

The relationship between rs58777844 KISS1R gene polymorphism and infertility in women from Guilan province

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Abstract:

Introduction:

KISS1R gene is responsible for wide ranges of diseases including cancer and infertility. Mutations in the KISS1R gene are one of the most important potential causes of infertility. KISS1R has an important role in regulating the hypothalamic-pituitary-gonadal axis that regulates reproduction and maturation in vertebrates. This study aimed to evaluate the relationship between rs58777844 KISS1R gene polymorphism and female infertility in Guilan Province.

Materials & Methods:

Blood samples were obtained from 40 infertile women as cases and 40 healthy women as controls. Genomic DNA was extracted from peripheral blood leukocytes and allele specific PCR (AS-PCR) method was used for determining codon variations. Data were analyzed in Medcalc software (v 13.0).

Results:

Results showed a significant relationship in distribution of genotypes ($P=0.03$) and allele frequencies between the two groups ($p=0.0113$).

Conclusion:

Results showed a significant relationship in distribution of genotypes ($P=0.03$) and allele frequencies between the two groups ($p=0.0113$).

Keywords: Infertility, KISS1R Gene, Polymorphism, Women

Introduction

Infertility is a reproductive system disorder, defined as the inability to conceive after at least 12 months of regular and optimal sexual intercourse (1, 2). It is divided into primary and secondary infertility. Primary infertility is used for couples who had never been pregnant, while secondary infertility is applied to those who have had one pregnancy but whose later attempts have been unsuccessful. From among all infertile couples, male and female infertility

respectively comprises 30-40% and 40-50% of all infertilities. Based on this definition, from among 10-30% of the remaining infertile couples, both men and women are infertile, or the cause remains unknown (2).

Infertility has a multifactorial condition, including female and male factors (3). Female factors include medical disorders, genetic disorders, factors caused by environmental toxins, cancer, infections,

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autoimmune diseases, and digestive disorders, e.g. inflammatory bowel disease and celiac disease (4). Hormonal causes of female infertility include disorders in reproductive organs as well as disorders of endocrine glands, non-reproductive disorders, and disorders in non-endocrine organs, e.g. liver and kidneys. These non-endocrine organs are engaged in the release and metabolism of reproductive hormones, thereby disrupting the maturation of ovum and ovulation (5). Injuries of fallopian tubes due to infections, endometriosis, and acquired or congenital uterine abnormalities are the major anatomical problems leading to female infertility (6). Causes of infertility, including hormonal problems and obesity as well as abnormalities in germ cells (ovum and sperms), can all be affected by genetic factors. Studies on specific genes in humans and laboratory models have somehow clarified the effect of genetic factors on infertility (7, 8). The discovery of KISSPEPTIN and its receptor in 1996 shed light on the physiology of the hypothalamic–pituitary–gonadal (HPG) axis. The KISSPEPTIN system is completely preserved in all vertebrates, except birds. So far, numerous studies have been conducted on the roles of KISSPEPTIN, most of which hold it responsible in the HPG axis. Lack of the KISS1 gene or its receptors delays puberty and hypogonadotropic hypogonadism, while the presence of KISS1 peptides induces the release of luteinizing hormone (LH) which is effective in fertility (9). The outcomes of KISSPEPTIN directly affect the neurons of gonadotropin-releasing hormone (GnRH), thereby causing the secretion of LH and follicle-stimulating hormone (FSH) from the gonads. KISSPEPTIN is a strong stimulant

