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KISS1R polymorphism rs587777844 (Tyr313His) is linked to female infertility

Z Siahpoosh[a], M Farsimadan[a], M Pazhohan[b], H Vaziri[a] and M Mahmoudi Gomari[c]

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

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Infertility is a reproductive system disorder, defined as the failure to become pregnant after 12 months of regular, unprotected intercourse, or to produce a viable infant [1,2]. Female infertility might be associated with a number of factors, including uterine, cervical, uterine, tubal abnormalities, chromosomal abnormalities, endocrine disorders, and immune defects, but 15–30% of cases remain unexplained [3]. As many aspects of these defects are strongly influenced by genetic factors, numerous studies attempted to identify candidate genes [4]. Specific genes and mutations can increase hormonal problems and may eventually lead to infertility [4]. *KISS1* and *KISS1R* are two of these genes [5–7].

Gonadotropin hormone-releasing hormone (GnRH) is the key regulator of the reproductive axis. The secretion of GnRH regulates the secretion of two significant hormones, gonadotropins follicle stimulating hormone (FSH) and luteinising hormone (LH), from cells in the anterior pituitary which play essential roles in endocrine function and gamete maturation. Significant advancements in reproductive biomedicine reveal an important role for kisspeptin in reproduction and discovery of a kisspeptin-neurokinin-dynorphin neuronal network in the hypothalamus. *KISS1* maps to chromosome 1q32 and contains three exons, with two of them translated into 145 amino and cleaved into an amidated C-terminal 54 amino acid product, kisspeptin-54 and shorter biologically active peptides, kisspeptin-14, −13, and − 10 [5,6]. The kisspeptin receptor, *KISS1R*, located at 19p13.3, consists of 5 exons and 4 introns and encodes a protein of 398 amino acids with seven transmembrane domains. Several studies have reported *KISS1* and *KISS1R* mutations that result in reproductive disorders such as idiopathic hypogonadotropic hypogonadism, precocious puberty, polycystic ovarian syndrome that may lead to infertility, but none has examined a potential role for *KISSR1* polymorphism rs587777844, responsible for a Tyr313His transformation [8–11]. Accordingly, we hypothesised a link between *KISSR1* polymorphism rs587777844 and female infertility.

Blood samples from 100 infertile women (mean [SD] age 99 [99], The mean and SD of the ages of controls is 26.14±0.20 and for the cases is 26.47±0.21) and 1000 healthy controls (age 99 [99], $p = 0.999$, The P value for the difference is 0.18) were collected from Guilan University Hospital, Rasht, Iran. The infertile group of 100 women reported no pregnancy after two years of unprotected regular sexual intercourse. The sexual partners of the women had no fertility disorders with normal semen parameters. Control women had at least one natural pregnancy without taking medications or using assisted reproductive technologies. An informed consent was obtained from all subjects. The study was approved by the ethics committee of Alzahra Hospital (no.: IR.GUMS.REC.1397.387).

Two ml of peripheral blood samples were collected from each into EDTA-coated venojects and were stored at −20°C. DNA was extracted using the Gpp Solution Extraction Kit (Gene Pazhouhan Co., Iran). DNA quality was determined with electrophoresis on 1.8% agarose gel stained by ethidium bromide (0.5 mg/ml). After checking extraction product quality, DNA was moved to the freezer at −70°C. PCR amplification was carried out by utilising primers designed by Oligo7 Software (version 7.54, Molecular Biology Insights Inc., Cascade, CO, USA) including Forward (F): 5′-CAGGAGGGGCGGT GCGAGGGG-3′ and Reverse (R): 5′-GGGTTCAGCGCG GAGTTGCTGGA-3′ for control allele (PCR product = 289 bp), F: 5′-CAGGAGGGGCGGTGCGAGGGG −3′ and R: 5′-GGGTTCAGCGCGGAGTTGCTGG −3′ for mutant allele (PCR product = 289 bp). PCR reaction mixture (25 µl) contained approximately 30 ng of genomic DNA, 1x PCR buffer, 0.2 mM dNTP, 1.5 mM of $MgCl_2$, 0.5 µM of primers, and 2 units of Taq DNA Polymerase (Bio flux, Japan). DNA amplification by PCR was performed by the following steps: initial denaturation at 95°C for 5 min, 35 cycles of denaturation of 95°C for 45 s, annealing at 54°C for 30 s, and 53°C for 30 s, extension at 72°C for 45 s, and final elongation at 72°C for 5 min. PCR products were separated on 2% agarose gel and visualised by ethidium bromide staining. PCR products showed that individuals who had two 298-base pair

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

segments amplified with the primers related to mutant and wild alleles were heterozygous (T/C). Those who only had the 298-base pair segment amplified with the primer related to the wild-type allele were healthy homozygous (T/T). Finally, those who only had the 298bp PCR product amplified with the primer related to the mutant allele were affected homozygous (C/C).

To evaluate the biological function of rs587777844 polymorphism of *KISS1R* gene, in silico analysis was also conducted. Different bioinformatics tools were used to assess the effects of this polymorphism on the structure of the protein. Statistical analyses were assessed by SPSS ver. 22. Allelic and genotypic frequencies were measured in both groups. The difference in genotype distribution between the affected and control groups was found using the chi-squared test. A value of $P < 0.05$ was assumed as statistically significant.

