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Association analysis of KISS1 polymorphisms and haplotypes with polycystic ovary syndrome

M Farsimadan[a], F Moammadzadeh Ghosi[b], S Takamoli[a] and H Vaziri[a]

[a]Department of Biology, Faculty of Sciences, University of Guilan, Rasht/Iran; [b]Department of Medical Microbiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

ABSTRACT

Introduction: *KISS1* play an essential role in human reproductive functions by regulating the hypothalamic-pituitary-gonadal axis. Loss-of-function mutations in this gene have been frequently identified in patients with different reproductive disorders. We hypothesised links between *KISS1* polymorphisms and polycystic ovary syndrome (PCOS).

Materials and methods: In order to find links between *KISS1* polymorphisms rs4889 C > G, rs12998 G > A, and rs35431622 A > G with PCOS, 770 blood samples were obtained from 385 control and 385 PCOS women. DNA was extracted, and genotyped for *KISS1* variants by PCR.

Results: rs12998 G > A was linked to PCOS in dominant ($p < 0.001$), recessive ($p < 0.001$), co-dominant ($p < 0.001$), and allelic models ($p < 0.001$). In addition, rs4889 C > G was linked in recessive, dominant, co-dominant, and allelic models ($p < 0.001$). rs35431622 A > G was not linked to PCOS. Further analysis indicated that C-G-G haplotype was more common and G-A-G haplotype was less prevalent in cases compared with controls.

Conclusion: *KISS1* variants rs12998 G > A and rs4889 C > G may be linked to the pathophysiology of PCOS.

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KEYWORDS
Polycystic ovary syndrome; *kiss1* gene; polymorphism

Introduction

Infertility affects about 15% of couples throughout the world and female infertility constitutes 37% of all infertility cases [1,2]. Polycystic ovary syndrome (PCOS), a common cause of infertility, affects 6–10% of women in reproductive age [3]. PCOS is a heterogeneous disorder characterized by hyperandrogenism, anovulation, and enlarged polycystic ovaries [4]. Women with this syndrome display menstrual irregularity, hair growth, acne, and overweight. A number of factors, including genetic, endocrine, and physiological factors are considered as major causes of PCOS. Over recent years, considerable attention has been directed at understanding the multifactorial aetiology of PCOS. A number of studies have been focused on investigating the hypothalamic-pituitary-gonadal (HPG) axis genetic factors related to PCOS and reported the disruption in the function of the HPG axis resulting from increased frequency and amplitude of the hypothalamic GnRH pulse generator. Various functional polymorphisms have been identified in genes that control the HPG axis that regulate the proper function and development of the female reproductive system [5,6]. *KISS1*, as one of these genes, is considered to have a vital regulatory role in gonadotropin secretion of the HPG axis. Different studies showed that SNPs in the *KISS1* and its receptor *KISS1R* could disrupt the HPG axis function and may play a significant role in the etiopathogenesis of PCOS.

KISS1, located at 1q32, consists of three exons [7]. Its product, kisspeptin, also known as metastine, is a 54-amino acid peptide first discovered while searching for melanoma metastasis-suppressor genes [8]. Different studies indicate that binding of kisspeptin to its receptor (GPR54) in GnRH neurons in the hypothalamus activate the HPG axis and lead to the stimulation of gonadotropin release, which in turn binds to the GnRH receptors in the pituitary and influences the release of LH and FHS, so playing a critical role in the maintenance of reproductive function [9]. Different mutations in exons of *KISS1* would deregulate the kisspeptin-GPR54 signalling pathway, resulting in disruption of GnRH secretion may increase the development of PCOS. We hypothesised links between certain *KISS1* polymorphisms and PCOS.

