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Case Report

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Three Siblings with Idiopathic Hypogonadotropic Hypogonadism in a Nonconsanguineous Family: A Novel *KISS1R/GPR54* Loss-of-Function Mutation

Running Head: A compound heterozygous mutation of KISS1R gene

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What is already known on this topic?
KISS-1 and its receptor, KISS1R, (formerly called GPR54) play key roles in the initiation of puberty. Kisspeptin that is a peptide encoded by the KISS1 gene and its receptor are essential to stimulate GnRH release from the hypothalamus to stimulate pituitary gonadotrophins secreation to initiate puberty. So, the function of KISS-1 and KISS1R in the hypothalamus is very important for the onset and progression of puberty. Loss of function mutations in KISS1R gene can cause NIHH. Up to now, more than 20 different mutations have been reported. Most of them were loss of function mutations.

What this study adds?
We found a compound heterozygous mutation of KISS1R gene to cause normosmic idiopathic hypogonadotropic hypogonadism as well as incomplete puberty. In previous studies, the loss-of-functional mutations of KISS1R/GPR54 which were inherited as autosomal recessive mutations are reported in consanguineous families. We identified these mutations in a non-consanguineous family which illustrates different phenotypic spectrum of KISS1R/GPR54. We recommend genetic counselling for families with KISS1R mutations, even when there is no consanguinity.

Abstract
Context: Idiopathic hypogonadotropic hypogonadism (IHH) is a rare disease caused by defects in the secretion of Gonadotropin releasing hormone (GnRH) or the action of GnRH on the pituitary gonadotrophes. *KISS1R* is one of the genes, when mutated, cause IHH, and mutations of this gene are responsible for about 2-5% of patients with normosmic IHH (NIHH).
Objective: In this report, we aim to present three siblings who have NIHH due to a compound heterozygous *KISS1R* mutation.
Patients: Genetic studies were carried out in the 14 year old index case with IHH and three siblings, two of whom have incomplete puberty.
Methods: Genomic DNA was extracted from peripheral leukocytes and *KISS1R* gene was sequenced by using standard PCR amplification procedures.
Results: In molecular analysis of the index case, a compound heterozygous mutation was determined in *KISS1R* gene (c.969C>A (p.Y323X) (known pathogenic) and (c.170T>C (p.L57P) (novel)). Mutation c.170T>C (p.L57P) was inherited from the mother while c.9C>A (p.Y323X) was inherited from the father. Also the same genotype was found in two of the three siblings.
Conclusions: A compound heterozygous mutation of *KISS1* gene causes normosmic idiopathic hypogonadotropic hypogonadism and also incomplete puberty in a non- consanguineous family.
Keywords: Kisspeptin, *KISS1R*, hypogonadotropic hypogonadism, delayed puberty

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Introduction
Idiopathic hypogonadotropic hypogonadism (IHH) is a rare genetic disorder, which is caused by defects in the

secretion of Gonadotropin releasing hormone (GnRH) or the action of GnRH on the pituitary gonadotrophes (1). The increased frequency and amplitude of the pulsatile secretion of the GnRH is essential for the initiation of normal pubertal development. The failure of pulsatile secretion of GnRH from the hypotalamus result in impairment of pubertal development and reproductive function which is referred as IHH. The clinical presentation of IHH may manifest as absent or incomplete puberty, cryptorchidism, small penis and infertility in males while amenorrhea, absence of breast development and infertility in females. IHH is divided into two major groups: Kallmann syndrome (KS) and normosmic IHH (NIHH) (2). The incidence of IHH is approximately 1–10 in 100 000 live births, Kallmann Syndrome is approximately 60% of this (3). NIHH results from the dysfunction of the normally situated GnRH neurons in the hypothalamus. Patients with NIHH typically do not have any accompanying congenital anomaly. Until today about 50 genes have been reported to be associated with IHH (2). But a smaller number of these genes are responsible to the pathogenesis of NIHH (1-4). Pathogenic mutations can be detected in about half of the IHH cases (1,2). *KISS1R* is one of the genes which cause NIHH, and mutations of this gene are responsible about 2-5% of patients with NIHH (4,5). *KISS-1* and its receptor, *KISS1R*, (formerly called GPR54) play key roles in the initiation of puberty. Kisspeptin that is a peptide encoded by the *KISS1* gene and its receptor are essential to stimulate GnRH release from the hypothalamus to stimulate pituitary gonadotrophins secreation to initiate puberty. So, the function of KISS-1 and KISS1R in the hypothalamus is very important for the onset and progression of puberty. Loss of function mutations in KISS1R gene can cause NIHH. Up to now, more than 20 different mutations have been reported. Most of them were loss of function mutations (6).
Here, we present three siblings with NIHH due to a compound heterozygous mutation including c.969C>A (p.Y323X) and novel c.170T>C (p.L57P) in *KISS1R* in a non- consanguineous family.

