Clinical and genetic features of 64 young male paediatric patients with congenital hypogonadotropic hypogonadism

Yi Wang1,2,3 | Chunxiu Gong1,2,3✉ | Miao Qin1,2,3 | Ying Liu1,4 | Yuanyuan Tian1,2,3

1National Center for Children’s Health, Capital Medical University, Beijing, China
2Department of Endocrinology, Genetics, Metabolism and Adolescent Medicine, Beijing Children’s Hospital, Capital Medical University, Beijing, China
3Beijing Key Laboratory for Genetics of Birth Defects, Beijing Children’s Hospital, Capital Medical University, Beijing, China
4Department of Pharmacy, Beijing Children’s Hospital, Capital Medical University, Beijing, China

Correspondence
Chunxiu Gong, National Center for Children’s Health, Capital Medical University, Beijing, China.
Email: chunxiugong@sina.com

Funding information
the National Key Research and Development Program of China, Grant/Award Number: 2016YFC0901505; Beijing Municipal Science and Technology Funding, Grant/Award Number: Z151100030915103

Summary
Context: The diagnosis of congenital hypogonadotropic hypogonadism (CHH) in pre-puberty has always been challenging. Here, we aimed at studying the clinical and genetic features of paediatric CHH, especially the phenotype of hypospadias and dual defects (patients showing hypothalamic and/or pituitary defects and testicular hypoplasia), so as to have a better understanding of CHH.

Design: The clinical and genetic features of patients with CHH were analysed, and the relationships between hypospadias, dual defects and genetics were investigated.

Patients: Patients who visited Beijing Children’s Hospital and were positively diagnosed with CHH.

Measurements: The collected data included sex hormones, MRI of the olfactory bulb, human chorionic gonadotrophin (hCG) test and genetic testing. We analysed clinical features and genetic results, especially hypospadias and dual defects, and compared the stimulated testosterone (T) levels in patients with and without cryptorchidism.

Results: Sixty-four patients were positively diagnosed, and forty-seven (73.4%) had Kallmann syndrome (KS). Four patients (6.3%) had hypospadias, including 2 KS. Micropenis combined with cryptorchidism was the most common phenotype (39%). Approximately two-third of patients showed a poor response to hCG; 15 cases were diagnosed with dual defects, and there were no significant differences between those with and without cryptorchidism. Twenty-six cases (51%) of 51 patients were identified as having classical HH mutations, affecting 10 different genes, with oligogenic mutations in 5 cases (9.8%). The most common mutations were in PROKR2 (17.6%), FGFR1 (13.7%) and CHD7 (7.8%). The frequency of PROKR2 mutations was higher in dual HH when compared to other HH cases (6/15 vs 3/36, P = .021).

Conclusions: Micropenis and/or cryptorchidism can serve as important signs for the diagnosis of HH in paediatrics, and the coexistence of hypospadias does not exclude the diagnosis of CHH, including KS or normosmic isolated HH (nHH). Testicular function may be impaired earlier than expected, and PROKR2 mutations need to be evaluated to identify presumed dual defects.

KEYWORDS
congenital hypogonadotropic hypogonadism, dual HH, gene, hypospadias
Congenital hypogonadotropic hypogonadism (CHH) results from deficiency in the production, secretion or action of gonadotrophin-releasing hormone (GnRH). Patients with CHH presented with micropenis and/or cryptorchidism, absent or delayed puberty or infertility. Some patients also exhibit other developmental anomalies, including cleft lip, dental agenesis, ear anomalies, congenital hearing impairment, renal agenesis, bimanual synkinesis. Patients presenting with anosmia or hyposmia is termed as Kallmann syndrome (KS). CHH affects approximately 1-10 per 100,000 newborns, with approximately two-third and one-third of cases arising from KS and idiopathic HH, respectively. CHH is genetically heterogeneous, with more than 31 different genes being implicated in Kallmann syndrome and/or nHH to date, which represents 50% of all cases, according to the CHH consensus published in 2015. However, the list of candidate genes continues to grow.

Hypogonadism can be divided into 3 types: central (hypothalamic-pituitary), primary (testicular) and combined forms (hypothalamic-pituitary and testicular, the so-called dual defect form). CHH occurs due to the impaired production of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secondary to GnRH deficiency or due to pituitary defects (central hypogonadism), while deficiency of GnRH or gonadotrophins may lead to the failure of testicular development. However, in some cases, both the hypothalamic-pituitary axis and the testes are affected simultaneously. These “dual” conditions are characterized by a lack of gonadotrophin elevation during puberty or adulthood despite the low testicular secretion of androgens and/or inhibit B. Hitherto, the presence of dual defects in CHH subjects has only been demonstrated in one previous study, which was in the context of GnRH pump therapy. A systematic analysis of the presence of dual defects in CHH men is lacking. In this study, we utilized the human chorionic gonadotrophin (hCG) stimulation test to assess potential testicular defects in paediatric CHH subjects.