Methods

We tested our hypothesis in 770 women: 385 cases and 385 controls. The criteria for the diagnosis of the PCOS patients was based on two of the three features of the Rotterdam 2003 criteria: (I) oligomenorrhea (menstrual period length > 35 days) or ame-

CONTACT H Vaziri drhamidrezavaziri00@gmail.com; vaziri@guilan.ac.ir Department of Biology, Faculty of Sciences, University of Guilan, Rasht/Iran.

norrhoea (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 containing 12 follicles measuring 2–9 mm in diameter and/or increased ovarian volume of at least 10 ml). Exclusion criteria were other reasons of hyperandrogenism or menstrual irregularity such as Cushing's syndrome, prolactinoma, and congenital adrenal hyperplasia, pregnancy, the first postpartum year, and receipt of any hormonal medicine three months prior to recruitment. Healthy controls had a regular menstrual cycle (menstrual period up to 7 days and menstrual cycle of 21–35 days), normal androgen levels, no symptoms of hirsutism, and no history of PCOS. The study was approved by the high graduate committees of the Faculty of Medicine/University of Guilan, Rasht, Iran. The informed consent documents were obtained from all subjects prior to sampling.

Blood samples were collected in tubes containing EDTA and stored at −20 °C until use. Genomic DNA was extracted from white blood cells with the Blood and Cell Culture DNA kit (Sinacolon, Iran) according to the manufacturer's protocol. DNAs were analysed by electrophoresis on 2% agarose gels with ethidium bromide staining. Concentrations of extracted DNAs were determined by Nanodrop. Three SNPs were chosen for the genotyping process. Table 1 shows information about the position and types of selected SNPs. The *KISS1* products were amplified from genomic DNA by PCR and genotyped by a restriction fragment length polymorphism (RFLP). The PCR conditions started with an initial denaturation at 94 °C for 5 minutes, 35 cycles of denaturation of molecules at 94 °C for 1 minute, annealing at 59 °C for rs4889, 56 °C for rs12998, and 60 °C for rs35431622 for 1 minute, and the products were under extension at 72 °C for 40se, and then a final extension at 4 °C for 5 minutes in Biorad Thermocycler. Subsequently, we performed RFLP analysis with the conditions summarized in Table 1. PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining.

All statistical analyses were done using both SNPAlyze software (ver.8.1, Dynacom, Japan) and SPSS (ver.22). Allele and genotype frequencies of the *KISS1* SNPs among control and case groups were compared and checked using Pearson $\chi 2$ statistic. Moreover, deviations from Hardy–Weinberg equilibrium (HWE) were tested using a $\chi 2$ goodness-of-fit test. Analyses were also performed assuming recessive, codominant, and dominant models of inheritance and crude odds ratio (OR), their 95% CI ranges. Haplotype analyses were done using rs4889, rs12998, and rs35431622 variants for all study samples according to the maximum-likelihood method with an expectation–maximization algorithm. In addition, pairwise LD coefficients of |D′| and r2 were assessed using SNPAlyze software version 8.1 (DYNACOM, Japan). LD analyses were done based on Hardy-Weinberg equilibrium model. The significance level of the statistical tests was selected to be <0.05.

Results

In the present study, the DNA of 385 (mean/SD age 28.1 ± 0.3) patients and 385 healthy controls (mean/SD age 28.6 ± 0.20)(P = 0.28) were analysed to perform the association of *KISS1* gene variants with PCOS. No statistically significant difference was observed in BMI (Kg/m2) of patients in comparison with controls (26.6 ± 5.7 vs. 26.1 ± 4.8)(p = 0.13). The results revealed that the PCOS group had higher rates of FSH compared to controls (6.5 ± 2.2 IU/L vs. 5.9 ± 2.5(p = 0.022) and also significantly higher levels of LH among patients indicating a noticeable endocrine disturbance among them (15.6 ± 4.1 IU/L vs. 5.7 ± 1.2)(p < 0.001). Further analysis revealed that in the PCOS group, 202 women had experienced pregnancy among which 181 had at least one successful pregnancy, whereas in controls 258 females had experienced pregnancy and 242 individuals had a history of at least one successful pregnancy (89% vs. 93%) (p = 0.10). Of 181 PCOS women, 50 (27.6%) reported only one successful pregnancy and 131 (72.3%) patients had more than one successful pregnancy. In comparison, from 242 controls, 58 (24%) and 184 (76%) women reported to have only one and more than one successful pregnancy, respectively [(50 vs. 58) (p = 0.39)] [(131 vs.184) (p = 0.39)]. Also, a significant association was found for the history of miscarriage in PCOS women compared to controls [39(19%) vs. 26(9%) (p = 0.005)]. The number of premature deliveries was significantly higher in the PCOS group compared to controls [25 (12.3%) vs. 11(4%) (p = 0.001)].

