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ORIGINAL ARTICLE

Male Endocrinology

Gonadotropin treatment for male partial congenital hypogonadotropic hypogonadism in Chinese patients

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Partial congenital hypogonadotropic hypogonadism (PCHH) is caused by an insufficiency in, but not a complete lack of, gonadotropin secretion. This leads to reduced testosterone production, mild testicular enlargement, and partial pubertal development. No studies have shown the productivity of spermatogenesis in patients with PCHH. We compared the outcomes of gonadotropin-induced spermatogenesis between patients with PCHH and those with complete congenital hypogonadotropic hypogonadism (CCHH). This retrospective study included 587 patients with CHH who were treated in Peking Union Medical College Hospital (Beijing, China) from January 2008 to September 2016. A total of 465 cases were excluded from data analysis for testosterone or gonadotropin-releasing hormone treatment, cryptorchidism, poor compliance, or incomplete medical data. We defined male patients with PCHH as those with a testicular volume of ≥ 4 ml and patients with a testicular volume of < 4 ml as CCHH. A total of 122 compliant, noncryptorchid patients with PCHH or CCHH received combined human chorionic gonadotropin and human menopausal gonadotropin and were monitored for 24 months. Testicular size, serum luteinizing hormone levels, follicle-stimulating hormone levels, serum total testosterone levels, and sperm count were recorded at each visit. After gonadotropin therapy, patients with PCHH had a higher spermatogenesis rate (92.3%) than did patients with CCHH (74.7%). During 24-month combined gonadotropin treatment, the PCHH group took significantly less time to begin producing sperm compared with the CCHH group (median time: 11.7 vs 17.8 months, $P < 0.05$). In conclusion, after combined gonadotropin treatment, patients with PCHH have a higher spermatogenesis success rate and sperm concentrations and require shorter treatment periods for sperm production.

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INTRODUCTION

Congenital hypogonadotropic hypogonadism (CHH) is defined by isolated deficiency or dysfunction of gonadotropin-releasing hormone (GnRH).¹ CHH is clinically characterized by absent or incomplete development during puberty, resulting in small testes by the age of 18 years and infertility in adults. These patients have low circulating testosterone levels with low gonadotropin levels, whereas other pituitary hormones are normal.² Pulsatile GnRH or combined gonadotropin therapy is administered to treat infertility in patients with CHH. Because CHH presents with a wide range of gonadotropin deficiency, the clinical spectrum of associated reproductive phenotypes is wide. For most patients with CHH, puberty never occurs (absent puberty). In a less common situation, puberty is initiated and then arrested (partial puberty) in CHH.^{1–6}

According to the degree of pubertal development, patients with CHH can be divided into partial CHH (PCHH) and complete CHH (CCHH).⁷ Patients who present with partial pubertal development can be diagnosed with PCHH. There is currently no definite diagnostic standard for PCHH. In this study, we defined male PCHH patients as those with a testicular volume of ≥ 4 ml and patients with a testicular volume of < 4 ml as CCHH. PCHH typically presents with mild gonadotropin deficiency

and partial pubertal development.^{8,9} Mutations in gonadotropin-releasing hormone receptor gene (*GNRHR*), fibroblast growth factor receptor 1 gene (*FGFR1*), tachykinin 3 (*TAC3*), and tachykinin receptor 3 gene (*TACR3*) cause PCHH.¹⁰ Because of lack of clear diagnostic criteria, previous studies of PCHH were mainly limited to description of symptoms and characterization of mutant genes. There have been no reports on gonadotropin treatment for a Chinese population with PCHH.

In the present study, we retrospectively analyzed 587 patients with CHH who were treated in Peking Union Medical College Hospital, Beijing, China, from January 2008 to September 2016. Patients with poor compliance and cryptorchidism were excluded. We then compared the clinical features and treatment outcomes between patients with CCHH and those with PCHH who received combined human chorionic gonadotropin (HCG) and human menopausal gonadotropin (HMG) and were monitored for 24 months.

PATIENTS AND METHODS

Patients

Patients with all of the following criteria were diagnosed with CHH: a male patient without puberty development before 18 years, a serum

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testosterone level $<100 \text{ ng dl}^{-1}$ (3.5 nmol l^{-1}) with low or inappropriately normal gonadotropin levels, normal levels of other pituitary hormones, and negative findings by sellar magnetic resonance imaging (MRI).