for the release of gonadotropin, acting as a link between steroid hormones and the secretion of GnRH. In humans, it stimulates the release of gonadotropin and GnRH in hypothalamus. Of course, KISSPEPTIN and its receptors (KISS1R) have other physiological roles too, e.g. controlling the reproductive and fertility axis, precocious and delayed puberty, and seasonal reproductive activities, inhibiting metastasis, and releasing calcium (10, 11). KISS1 gene is located on the long arm of chromosome 1. This gene has four exons, while only the third and fourth are transcribed. The peptides resulting from this gene are known as KISSPEPTIN and belong to a large family of peptides called RFamide, all of which have the general motif Arg-Phe-NH₂ or R-F-NH₂ in their N-terminus. Based on the mRNA, a preprokisspeptin with the length of 145 amino acids is created. By proteolytic cleavage and other chemical processes, Kp54 (KISSPEPTIN), Kp13, Kp14, and Kp10 are generated (12) with slightly different potentials for activating the relevant receptor. This difference is the result of the difference in the length of N-terminal amino acids (9, 13, 14). KISSPEPTIN was introduced as a ligand for the orphan gene GPR54 in 2001. Before that, no ligand had been identified for this gene. As already mentioned, this receptor was named GPR54 in rats, while its human homologs were named AXOR12 or hOT7T175. For the sake of simplicity, we refer to this receptor the KISSPEPTIN receptor (KISS1R) (15, 16, 17). The gene of this receptor is located on the short arm of chromosome 19, region 1, band 3, and sub-band 3 (19p13.3), and consists of 5 exons and 4 introns. The fifth exon is the longest and the site of most mutations (11, 18). The protein produced

from this gene has 398 amino acids, includes seven transmembrane sections in its structure, and belongs to the large family of rhodopsins (11· 18).

Genetic factors have an important role in various diseases, including infertility. One of these factors is mutations in KISS1R. The present study aimed to examine the effect of rs58777844 polymorphism on infertility. This polymorphism is located in locus 1098 of the gene and codon 313 of the KISS1R protein. In this locus, a missense mutation (Missense) occurs, changing base T to C and thus turning codon TAC to CAC. As a result of this alteration, the amino acid tyrosine (T) is converted to histidine. The locus of this amino acid is in exon 5 and the beginning of the 7th transmembrane section and is highly protected. Based on the protected locus of the amino acid 313 and the important role of this protein in the reproductive axis, we investigated the role of this polymorphism in infertility among women in Gilan, Iran.

Materials and Methods

In this case-control study, we collected blood samples from two groups: affected (infertile) individuals and healthy (fertile) controls. The infertile group included 40 women with idiopathic infertility, selected after being diagnosed and recommended by a gynecologist. The healthy group comprised 40 healthy women. The subjects aged 20-35 years old. Sampling was performed from September 2014 to March 2015 from individuals visiting the Alzahra Education, Research, and Remedial Center and Mehr Medical Laboratory, Rasht, Iran. The healthy controls had passed at least one period of natural pregnancy without taking medications or using assisted reproductive technologies. We obtained written consent from all the subjects in accordance with the

Declaration of Helsinki. In the next step, we collected 2 mL of blood from every subject. To prevent coagulation, blood was stored in vials containing EDTA. The vials were then moved to a freezer at -20 °C to be used for DNA extraction in the following step.

Genomic DNA Extraction from Blood

To extract DNA from the collected blood samples, we used the Gpp Solution Extraction Kit (Gene Pazhouhan Co., Iran) according to the provided guidelines. We examined the quality of the extracted DNA using horizontal electrophoresis. The extracted DNA samples were added to wells containing 1% agarose gel. Afterwards, we imaged the gel using Gel Doc with UV light. After quality assessment, DNA was moved to the freezer at -70 °C.

Polymerase chain reaction (PCR)

We employed the 48-well thermocycler device (BioRad) in this reaction. The materials utilized in PCR as well as their concentration and amount were as follows: 6 µL of master (1X), 2 µL of template DNA, 0.5 µL of (10Pmol) forward primer, 0.5 µL of (10Pmol) reverse primer, and 3.5 µL of sterile distilled water (total: 12.5 µL). To prepare the PCR solution, we used 0.2 mL microtubes. We poured the mentioned materials in the tubes and stirred them by pipetting. We amplified the noted gene segment by primers. To amplify the 298-base pair segment related to the healthy allele, the sequence of forward and reverse primes was CAGGAGGGGCGGTGCGAGGGG and GGGTTCAGCGCGGAGTTGCTGGA, respectively. To amplify the 298-base pair segment related to the mutant allele, the sequence of forward and reverse primes was CAGGAGGGGCGGTGCGAGGGG