Table 1. General information of selected SNPs obtained from dbSNP.

SNP	Position	Location	Codon change	A.A change	Type of variant	Primer sequences	Enzyme	Products length (bp)
rs4889	chr1:204,190,659	Exon 3	CCC → CTC	Pro81Arg	Missense	F:5 - CAAAGCCATCTTTCCCGGAC −3 R: 5 - GCCGAAGGAGTTCCAGTTGT −3′	BseYI	304, 192, 112
rs12998	chr1:204,192,819	Exon 1	GAG → AAG	Glu20Lys	Missense	F:5 - ACTGTCCCTTTTGCACTGG-3 R: 5 - GGAAAGCTCATTTTGCAACAAC-3′	NlaIV	291, 184, 107
rs35431622	chr1:204,190,794	Exon 2	CAG → CGG	Gln36Leu	Missense	F:5 - ATCCCAGCTAAGGTGATCGTG −3 R: 5 - TTGTAGTTCGGCAGGTCCTTC −3′	NaeI	392, 232, 160

Allele and genotype distribution frequencies of the three SNPs in the case and control groups are shown in Table 2. The results revealed that rs12998 G > A had a significant association with PCOS under the dominant, recessive, co-dominant, and allelic model. Also, rs4889 C > G showed a significant association with PCOS under the recessive, co-dominant, and allelic model, respectively; however, rs35431622 A > G did not indicate any association with PCOS.

Statistical analyses indicated that the frequencies of SNP alleles in the control group are in accordance with the HWE except for rs4889.

Also, to evaluate the association of *KISS1* variants with PCOS, haplotype analyses were done using, rs4889, rs12998, and rs35431622 variants for all study samples. Haplotype analyses among case and control groups were done according to the maximum-likelihood method with an expectation–maximization algorithm. P values were assumed by comparing haplotype frequencies between case and control subjects based on 10,000 replications. We found significant negative associations between seven haplotypes and PCOS status. The C-G-G and G-G-A haplotypes, corresponding to rs4889, rs12998, and rs35431622, were the most prevalent haplotypes among cases and controls. The frequencies of estimated haplotypes between cases and controls are shown in Table 3.

Additionally, there was no LD between the rs4889, rs12998, and rs35431622 SNPs based on the measured D′ and r^2 parameters (Table 4).

Discussion

Kisspeptin and its receptor play an essential role in the regulation of endocrine function. Soon after the discovery of this neuropeptide, it was reported that they are significant regulators of the GnRH secretion and reproductive axis. Researchers in various studies indicate the significant role of kisspeptin in the onset of puberty in mammals by stimulating the secretion of gonadotropin-releasing hormone [10]. Loss of function mutations in *KISS1* and its receptor GPR54 lead to increased infertility, hypogonadotropic hypogonadism, and central precocious puberty [11,12].

In women, reproductive function depends on proper development and regulation of the hypothalamic pituitary gonadal (HPG) axis. Kisspeptin directly stimulates gonadotropin-releasing hormone neurons to secrete GnRH at the onset of puberty and to initiate the preovulatory surge of LH during reproductive cycles; therefore, it might be potentially involved with the development of PCOS. The results of the present study showed that rs12998 G > A and rs4889 C > G had a significant association with PCOS; however, rs35431622 A > G did not indicate any association with PCOS.

