Clinical characteristics and follow-up of 5 young Chinese males with gonadotropin-releasing hormone deficiency caused by mutations in the KAL1 gene

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Abstract

Isolated gonadotropin-releasing hormone (GnRH) deficiency (IGD) pertains to a group of genetic disorders consisting of anosmic hypogonadotropic hypogonadism (Kallmann syndrome, KS) and normosmic idiopathic hypogonadotropic hypogonadism (nIHH). KS is genetically heterogeneous. We hereby present 5 young male patients with GnRH deficiency caused by mutations in the KAL1 gene. Their ages ranged from 9 months to 16 years. They were referred to our department for an endocrine consultation for micropenis. Hormone assays showed low circulating gonadotropins and testosterone. Molecular studies revealed KAL1 mutations in all cases, three reported nonsense sequence variants in the KAL1 gene were detected in 4 patients, respectively (c.784C>T(p.Arg262*), c.1267C>T(p.Arg423*), and c.1270C>T(p.Arg424*)), and one patient harbored a novel hemizygous sequence variant[c.227G>A(p.Trp76*)]. Only one patient presented short stature without growth hormone deficiency and anosmia. Another patient had bilateral eyelid ptosis, trichiasis, and refractive error. This is the first report on the co-occurrence of a KAL1 gene mutation and tent-like upper lip in four patients. All of our cases had normal olfactory bulbs and showed no renal agenesis, cleft lip/palate, and hearing impairment. These cases expand our knowledge of the phenotype associated with KAL1 sequence variations, although the precise mechanism by which KAL1 gene influences the development of this phenotype is still unknown.

1. Introduction

The hypothalamic–pituitary–gonadal (HPG) axis plays a crucial role in the development and progression of puberty. The pulsatile secretion of gonadotropin-releasing hormone (GnRH) into the hypophyseal-portal vessels controls the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary gland, which then stimulates the gonads to produce sex steroids and gametes. Isolated GnRH deficiency (IGD) is a rare disorder with an estimated incidence of one case per 48,000 births. It is caused by a defect in the HPG axis, resulting in low levels of sex steroids and a delay or absence of puberty. IGD is divided into anosmic hypogonadotropic hypogonadism (Kallmann syndrome, KS) and normosmic idiopathic hypogonadotropic hypogonadism (nIHH) (Fathi and Luo, 2013). About 60% of patients with IGD present with anosmia or hyposmia (KS) by total or partial defects of the olfactory bulb due to the fact that the GnRH-releasing neurons are primarily derived from progenitor cells in the nasal compartment and migrate along the fibers derived from the olfactory system across the cribiform plate to the forebrain (Sykiotis et al., 2010).

KS is the most frequent cause of congenital hypogonadism. It is characterized by the association of optic atrophy, deafness, a cleft lip, renal malformations, cryptorchidism, and neurological anomalies. This disorder is a form of hypogonadotropic hypogonadism (HH), which is a condition affecting the production of hormones that facilitate sexual development. Males with hypogonadotropic hypogonadism are often born with an unusually small penis (microgenitum) and undescended testes (cryptorchidism). They also present with delayed or incomplete puberty. KS and other syndromes causing a congenital deficiency of GnRH are characterized by low levels of LH and FSH with low levels of sexual steroids (testosterone and estradiol). In some sporadic cases of hypogonadotropic hypogonadism, etiologies that may disrupt the communication pathway between the hypothalamus and pituitary should be excluded. Mutations in at least 19 genes contribute to the molecular basis of IGD. The genetic defects are classified according to pathophysiology: defects in the neurodevelopmental pathway (KAL1, NELF, and...
At birth, his weight was 2450 g (about 3rd percentile). The height of father and mother was 158 cm and 157 cm, respectively. He was born by spontaneous delivery after an uneventful pregnancy at 35 weeks of preterm gestation to a nonconsanguineous and healthy couple. The newborn's weight and length were normal [weight: 3430 g (more than 50th percentile); length: 50 cm (more than 50th percentile)], except for the presentation of a micropenis (15 mm; −2SD) (Fig. 1A), undescended testes, and bilateral testicular hypoplasia (mean testicular volume: 0.33 ml; (sonography): −3SD). The patient showed no other congenital anomalies (anosmia, hearing loss, missing teeth). Hormone assays conducted at age 18 months showed low circulating gonadotropins [FSH: 0.18 (normal range: 0.2–3.5); LH: 0.04 IU/L (0.5–6.5)], testosterone: 0.1 (normal range: 0.5–4.8), and low testicular peptide levels (AMH: 69 ng/ml; normal range: 80–154) (Table 1). Sense of smell was not assessed because of his young age. MRI performed at the age of 19 months revealed normal olfactory bulbs. His parent declined KAL1 carrier testing of the patient's younger brother.

