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Relationship between idiopathic female infertility with KISS1receptor gene mutation in northern Iranian women

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Abstract

Background: The kiss peptins and its receptor G protein coupled receptor (GPR54) or KISS1 receptor system are being described as key signaling molecules for reproductive function in animal models and humans. They play essential roles in regulation of the hypothalamic-pituitary- gonadal (HPG) axis and the onset of puberty and fertility.

Objective: This study was performed to delineate the association of T305C (Leu 102 Pro) KISS1 receptor gene mutation with idiopathic female infertility in Iranian women.

Methods: In this study, 140 healthy women with at least one child and no history of infertility and abortion and 130 idiopathic infertile women were recruited for this study. By using allele specific PCR (AS-PCR) method, the allele and genotype frequencies among infertile and healthy women were determined.

Results: The gene frequencies of the 305 T and C allele of the KISS1 receptor were 45% and 54% among infertile women and 50% and 50% among healthy controls, respectively. The distribution of genotype frequencies in the patients and controls was as follows: TT (Leu/Leu) was 15% and 0%, TC (Leu/Proline) was 60% and 100% and CC (Pro/Pro) was 24% and 0% respectively. Structural analysis was performed using the MedCalc program (version 12). Our results suggests that significant association were not observed in genotype (P=0.8) and allelic (P=0.6) distribution between cases and controls.

Conclusions: The data presented show that mutant allele C is not a risk factor for infertility, suggesting that the presence of KISS1 receptor T305C mutation is probably not associated with idiopathic female infertility in this population (P>0.05).

Keywords: Infertility, KISS1 receptor, Mutation

Introduction

Infertility is an important medical/social problem that many couples suffer in the worldwide. According to world health organization (WHO) definition, infertility is defined as an inability of the couples to conceive after one year of regular intercourses without using any contraceptive methods (this time is six months for women who are more than 35 years old) (Practice Committee of the Americal Society for Reproductive Medicine, 2008). 15% of all couples are infertile, but female factors accounts for more than 50 percent of cases (Estevens et al., 2012).The proportion of female infertility cases are

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associated with several defect such as ovulation abnormalities, tubal defects, obesity and neuroendocrine system disorders. Approximately, 10-15 percent of all couples will experience primary infertility (refers to couples who have not been pregnant after at least one year of unprotected intercourse) or secondary infertility that means debility to conceive after one successful pregnancy (Healy et al., 1994; Thonneau et al., 1989). The reason of 10-15 percent of infertility in women is unanswered that this is considered as an idiopathic female infertility (Gordon, 2010). There are several genes that affect female fertility. One of those genes is *KISS1* and *KISS1* receptor gene. *KISS1* gene has been localized on chromosome 1 and encodes 145 amino acids that can be cleaved to different lengths of kisspeptins (kp10, kp13, kp14 and kp54) (Cerrato and Seminara, 2007). The kp54 is originally called metastin because of its ability to suppress tumor metastasis (Gottsch et al., 2009, 2009). The importance of kps as a key regulator and mediator of reproductive biology in several of mammals has been discovered since 2003 (Gianetti and Seminara, 2008). Kp' function via its G-protein coupled receptor (KISS1 receptor) is being determined (Lee et al., 1999). Evidence indicated that kp acts directly on hypothalamic, GnRH neurons, stimulates GnRH and gonadotropin secretion from anterior pituitary and being described as mediator of the puberty and reproductive axis (Kaur et al., 2012). *KISS1* receptor (GPR54) is highly expressed in placenta and pancreas and lowly expressed in peripheral leukocyte, spleen, thymus, adrenal gland and lymph node (Funes et al., 2003). Kp is originally expressed in placenta and have proven as a regulator of pregnancy. Circulating kisspeptin levels are low in males and non pregnant females but increase during conception (Reynolds et al., 2009). The *KISS1* receptor is assigned to short arm of chromosome 19 (19p13.3) comprising five exons and four introns (Muir et al., 2001). Several variations in this gene have been discovered. It is found in exon 2 at position 305 and determined an amino acid substitution of leucine by proline at position 102 in the corresponding protein. This substitution can be lead to hypogonadotropic hypogonadism in human (Tanenbaum-Rakover et al., 2007.But until now, the association between T305C mutation and idiopathic female infertility has not been studied. We investigated the distribution of the mutation in relation to infertility in Iranian women.