Genotyping of rs587777844 was performed by ASPCR method. The statistical analysis revealed a significant difference under recessive models of inheritance. Allele frequency also differed significantly across the groups. The statistical analysis of the genotypes and allele frequency of the SNP is shown in Table 1. Different in silico analysis predicted *KISS1R* rs587777844 variation to be damaging. PredictProtein and SNAP servers indicated that Tyr313His substitution could have significant effects on the protein structure (Scores: 69; Expected accuracy: 80%), which might be associated with the damaging effects on KR structure (Figure 1).

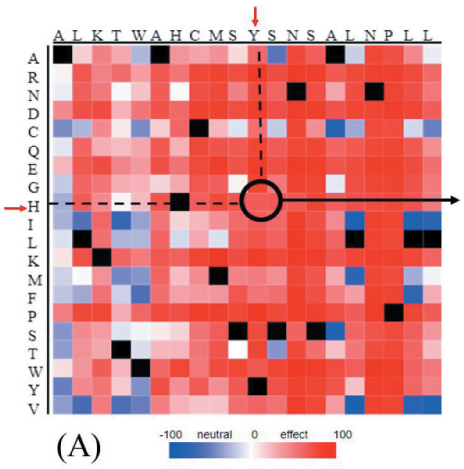
Kisspeptin and its receptor are significant regulators of the GnRH secretion and reproductive axis [7–9,12–14]. There may also be a role for KISS1 SNPs in polycystic ovary syndrome [10]. Different studies have investigated KISS1/KISS1R function but until now, there have been few statistical and modelling studies on *KISS1R* and its SNPs. In this study, the effect of rs587777844 polymorphism on KISS1R normal activity was evaluated both statistically and using modelling tools. In this point mutation, T changes to C and codon TAC turns to CAC. As a result of this missense mutation, tyrosine amino acid (T) is converted to histidine (H). This polymorphism is located at in exon 5 locus 937 of *KISS1R* gene and codon 313 of the KISS1R protein that is highly conserved.

Recently, the relationship between *KISS1R* genetic polymorphism and predisposition to some reproductive diseases has received considerable attention. Different *KISS1R* gene mutations, such as L148S [7], C223R [12], R297L [7], L102P [7], E232Q [13], R331X [7], and X339R [7] which are loss of function mutations, have led to the congenital hypogonadotropic hypogonadism phenotype. In addition, P196H [14], R386P [7], and P110T [8] polymorphisms are gain of function mutations and lead to precocious puberty. A study from Tunisia revealed that an autosomal dominant GPR54 mutation that was a substitution of proline for arginine at codon 386 (Arg386Pro) was associated with idiopathic central precocious puberty (CPP) [9]. Also, Pagani et al. in a very recent study discovered a new mutation in *KISS1R* gene (rs 350132) and considered it as an inducible factor in the pathogenesis of CPP [11].

Table 1. Allelic and genotypic frequency of Y313H polymorphism in infertile women and control group.

Genotype - Allele	Cases	Controls	P value
TT	0.30	0.40	**<0.001**
TC	0.48	0.58	
CC	0.22	0.02	
T	0.54	0.89	**0.029**
C	0.46	0.21	
Dominant (TT v. TC+CC)	1.55 (0.866-2.79)		0.138
Co-dominant (TC v. TT+CC)	1.62 (0.92-2.84)		0.08
Allelic level C v. T	1.98 29.9 (3.54-204.5)		**0.001**
Recessive (CC v. TT+TC)	1.98 (1.31-2.99)		**<0.001**

Data presented as frequency (N=100 per group) or as odd ration with 95% confidence interval.



Wildtype Amino Acid	Position	Variant Amino Acid	Predicted Effect	Score	Expected Accuracy
Y	313	A	effect	62	80%
Y	313	R	effect	91	95%
Y	313	N	effect	79	85%
Y	313	D	effect	89	91%
Y	313	C	effect	58	75%
Y	313	Q	effect	83	91%
Y	313	E	effect	89	91%
Y	313	G	effect	83	91%
Y	313	H	effect	69	80%
Y	313	I	effect	69	80%
Y	313	L	effect	74	85%
Y	313	K	effect	91	95%
Y	313	M	effect	52	75%
Y	313	F	effect	55	75%
Y	313	P	effect	92	95%
Y	313	S	effect	79	85%
Y	313	T	effect	84	91%
Y	313	W	effect	72	85%
Y	313	Y	neutral	-57	78%
Y	313	V	effect	65	80%

(B)

Figure 1. The results of the effect of Tyr313His substitution on protein functions evaluated by SNAP and PredictProtein. SNAP predicted KISS1R Tyr313His mutation to be effect (A). As the figure shows, the point mutations with the score ranged from −100 to 0 indicated in blue and white colour are neutral and the mutations with the score ranged from 0 to 100 in red colour shows the severity of the effect of point mutations. A graphic summary and result of the analysis is shown in the table (B).

KISS1 and *KISS1R* are the first genes involved in gonadotropin-dependent precocious puberty (GDPP) phenotypes in humans so far. Patients with *KISS1R* or *KISS1* mutations exhibited typical features of idiopathic GDPP and showed an adequate response to conventional GDPP treatment with GnRH agonists [9,15].

The complete deficiency was observed among all the patients in an investigation on a family in Israel who all had a homozygous mutation (F272S) in *KISS1R*. They indicated that this mutation in all the female patients was significantly associated with primary amenorrhoea and impuberism. The results of this study demonstrated the important role of this gene in female reproductive function [16]. The results of our study were also consistent with these findings and indicated the rs587777844 to be functional SNPs of *KISS1R* gene that might be linked to infertility. It was revealed that the presence of the C allele could be a risk factor for infertility among women.

This work represents an advance in biomedical science because it shows a link between infertility and the *KISS1R* (rs587777844) SNP.

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

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