This study includes CHH patients who met the following conditions: (1) patients did not receive any therapy before the earliest noted date in this study, including pulsatile GnRH, combined gonadotropins, and testosterone; (2) patients were azoospermic before treatment; (3) patients had no history of cryptorchidism; and (4) patients received HCG/HMG treatment for at least 24 months. The study was approved by the ethics committee of the Peking Union Medical College Hospital, Beijing, China. Informed consent has been obtained from each patient after full explanation of the purpose of this study.

Treatment and follow-up

HCG (2000–5000 U, Livzon Pharmaceutical Co., Zhuhai, China) was injected into the muscle twice weekly for 3 months followed by intramuscular HMG (75–150 U, Livzon Pharmaceutical Co.) injection twice weekly, combined to HCG. Regular follow-ups were conducted at 3-month intervals during the therapy. Gonadotropin dosages were adjusted to maintain plasma testosterone at about 300 ng dl^{-1} .

Luteinizing hormone (LH), follicle-stimulating hormone (FSH), testicular size (measured by Prader orchidometer), testosterone, and sperm count were measured at each visit. Plasma gonadotropins (FSH and LH) and testosterone were measured 48 h after HCG injection by chemiluminescence using a commercial kit (ACS 180 Automatic Chemiluminescence System, Bayer, Germany).

Semen samples were collected by masturbation and analyzed according to the standard World Health Organization method (before 2010, according to 1999 WHO method;¹¹ after 2010, according to 2010 WHO method¹²). Successful spermatogenesis was defined as the observation of one sperm by microscope in centrifugated seminal fluid.

Targeted next-generation sequencing

Blood samples were collected from 87 of 122 patients. Genomic DNA was extracted from the peripheral blood leukocytes using Qiagen DNA Blood kit (Qiagen, Hilden, Germany). A gene panel (NimblegenSeqCap EZ system, Roche, Basel, Switzerland) was designed to capture all exons and 10 bp flanking intron sequences of the 31 CHH-related genes (Supplementary Table 1). The DNA samples were subjected to massive parallel sequencing (100 bp paired-end reads) on an Illumina HiSeq2500 sequencing system (Illumina, Inc., San Diego, CA, USA) after hybridization to the capture array. Bioinformatic analysis including quality control, reads alignment, and variants calling (including single-nucleotide variations [SNVs] and small indels) was performed following the pipelines previously described.¹³

Statistical analyses

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Normal distributive data are expressed as the mean \pm standard deviation (s.d.), and nonnormal distribution data are listed as median (quartiles). Gonadotropins, testosterone, testicular size, sperm count, and sperm production periods were compared between groups with partial and complete CHH by independent sample's *t*-test. Cox regression models were built to analyze the predictors for successful spermatogenesis. The age at the start of HCG/HMG treatment, body mass index (BMI), height, peak LH and FSH after GnRH (triptorelin, 100 mg) stimulation, family histories of delayed puberty (0 = no, 1 = yes), and basal testicular volume were considered as variables in the Cox regression model and multivariate linear regression model.

Sperm concentrations were compared by Mann–Whitney U test. Kaplan–Meier analyses were used to estimate the median time to achieve a threshold sperm level. Statistical significance was set at $P < 0.05$.

RESULTS

Clinical characteristics of patients with CHH

From January 2008 to September 2016, a total of 587 patients with CHH were retrospectively evaluated. A total of 181 cases were excluded because of accepting testosterone replacement therapy or pulsatile GnRH therapy, 56 were excluded for cryptorchidism, and 228 were excluded for poor compliance or incomplete medical data. Therefore, this study included a total of 122 male patients with CHH who received combined gonadotropin treatment for at least 24 months (Figure 1). These patients were in overall good health with normal blood and urine routine test results and normal liver and renal function. Thyroid hormone, adrenal glucocorticoid, and growth hormone levels were all within normal range. Baseline median (quartiles) serum levels of LH were 0.24 ($0, 0.50$) IU l^{-1} and GnRH agonist-stimulated peak LH levels were 3.80 ($1.10, 6.60$) IU l^{-1} . Baseline median (quartiles) serum levels of FSH were 1.10 ($0.40, 1.30$) IU l^{-1} and GnRH agonist-stimulated peak FSH levels were 3.00 ($1.50, 4.90$) IU l^{-1} . The patients' mean testicular volume was 3.5 (s.d. = 3.3) ml (Table 1).