Materials and Methods

Study Subjects

The cases comprised of 140 idiopathic infertile patients who had been unable to conceive within a year (The control group consisted of 130 healthy women with at least one child. The

case and control groups have been matched by sex and age. All the participants (from the Guilan province) provided informed consent prior to sample retrieval. Patients with the history of amenorrhea, oligomenorhea, hyperprolactenemia, polycystic ovarian syndrome (PCOS), endometriosis, fibroids and antisperm antibody syndrome were excluded from the study after evaluation by infertility specialist. The study was approved by ethics Committee of Guilan University. This study was performed in compliance with the declaration of Helsinki regarding the use of human samples.

DNA extraction and genotyping of *KISS1* receptor T305C mutation

For genetic analysis, one ml of peripheral blood was collected in to EDTA (Ethylenediaminetetraacetic acid) as an anticoagulant. Genomic DNA was extracted from blood leukocyte by using a commercially standard DNA extraction kit (Gene Pajohan, Iran). DNA extraction was evaluated by agarose gel electrophoresis. Then the purified genomic DNA was maintained in -20 ^{0} C for molecular analysis. The presence of the *KISS1* receptor T305C mutation was detected by Allele-Specific-PCR (AS-PCR).Amplifications were carried out using primers designed by oligo primer analysis software (Version 7.54 Molecular Biology Insights, USA).The PCR amplification reaction was performed in 25 µl of total volume, including 23 ng of DNA extraction, 10mMdNTP, 1X PCR buffer (10 mM Tris HCL, 50 mM KCl and 0.1%Triton X-100), 1.5µM of MgCl2, 2.5 U Taq DNA polymerase (Biflux, Japan) and 0.5 µmol of each primer. The allelic variants of the gene were determined by using primers as follows: wild type forward primer 5'-**GTCCCCTTCACGGCCCTGCT-3'**, forward mutant primer was **5'GTCCCCTTCACGGCCCTGCC3** and reverse/forward mutant primer was **5'AAGTGCGCCTCTCCCCTC3'**.

The reaction was started with initial denaturing step at 95°Cfor 5min following by 35 cycles of denaturation annealing and extension was 95°C for 5min, 63°C for 45 s and 72°C for 30 s, respectively. Final extension at 72°C for 5 min was accomplished. The PCR product was electrophoresed on 2% agarose gel and visualized by ethidium bromide staining and then visualized by UV illumination. The quality of PCR product (121bp) is as shown in Figure 1.

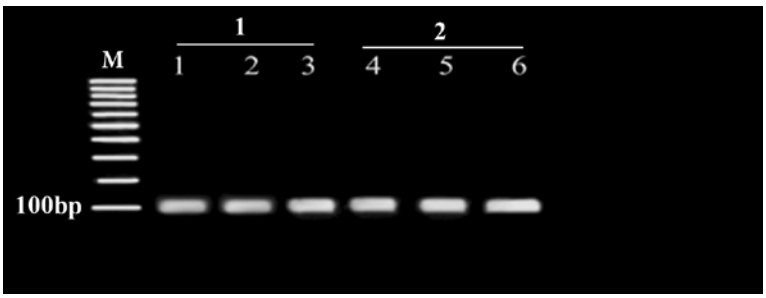

Figure 1. Agarose gel electrophoresis by ethidium bromide after allele-specific polymerase chain reaction (AS-PCR). Lane M: 100bp DNA marker; Lane 1 Fragments indicating the T allele for three heterozygous patients. Lane 2: fragments presenting the C allele for the same three heterozygous patient women.

Statistical analysis

To investigate the risk of female infertility among different, *KISS1* receptor genotype among different genotype, and 95% confidence intervals (95%CI) by logistic regression were calculated. The *P*-values and Hardy-Weinberg equilibrium was calculated by chi-square (χ^2) analysis. All statistical analyses were performed by Medcalc statistical software (version 12.1, Mariakerke, Belgium). A *P*-value of <0.05 was indicated statistically significant.

