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 secretion 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 KISS1R gene causes normosmic idiopathic hypogonadotropic hypogonadism and also incomplete puberty in a non-consanguineous family. Keywords: Kisspeptin, KISS1R, hypogonadotropic hypogonadism, delayed puberty

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 amenorhea, 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). KISS1 and its receptor, KISS1R, (formerly called GPR54) play key role 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 secretion to initiate puberty. So, the function of complete hypogonadism including microphallus, enuchoid habitus (upper segment/lower segment ratio<0.9, arm span>height), and also he had no pubic and axillary hair. Both testes 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), follicle-stimulating hormone (FSH), plasma testosterone (T), adrenocorticotropin (ACTH), dehydroepiandrosterone sulfate (DHEAS) and cortisol concentrations were normal. Clinical and hormonal characteristics of all cases including the proband are shown in the Table 1.

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 (0.6 SDS), and bone age was 14.0 years. He had typical signs of complete hypogonadism including microphallus, enuchoid habitus (upper segment/lower segment ratio<0.9, arm span>height), and also he had no pubic and axillary hair. Both testes 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), follicle-stimulating hormone (FSH), plasma testosterone (T), adrenocorticotropin (ACTH), dehydroepiandrosterone sulfate (DHEAS) and cortisol concentrations were determined by electro-chemiluminescence immunoassay (Table 1). We, also, performed 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 axillary hair; menarche 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 (37x18x11 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 hormone levels confirmed incompletely pubertal. The youngest sister, who was 5 years old, had tanner stage 1 breast development and hormone 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 boundaries 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 andcryptorchidism. 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 of GnRH from the hypothalamus.

Nimri et al. reported two highly consanguineous families of Israeli-Arab origin. Among the 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 another one, 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.

Acknowledgement; we also thank to Dr. Topaloğlu for his valuable contribution.

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
Surgical and Medical Practices: Filiz Hazan
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Conflict of Interest: No conflict of interest

Financial Disclosure: No financial disclosure

References
| Table-1. Clinical and Hormonal Characteristics of the Proband and Siblings |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Age at diagnosis (year)** | 14                          | 14                          | 12                          | 5                           |
| **Sex**                     | Male                        | Female                      | Female                      | Female                      |
| **Physical examination**    | Tanner stage 1              | Tanner stage 2               | Tanner stage 1              | Tanner stage 1              |
|                             | Stretched penis length 4 cm | Amenorrhea                   |                             |                             |
|                             | Testis sizes 3cc/3 cc       |                             |                             |                             |
| **Laboratory**              | FSH: 0.9 IU/ml              | FSH: 4.06 mIU/ml             | FSH: 0.86 mIU/ml            | FSH: 1.51 mIU/ml            |
|                             | LH: 0.13 IU/ml              | LH: 1.21 mIU/ml             | LH: 0.07 mIU/ml             | LH < 0.07 mIU/ml           |
| **Total T**: 15 ng/dl       |                             |                             |                             |                             |
| **ACTH**: 34 g/ml           |                             |                             |                             |                             |
| **Kortizol**: 15 µg/dl      |                             |                             |                             |                             |
| **170H progesteron**: 0.11 ng/ml |                |                             |                             |                             |
| **AMH**: 51.20 ng/ml (2-30.7) |                |                             |                             |                             |
| **Imaging**                 | Pelvic USG:                 | Pelvic USG and pelvic MR:   | Pelvic USG:                 | Pelvic USG:                 |
|                             | Uterus 47 x 18 x 11 mm      | small uterus and ovaries    | Uterus 33 x 15 x 9 mm      | Uterus 33 x 15 x 9 mm      |
|                             | Right ovary 24 x 18 x 14 mm |                             | Right ovary 19 x 15 x 9.5 mm|                             |
|                             | Left ovary 20 x 16 x 14 mm  |                             | Left ovary 22 x 11 x 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.

KISS1R gene heterozygous p.Y323X (c.969C>A) mutation

KISS1R gene heterozygous p.L57P (c.170 T>C) mutation