Case report

A Novel KISS1R Loss-of-Function Variant in a Chinese Child with Congenital Hypogonadotropic Hypogonadism

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What is already known on this topic?
Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder, resulting from impaired production, secretion, or action of gonadotropin-releasing hormone (GnRH). Mutations of the KISS1R (GPR54) gene can result in CHH. Minipuberty is a critical period for genital development due to the activation of the GnRH axis in the initial postnatal months.

What this study adds?
A novel compound heterozygous mutation of the KISS1R gene causing CHH in a Chinese boy was reported. The report adds to the spectrum of mutations in the KISS1R gene seen in children with CHH. Evaluation of minipuberty in male newborns and infants who present with micropenis with or without undescended testes can help in the early diagnosis and possible early treatment of nCHH.

Abstract
Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder, resulting from impaired production, secretion, or action of gonadotropin-releasing hormone (GnRH). Variants of the KISS1R can result in CHH. Here we describe a Chinese boy with CHH, caused by a novel compound heterozygous variant in the KISS1R. A male infant presented to the pediatric urological surgeon at three months of age, for micropenis. Investigations done at this time revealed low levels of serum gonadotropins and testosterone, suggesting a lack of minipuberty. Topical application of dihydrotestosterone gel was recommended, but the parents refused treatment. The child was brought to our hospital at 3.3 years of age for the same complaint. A diagnosis of CHH was considered, and next generation sequencing revealed a compound heterozygous variant including a novel c.182C>A (p.S61*) and a c.418C>T (p.R140C) in the KISS1R. We describe a novel compound heterozygous variant in the KISS1R in a boy with CHH, born to non-consanguineous Chinese parents. Our report adds to the spectrum of variants in the KISS1R seen in children with CHH.

Keywords: Hypogonadotropic Hypogonadism, KISS1R, minipuberty
Introduction
Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder caused by a defect in the production, secretion, or action of gonadotropin-releasing hormone (GnRH), which regulates the reproductive axis. CHH may present with reproductive symptoms such as cryptorchidism, micropenis, absent or incomplete puberty, infertility, amenorrhea, and a lack of breast development, and with non-reproductive features, such as bimodal synkinesis, abnormal eye movements, agenesis of the corpus callosum, unilateral or bilateral renal agenesis, cleft lip or palate, alteration of digital bones, and daltonism [1]. CHH can be broadly divided into two categories, those resulting from the abnormal embryonic migration of GnRH neurons from the olfactory placode to the forebrain and associated with anosmia/hyposmia (Kallmann syndrome [KS]), and those causing pure neuro-endocrine impairment of GnRH secretion or action namely normosmic CHH (nCHH). The incidence of CHH is uncertain. KS has an incidence of 1:125,000 in female and 1:30,000 in male as reported in a recent epidemiological study [2]. CHH is seldom diagnosed during childhood, a physiologically hypogonadal period. However, minipuberty is a window for early diagnosis of CHH. Minipuberty is a critical period for genital development due to the activation of the GnRH axis in the initial postnatal months. In boys, there is a rise in the serum testosterone (T) and gonadotropin levels, which peak at three months of age almost approaching adult male levels. This is followed by a decline to low prepubertal levels by about six months. Due to the impaired activation of the GnRH axis in minipuberty, low serum T and gonadotropin levels can be observed in infants affected with CHH [3].

Variants of the KISS1R are found in approximately 5% of patients with nCHH [4]. More than 30 different KISS1R variants have been reported so far [5]. With the widespread use of genetic testing, genetic causes of CHH are being increasingly identified [1]. We present a boy with nCHH born to non-consanguineous, Chinese parents, where a compound heterozygous variant was identified on genetic testing. This included a novel c.182C>A (p.S61*) (pathogenic) variant and a c.418C>T (p.R140C) (Variant of uncertain significance, VUS) variant in the KISS1R.