Results

Genotypes for the *KISS1* receptor mutation were identified in 140 patients and 130 controls. PCR products for both alleles were 118 bp in size, which presented in Figure1 as amplified for three heterozygote patients. The clinical manifestations of the infertile patients are summarized in Table 1. Age distribution was not significantly different among patients and controls, and no family history of infertility existed among infertile women. Frequency of intercourses among infertile women was similar to normal controls. The association of the *KISS1* receptor genotype with the risk of female infertility is shown in Table 2. The T305C T and C allele frequencies were 45% and 54% for patients and 50% and 50 % among the normal controls. However, the genotype frequencies were found to be null for TT and CC and 100% for TC among the normal controls, and 15% for TT, 60% for TC and 24% for among the patients. Genotype distribution for this mutation did not deviate from Hardy-Weinberg equilibrium. There were no significant differences in allele distribution of T305C *KISS1* receptor mutation between infertile women and healthy controls (P=0.6).The genotype frequency of the mutation in the present study was not significant (OR=1.58, 95% CI=0.0303-83.06, P= 0.8). Therefore in the presence study, there was no significant difference between idiopathic infertile women compared to control women. The presence of the mutant allele C may not be regarded as a risk factor for female infertility in studied population.

Table 1. Clinical manifestation of patient with idiopathic infertility and controls

Clinical characteristics	Patients' n (%)	Controls n (%)
Age	27.25	28.35
Duration of infertility	1.5-4 years	-
Family history with infertility	-	-
Frequency of coitus	2-3	2-3

Table 2. *KISS1*receptor T305C genotype and allele frequencies

***KISS1* receptorT305C genotype**	**Group1 (controls) n%**	**Group 2 (patients) n%**	**OR (95%CI)**	***p*-value**
Total no. of subjects				
TT	0 (0%)	20 (15%)	1.00 (Ref)	
TC	130 (100%)	78 (60%)	0.0147 (0.0009-0.2)	0.003
CC	0 (100%)	32 (24%)	1.58 (0.0303-83.06)	0.8
***KISS1*receptor T305Calleles**	**Allele frequencies (%)**	**Allele frequencies (%)**		
T allele	130 (50%)	128 (45%)		
C allele	130 (50%)	142 (54%)		0.6

Discussion

Many different gene mutations in relation to fertility problems have been identified. In human*KISS1* receptor gene mutation was found in idiopathic hypogonadotropic hypogonadism. Hypogonadotropic hypogonadism is a deficiency of pituitary hormone of FSH and LH which deteriorates puberty and reproductive function (Teles et al., 2008). Kisspeptins are a family of peptide hormone which plays a crucial role in regulation of hypothalamic-pituitary- gonadal axis, thus in turn influencing fertility and reproduction. The role of kisspeptin as a gatekeeper of puberty has been described in 8 years old girls who presented with idiopathic precocious puberty (de Tassigny et al., 2010).

Experimental studies show that the *KISS1* receptor knockout female mice do not undergo pubertal development, poor gonadal growth and impaired gametogenesis. Female mice did not show normal estrus cycle and formation of corporalutea in their ovaries did not occur. Proline substitution of hydrophobic residue such as leucine induces conformational changes in the receptor. This substitution within the first loop inhibits the phospholipase C pathway and leads to deficiency in cellular action of GnRH neurons then block activation phosphorylation cascade (Gether, 2000).

According to this study, the possible association between *KISS1* receptor T305C mutation (codon 102) with female infertility was explained for the first time. Our data suggest that mutant allele C is not a risk factor for infertility, showing the presence of *KISS1*

receptor T305C mutation may not be associated with idiopathic female infertility in this study (P>0.05). Our result also suggests that there is no significant association between the genotype (P=0.8) and allelic (P=0.6) distribution between cases and controls.

We have also shown that the substitution in extracellular loop may not be a risk factor for female infertility in our study. However it is indicated that the frequency of C (mutant) allele is more than the T (wild type) allele, so the association between this gene variant and idiopathic female infertility can be considered as a polymorphism in female patient, but further analyses of T305C mutation in different set of population is required to evaluate its relation with idiopathic female infertility.

In conclusion, the presence of KISS1 receptor T305C mutation may not be associated with idiopathic female infertility in this study (P>0.05). Likewise, it appears that the mutant allele C is not a risk factor for infertility. This information may open a route for further studies concerning of different KISS1 receptor gene mutations in larger population.

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Conflicts of interests:

There is no conflict of interest in this research.

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