Male neonates and infants suffering from CHH may present with micropenis and/or cryptorchidism, which makes it possible to identify CHH in early childhood. Currently, genetic testing provides further evidence for the prepubertal diagnosis of CHH. In contrast, hypospadias is caused by abnormal urethral closure during the early weeks of embryonic development, while urethral closure is often regulated by testosterone induced by hCG instead of gonadotrophins. Therefore, patients with hypospadias are often excluded from the diagnosis of CHH. However, we have previously described KS patients with hypospadias in clinical practice and observed that some genes that are involved in CHH could also be candidate genes for hypospadias. The same signalling pathway plays a critical role in GnRH neuron ontogeny and development of the olfactory system. Recently, both Ayers et al and Eggers et al suggested that some patients with 46,XY disorders of sexual development (DSD) of unclear origin or showing isolated hypospadias carried CHH genes. One possible reason for this interesting phenomenon is that CHH genes could contribute to DSD, but another possibility is that these patients may be diagnosed with CHH during follow-up. We have observed some patients with CHH who presented with micropenis with or without cryptorchidism or hypospadias; hence, we deduce that hypospadias may be a rare sign of CHH.

In this study, we present data of 64 male CHH cases whose clinical information was examined by the same paediatric endocrinologist and systematically analysed according to the clinical features and genetic results. In addition to establishing the phenotypic spectrum, we also analysed the prevalence of the dual defect in CHH subjects and correlated them with the genetic results.

2 | PATIENTS AND METHODS

2.1 | Patients

We selected 64 male CHH Han-Chinese patients who were seen at the Endocrinology outpatient clinic at Beijing Children’s Hospital between January 2008 and August 2016 for suspected CHH. These 64 cases were 0-18 years of age when diagnosed and were from nonconsanguineous pedigrees. They were confirmed to be CHH according to clinical features and family history, karyotype, gonadotrophin and sex hormone profiles, MRI of the olfactory bulb and ultrasonographic examination of the pelvis and testes. We also performed HCG stimulation testing and genetic analysis (see below). The younger patients were also confirmed by positive genes. The research was approved by the Ethics Committee of Beijing Children’s Hospital, Capital Medical University, and written informed consents were signed by the patients’ parents or legal guardians.

2.2 | Inclusion criteria

All patients met the following criteria: (i) no signs of puberty (testicular volume < 4 mL) with or without micropenis/cryptorchidism/ hypospadias, and baseline sex hormones (serum testosterone ≥ 0.69 nmol/L) and gonadotrophins (LH, FSH) at a prepubertal levels, except for those with stalled puberty. For those with bone age > 12 years, the LHRH stimulation test results were referenced; (ii) no space-occupying lesions on MRI of pituitary and hypothalamic areas, and patients in different age groups had more specific restrictions; (iii) KS diagnosis was made by anosmia/hyposmia, MRI of olfactory bulb or KS family history; (iv) follow-up to rule out growth hormone deficiency (GHD).

For patients aged ≥ 14 years: (i) bone age ≥ 12 years; (ii) completed 2 years of follow-up or underwent a LHRH stimulation test to confirm the diagnosis. For patients aged > 6 m and < 14 years: (i) diagnosed KS by MRI olfactory bulb or KS family history; (ii) bone age concordant with chronic age; (iii) genetic testing supporting the diagnosis, and genes suggesting pathogenesis ((a) nonsense, frameshift and splicing site mutations; (b) missense mutations predicting pathogenesis with POLYPHEN-2, SIFT and MUTATIONTASTER software packages; (c) mutations of MAF < 5% in East Asian people in ExAC database; (d) mutational sites reported to be pathogenic in ClinVar). For patients aged ≥ 6 m: (i) no minipuberty, LH, FSH, T levels were low and (ii) genetic testing was necessary.
2.3 Exclusion criteria

The exclusion criteria were as follows: (i) any ascertained reasons (e.g., chromosomal abnormality, trauma, surgeries, congenital adrenal hyperplasia (CAH), NR5A1-related micropenis/cryptorchidism) or other ascertained diseases (e.g., Prader-Willi syndrome (PWS) accompanied by sex agenesis, multiple pituitary hormone deficiency); (ii) presence of chronic systemic diseases (e.g., uraemia, thalassaemia, poorly controlled diabetes); (iii) protein-energy malnutrition; (iv) eating disorders (e.g., anorexia nervosa, bulimia); (v) intracranial lesions or pituitary tumours (possibly following surgery).

2.4 Human chorionic gonadotrophin and LHRH stimulation tests

The hCG standard and LHRH stimulation tests were performed as previously described. For the hCG prolonged test, hCG 1000 U was injected every other day for a total of 10 doses and serum testosterone level checked on the 20th day. A serum testosterone ≥3.47 nmol/L (100 ng/dL) was considered a good response, and a level of <3.47 nmol/L was considered a poor response.