Spermatogenesis in patients with PCHH and those with CCHH

Patients were divided into PCHH ($n = 39$) and CCHH ($n = 83$) groups, according to their testicular volume. The PCHH group had significantly larger basal testicular volume ($P < 0.001$), higher basal LH levels ($P < 0.001$), and peak LH levels after triptorelin stimulation ($P = 0.001$) compared with the CCHH group. Patients in the PCHH group had a higher height ($P = 0.001$) and height after treatment ($P = 0.004$) compared with those in the CCHH group. However, the two groups had a similar age of initiating treatment, BMI, rate of a family history, rate of dysosmia, and basal FSH and peak FSH levels after triptorelin stimulation (Table 1).

Patients in the PCHH group had a higher spermatogenesis rate ($P = 0.023$) and a shorter time of first sperm appearance compared with those in the CCHH group ($P < 0.001$). The median (quartiles) sperm concentrations at 6, 12, 18, and

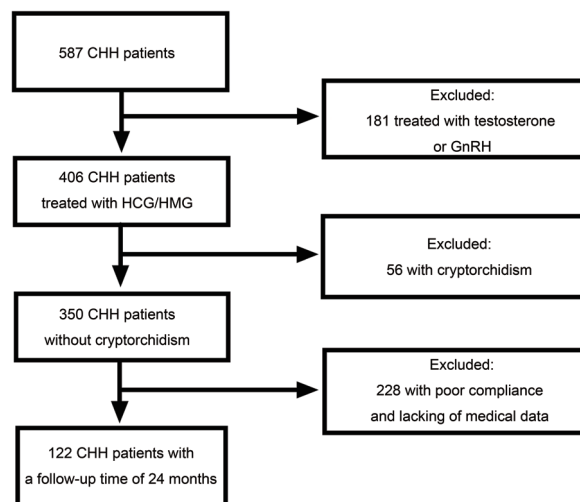


Figure 1: Flowchart of screening patients. CHH: congenital hypogonadotropic hypogonadism; GnRH: gonadotropin-releasing hormone; HCG: combined human chorionic gonadotropin; HMG: human menopausal gonadotropin.

Table 1: Baseline features of congenital hypogonadotropic hypogonadism patients

Baseline features	All patients (n=122)	CCHH (n=83)	PCHH (n=39)	P ^a	S subgroup (n=98)	N subgroup (n=24)	P ^b
Age of initiating treatment (year), mean±s.d.	20.6±4.3	20.3±3.9	21.3±4.9	0.266	20.8±4.3	19.8±4.0	0.281
BMI (kg m ⁻²), mean±s.d.	22.3±3.9	22.2±4.1	22.7±3.5	0.483	22.3±3.9	22.5±3.9	0.84
Basal LH (IU l ⁻¹), median (quartiles)	0.24 (0, 0.50)	0.10 (0, 0.20)	0.50 (0.20, 1.30)	<0.001	0.24 (0, 0.60)	0.10 (0, 0.25)	0.272
Peak LH (IU l ⁻¹), median (quartiles)	3.80 (1.10, 6.60)	2.40 (0.60, 3.80)	6.70 (2.80, 12.30)	0.001	3.81 (1.05, 6.80)	3.81 (1.26, 4.20)	0.875
Basal FSH (IU l ⁻¹), median (quartiles)	1.10 (0.40, 1.30)	1.10 (0.40, 1.25)	1.20 (0.62, 2.01)	0.062	1.07 (0.40, 1.30)	1.25 (0.40, 1.31)	0.894
Peak FSH (IU l ⁻¹), median (quartiles)	3.00 (1.50, 4.90)	2.60 (1.34, 4.00)	4.10 (1.99, 7.15)	0.06	3.80 (1.72, 4.00)	3.96 (2.38, 4.51)	0.634
Rate of family history (0, 1) (%)	4.9	3.6	7.7	0.334	4.1	8.3	0.334
Basal testicular volume (ml), mean±s.d.	3.5±3.3	1.8±0.7	7.0±3.9	<0.001	3.8±3.6	2.3±1.2	0.042
Dysosmia (0, 1) (%)	38.52	42.17	31.77	0.486	36.73	46.83	0.416
Height (cm), mean±s.d.	173.4±6.7	172.1±6.6	176.3±6.1	0.001	174.0±6.8	171.2±5.7	0.064
Height after treatment (cm), mean±s.d.	176.3±5.8	175.3±5.7	178.6±5.5	0.004	176.7±5.9	175.0±5.2	0.203
Spermatogenesis (0, 1) (%)	80.3	74.7	92.3	0.023	–	–	–
Time of first sperm appearance (month), mean±s.d.	15.9±7.0	17.8±6.2	11.7±6.8	<0.001	–	–	–
Prior androgen treatment (0, 1) (%)	52.5	65.1	25.6	<0.001	52.0	54.1	0.853