Case 2. The patient was an 18-month-old male. Ultrasound examination during the 24th week of gestation showed a normal fetus. Normal delivery took place at the 39th week of gestation. At delivery, the newborn's weight and length were normal [weight: 3430 g (more than 50th percentile); length: 50 cm (more than 50th percentile)], except for the presentation of a micropenis (Fig. 1B); no other physical malformation was detected. Hearing tests conducted at 2 months of age showed normal hearing. His motor development was normal, with independent sitting and walking achieved on time (6 and 12 months, respectively). At the age of 12 months, the patient developed cryptorchidism of the left testis. He was treated with HCG (500 U i.m., twice a week), and after 4 weeks, the testes descended into the scrotums. Throughout the childhood, the patient manifested a slower growth rate. The patient was referred to local endocrinologist because of short stature at the age of 14. At that time, his height was 142.5 cm (less than 3rd percentile) and his weight was 30 kg (less than 3rd percentile). The patient was referred for a conventional chromosomal analysis, which showed normal male karyotype (46, XY). His brain MRI scans were unremarkable. Bone age assessment based on left carpal X-ray was delayed in terms of chronological age. A stimulated growth hormone test was performed; the results showed that his growth hormone peak value was 16.2 ng/mL. The patient was diagnosed to have idiopathic short stature (ISS) with familial short stature. He was administered growth hormone (1.5 U/kg/d) for 3 months, and then his parents decided to stop the growth hormone therapy.

Physical examination showed a height of 146.2 cm, and weight of 32 kg at the age of 15.6 years old. The mean arterial pressure (MAP) of the patient was 115/65 mm Hg. He presented with a small penis, male phenotype at Tanner I stage, and no goiter. He did not tolerate exposure to different scents or aromas during childhood compared to the other family members. Due to the reported decreased sensitivity to smell, objective olfactory analyses were performed, the registration of olfactory-evoked potentials (OEPs) showed a considerable decrease in the amplitudes that were symmetrical on both sides. Endocrinological tests done at the age of 15.6 years old showed normal results for TSH, free triiodothyronine (FT3), FT4, prolactin (PRL), and Adrenocorticotropic Hormone (ACTH). However, FSH, LH and testosterone levels were lower than normal (Table 1). After being diagnosed with Kallmann Syndrome, the patient was first treated with HCG (2000 U i.m., twice a week for 4 weeks). A second HCG injection (2000 U i.m., twice a week for 4 weeks) was given 3 months later. Then he was started on hormone replacement with intramuscular injections of testosterone derivatives for 12 months after 6 months of being diagnosed. His secondary sexual characteristics developed satisfactorily after treatment for 1.5 years. His penis became larger and his pubic hair appeared. His height increased by near 12 cm within 1.5 years, and his height at the last follow-up measured 158 cm when he was 17.1 years old.

Case 3. The patient was first referred to our clinic at the age of 15.6 years old. He was born by spontaneous delivery after an uneventful pregnancy (G1P1) at 35 weeks of preterm gestation to a nonconsanguineous and healthy 23-year-old mother and a 25-year-old father of Han ethnicity. The height of father and mother was 158 cm and 157 cm, respectively. At birth, his weight was 2450 g (about 3rd–10th percentile), length 48 cm (50th percentile), and his Apgar score was 7 and 10 at 1 and 5 min, respectively. Physical examination after birth revealed a micropenis (Fig. 1B); no other physical malformation was detected. Hearing tests conducted at 2 months of age showed normal hearing. His motor development was normal, with independent sitting and walking achieved on time (6 and 12 months, respectively). At the age of 12 months, the patient developed cryptorchidism of the left testis. He was treated with HCG (500 U i.m., twice a week), and after 4 weeks, the testes descended into the scrotums. Throughout the childhood, the patient manifested a slower growth rate. The patient was referred to local endocrinologist because of short stature at the age of 14. At that time, his height was 142.5 cm (less than 3rd percentile) and his weight was 30 kg (less than 3rd percentile). The patient was referred for a conventional chromosomal analysis, which showed normal male karyotype (46, XY). His brain MRI scans were unremarkable. Bone age assessment based on left carpal X-ray was delayed in terms of chronological age. A stimulated growth hormone test was performed; the results showed that his growth hormone peak value was 16.2 ng/mL. The patient was diagnosed to have idiopathic short stature (ISS) with familial short stature. He was administered growth hormone (1.5 U/kg/d) for 3 months, and then his parents decided to stop the growth hormone therapy.