2.5 DNA sequencing

Overall, there were 164 genes (all CHH genes listed in reference 3 were included) in our gonadal panel, and the genes were obtained by referencing the OMIM and HGMD databases and entering the keywords "congenital hypogonadotropic hypogonadism," "idiopathic GnRH deficiency," "complex hypogonadism," "Kallmann syndrome," "gonad dysgenesis," "micropenis," "cryptorchidism," "hypospadias" and "disorders of sex development" in Pubmed. DNA was extracted from peripheral blood leucocytes of patients and their parents and siblings, and the NEXTSEQ 500 sequencer (Illumina Corporation, San Diego, CA, USA) was used to filter out all possible pathogenic missense, frameshift and splice site mutations. Design primers and Sanger sequencing were used to verify the mutations of samples from the same family. Missense mutations were assessed for predicted pathogenicity using POLYPHEN-2, SIFT and MUTATIONTASTER software packages; both frameshift and splicing site mutations were considered pathogenic mutations. Each mutation was contrasted to those in EXAC, SNP databases and mutations of minor allele frequency (MAF) <1% in East Asian people. In addition, we checked the pathogenicity of the mutations in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar).

2.6 Hormone detection

Luteinizing hormone, FSH and testosterone were detected using enzyme-enhanced chemiluminescence immunoassay (Immulite 2000, Siemens Corporation, Munich, Germany).

2.7 Statistical analysis

A chi-square test of four-grid data was used when comparing testosterone levels after the hCG stimulation test and PROKR2 frequency between dual and pure HH. Data analysis was performed using STATISTICAL PRODUCT AND SERVICE SOLUTIONS 17.0 software (spss, International Business Machines Corporation, Armonk, NY, USA).

3 RESULTS

3.1 Demographic summary

A total of 64 cases were diagnosed definitively using the inclusion criteria. Forty-seven patients were diagnosed with KS (73.4%), and 17 had nHH without any special syndrome features (e.g., Charge syndrome, PWS). One case was diagnosed within 6 months of birth, 41 cases were between 6 months and 14 years, and 22 cases were between 14 and 18 years.

3.2 Clinical features

External genitalia morphology included isolated micropenis (35%), delayed puberty (12%), isolated cryptorchidism alone (7%) and mixed form (46%) (see Figure 1 and Table S1). Micropenis combined with cryptorchidism was the most common form (39%), followed by isolated micropenis. However, all forms of cryptorchidism, including the combined form, were the most common feature of CHH in our study (51.6%). There was no significant difference when comparing testosterone