Table 2. Allele and genotype distribution frequencies of every three SNPs in the case and control groups.

SNP	Model	Cases (n, %)	Controls (n, %)	OR (95% CI)	P-value
rs4889	Allele C	486 (63.1)	354 (46.0)	0.5 (0.40-0.610)	<0.001
	Allele G	284 (36.9)	416 (53.0)		
	Co-dominant (CG v CC+GG)	240 (62.3)	177 (46.0)	1.94 (1.45-2.59)	<0.001
	Dominant (CC v CG+GG)	123 (31.9)	89 (23.1)	0.64 (0.46-0.88)	0.006
	Recessive (GG v CC+CG)	22 (5.8)	119 (30.9)	0.13 (0.08-0.21)	<0.001
		P< 0.01[a]	P= 0.14[b]		
rs12998	Allele G	488 (63.4)	626 (81.3)	2.47 (1.96-3.12)	<0.001
	Allele A	282 (36.6)	144 (18.7)		
	Co-dominant (GA v GG+AA)	167 (43.4)	111 (28.8)	1.89 (1.40-2.54)	<0.001
	Dominant (GG v GA+AA)	161 (41.8)	257 (66.7)	2.79 (2.08-3.74)	<0.001
	Recessive (AA v GG+GA)	57 (14.8)	17 (4.4)	3.96 (2.14-6.59)	<0.001
		P= 0.2[a]	P= 0.26[b]		
rs35431622	Allele A	408 (53.0)	406 (52.7)	0.99 (0.81-1.20)	0.91
	Allele G	362 (47.0)	364 (47.3)		
	Co-dominant (AG v AA+GG)	187 (48.6)	177 (46.0)	1.11 (0.83-1.47)	0.76
	Dominant (AA v AG+GG)	110 (28.6)	114 (29.6)	1.05 (0.77-1.43)	0.75
	Recessive (GG v AA+AG)	88 (22.8)	94 (24.4)	0.91 (0.65-1.27)	0.61
		P= 0.61[a]	P= 0.12[b]		

[a] indicates HWE p-value for cases, [b] indicates HWE p-value for controls

Table 3. Distribution of haplotype blocks in PCOS patients and controls.

rs4889	rs12998	rs35431622	Overall frequency	Case frequency	Control frequency	OR (95% CI)	P-value
C	G	A	0.20	0.20	0.21	0.89 (0.68–1.16)	0.47
C	G	G	0.19	0.22	0.18	1.44 (1.15–1.80)	0.001
G	G	A	0.17	0.12	0.22	0.48 (0.37–0.64)	<0.001
G	G	G	0.15	0.09	0.20	0.17 (0.11–0.24)	<0.001
C	A	A	0.07	0.11	0.02	4.92 (2.67–9.04)	<0.001
G	A	A	0.07	0.09	0.06	1.54 (1.16–2.06)	0.003
C	A	G	0.06	0.10	0.03	3.79 (2.12–6.78)	<0.001
G	A	G	0.05	0.05	0.06	1.35 (0.81–2.24)	0.24

Three SNPs (including rs4889, rs12998, and rs35431622) used to analyse haplotypes. Eight haplotypes with a frequency of more than 0.05% were found.

Table 4. Evaluation of linkage disequilibrium between KISS1 SNPs.