^aCompared between CCHH and PCHH groups, $P < 0.05$ is defined as significant difference. ^bCompared between S subgroup and N subgroup, $P < 0.05$ is defined as significant difference. Peak LH means highest LH level after intramuscular injection of triptorelin 100 µg. Peak FSH means highest FSH level after intramuscular injection of triptorelin 100 µg. BMI: body mass index; LH: luteinizing hormone; FSH: follicle-stimulating hormone; s.d.: standard deviation; CCHH: complete congenital hypogonadotropic hypogonadism; PCHH: partial congenital hypogonadotropic hypogonadism; N subgroup: nonspermatogenesis subgroup; S subgroup: spermatogenesis subgroup; –: data not applicable

24 months were $0 (0, 3.50) \times 10^6 \text{ ml}^{-1}$, $0 (0, 6.67) \times 10^6 \text{ ml}^{-1}$, $2.25 (0, 16.35) \times 10^6 \text{ ml}^{-1}$, and $9.99 (0, 22.01) \times 10^6 \text{ ml}^{-1}$ in the PCHH group and $0 (0, 0) \times 10^6 \text{ ml}^{-1}$, $0 (0, 0) \times 10^6 \text{ ml}^{-1}$, $0 (0, 3.43) \times 10^6 \text{ ml}^{-1}$, and $0.49 (0, 16.45) \times 10^6 \text{ ml}^{-1}$ in the CCHH group. Sperm concentrations in the PCHH group were significantly higher than those in the CCHH group at 6, 12, and 14 months ($P < 0.05$). The PCHH group also tended to produce higher sperm concentrations than did the CCHH group. During the follow-up, 50% of the patients with PCHH produced their first sperm within 12 months, whereas the CCHH group took until the 18-month follow-up to produce their first sperm (Figure 2).

Kaplan–Meier analysis showed that the median time to begin sperm production in the PCHH and CCHH groups was 12.9 (95% CI: 10.7–15.0) months and 17.7 (95% CI: 16.4–19.0) months, respectively ($P = 0.01$, Figure 3a). Patients with PCHH showed a tendency for a shorter time of beginning sperm production than did those in the CCHH group. The median time to reach a sperm threshold $\geq 5 \times 10^6 \text{ ml}^{-1}$ was 16.2 (95% CI: 14.0–18.5) months in the PCHH group and 21.4 (95% CI: 20.3–22.5) months in the CCHH group ($P = 0.004$, Figure 3b). Reaching a sperm threshold $\geq 10 \times 10^6 \text{ ml}^{-1}$ took a median time of 24 (95% CI: 15.8–32.2) months in the PCHH group (Figure 3c). These data were not available for the CCHH group ($P = 0.01$). The estimated median time to reach a sperm concentration $\geq 15 \times 10^6 \text{ ml}^{-1}$ could not be obtained for either group because of the limited number of patients who produced sperm above this concentration ($P = 0.046$, Figure 3d).

Subgroup analysis

A total of 80.3% (98/122) of patients with CHH achieved successful spermatogenesis after gonadotropin therapy. According to the patients' testicular volume, they were divided into the nonspermatogenesis subgroup ($n = 24$) and the spermatogenesis subgroup ($n = 98$). The two subgroups of patients generally had similar clinical characteristics, except for basal testicular volume. Patients in the spermatogenesis subgroup had significantly larger basal testicular volume than did those in the nonspermatogenesis subgroup (mean ± s.d.: $3.8 \pm 3.6 \text{ ml}$ vs $2.3 \pm 1.2 \text{ ml}$, $P = 0.042$) (Table 1).