![FIGURE 1](wileyonlinelibrary.com)
| No. | Age (yrs) | Diagnosis | Smell | MRI | Micropenis | Length of penis (cm) | Cryptorchidism (L/R) | Testis volume (mL) | Hypospadias | Location |
|-----|-----------|-----------|-------|-----|------------|---------------------|---------------------|-------------------|-------------|----------|
| 1   | 0.58      | KS        | NA    | Abnormal | Yes        | 1.8                 | L + R               | NA                |            |          |
| 2   | 11        | KS        | Hyposmia | Abnormal | Yes        | 2.4                 | R                   | <1                |            |          |
| 3   | 11        | KS        | Hyposmia | NA    | KS         | 3.5                 | R                   | NA                |            |          |
| 4   | 12        | KS        | Hyposmia | Abnormal | Yes        | 3.5                 | L                   | 2                 |            |          |
| 5   | 13        | KS        | Hyposmia | Abnormal | Yes        | 3.4                 | No                  | 2                 |            |          |
| 6   | 14.58     | KS        | Hyposmia | Abnormal | Yes        | 3.5                 | No                  | 1                 |            |          |
| 7   | 15.5      | KS        | Hyposmia | Abnormal | Yes        | 3                  | No                  | 3                 |            |          |
| 8   | 15.75     | KS        | Hyposmia | Abnormal | Yes        | 2.5                 | R                   | 2                 |            |          |
| 9   | 7.83      | nHH       | Normal | NA    | Yes        | 0.8                 | R + L                | 1                 |            |          |
| 10  | 9         | nHH       | Normal | Normal | Yes        | 2.5                 | R + L                | NA                |            |          |
| 11  | 9         | nHH       | Normal | NA    | No         | 3.7                 | R + L                | 1-2               | Yes        | Root of penis |
| 12  | 10.17     | nHH       | Normal | Normal | No         | 4.5                 | R + L                | 1                 |            |          |
| 13  | 14.25     | nHH       | Normal | Normal | Yes        | 3                  | No                  | 1                 |            |          |
| 14  | 14.42     | nHH       | Normal | NA    | No         | 7                  | No                  | 2-3               |            |          |
| 15  | 16.83     | nHH       | Normal | Normal | Yes        | 4                  | No                  | 3                 |            |          |
| 16  | 1.58      | KS        | NA    | Abnormal | Yes        | 2                  | R                   | 1                 |            |          |
| 17  | 2.5       | KS        | Hyposmia | Abnormal | Yes        | 2.5                 | R                   | 1                 |            |          |
| 18  | 5.4       | KS        | Normal | Abnormal | Yes        | 2                  | L + R                | <1                |            |          |
| 19  | 6.33      | KS        | NA    | Abnormal | KS         | 2.3                 | L + R                | NA                |            |          |
| 20  | 7         | KS        | Normal | Abnormal | No         | 3.6                 | L + R                | 2                 |            |          |
| 21  | 9         | KS        | Hyposmia | Abnormal | Yes        | 2.6                 | No                  | 2                 | Yes        | Root of penis |
| 22  | 9.58      | KS        | Hyposmia | Abnormal | Yes        | 2.5                 | L + R                | 2                 |            |          |
| 23  | 10.17     | KS        | Hyposmia | NA    | Yes        | 1.5                 | L + R                | 1                 | Yes        | Root of penis |
| 24  | 11.08     | KS        | Hyposmia | NA    | Yes        | 3                  | No                  | 3                 |            |          |
| 25  | 11.33     | KS        | Hyposmia | Abnormal | Yes        | 3.1                 | No                  | 1                 |            |          |
| 26  | 11.67     | KS        | Hyposmia | Abnormal | Yes        | 3.5                 | No                  | 2                 |            |          |
| 27  | 11.83     | KS        | Normal | Abnormal | Yes        | 3                  | R                   | 1                 |            |          |
| 28  | 11.83     | KS        | Hyposmia | NA    | Yes        | 4                  | R                   | 3                 |            |          |
| 29  | 12        | KS        | Hyposmia | Abnormal | Yes        | 3.4                 | No                  | 1                 |            |          |
| 30  | 12.25     | KS        | Hyposmia | NA    | Yes        | 2.5                 | No                  | 1                 |            |          |
| 31  | 12.67     | KS        | Normal | Abnormal | No         | 4.9                 | R + L                | 3-4               |            |          |
| 32  | 13        | KS        | Hyposmia | NA    | Yes        | 3.8                 | L                   | 1                 |            |          |
| 33  | 13        | KS        | Hyposmia | Abnormal | No         | 7.5                 | No                  | 2                 |            |          |
| Associated phenotypes                                                                 | Family history | Basic LH (IU/L) | Basic FSH (IU/L) | T (nmol/L) | T after hCG standard test (nmol/L) | T after hCG prolonged test (nmol/L) |
|--------------------------------------------------------------------------------------|----------------|----------------|------------------|------------|-----------------------------------|-----------------------------------|
| Facial anomalies                                                                      | NA             | 0.23           | 1.15             | <0.69      | <0.69                             | 3.40                              |
| Facial freckles, childish face                                                        | NA             | <0.1           | 0.15             | NA         | <0.69                             | 0.29                              |
| Obesity, left little finger deformity                                                 | NA             | <0.1           | 0.17             | <0.69      | 0.82                              | <0.69                             |
| Rhinitis                                                                             | NA             | 1.08           | 23.7             | <0.69      | <0.69                             | <0.69                             |
| Congenital ptosis                                                                    | NA             | <0.1           | 0.11             | <0.69      | 0.71                              |                                   |
| Obesity, breast development                                                          | CDGP           | 0.25           | 0.64             | <0.69      | <0.69                             | 3.25                              |
| Congenital hearing impairment                                                        | No             | 0.1            | 2.5              | NA         | 4.93                              |                                   |
| Obesity, left little finger deformity and obesity, high palate, nostrils valgus, the 4th and 5th toes short | NA             | <0.1           | 0.46             | <0.69      | 5.03                              |                                   |
| Low hairline, xiphoid sunken, humpback                                               | No             | 0.32           | 3.16             | NA         | 4.34                              |                                   |
| Enuresis, spine bifida                                                               | CDGP           | 0.47           | 2.33             | NA         | 2.24                              | 10.36                             |
| Slender body, triangular face, outer corner slant, high palate, right michitsura palm, bimanual short index finger | NA             | 1.2            | 2.33             | NA         |                                   | 6.77                              |