Case presentation
The proband, born to non-consanguineous Chinese parents, was noticed to have a small penis since birth. A pediatric urologist was consulted for the same complaint when the infant was three months old. Investigations showed low levels of serum T and gonadotropins – follicle stimulating hormone (FSH) and luteinizing hormone (LH) (T<0.03ng/ml, FSH<1.1mIU/ml, and LH<0.1IU/l). The karyotype was 46, XY. An absence of minipuberty was diagnosed and dihydrotestosterone topical gel was recommended for treatment of micropenis. However, the parents refused treatment at this time.

At 3.3 years of age, the boy was referred to our hospital for investigation of micropenis. There was no family history of any reproductive problems or non-reproductive features associated with CHH. The child could perceive smell. On physical examination, he had a micropenis with stretched length of 1.5cm (<-4SD) [6]. Hypospadias or cryptorchidism was not found. The right testicular volume was 1ml and the left 0.5ml. There were no dysmorphic features on general examination. Neurological examination was normal.

Investigations showed a basal serum T level of <0.07ng/mL, LH <0.1IU/L and FSH 1.9IU/L. The GnRH stimulation test showed an LH peak of 3.9 IU/L at 30 min and an FSH peak of 26.7 IU/L at 60 min (Table 1). The results of the 3-day human chorionic gonadotropin (hCG) stimulation test are shown in Table 1. Serum testosterone increased by 5-10 times on stimulation, which is considered a good response. Serum thyroid stimulating hormone (TSH), free thyroxine (FT4), prolactin, adrenocorticotropic hormone (ACTH), morning cortisol, and insulin-like growth factor-1 (IGF-1) levels were all within the normal range.
An ultrasound scan of the scrotum revealed a right testis of 12 x 6 x 8 mm and a left testis of 11 x 5 x 10 mm. Bone age was 2.2 years (10th-25th percentile) assessed by Tanner-Whitehouse 2 (TW2) method. Magnetic resonance imaging (MRI) of the brain detected multiple abnormal signals (presenting with slightly longer signals on T1 and T2 weighted images, and slightly higher signal on the flair sequence) in the centrum semiovale and lateral ventricles. The upper edge of the pituitary was sunken. The olfactory bulb and olfactory tract were normal.

Whole exome sequencing and Sanger sequencing revealed a compound heterozygous variant in the KISS1R. Variant c.182C>A (p.S61*) was inherited from the mother while c.418C>T (p.R140C) was inherited from the father (Figure 1). After annotation via InterVar and classification according to the American College of Medical Genetics and Genomics (ACMG) guideline, the c.182C>A (NM_032551.5) was classified as pathogenic with evidence levels PVS1+PM2+PP3, while c.418C>T was classified as VUS with evidence levels PM1+PM2+PP3. A final diagnosis of nCHH was made and treatment with hCG recommended to induce penile growth. However, the parents refused treatment this time as well. At 4.6 years of age, the boy was again referred to our hospital for the same complaint. At this visit, the parents opted to use dihydrotestosterone topical gel to treat micropenis rather than hCG or intramuscular testosterone. The child started local application of 2.5% dihydrotestosterone topical gel (0.2-0.3 mg/kg/day) and is due for follow-up after three months.

**Discussion**

Interaction of the KISS1R protein and its natural ligand Kisspeptin-1 plays a critical role in initiation and development of puberty. Loss-of-function variants of KISS1R responsible for nCHH in humans were first reported in 2003 [7]. Most of the previous cases of nCHH associated with KISS1R were homozygous variants described in adults and teenagers born of consanguineous parents [5].