Cox-related regression analysis (including the variables of age, BMI, basal LH, peak LH, family history, and basal testicular volume)

showed that larger basal testicular volume ($\beta = 0.082$, $P = 0.041$) was the only favorable predictor for a shorter time to produce sperm (Table 2).

Mutational analysis

A total of 87 patients underwent targeted next-generation sequencing. Thirty-three patients with CHH had CHH-related variants by targeted next-generation sequencing with a detection rate of approximately 37.9% (33/87). *FGFR1*, Kallmann syndrome 1 gene (*KALI*), prokineticin receptor 2 gene (*PROKR2*), and chromodomain helicase DNA binding protein 7 (*CHD7*) mutations were found in the PCHH and CCHH groups. Luteinizing hormone beta polypeptide gene (*LHB*) and NMDA receptor synaptonuclear signaling and neuronal migration factor gene (*NELF*) mutations were only found in the PCHH group. *GNRHR*, kisspeptin receptor gene (*KISS1R*), fibroblast growth factor 8 gene (*FGF8*), and prokineticin 2 gene (*PROK2*) mutations were found in the CCHH group. However, these mutations were not detected in the PCHH group (Table 3).

DISCUSSION

We found that compliant, noncryptorchid patients with PCHH responded better to gonadotropin therapy for spermatogenesis than did patients with CCHH. During 24 months of combined gonadotropin treatment, patients with PCHH required less time to achieve sperm and produced higher sperm counts than did those with CCHH.

We defined PCHH as a testicular volume of $\geq 4 \text{ ml}$. This definition was based on the following considerations. First, the marker of onset of puberty is the size of testicular volume. Most previous studies used testicular volume ($\geq 4 \text{ ml}$) as the standard,^{1–3,5–7} while a few studies used testicular volume ($>3 \text{ ml}$) as the standard.^{14,15} Testicular volume is assessed by the Prader orchidometer. Therefore, a testicular volume of $\geq 4 \text{ ml}$ or $>3 \text{ ml}$ represents the same group of patients. In this study, we chose the criterion for testicular volume that was used by most recent studies ($\geq 4 \text{ ml}$).^{1–3,5–7} Second, patients with CHH with a large testicular size have a higher chance of reversal of function of the hypothalamus–pituitary–testis axis.^{2,16} Raivio *et al.*¹⁶ described 15 patients with reversal of CHH, 11 of whom had testicular volumes $\geq 4 \text{ ml}$. Another study showed that 12 of 16 patients with CHH who experienced reversal had testicular volumes $\geq 4 \text{ ml}$.¹⁷

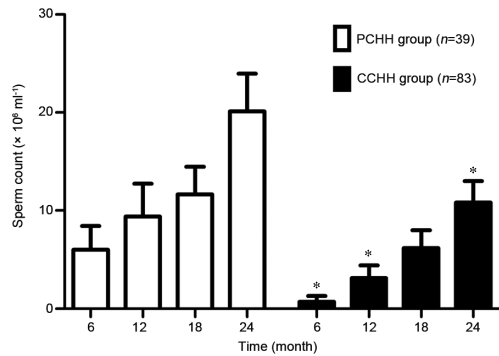


Figure 2: Mean sperm count increased during gonadotropin therapy in both groups. During 24-month combined gonadotropin therapy, PCHH group had a higher sperm concentration than CCHH group. The differences between two groups were statistically significant ($P < 0.05$) at 6, 12, and 24 months. CCHH: complete congenital hypogonadotropic hypogonadism; PCHH: partial congenital hypogonadotropic hypogonadism.