(Continues)
| No. | Age (yrs) | Diagnosis | Smell  | MRI      | Micropenis | Length of penis (cm) | Cryptorchidism (L/R) | Testis volume (mL) | Hypospadias | Location |
|-----|-----------|-----------|--------|----------|------------|----------------------|----------------------|-------------------|-------------|----------|
| 34  | 13.25     | KS        | Hyposmia | Abnormal | Yes        | 3.5                  | No                   | 1                 |             |          |
| 35  | 13.42     | KS        | Hyposmia | Abnormal | No         | 8                    | No                   | <4                |             |          |
| 36  | 13.42     | KS        | Normal  | Abnormal | Yes        | 3                    | No                   | 3                 |             |          |
| 37  | 13.4      | KS        | NA      | NA       | Yes        | 3                    | R                    | <1                |             |          |
| 38  | 13.58     | KS        | Hyposmia | Abnormal | Yes        | 3                    | R + L                | 2                 |             |          |
| 39  | 13.75     | KS        | Normal  | Abnormal | Yes        | 3.1                  | R                    | 2                 |             |          |
| 40  | 13.75     | KS        | Hyposmia | Abnormal | Yes        | 2                    | No                   | 2                 |             |          |
| 41  | 14        | KS        | Hyposmia | Abnormal | Yes        | 4                    | No                   | 3                 |             |          |
| 42  | 14        | KS        | NA      | NA       | Yes        | 3                    | No                   | 1                 |             |          |
| 43  | 14.08     | KS        | Normal  | Abnormal | Yes        | 4                    | R + L                | 4                 |             |          |
| 44  | 14.33     | KS        | Anosmia | Abnormal | Yes        | 3.6                  | No                   | 3                 |             |          |
| 45  | 14.58     | KS        | Hyposmia | Abnormal | Yes        | 4.5                  | R + L                | NA                |             |          |
| 46  | 15.17     | KS        | Anosmia | Abnormal | Yes        | 5                    | R + L                | 2                 |             |          |
| 47  | 15.25     | KS        | Anosmia | Abnormal | Yes        | 3                    | No                   | 5                 |             |          |
| 48  | 15.3      | KS        | NA      | Abnormal | No         | 5                    | No                   | 2                 |             |          |
| 49  | 15.5      | KS        | Hyposmia | Abnormal | Yes        | 3                    | R + L                | 1.5               |             |          |
| 50  | 15.5      | KS        | Normal  | Abnormal | Yes        | 4.1                  | No                   | 1-2               |             |          |
| 51  | 15.67     | KS        | Normal  | Abnormal | Yes        | 4                    | No                   | 2                 |             |          |
| 52  | 15.67     | KS        | Anosmia | Abnormal | Yes        | 3.2                  | R                    | 1-2               |             |          |
| 53  | 16        | KS        | Hyposmia | Normal   | Yes        | 3                    | No                   | 2                 |             |          |
| 54  | 16.67     | KS        | Normal  | Abnormal | Yes        | 4.3                  | No                   | 1                 |             |          |
| 55  | 0.5       | nHH       | NA      | NA       | Yes        | 0.8                  | R                    | 1-2               |             |          |
| 56  | 0.92      | nHH       | NA      | NA       | Yes        | 1.2                  | R + L                | NA                |             |          |
| 57  | 1.33      | nHH       | NA      | NA       | Yes        | 2.1                  | L                    | 2                 | Yes         | Root of penis |
| 58  | 3.08      | nHH       | NA      | Normal   | Yes        | 2.3                  | No                   | 2                 |             |          |
| 59  | 5         | nHH       | Normal  | Normal   | Yes        | 2.2                  | No                   | 1                 |             |          |
| 60  | 5.25      | nHH       | Normal  | NA       | Yes        | 2                    | No                   | 1-2               |             |          |
| 61  | 11.25     | nHH       | Normal  | Normal   | Yes        | 4                    | L                    | 2                 |             |          |
| 62  | 13.83     | nHH       | Normal  | Normal   | Yes        | 5.3                  | No                   | 6                 |             |          |
| 63  | 15.92     | nHH       | Normal  | Normal   | Yes        | 5.5                  | R + L                | 3                 |             |          |
| 64  | 16.08     | nHH       | Normal  | Normal   | Yes        | 4                    | No                   | 1                 |             |          |

Levels after the hCG standard or prolonged stimulation tests among patients with or without cryptorchidism ($P > 0.05$); see Table S2.

Overall, 4 (6.3%) of 64 cases had hypospadias, including 2 KS (cases 21 and 23) and two with micropenis, cryptorchidism and hypospadias (cases 23 and 57); one case had micropenis combined with hypospadias (case 21); and another, cryptorchidism with hypospadias (case 11). No patient had hypospadias alone. There were 18 (28.1%) of 64 cases accompanied by another anomaly, such as hearing impairment, renal agenesis, synkinesis, pectus carinatum, spina bifida, dental dysplasia and mild facial anomalies. There were 11 self-reported KS patients with a normal sense of smell, although they were found to have olfactory bulb and/or olfactory tract dysplasia by MRI. In
contrast, another reported hyposmia but had normal olfactory bulb and/or olfactory tract by MRI. See Table 1.

There were 15 cases concordant with the dual defects, as assessed by the hCG standard test and confirmed by the prolonged test. The rate of dual form was 23.4% (15/64) in this paediatric group, of whom eight were over 14 years old and had normal bone age.