andGGGTTTCAGCGCGGAGTTGCTGG, respectively. The utilized primers were manufactured by Tag Copenhagen Co. The 298-base pair segment related to the healthy allele was amplified in the thermocycler as follows: Cycle 1 for the initial denaturation: once for 5 min at 94 °C; Cycle 2 including three steps: denaturation, binding the primer to the template strand, and polymerase expansion: 35 times, each for 30 sec at 94, 66.5, and 72 °C, respectively; Cycle 3 for the final expansion: once for 10 min at 72 °C; Cycle 4 for maintaining the products: once at 4 °C. The steps of the expansion program for the 298-base pair segment related to the deleterious allele in the thermocycler device were similar to those of the mutant allele, while the temperature of binding to the template strand was 67.5 °C.

In the agarose gel of PCR results, individuals who had two 298-base pair segments with respect to the primers related to mutant and wild alleles were heterozygous (T/C). Those who only had the 298-base pair segment with respect to the primer related to the wild allele were healthy homozygous (T/T). Finally, those who only had the 298-base pair segment with respect to the primer related to the mutant allele were affected homozygous (C/C).

Statistical analysis

We used MedClac 13.0 to interpret the results. Allele and genotype frequency was measured in both healthy and affected groups. Afterwards, we determined the difference in genotype distribution between the affected and control populations using the chi-squared test. The significance level was ≤ 0.05 .

Results

PCR Results: We examined the quality of allele-specific PCR (AS-PCR) products using agarose gel and gel imaging (Figure 1). PCR products were placed on 2% agarose gel together with a marker with the molecular weight of 100 base pairs.

Genotypic and allelic examination of KISS1R gene polymorphism and its statistical analysis results

In the present study, we examined 80 women including 40 infertile ones as the affected group and 40 fertile ones as the control group, aged 20-35 years. In the affected group, 13 (32.5%) were T/T homozygous, 20 (50%) were T/C heterozygous, and 7 (17.5%) were C/C homozygous. On the other hand, in the healthy group, 22 (55%) were T/T homozygous, 18 (45%) were T/C heterozygous, and 0 (0%) were C/C homozygous.

Based on the chi-squared test, there was a significant difference in genotype frequency between the two groups ($p=0.03$). Thus, there is a significant relationship between the rs58777844 polymorphism of KISS1R gene and infertility among a population of women in Guilan, Iran (Table 1). The examination of the population's allele frequency showed that there were 46 (57.5%) T alleles and 34 (42.5%) C alleles in the affected population, while there were 62 (77.5%) T alleles and 18 (22.5%) C alleles in the control population. The statistical analysis of the allele frequency of the noted polymorphism using chi-squared test yielded the odd-ratio=3.78 and $\chi^2=6.41$, $p=0.0113$. As a result, similar to genotypic difference, allele frequency differs significantly across the two affected and control groups (Table 2).

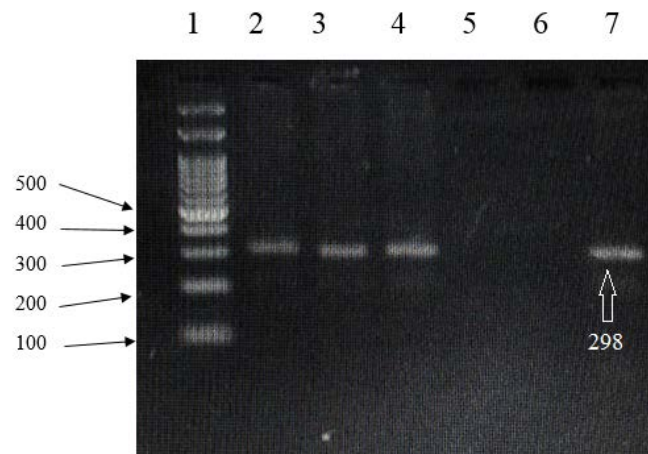


Figure 1: Image of 2% agarose gel from the 298-base pair segment. The weight marker was in well 1, while the heterozygous (T/C) sample was in wells 2 and 3, and a band was created with both primers. The sample in wells 4 and 5 was dominant homozygous (T/T) and created a band only with the wild primer. The sample in wells 6 and 7 was recessive homozygous (C/C) and created a band only with the mutant primer.