Case Report

The proband who was referred to our outpatient clinic due to the failure of puberty was 14 years-old boy at admission. He was the first child of healthy, non-consanguineous Turkish parents. He had three sisters whose ages were fourteen, twelve and five years old. He had microphallus and bilateral undescending testicles in newborn period. Therefore, bilateral orchiopexia was performed when he was one and half years old. On physical examination, height was 165.3 cm (0.14 SDS), weight was 62 kg (0.94 SDS), and bone age was 14.0 years. He had typical signs of complete hypogonadism including microphallus, enuchoid habitus (uper segment/lower segment ratio<0.9, arm span>height), and also he had no pubic and axillary hair. Both testicles were intrascrotal and the testis sizes were 3cc, bilaterally. He had a normal sense of smell on olfactometry. He had no craniofacial stigmata or other morphological abnormalities on physical examination. His karyotype was 46 XY. Basal serum luteinizing hormone (LH), follicule-stimulating hormone (FSH), plasma testosterone (T), adrenocorticotropin (ACTH), dehydroepiandrosterone sulfate (DHEAS) and cortisol concentrations were determined by electro-chemiluminescence immunoassay (Table 1). We, also, peformed a IV GnRH-stimulation test to obtain stimulated FSH and LH levels at 0, 20, 40, and 60 minutes confirm the diagnosis of hypogonadotropic hypogonadism. Magnetic resonance imaging (MRI) of the central nervous system (CNS) revealed normal findings.
The oldest sister, who was 14 years old, had a breast development corresponding to Tanner stage 2. She had no pubic and axiller hair; menarch had never occurred. The bone age was 14 years. Her breast development first appeared when she was 10 years old and after that there was no further progression in pubertal stages. Pelvic sonography revealed a uterus (47x18x11 mm) and two small ovaries (24x18x14 and 20x18x14 mm). Hormone assays were as followed: Basal FSH 4.06 mIU/ml, LH 1.21 mIU/ml, and estradiol 14 pg/ml. The second sister of the proband was 12 years old and she had no sign of pubertal development. Pelvic sonography showed a small uterus and small ovaries. Evaluation of basal and GnRH stimulated hormon levels confirmed incompleted puberty. The youngest sister, who was 5 years old, had tanner stage 1 breast development and hormon profile were prepubertal. Karyotype analysis of all three sisters were 46,XX.
Genomic DNA was extracted from peripheral leukocytes and the promoter region, the three coding exons and exon-intron boundries of the *KISS1R* gene (NM_032551) were amplified by polymerase chain reaction (PCR) and sequenced. In the index case, we found a compound heterozygous mutation in the *KISS1R*, one of these was a nonsense variant (c.969C>A, p.Y323X) which was known as an inactivating mutation to cause NIHH and the other was a novel missense variant (c.170T>C, p.L57P)(figure1). This novel missense variant was evaluated for functional impact using a variety of *in-silico* prediction tools including SIFT, Polyphen2 and Mutation Taster which support for a disease causing effect of this mutation (7-9). Molecular analysis of the parents showed that both parents were heterozygous carriers. While the mutation c.969C>A (p.Y323X) was inherited from the father, c.170T>C (p.L57P) was inherited from the mother. Genetic analysis of both sisters who were 12 and 14 years old revealed the same compound heterozygous mutation whereas the genetic analysis of the youngest one was normal. Clinical and hormonal characteristics of all cases including the proband are shown in the Table 1. Informed consent from the parents of the patients was provided.

Discussion

Timing of pubertal onset is related to increased GnRH pulses which activates the rising of gonadotropins and sex