### 3.3 Genetic results

In the 51 patients who had genetic testing and Sanger sequencing verification, we found 10 classical CHH gene mutations listed in the CHH consensus of 2015. The positive detection rate of gene mutations was 26 (51.0%) of 51 patients; see Tables S1 and
S3. Twenty-one cases (41.1%) were single allele mutations and five were oligogenic (9.8%, PROKR2/PROK2, PROKR2/FGFR1, PROKR2/CHD7, FGFR1/SPRY4 and IL17RD/KAL1). The mutant genes of KS and nHH are shown in Figure 2. The most commonly mutated genes were PROKR2 (17.6%, 9 cases, KS/nHH = 6/3), FGFR1 (13.7%, 7 cases, KS/nHH = 5/2) and CHD7 (7.8%, 4 cases, KS/nHH = 2/2), see Table S3. Two cases (cases 57 and 59) were compound heterozygous mutations of the same gene (KISS1R, CHD7).

3.4 | Relationship between hypospadias and genotype

Case 7 had a FGF17 (p.Pro120Leu) missense mutation. He had hyposmia, and the MRI showed an abnormality. Two cases (23 and 57) carried CHD7 missense mutations: one was diagnosed as KS and the other had CHD7 compound heterozygous mutations (p.Ala2732Thr; p.Ala2866Val), and his father had delayed puberty. Case 11 had a negative genetic result, and he was 14 years old and presented with stalled puberty that began at the age of 12 with testes 4-5 mL, but showed no progression in the following 2 years. See Figure 3 and Table S4.

3.5 | Relationship between dual defects and genotype

Six of the 15 dual CHH patients carried the PROKR2 mutation. This was a higher frequency compared to the other 36 patients (6/15 vs 3/36, P = .021). Furthermore, 4 PROKR2 mutations (including 2 KS) were at the same allele, p.Trp178Ser. The other 2 dual patients (including 1 KS) carried the FGFR1 mutation; see Figure 3. However, 4 cases had negative results, and 3 patients with KS did not undergo genetic testing.

![Figure 2: Mutant genes distribution in 64 cases including 47 KS and 17 nHH](image)

Note: NA: Didn't do genetic test. No: No CHH gene mutation was found. [Colour figure can be viewed at wileyonlinelibrary.com]

![Figure 3: The correlation between various clinical phenotypes and genotype of 4 cases with hypospadias and 15 dual HH.](image)

Note: #: Means compound heterozygous mutation. *: p.Trp178Ser. There were 6 dual cases had same gene PROKR2 mutations, which was higher than that in pure HH patients (3/36, p = 0.021), and 4 of them at the same mutation site. NA: Didn’t do gene test. None: No CHH gene mutation was found. [Colour figure can be viewed at wileyonlinelibrary.com]
The 64 young male paediatric patients included in this study were diagnosed with HH, and their clinical and genetic data were studied over 8 years in our hospital. The proportion of patients with KS was 73.4%, which is similar to that previously reported by endocrinologists in teenage and adult healthcare institutions.\textsuperscript{21} In our study, we also found some patients in whom HH was combined with hearing impairment, renal agenesis and synkinesis. The sense of smell of 12 patients with KS was inconsistent with MRI analysis of the olfactory bulb, which has been reported previously.\textsuperscript{15,22,23} Mutations in the genes PROKR2 (17.6%), FGFR1 (13.7%), CHD7 (7.8%) and KAL1 (5.9%) were found to be common in the patients of this paediatric study, whereas FGFR1, CHD7, GNRHR and KAL1 were the most common genetic causes in a previous study of adult patients where the proportion each was no more than 10%.\textsuperscript{24} This might indicate that those diagnosed in a paediatric clinic have more severe phenotypes.

The process of embryonic development establishes the hypothalamo-pituitary-gonadal axis after the completion of sex differentiation, and therefore, central hypogonadism does not lead to ambiguous genitalia or hypospadias.\textsuperscript{2} However, after we analysed the molecular genetics of hypospadias, we found that genes such as FGF8, FGF10, FGFR2, CHD7 and SPRY4 could cause both hypospadias and CHH,\textsuperscript{10,11} as has also been reported by another study.\textsuperscript{16} KAL1 and HS6ST1, 2 other genes known to be mutated in CHH, also encode important components of the FGF8-FGFR1 signalling system. IL17RD is one of the FGF8 synexpression group members, and FGFR1 encodes a member of the so-called FGF8 subfamily and shares homology with FGF8.\textsuperscript{15} In our study, we found 4 patients presenting with hypospadias, of whom one had an FGF17 mutation and two had CHD7 mutations (one was p.Thr1083Ala and the other was p.Ala2732Thr and p.Ala2866Val). CHD7 was a candidate gene for causing hypospadias.\textsuperscript{11,25} Similar to previous reports,\textsuperscript{16,17} CHH genes could be found in patients with hypospadias, but the patients were so young that they could not be diagnosed with CHH. However, 2 patients (aged 10.5 and 12.1 years) in our study presented with hyposmia: one harboured an FGF17 mutation and the other a CHD7 mutation, which supported the diagnosis of KS; hence, we consider that hypospadias in children does not rule out the diagnosis of CHH, although it is rare.