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Case 4 and Case 5. Case 4 (14 years old) and Case 5 (16 years and 7 months old) were referred to our hospital based on the observation of a micropenis right after birth. Physical examination showed micropenis measuring <2.0 cm with no other malformations (Figs. 1C and D). No clinically apparent malformations of the midline were discovered in Case 4. Case 5 presented with bilateral eyelid ptosis, strabismus, and refractive error from the age of 2-year-old till now. Testing of pulsatile LH secretion showed no significant pulses in Cases 4 and 5. Other antepituitary functions (FT4, FT3, TSH, ACTH, and peak cortisol) were normal, as was prolactin (Table 1). T tests B ultrasound results showed less than 1 mL of testes volume (normal volume: >10 mL). Olfactometry confirmed that the patient had normosmia. Brain MRI showed no agenesis of the olfactory bulbs, a normal antepituitary size, and a normal pituitary stalk. Audiometry showed normal hearing. Micropenis was not corrected by testosterone treatment in Cases 4 and 5. Combination therapy with recombinant human pituitary gonadotropins (LH and FSH) was therefore recommended, and was initiated 3 months ago for Case 4. This treatment resulted in a marked increase in testicle size (from 0.9 to 2.3 mL upon sonography) and penis length (from 2 to 4 cm). Case 5 refused to receive gonadotropin treatment.

Patients with KS often showed a cleft lip and cleft palate. However, in Case 4 and Case 5, their upper lips were thicker than normal, and the angles of their mouths were oriented downward (Fig. 1).
Table 1
Summary of clinical findings in Kallmann syndrome patients.

| Patients | NO.1 | NO.2 | NO.3 | NO.4 | NO.5 |
|----------|------|------|------|------|------|
| Sex      | Male | Male | Male | Male | Male |
| Age of first administration (months) | 5 | 18 | 15.6 | 14 | 16.6 |
| Height (cm) (percentile) | 67 (50th) | 83 (50th–75th) | 146.2 (1st) | 153 (25th–50th) | 167.8 (25th–50th) |
| Weight (kg) (percentile) | 7.5 (50th) | 12.5 (50th–75th) | 32 (1st) | 42 (25th–50th) | 67 (75th–95th) |
| Sense of smell | No data available | No data available | Hyposmia | Normosmic | Normosmic |
| Sexual maturation* (Tanner stage) | G1P1B1 | G1P1B1 | G1P1B1 | G1P1B1 | G1P1B1 |
| Testicular volume and cryptorchidism** (right/left) | 6 × 4 × 5 mm/8 × 5 × 6 mm | 10 × 5 × 5 mm/7 × 4 × 4 mm ** | 14 × 5 × 7 mm/13 × 9 × 5 mm**; | No data available | 14 × 5 × 7 mm; 13 × 9 × 5 mm; |
| Renal malformation, dental agenesis, synkinesis, short fourth metacarpal | No | No | No | No | No |
| Other signs | No | No | No | No | No |
| Other signs | Bilateral eyelid ptosis, trichiasis and refractive error |

Summary of laboratorial tests in the first assessment

| Test                  | NO.1         | NO.2 | NO.3 | NO.4 | NO.5 |
|-----------------------|--------------|------|------|------|------|
| FSH (IU/L)            | <0.03        | 2.11 | <0.3 | 0.9  | 0.3  |
| LH (IU/L)             | <0.01        | 0.44 | 0.07 | 0.5  | 0.06 |
| Testosterone ng/ml    | <0.1         | 0.01 | 0.07 | 0.07 | 0.31 |
| TSH, ACTH, COR        | Normal       | Normal | Normal | Normal | Normal |
| MRI brain             | 46, XY       | 46, XY | 46, XY | 46, XY | 46, XY |
| KAL1 gene mutation    | c.1270C>T, p.Arg424* | c.227G>A, p.Trp76* | c.1267C>T, p.Arg423* | c.784C>T, p.Arg262* | c.1270C>T, p.Arg424* |
| Follow-up             | HCG 500 U; im q5d 10 times, His penis became enlarged | After 2 years, his little brother was born and had similar small penis. The parents declined to do genetic analysis of the younger brother till now. | He was treated with HCG (2000 U im, twice a week for 4 weeks). A second HCG injection treatment was given 10 months later. Then he was started with testosterone derivatives (im) for 12 months, the total duration (HCG + testosterone derivatives) till the last follow up was 18 months. His penis became larger. His pubic hair appeared, his height was 158 cm at the last follow up when he was 17.1 years old. | He was treated with HCG (1500 U im, twice a week), each treatment duration continued to 5 weeks, and he had finished 3 durations. | Declined to answer |