hormones. Interaction of kisspeptins and their corresponding receptor has been reported to have a critical role in initiation and development of puberty. Inactivating mutations of *KISS1R* lead to NIHH (10-12).
Kisspeptin which is a very potent stimulator for GnRH secretion is expressed from the neurons which located at two different parts of mammalian hypothalamus, preoptic area and arcuate nucleus (13). It is not only a potential stimulator of GnRH but also a mediator of positive and negative feedback effects on sex steroids (14).
More than 20 mutations in the *KISS1R* (*GPR54*) gene had been previously described in the literature, and these mutations were found to have variable clinical manifestation (15,16).
Recently, Topaloğlu et al reported an inactivating mutation of *KISS1 to* cause complete normosmic idiopathic hypogonadotropic hypogonadism in a large consanguineous family from Turkey. The probound was 14.9 years-old. She had no breast development and her pelvic Pelvic ultrasonography revealed a hypoplastic uterus and ovaries lacking follicles. The affected three sisters of the proband had no spontaneous breast development. All four affected sisters were otherwise healthy and had a normal sense of smell (17).
Demirbilek et al. identified a homozygous nonsense mutation (p.Y323X) in *KISS1R* gene in three nonconsanguineous families with NIHH. One male presented with absence of pubertal onset and severe penoscrotal hypospadias and cryptorchidism. Two other males had absence of pubertal onset. Two of four female cases required replacement therapy for pubertal onset, while the other two females had spontaneous pubertal onset but incomplete maturation. This was a nonsense c.C969A (Y323X) mutation (18).
A similar nonsense mutation which were at position 969 of the nucleotide sequence in the *KISS1R* gene (c.C969>A) located on the short arm of chromosome 19 (19p13.3) has been reported in a case of normosmic IHH in a female patient from a consanguineous family (1). This nonsense mutation results in the creation of a stop codon that leads to incomplete production of the kispeptin. This truncated KISS1R protein fails to signal the release ofGnRH from the hypothalamus.
Nimri et al.reported two highly consanguineous families of Israeli-Arab origin. Among their patients, had evidence for complete hypogonadotropic hypogonadism. Cryptorchidism and relatively short penile length were noted in all male patients at birth. A novel loss-of- function mutation in the *GPR54* gene in six members of the family has been identified (15).
Breuer et al describe a novel severe homozygous *KISS1R* splice site mutation in three siblings in a consanguineous Palestinian family with IHH. They had normal neonatal external genitalia presented with no pubertal development, normosmia, and a low response to GNRH stimulation (16).
KISS1R mutations which have been reported before include point mutations, deletion, insertion, acceptor splice site mutation and missense mutation. Hereby, we described a compound heterozygous mutation in *KISS1R* gene in a non-consanguineous family. One of these was a known pathogenic nonsense variant (c.969C>A, p.Y323X) and the other was a novel missense variant (c.170T>C, p.L57P). The proband had NIHH, whereas two sisters had incomplete pubertal development and the other sister was prepubertal. Previously described inactivating mutations associated with the *KISS1R* gene have been homozygous from consanguineous marriages. In this report, we described *KISS1R* gene mutation in a non- consanguineous family, for the first time.
Thus, the inadequacies of kisspeptin receptor can manifest itself in different clinical entities. In this study, although three siblings have the same inactivating compound heterozygous mutation, one of them has incomplete puberty and amenorrhea while the remaining two have NIHH.
In conclusion, we found a compound heterozygous mutation of *KISS1R* gene to cause normosmic idiopathic hypogonadotropic hypogonadism as well as incomplete puberty. In previous studies, the loss-of-functional mutations of *KISS1R/GPR54* which were inherited as autosomal recessive mutations are reported in consanguineous families. We identified these mutations in a non-consanguineous family which illustrates different phenotypic spectrum of *KISS1R/GPR54*. We recommend genetic counselling for families with *KISS1R* mutations, even when there is no consanguinity.

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Ethics

Ethics Committee Approval: This manuscript is a case report. Therefore we didn't approve for ethics committee.
Informed Consent: Inform consent from the parents of the patients was obtained verbally.

Authorship Contributions

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Table-1. Clinical and Hormonal Characteristics of the Proband and Siblings

	Patient 1 (Proband)	***Patient 2***	***Patient 3***	***Patient 4***
Age at diagnosis (year)	14	14	12	5
Sex	Male	Female	Female	Female
Physical examination	Tanner stage 1 Stretched penil length 4 cm Testis sizes 3cc/3 cc	Tanner stage 2 Amenorrhea	Tanner stage 1	Tanner stage 1
Laboratory	**FSH:** 0,9 IU/ml **LH:** 0,13 IU/ml **Total T:** 15 ng/dl **ACTH:** 34 g/ml **Kortizol:** 15 µg/dl **17OH progesteron:** 0,11 ng/ml **AMH:** 51,20 ng/ml (2-30,7)	**FSH:** 4.06 mIU/ml **LH:** 1.21 mIU/ml **Estradiol**: 14 pg/m	**FSH**: 0.86 mIU/ml **LH:** 0.07 mIU/ml **Estradiol** < 10 pg/ml	**FSH:** 1.58 mIU/ml **LH** < 0.07 mIU/ml **Estradiol** < 10 pg/ml
İmaging	Testicules are in scrotum, bilaterally Right testis 16 x 9 x 9 mm, Left testis 15 x 8 x 8 mm	Pelvic USG: Uterus 47 x 18 x 11 mm Right ovary 24 x 18x 14 mm Left ovary 20 x 18 x 14 mm	Pelvic USG and pelvic MR: small uterus and ovaries	Pelvic USG: Uterus 33x 15 x 9 mm Right ovary 19x 15 x 9.5 mm Left ovary 22x 11x 9 mm
Karyotype	46 XY	46 XX	46 XX	46 XX
Genetic analysis	c.969C>A (p.Y323X) and novel c.170T>C (p.L57P) compound heterozygous mutation in KISS1R gene	c.969C>A (p.Y323X) and novel c.170T>C (p.L57P) compound heterozygous mutation in KISS1R gene	c.969C>A (p.Y323X) and novel c.170T>C (p.L57P) compound heterozygous mutation in KISS1R gene	Normal

Figure 1.