In this case report, we describe a prepubertal boy with micropenis, a lack of minipuberty and delayed bone age, suggestive of CHH. Considering CHH remains a diagnosis of exclusion[10]. Malnutrition, any chronic disease, or excessive exercise were not found, so functional causes were excluded. Structural causes such as tumors, apoplexy, surgery, or infiltrative diseases were ruled out by brain MRI. Combined pituitary hormone deficiency was also excluded by normal range of TSH, FT4, prolactin, ACTH, morning cortisol and IGF-1. So the diagnosis of CHH was considered. Whole exome sequencing revealed a compound heterozygous variant in the KISS1R, including a novel c.182C>A (p.S61*) (pathogenic) variant and a c.418C>T (p.R140C) (VUS) variant. Loss-of-function variants of KISS1R are responsible for nCHH in humans. The boy could perceive smell. The MRI also showed normal olfactory bulb and olfactory tract. Since KISS1R is involved in GnRH neurons activation and not their migration. When clinical data and genetic results are matched, these variants are most likely to explain the disease of nCHH.

The proband was the only child of his parents. He presented with micropenis. Patients with kisspeptin receptor insufficiency may manifest with either complete or partial gonadotropic deficiency. Micropenis in these patients is due to fetal testosterone deficiency resulting from absence of testicular stimulation by gonadotropins during the second and third parts of the pregnancy [8]. Hormonal evaluation revealed low gonadotropins and testosterone concentrations at three months of age, supporting a diagnosis of a lack of minipuberty. Minipuberty is the transient sex-specific activation of the hypothalamic-pituitary-gonadal (HPG) axis during the first six months of life in boys [9]. A lack of minipuberty in infancy provides a valuable opportunity for the diagnosis of CHH, since childhood is a period of physiological hypogonadism [10].

We found a compound heterozygous variant in the KISS1R. The first variant was a nonsense variant which cause an early termination for translation and classified as pathogenic according to the ACMG guideline. The second variant a missense variant, its frequency was extreme low in gnomAD database (0.000006571 for whole database and 0.0001924 for East Asian subgroup of gnomAD database), and relative higher (0.1445%) in South Asia subgroup of the GenomAsia database (1 allele count in total 692). The frequency difference between GenomAsia...
and gnomAD maybe due to the total cohort number: gnomAD (5198), GenomAsia (692). Comprehensively considering other evaluation results: cohort>1000, pathogenic variant was detected at the trans position in AR disease, multiple protein prediction software predicted that the variation was deleterious and genotype is correlated with phenotype, the variant as classified as VUS. However, no functional studies were performed to demonstrate the pathogenicity of these variants. Our patient will need long term follow-up to observe the efficacy of dihydrotestosterone topical gel. Multiple abnormal signals in the centrum semiovale and lateral ventricles, and a sunken upper edge of the pituitary were observed in MRI of the brain, which are unable to be explained at present.

In conclusion, we report a novel compound heterozygous variant of the KISS1R causing nCHH in a Chinese boy. Evaluation of minipuberty in male newborns and infants who present with micropenis with or without undescended testes can help in the early diagnosis and possible early treatment of nCHH. Genetic testing is also recommended to assist the diagnosis of CHH and to provide genetic counseling to the family. Our case adds to the increasing spectrum of CHH caused by variants in the KISS1R.

References


Table 1. GnRH test and hCG stimulation test

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>LH (IU/L)</th>
<th>FSH (IU/L)</th>
<th>Testosterone (ng/mL)</th>
<th>Dihydrotestosterone (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>&lt;0.1</td>
<td>2.0</td>
<td>&lt;0.07</td>
<td>&lt;50</td>
</tr>
<tr>
<td>30 min</td>
<td>3.9</td>
<td>21.7</td>
<td>2.19</td>
<td>69.61</td>
</tr>
<tr>
<td>60 min</td>
<td>3.5</td>
<td>26.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td>2.5</td>
<td>25.1</td>
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<td></td>
</tr>
<tr>
<td>120 min</td>
<td>1.9</td>
<td>23.6</td>
<td></td>
<td></td>
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</table>

GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin
Figure 1. Circles represent females and squares males. Half-shaded symbols indicate unaffected heterozygous and solid symbols affected subjects. The proband, subject II-1 (arrow), was compound heterozygous for KISS1R variants R140C/S61*. The unaffected father (I-1) was heterozygous for the R140C variant and the unaffected mother (I-2) was heterozygous for S61*.