Table 1: Number and percentage of polymorphism genotypes in infertile and healthy women

Odds-Ratio (95%-CI)	p-value	Percentage (number) Control group (n=40)	Percentage (number) Affected group (n=40)	Genotype	Gene
(Ref) 1.00	$\chi^2=9.42$	55 (22)	32.5 (13)	T/T	KISS1R Tyr313His
4.06 (0.73-4.79)	0.1	45 (18)	50 (20)	T/C	
472.14 (1.32-473.46)	0.03	0.0 (0)	17.5 (7)	C/C	

Table 2: Allele frequency of healthy and affected individuals with analysis results

Odds-Ratio (95%-CI)	p-value	Percentage (number) Control group	Percentage (number) Affected group	Allele	Polymorphism
(Ref) 1.00	0.0113	77.5 (62)	57.5 (46)	T	KISS1R Tyr313His
3.78 (1.28-5.06)	$\chi^2=6.41$	22.5 (18)	42.5 (34)	C	

Discussion

Since infertility is a multifactorial condition, we need research on different populations and races in order to determine the role of and interaction between other genetic and environmental factors. The interaction between other genes in the

puberty and reproductive axis may alter the effect of this KISS1R polymorphism. Furthermore, understanding the precise mechanism of the effect of genetic diversity in KISS1R gene on infertility may lead to accurate conclusions. In sum, infertility is a

multifactorial syndrome caused by various genetic and non-genetic factors, e.g. epigenetic factors (19). It results in a wide range of social, mental, physical, and economical problems (20).

In the present study, we observed a significant genotypic difference between the two affected and control groups. The distribution of the noted allele differed across the two groups. Considering its odds-ratio of 3.78, the presence of the C allele can be a risk factor of infertility among women in Gilan, Iran.

We believe that, with the mentioned mutation and the incorrect folding of KISS1R protein, this receptor cannot work properly, negatively affecting the female fertility axis. So far, no extensive study has been conducted on the effect of rs58777844 polymorphism of KISS1R gene on female infertility. Only one study (Brioude et al.) was performed on a family suffering from congenital hypogonadotropic hypogonadism, leading to the discovery of this SNP (21).

Recently, the relationship between genetic polymorphism and predisposition to some diseases has received considerable attention. KISS1R and its ligand KISS1 play a role in the direct regulation of the HPG and reproductive axes. In humans, delayed puberty and hypogonadotropic hypogonadism are related to the absence of KISS1R protein or the presence of the inactive protein (22).

Different mutations were observed in studies on KISS1R gene, e.g. L148S (18), C223R (23), R297L (18), L102P (18), E232Q (24) R331X, X339R (18), deletion of GCA in locus -2 to -4, and insertion of ACCGGCT (24), which result in the congenital hypogonadotropic hypogonadism phenotype. In addition, P196H (25),

R386P (18), and P110T (26) polymorphisms lead to precocious puberty.

Conclusion

Based on the results, the rs58777844 polymorphism of KISS1R gene can be a risk factor for infertility in the studied population. This polymorphism is located in locus 1098 of the gene and codon 313 of the KISS1R protein. In this locus, a missense mutation occurs, changing base T to C and thus turning codon TAC to CAC. Following these variations, the amino acid tyrosine (Y) with a polar side chain is converted to the positively-charge amino acid histidine (H). The locus of this amino acid is in exon 5 and the beginning of the 7th transmembrane section and is highly protected. Based on the protected locus of the 313th amino acid and the determining role of this protein in the reproductive axis, we confirmed the role of this pathogenic polymorphism in a population of infertile women in Gilan, Iran. As infertility is a multifactorial condition, genetic composition may affect polymorphism and predisposition to infertility. Nevertheless, results may vary by significantly changing the gene pool or population size. The phenotypic effect of gene polymorphisms is always affected by genetic and environmental factors, and the interaction between other genes in the puberty and reproductive axis may alter the effect of KISS1R gene polymorphism.