	D′ statistic		r^2 statistic		P value
SNPs	rs12998	rs35431622	rs12998	rs35431622	0.38[a]
rs4889	0.03	0.005	0.004	0 < 001	0.82[b]
rs35431622	0.03	-	0.004	-	0.40[c]

[a](rs4889 vs. rs12998), [b](rs4889 vs. rs35431622), [c](rs12998 vs. rs35431622)

Recently, the relationship between *KISS1* polymorphisms and predisposition to some reproductive diseases has received considerable attention. Kisspeptins have been considered as the most potent stimulators of GnRH/gonadotropin secretion and the key regulator of LH pulsatile release, which might be potentially involved with the development of PCOS; however, only a few studies have assessed the effects of *KISS1* SNPs in the pathophysiology of PCOS. Daghestani et al. investigating the effects of kisspeptin on PCOS, identified three novel polymorphisms rs1213704663 C > G, rs1481572212 T > G, and rs775770652 G > A in *KISS1* in women who suffered from PCOS; however, only rs1213704663 C > G was found to be significantly associated with PCOS [13]. Another study indicated that two other SNPs in this gene, rs12998 and rs35431622, had no significant influence on PCOS, but rs372790354 revealed a significant association with the values of LH and kisspeptin in PCOS women under the dominant model [14]. However, these studies suffer from the limited number of samples which might significantly affect their findings. Another study among Sri Lankan women revealed no significant difference in the frequency of the two identified *KISS1* polymorphisms including rs5780218 and rs4889 in PCOS patients and controls [15]. Contrasting results were observed in an investigation in Saudi Arabia: they found rs4889 polymorphism to be a factor contributing to PCOS [16]. Vaziri et al. evaluated the association of rs35431622 A > G with idiopathic infertility among women and found this polymorphism did not have any relationship with increased idiopathic infertility [17].

KISS1 and *KISS1R* are the first genes involved in gonadotropin-dependent precocious puberty (GDPP) phenotypes in humans. Patients with *KISS1* mutations exhibit typical features of idiopathic GDPP and show an adequate response to conventional GDPP treatment with GnRH agonists [18,19]. Different investigations have studied *KISS1* mutations in disorders of puberty. In a very recent study on a large group of girls, Li et al. evaluated the association of *KISS1* polymorphism with the risk of central precocious puberty (CPP) and their results suggested that the mutation in rs5780218 increases the risk of CPP [20]. Similarly, the results of another study among Chinese girls diagnosed with CPP uncovered several potentially polymorphisms in *KISS1* and reported the frequency of these SNPs to be significantly higher among patients compared with controls [21]. Likewise, Silveira et al. demonstrated two different missense mutations in *KISS1* be associated with a higher risk of isolated hypogonadotropic hypogonadism (IHH) and CPP [11]. However, some studies failed to show any relationship between the *KISS1* polymorphisms and increased risks of CPP [22,23] and IHH [24].

This work represents an advance in biomedical science because it shows a link between PCOS and both individual and combined (haplotype) *KISS1* SNPs that may have a place in early diagnosis.

Summary table

What is known about this subject:
- Kisspeptin plays a critical role in the maintenance of reproductive function by regulating the release of LH and FHS.
- Different mutations in the *KISS1* gene could disrupt the GnRH secretion and may increase the development of PCOS.

What this paper adds:
- A strong link between PCOS and the *KISS1* SNPs.
- The rs12998 G>A and rs4889 C>G variants show a significant association with PCOS.
- The rs35431622 A>G variant is not linked to PCOS.

Disclosure statement

The authors declare no conflict of interest.

ORCID

M Farsimadan http://orcid.org/0000-0001-5917-7845
H Vaziri http://orcid.org/0000-0001-6325-8349