Notes: *G, external genitalia; P, pubic hair; B, breast development; ** cryptorchidism.
2.2. Sanger sequencing of the KAL1 gene

The genomic DNA of the patient and parents was isolated from peripheral blood samples using a QIAamp Blood DNA Mini kit® (Qiagen GMBH, Hilden, Germany). All of the exons and exon-intron boundaries of the KAL1 (GenBank accession number: NM_000216.2) gene were amplified by PCR (TaKaRa, Dalian, China) using primers listed in Table 2. The primers were designed using the UCSC ExonPrimer online software (http://genome.ucsc.edu/index.html). The products were examined on a 1% agarose gel and purified with a QIAquick Gel Extraction Kit (Qiagen GMBH, Hilden, Germany). The resulting DNA was sequenced via the ABI3730XL sequencer (Applied Biosystems, Foster City, CA, U.S.).

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3. Results

3.1. Sequencing of KAL1 gene

All the patients were evaluated by direct nucleotide sequencing. Cases 1 and 5 harbored a documented hemizygous nonsense mutation, c.784C>T (p.Arg264*). Cases 2–5 harbored documented hemizygous nonsense mutations, c.1267C>T (p.Arg424*), c.1789C>T (p.Arg423*), and c.1375C>T (p.Arg262*). The patient was hemizygous for a mutation designated as c.227G>A in exon 2. Sequence analysis indicated that the c.227G>A mutation at codon 76 of exon 2 was a nonsense mutation that led to the early termination of the protein translation process (p.Trp76*). Targeted testing showed that his mother was heterozygous for this same sequence variant (Fig. 2). All detected sequence variants in the KAL1 gene of these cases were nonsense.

4. Discussion

HH is characterized by a defective development of the GnRH pulse generator (Grumbach, 2005). KS and normosmic HH (nHH) share anatomical and genetic etiologies with common features. Mutations in genes such as FGFRI, FGF8, PROKR2, and PROKR2 have been shown both in KS and in nHH, confirming that KS and nHH are two different phenotypes of the same pathology (Lewkowitz-Shpuntoff et al., 2012). Mutations in several genes/pathways affecting GnRH neuronal migration (neurodevelopmental genes) have been identified in KS patients.

The KAL1 gene is one of the main genes that cause KS. We have described five cases of HH in which the diagnoses were strongly suspected during childhood. The molecular identification of mutations in the KAL1 gene corroborated the diagnosis. To date, nearly 161 mutations have been identified in the KAL1 gene, including missense/nonsense mutations, splicing mutations, small insertions/deletions, gross deletions, and complex rearrangements (data from HGMD). The proportion of missense/nonsense mutations accounted for nearly 40% of the total number of reported sequence variants. Sequencing all exons of KAL1 gene of all our patients was performed. In our 5 patients, 4 variations were identified, and three variants were causative for KS in the previous studies (p.Arg424*, p.Arg423*, p.Arg263*; Sato et al., 2004; Hardelin et al., 1993; Söderlund et al., 2002). In Case 2, we identified a novel hemizygous mutation in exon 2, namely, c.227G>A (p.Trp76*) that is predicted to result in a truncated protein that results from the formation of a premature stop codon or nonsense-mediated RNA decay (NMD). Interestingly, all the detected variations were nonsense.

Only less than 20% of KS patients display a mutation in KAL1, indicating that the KAL1 gene may be not the only disease causative gene. Hemizygous sequence variants in the KAL1 gene, p.Arg191* and p.Cys13*, a heterozygous FGFRI variant, p.Arg250Trp; and a homozygous PROKR2 variant, p.Tyr113His were previously detected in five out of seven Chinese KS pedigrees (Gu et al., 2015). Classical monogenic...
inheritance does not explain the full range of genetic inheritance patterns for KS and HH, which in turn suggests that KS is not only a monogenic Mendelian disease, but rather a digenic or potentially oligogenic condition.