In the first trimester of gestation, primordial germ cells develop gradually into Leydig cells under the regulation of genes and placental hCG, resulting in the secretion of testosterone and its deoxidation to DHT to promote the development of external genitalia. In the second trimester, LH acts on Leydig cells to produce testosterone/insulin-like factor 3 (INSL3), while FSH induces immature Sertoli cell proliferation and anti-Mullerian hormone (AMH)/inhibin B secretion.\textsuperscript{26} The maturity of the HPG axis determines the phenotype of patients with CHH at birth. Generally, patients with CHH diagnosed during adulthood have delayed puberty, with a large variation in severity, from temporary delay with re-entry into sexual development to infertility.\textsuperscript{3} However, most paediatric patients presented with micropenis and/or cryptorchidism at birth,\textsuperscript{7} which suggests that paediatric patients have more severe phenotypes. Dual HH patients may have impaired descent, development and maturation of the testes at birth or even earlier. In this study, 90% showed atypical genitalia, 61.9% had a poor response to the hCG standard test and 23.8% were diagnosed as dual defects. This indicated that testicular development had been affected more or less in early life. Thus, paediatric endocrinologists should evaluate and confirm the dual defects of CHH when they visit clinics as early as possible, as patients might benefit from early treatment.

In this study, we were lacking data on AMH and INH-B; only testosterone levels after hCG were evaluated. We presume that if we had also evaluated Sertoli cell function, the rate of dual defects would have been higher. Some patients with CHH could have testicular hypoplasia as they lack stimulation by LH/FSH; testosterone levels after the hCG test revealed the development of Leydig cells; therefore, the hCG test, especially the prolonged test, could help us understand the severity of HH. On the other hand, the hCG test has its limitations in evaluating testicular function as testosterone levels do not assess the function of Sertoli cells.

We found that six of 15 dual patients carried the same PROKR2 mutation, while several studies have reported that loss of Prokr2 compromises the integrity of the testicular vasculature, ultimately rendering boys more susceptible to reproductive anomalies postnatally\textsuperscript{27} and small testes compared to boys who did not carry mutations in PROKR2.\textsuperscript{28} In addition, the study showed no obvious differences in the testes of Prokr2\textsuperscript{17} heterozygous mice compared with wild-type mice.\textsuperscript{27} In our study, we found a high frequency of PROKR2 mutations, and some patients carried the same mutation, especially in dual patients. There were 2 patients harbouring only an FGFR1 mutation. So far, we have been unable to explain the pathogenicity of dual defects and believe that the mechanism should be studied further.

In conclusion, KS-related phenotypes are the main basis of CHH diagnosed during childhood. Micropenis alone or in combination with cryptorchidism provides an important clue for CHH. Paediatric patients may be found to have dual defects in CHH, and hypospadias cannot exclude a diagnosis of CHH. PROKR2 (17.6%), FGFR1 (13.7%), CHD7 (7.8%), KAL1 (5.9%) and IL17RD (5.9%) are common CHH genes. Testicular function may be impaired earlier than expected, so it is meaningful to evaluate dual defects in children and follow up their infertility into adulthood. For adolescents, a genetic test could confirm the diagnosis, while for young patients below 14 years of age, and especially for infants up to 6 months of age, genetic testing may be obligatory.

**ACKNOWLEDGEMENTS**

Chunxiu Gong and Yi Wang designed the research. Yi Wang, Miao Qin and Ying Liu collected data. Yi Wang wrote the report, and Chunxiu Gong revised it and provided many excellent ideas. All authors have read and modified the manuscript and approved the final submitted version. Chunxiu Gong is responsible for the accuracy of all data and viewpoints. The study was supported by the Public Health Project for Residents in Beijing (Z151100003915103) and the National Key Research and Development Programme of China (2016YFC0901505). We thank the patients and their parents or legal guardians. We thank
the doctors and nurses of the Department of Endocrinology. We also thank Professor Balasubramanian Ravikumar from Massachusetts General Hospital, Harvard Medical School, Harvard University, who gave us many good suggestions on the preparation of this manuscript.

CONFLICT OF INTEREST

Yi Wang and Chunxiu Gong equally contributed to this article. All authors declare that they have no conflict of interest.