References

[1] Farsimadan M, Motamedifar M. The effects of human immunodeficiency virus, human papillomavirus, herpes simplex virus-1 and −2, human herpesvirus-6 and −8, cytomegalovirus, and hepatitis B and C virus on female fertility and pregnancy. Br J Biomed Sci. 2020;1–11. DOI:10.1080/09674845.2020.1803540
[2] Farsimadan M, Motamedifar M. Bacterial Infection of the Male Reproductive System Causing Infertility. J Reprod Immunol. 2020;103183. DOI:10.1016/j.jri.2020.103183
[3] Cena H, Chiovato L, Nappi RE. Obesity, polycystic ovary syndrome and infertility: a new avenue for GLP-1 receptor agonists. J Clin Endocrinol Metab. 2020. DOI:10.1210/clinem/dgaa285
[4] Wang L, et al. Altered circulating inflammatory cytokines are associated with anovulatory polycystic ovary syndrome (PCOS) women resistant to clomiphene citrate treatment. Med Sci Monit. 2017;23:1083.
[5] Haqiqi H, et al. Association of FSHR missense mutations with female infertility, in silico investigation of their molecular significance and exploration of possible treatments using virtual screening and molecular dynamics. Anal Biochem. 2019;586:p. 113433.
[6] Jodeiryzaer S, et al. Association of oestrogen receptor alpha gene SNPs Arg157Ter C> T and Val364Glu T> A with female infertility. Br J Biomed Sci. 2020;1–3.

[7] Fratangelo F, Carriero MV, Motti ML. Controversial role of Kisspeptins/KiSS-1R signaling system in tumor development. Front Endocrinol (Lausanne). 2018;9:192.
[8] Lee J-H, et al. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. JNCI. 1996;88: 1731–1737.
[9] Colledge WH. Kisspeptins and GnRH neuronal signalling. Trends Endocrinol Metab. 2009;20:115–121.
[10] Messager S, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Nat Acad Sci. 2005;102:1761–1766.
[11] Silveira LG, et al. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab. 2010;95:2276–2280.
[12] Siahpoosh Z, et al. KISS1R polymorphism rs587777844 (Tyr313His) is linked to female infertility. Br J Biomed Sci. 2021.
[13] Daghestani MH, et al. Relevance of KISS1 gene polymorphisms in susceptibility to polycystic ovary syndrome and its associated endocrine and metabolic disturbances. Br J Biomed Sci. 2020. DOI: 10.1080/09674845.2020.1726662. just-accepted.
[14] Daghestani MH, et al. Influence of KISS1 gene polymorphisms on the risk of polycystic ovary syndrome and its associated variables, in Saudi women. BMC Endocr Disord. 2020;20:p. 1–10.
[15] Branavan U, et al. Association of Kiss1 and GPR54 gene polymorphisms with polycystic ovary syndrome among Sri Lankan women. Biomed Res Int. 2019;2019. DOI:10.1155/2019/6235680.
[16] Albalawi FS, et al. rs4889 polymorphism in KISS1 gene, its effect on polycystic ovary syndrome development and anthropometric and hormonal parameters in Saudi women. J Biomed Sci. 2018;25: p. 50.
[17] Vaziri H, Rafeie A, Siapoosh Z. Q36R (rs 35431622) Polymorphism in KISS1 gene and idiopathic female infertility in a Northern Iranian population. Gene Cell Tissue. 2017;4.
[18] Silveira LFG, Trarbach EB, Latronico AC. Genetics basis for GnRH-dependent pubertal disorders in humans. Mol Cell Endocrinol. 2010;324:30–38.
[19] Teles MG, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med. 2008;358:709–715.
[20] Li D, et al. Association of polymorphisms in the Kisspeptin/GPR54 pathway genes with risk of early puberty in Chinese girls. J Clin Endocrinol Metab. 2020;105:e1458–e1467.
[21] Luan X, et al. Association study of the polymorphisms in the KISS1 gene with central precocious puberty in Chinese girls. Eur J Endocrinol. 2007;157:113–118.
[22] Tommiska J, et al. LIN28B, LIN28A, KISS1, and KISS1R in idiopathic central precocious puberty. BMC Res Notes. 2011;4:1–4.
[23] Krstevska-Konstantinova M, et al. Mutational analysis of KISS1 and KISS1R in idiopathic central precocious puberty. J Pediatr Endocrinol Metab. 2014;27:199–201.
[24] Zhang Y, et al. Mutations in KISS1 are not responsible for idiopathic hypogonadotropic hypogonadism in Chinese patients. J Assist Reprod Genet. 2015; 32:375–378.