages dysregulation in GnRH secretion, which further leads to the hypersecretion of LH. Subsequently, the condition of hyperandrogenism with a high level of testosterone is induced by the direct action of high stimulation of LH on gonads. Eventually, all these conditions may favor the increase in the risk of PCOS.

Many recent reports have suggested the role of altered behavior of kisspeptin as a major cause in inducing hyperandrogenism associated with PCOS [3, 37]. In this present study, we have suggested the role of SNP rs372790354 of the KISS1 gene in creating a disturbance in the functional activity of kisspeptin with hypersecretion of LH, which in turn may be responsible for increasing the risk of PCOS. No other previous studies are available in the literature to compare the results of our study. However, the association of this polymorphism with PCOS should be confirmed by further studies, as the sample size in this study is relatively small and does not give strong evidence for these related findings.

The other identified SNPs rs12998 G/A, and rs35431622 A/T showed no significant association with the risk of PCOS. They did not exhibit any influence on the endocrine and obesity-linked parameters in PCOS patients and controls.

Conclusion
In conclusion, the current study reports three novel SNPs (rs372790354 G/A, rs12998 G/A, and rs35431622 A/T) in the KISS1 gene, among women population of Saudi Arabia and shows a significant influence of rs372790354 G/A on the risk of PCOS. Furthermore, this novel SNP shows a considerable impact on the levels of LH, kisspeptin, and WHR in PCOS women. These findings may improve the understanding of PCOS in regards to its multifactorial etiology, especially those associated with KISS1 gene polymorphisms and their influence on the levels of biochemical and demographic factors. This study has the drawback that it was conducted on only a small number of samples. Hence, further, more extensive studies in different populations are required to confirm this association.

Abbreviations
BMI: Body mass index; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; UTR: Untranslated region; MAF: Minor allele frequency; SNP: Single nucleotide polymorphisms

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Authors’ contributions
MHD, ASW, MD in conception and design of the study, obtained all the needed chemicals, kits and other requirements. MHD conducted all experimental analysis. MHD® contributed in selection of patients, obtained relevant information regarding questionnaire. ASW and MHD conducted the data analysis and interpretation of the data; KA and ASW contributed in preparation and final editing of the manuscript. FSA and LMN were involved in manuscript preparation. 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.

Ethics approval and consent to participate
The study was approved by the Ethical Committee of the Institutional Review Board (IRB), Umm Al-Qura University, Makkah, Saudi Arabia (IRB No. 235). Written informed consent was obtained from all participants.
Consent for publication
Not applicable.

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

Author details
1Department of Zoology, Female Center for Scientific and Medical Colleges, King Saud University, Riyadh, Saudi Arabia. 2Department of Obstetrics & Gynaecology, Umm-Al-Qura University, Makka, Saudi Arabia. 3Department of Surgery, King Abdulaziz Medical City, National Guard Health Affairs, Jeddah, Saudi Arabia. 4Department of Biotechnology, Integral University, Lucknow, India. 5Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia. 6Central Laboratory, Female Center for Scientific and Medical Colleges, King Saud University, Riyadh, Saudi Arabia.

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References
1. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25(2):544–51.
2. Hayek SE, Bitar L, Hamdar LH, Mirza FG, Daoud G. Poly cystic ovarian syndrome: an updated overview. Front Physiol. 2016;7:124.
3. Leo VD, Musacchio MC, Cappelli V, Masaro MG, Morgante G, Petroglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. Reprod Biol Endocrinol. 2016;14(38):1–17.
4. Khan MJ, Ullah A, Basit S. Genetic basis of polycystic ovary syndrome (PCOS): current perspectives. Appl Clin Genet. 2019;12:249–60.
5. Umayal B, Chandrasekharan NV, Wijesundera WS, Wijeyaratne CN. Polycystic ovary syndrome: Genetic Contributions from the hypothalamic-pituitary-gonadal Axis. Int Arch Endocrinol Clin Res. 2018;40(3):1–8.
6. Välenburg O, Lutterinlen AG, Piersma D, Hofman A, Themmen APN, de Jong FH, et al. Genetic polymorphisms of GnRH and gonadotrophin hormone affect the phenotype of polycystic ovary syndrome receptors. Hum Reprod. 2009;24(8):2014–22.
7. Prapas N, Karkanaki A, Prapas I, Kalogiannidis I, Panidis D. Genetics of polycystic ovary syndrome. Hippokratia. 2009;13(4):216–23.
8. Nejad SZ, Tehrani FR, Vakili AZ. The role of kisspeptin in female reproduction. Int J Endocrinol Metab. 2017;15(3):1–11.
9. Albalawi FS, Daghestani MH, Daghestani MH, Eldali A, Warsy AS. rs4889 polymorphism in the KISS1 gene, its effect on polycystic ovary syndrome. J Biomed Sci. 2018;25(50):1–10.
10. Trevisan CM, Denice M, Christofolinia Caio P, Barbosaa Keith A, Biancoa CB. Kisspeptin/GPR54 system: what do we know about its role in human reproduction? Cell Physiol Biochem. 2018;49:1259–76.
11. Johansson J, Stener-Victorin E. Review Article Polycystic Ovary Syndrome: an updated overview. Front Physiol. 2016;25:207–13.
12. Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev. 2012;92(1):323–316.
13. Chitme HR, Al Azazi EAK, Al Abri AM, Al Buasmi BM, Salam ZKA, Al Taie MM, et al. Anthropometric and body composition analysis of infertile women with polycystic ovary syndrome. J Taibah Univ Med Sci. 2017;12(2):139–45.
14. Zeng Q, He Y, Dong S, Zhao X, Chen Z, Song Z, et al. Optimal cut off values of BMI waist circumference and waist:height ratio for defining obesity in Chinese adults. Br J Nutr. 2014;112(1):1735–44.
15. Lu DF, Guo XY. Parameters of obesity in polycystic ovary syndrome. J Rare Dis Res Treat. 2016;2(1):19–22.
16. Zhong Q, He Y, Dong S, Zhao X, Chen Z, Song Z, et al. Optimal cut off values of BMI waist circumference and waist:height ratio for defining obesity in Chinese adults. Br J Nutr. 2014;112(1):1735–44.
17. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81:19–25.
18. Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(6):618–37.
19. Umayal B, Chandrasekharan NV, Wijesundera WS, Wijeyaratne CN. Association of Kiss1 and GPR54 polymorphisms with Polycystic Ovary Syndrome among Sri Lankan women. J Taibah Univ Med Sci. 2017;12(2):19–20.