ORCID

Chunxiu Gong http://orcid.org/0000-0002-1262-7383

REFERENCES

1. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. Nat Rev Endocrinol. 2009;5:569-576.
2. Quinton R, Duke VM, Robertson A, et al. Idiopathic gonadotrophin deficiency genetic questions addressed through phenotypic characterization. Clin Endocrinol (Oxf). 2001;55:163-174.
3. Boehm UBP, Dattani MT, et al. European consensus statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment. Nat Rev Endocrinol. 2015;11:547-564.
4. Quaynor SD, Bosley ME, Duckworth CG, et al. Targeted next generation sequencing approach identifies eighteen new candidate genes in normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Mol Cell Endocrinol. 2016;437:86-96.
5. Grinspon RP, Loreti N, Braslavsky D, et al. Spreading the clinical window for diagnosing fetal-onset hypogonadism in boys. Front Endocrinol (Lausanne). 2014;5:51.
6. Sykiotis GPHX, Avbelj M, et al. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. J Clin Endocrinol Metab. 2010;95:3019-3027.
7. Vizeneux A, Hilfliger A, Boullard J, et al. Congenital hypogonadotropic hypogonadism during childhood: presentation and genetic analyses in 46 boys. PLoS One. 2013;8:e77827.
8. Xu C, Lang-Muritano M, Phan-Hug F, et al. Genetic testing facilitates prepubertal diagnosis of congenital hypogonadotropic hypogonadism. Clin Genet. 2017;92:213-216.
9. Qin MGC, Qi Z, et al. Children with idiopathic hypogonadotropic hypogonadism clinical data analysis and mutations analysis of KAL1 and FGFR1 gene. Zhonghua Er Ke Za Zhi. 2014;52:942-947.
10. Beleza-Meireles A, Lundberg F, Lagerstedt K, et al. FGFR2, FGFR8, FGFI0 and BMP7 as candidate genes for hypospadias. Eur J Hum Genet. 2007;15:405-410.
11. Bouty A, Ayers KL, Pask A, Heloury Y, Sinclair AH. The genetic and environmental factors underlying hypospadias. Sex Dev. 2015;9:239-259.
12. Carmichael SL, Ma C, Choudhry S, Lamer EJ, Witte JS, Shaw GM. Hypospadias and genes related to genital tubercle and early urethral development. J Urol. 2013;190:1884-1892.
13. Dode C, Levilliers J, Dupont JM, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003;33:463-465.
14. Falardeau J, Chung WC, Beenken A, et al. Decreased FG8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest. 2008;118:2822-2831.
15. Miraoui H, Dwyer AA, Sykiotis GP, et al. Mutations in FG17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. Am J Hum Genet. 2013;92:725-743.
16. Ayers KL, Bouty A, Robevska G, et al. Variants in congenital hypogonadotropic hypogonadism genes identified in an Indonesian cohort of 46,XY under-virilised boys. Hum Genomics. 2017;11:11.
17. Eggers S, Sadedin S, van den Bergen JA, et al. Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. Genome Biol. 2016;17:243.
18. Bang AK, Nordkap L, Almstrup K, et al. Dynamic GnRH and hCG testing: establishment of new diagnostic reference levels. Eur J Endocrinol. 2017;176:379-391.
19. Dunkel LPJ, Sorva R. Single versus repeated dose human chorionic gonadotropin stimulation in the differential diagnosis of hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 1985;60:333-337.
20. Santoro NFM, Crowley WF Jr. Hypogonadotropic disorders in men and women: diagnosis and therapy with pulsatile gonadotropin-releasing hormone. Endocr Rev. 1986;7:11-23.
21. Young J. Approach to the male patient with congenital hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2012;97:707-718.
22. Goncalves CI, Fonseca F, Borges T, Cunha F, Lemos MC. Expanding the genetic spectrum of ANOS1 mutations in patients with congenital hypogonadotropic hypogonadism. Hum Reprod. 2017;32:704-711.
23. Valdes-Socin H, Rubio Almanza M, Tome Fernandez-Ladreda M, Debray FG, Bours V, Beckers A. Reproduction, smell, and neurodevelopmental disorders: genetic defects in different hypogonadotropic hypogonadal syndromes. Front Endocrinol (Lausanne). 2014;5:109.
24. Sykiotis GPPL, Hughes VA, Au M, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. Proc Natl Acad Sci USA. 2010;107:15140-15144.
25. Wang Y, Li Q, Xu J, et al. Mutation analysis of five candidate genes in Chinese patients with hypospadias. Eur J Hum Genet. 2004;12:706-712.
26. Rey RA, Grinspon RP, Gottlieb S, et al. Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. Andrology. 2013;1:3-16.
27. Svingen T, McClelland KS, Masumoto K, et al. Prokr2-deficient mice display vascular dysmorphology of the fetal testes; potential implications for Kallmann syndrome aetiology. Sex Dev. 2011;5:294-303.
28. Matsumoto S, Yamazaki C, Masumoto KH, et al. Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. Proc Natl Acad Sci USA. 2006;103:4140-4145.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Wang Y, Gong C, Qin M, Liu Y, Tian Y. Clinical and genetic features of 64 young male paediatric patients with congenital hypogonadotropic hypogonadism. Clin Endocrinol (Oxf). 2017;87:757–766. https://doi.org/10.1111/cen.13451