Conflict of Interest

None to declare

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References:

- Warren BD, Kinsey WK, McGinnis LK, et al. Ovarian autoimmune disease: clinical concepts and animal models. *Cell Mol Immunol* 2014;11(6):510-21.
- Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology, 2009. *Hum Reprod* 2009 ;24(11):2683-2687.
- Hull M, Glazener C, Kelly N, Conway D, Foster P, et al. Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* 1985;291(6510):1693-7.
- Tinneberg H-R, Gasbarrini A. Infertility today: the management of female medical causes. *Int J Gynecol Obstet* 2013;123(2):25-30.
- Luciano AA, Lanzone A, Goverde AJ. Management of female infertility from hormonal causes. *Int J Gynecol Obstet* 2013;123(2):9-17.
- Abrao MS, Muzii L, Marana R. Anatomical causes of female infertility and their management. *Int J Gynecol Obstet* 2013;123(2):18-24.
- Weiss RV, Clapauch R. Female infertility of endocrine origin. *Arq Bras Endocrinol Metabol* 2014;58(2):144-52.
- Crujeiras AB, Casanueva FF. Obesity and the reproductive system disorders: epigenetics as a potential bridge. *Hum Reprod Update* 2015;21(2):249-61.
- Hameed S, Jayasena CN, Dhillon WS. Kisspeptin and fertility. *J Endocrinol* 2011;208(2):97-105.
- Seminara S, Crowley Jr W. Kisspeptin and GPR54: discovery of a novel pathway in reproduction. *J neuroendocrinol* 2008;20(6):727-31.
- Skorupskaitė K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. *Hum Reprod Update* 2014;20(4):485-500.
- Pinilla L, Aguilar E, Dieguez C, et al. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiol Rev* 2012;92(3):1235-1316.
- Kotani M, Dethoux M, Vandenberghe A, et al. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem* 2001;276(37):34631-6.
- Cvetković D, Babwah AV, Bhattacharya M. Kisspeptin/KISS1R system in breast cancer. *J Cancer* 2013;4(8):653.
- Lee J-H, Miele ME, Hicks DJ, et al. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J J Natl Cancer Inst* 1996;88(23):1731-7.
- Muir AI, Chamberlain L, Elshourbagy NA, et al. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem* 2001;276(31):28969-75.
- Reynolds RM, Logie JJ, Roseweir AK, et al. A role for kisspeptins in pregnancy: facts and speculations. *Reproduction* 2009;138(1):1-7.
- Roseweir A, Millar R. The role of kisspeptin in the control of gonadotrophin secretion. *Hum Reprod Update* 2009;15(2):203-12.
- Simpson JL. Genetic and Nongenetic Causes of Pregnancy Loss. *Women*. 2013;3(30):4.19
- Greil AL, Slauson-Blevins K, McQuillan J. The experience of infertility: a review of recent literature. *Sociol Health Illn*. 2010;32(1):140-62.
- Brioude F, Bouligand J, Francou B, et al. Two families with normosmic congenital hypogonadotropic hypogonadism and biallelic mutations in KISS1R (KISS1 receptor): clinical evaluation and molecular characterization of a novel mutation. *PLoS One* 2013;8(1): 53896.
- Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med* 2003;349(17):1614-27.
- Semple R, Achermann J, Ellery J, et al. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J ClinEndocrinol Metab* 2005;90(3):1849-55.
- Pankov YA. Kisspeptin and leptin in the regulation of fertility. *Mol Biol* 2015;49(5):631-7.
- Teles MG, Trarbach EB, Noel SD, et al. A novel homozygous splice acceptor site mutation of KISS1R in two siblings with normosmic isolated hypogonadotropic hypogonadism. *Eur J Endocrinol* 2010;163(1):29-34.
- Luan X, Zhou Y, Wang W, et al. Association study of the polymorphisms in the KISS1 gene with central precocious puberty in Chinese girls. *Eur J Endocrinol* 2007;157(1):113-8.