KS patients with KAL1 mutations may have a variety of associated disorders of neurologically urogenital nature, and the most frequent are mirror movements, renal anomalies, neurogenic deafness, midline anomalies (cleft lip or palate), skeletal anomalies of the hands or feet, dental abnormalities, deafness, and schizophrenia (Verhoeven et al., 2013). A previous epidemiological study has reported that KS patients present a wide range of clinical features (Bonomi et al., 2012). Four of our cases showed thicker upper lips without a cleft lip/palate. All of our cases showed no renal agenesis. This was in agreement with the findings of Costa-Barbosa et al., who observed that renal agenesis and cleft lip/palate were not statistically significant phenotypic predictors (Costa-Barbosa et al., 2013). Furthermore, the present study did not detect mirror movements in the KS patients. Only one patient had bilateral eyelid ptosis. Synkinesis is often thought to be observed in KAL1 genotypes with different prevalence rates ranging from 4.1% to 31% (Bonomi et al., 2012; Maione et al., 2013). No synkinesis was detected in our cases. These cases expand our knowledge of the phenotype caused by KAL1 mutations, but the precise mechanism by which the KAL1 gene contributes to this phenotype is still unknown.

Approximately 40% of patients with idiopathic HH have normal sense of smell (Fathi and Luo, 2013; Lewkowitz-Shpuntoff et al., 2012). However, only one adolescent had hyposmia, there were 2 patients who were too young to participate in the collection of smell data in our patients. MRI of olfactory structures of our HH patients confirmed normal olfactory bulbs. Koenigkam-Santos et al. showed that the olfactory bulb and sulcus agenesis were the most common findings in KS patients and demonstrated agreement between MRI findings and the smell test, especially the presence of bulb aplasia and anosmia (Koenigkam-Santos et al., 2011). However, there are also other reports that describe normosmic subjects with hypoplastic left olfactory bulbs (Hardelin et al., 1993r). Our MRI findings and the results of our smell test were not in agreement with above reports. KS subjects with olfactory bulb agenesis on MRI or harbored KAL1 mutations had the most significant changes in olfactory fossa measurements and angles (Maione et al., 2013). The precise mechanism underlying this disorder is unclear; however, oligogenicity might be a plausible reason.

The prevalence of cryptorchidism was 35% in KS patients and 10% in HH patients (Ghervan and Young, 2014). The presence of microepispadius, and/or cryptorchidism strongly argues for HH. Costa-Barbosa et al. performed a detailed comparative phenotypic evaluation between a group of KS subjects harboring known rare sequence variations (RSVs) in 8 genes (KAL1, NELF, CHD7, H6GST1, FGFR1, FGFR2, FGFR3, and PROK2) and a cohort of KS patients without RSVs. They found testicular volumes of male KS subjects with KAL1 RSVs were smaller than KS patients without RSVs ((1.5 ± 0.1 mL vs 3.7 ± 0.3 mL, P < .05) (Costa-Barbosa et al., 2013). Our cases had similar smaller-sized testes, and we found cryptorchidism in two patients.

The selection for the appropriate treatment for KS should therefore be individualized. Treatment protocols are basically a choice between androgen replacement to virilise, gonadotropin therapy to induce fertility, and luteinizing hormone releasing hormone (LHRH) analog administration for most physiological replacements. Considering the age of the patients, our therapeutic aim was to restore normal development of the genitals and secondary sex characteristics. In KS with cryptorchidism, chorionic gonadotropin therapy results in the elimination of cryptorchidism without surgery. An attempt to “milk” the testes downwards should also be conducted. The cryptorchidism in Cases 2 and 3 was resolved after HCG therapy. Cases 2 and 3 served as examples where meticulous clinical examination saved the patients from unnecessary surgical intervention. It is also appropriate to treat HH patients with microepispadius with androgens to enlarge the penis into that of the normal childhood range. Our experience with testosterone replacement in these patients was overwhelming in this regard.

5. Conclusions

In the present study, we identified four mutations in the KAL1 gene in five KS patients. Three variations had been reported in the literature that could lead to KS. In addition, we identified a novel nonsense mutation in one patient, namely, c.227G > A, which is predicted to result in a premature stop codon (p.Trp76*). This finding could be used in the development of a database of KAL1 gene mutations that disturb the development and function of the hypothalamic–pituitary–gonadal (HPG) axis. Unidentified gene mutations could partially account for the phenotypes of our patients with tent-like lips and eyelid ptosis.

Conflict of interest statement

The authors declare no conflict of interest related to this study.

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