Table 2: Predictors for spermatogenesis (correlated Cox analysis)

Predictors	β	HR	P	95% CI	
				Lower bound	Upper bound
Age of initiating treatment	-0.019	0.981	0.474	0.932	1.034
BMI	-0.024	0.976	0.373	0.926	1.029
Basal LH	-0.049	0.952	0.682	0.751	1.206
Peak LH	-0.002	0.998	0.902	0.969	1.028
Family history (0, 1)	-0.065	0.937	0.901	0.335	2.617
Basal testicular volume	0.082	1.086	0.041*	1.003	1.175

* $P < 0.05$ is defined as statistical significance. BMI: body mass index; LH: luteinizing hormone; FSH: follicle-stimulating hormone; HR: hazard ratio; CI: confidence interval

Table 3: The frequency of known causal genes in the 87 probands

Gene	OMIM	Inheritance	Mutated rate, n (%)	
			PCHH (n=23)	CCHH (n=64)
<i>KAL1</i>	300836	XLR	1 (4.3)	1 (1.6)
<i>FGFR1</i>	136350	AD	2 (8.7)	5 (7.8)
<i>PROKR2</i>	607123	AD	1 (4.3)	5 (7.8)
<i>CHD7</i>	608892	AD	2 (8.7)	5 (7.8)
<i>LHB</i>	152780	AR	1 (4.3)	0 (0)
<i>NELF</i>	608137	AD	1 (4.3)	0 (0)
<i>KISS1R</i>	604161	AR	0 (0)	3 (4.7)
<i>GNRHR</i>	138850	AR	0 (0)	3 (4.7)
<i>FGF8</i>	600483	AD	0 (0)	1 (1.6)
<i>PROK2</i>	607002	AD	0 (0)	1 (1.6)

KAL1: Kallmann syndrome 1 gene; *FGFR1*: fibroblast growth factor receptor 1 gene; *PROKR2*: prokineticin receptor 2 gene; *CHD7*: chromodomain helicase DNA binding protein 7; *LHB*: luteinizing hormone beta polypeptide gene; *NELF*: NMDA receptor synaptonuclear signaling and neuronal migration factor gene; *KISS1R*: kisspeptin receptor gene; *GNRHR*: gonadotropin releasing hormone receptor gene; *FGF8*: fibroblast growth factor 8 gene; *PROK2*: prokineticin 2 gene; XLR: X-linked recessive; AD: autosomal dominant; AR: autosomal recessive; PCHH: partial congenital hypogonadotropic hypogonadism; CCHH: complete congenital hypogonadotropic hypogonadism

Third, baseline testicular volume is the most important predictor of gonadotropin-induced spermatogenesis. Larger testicular size correlates with a higher chance of spermatogenesis.^{18–20} Accordingly, we defined patients with a testicular volume of ≥ 4 ml as having PCHH and those with < 4 ml as having CCHH.

CHH is caused by defects in GnRH release, activity, or both.²¹ Partial activation of hypothalamus–pituitary–testis axis function could result from multiple defects. Fewer GnRH neurons in the hypothalamus

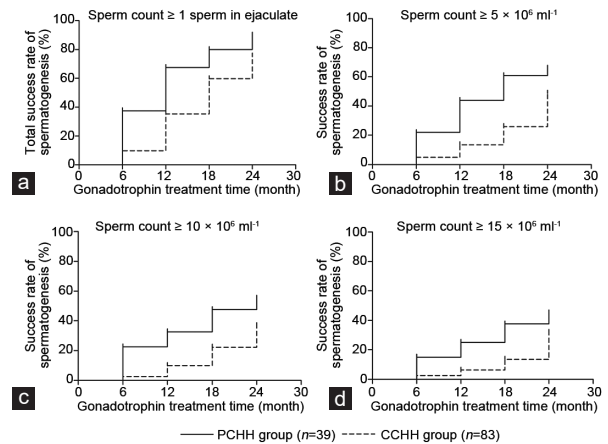


Figure 3: A time-to-event analysis showed that the PCHH group had a higher success rate of spermatogenesis than CCHH group. (a) The median time of begin sperm production (≥ 1 sperm) in PCHH group is shorter than that in CCHH group. (b) The median time of reaching a sperm threshold $\geq 5 \times 10^6 \text{ ml}^{-1}$ in PCHH group is shorter than that in the CCHH group. (c) The median time of reaching sperm threshold $\geq 10 \times 10^6 \text{ ml}^{-1}$ in PCHH group is shorter than that in the CCHH group. (d) The median time of reaching sperm threshold $\geq 15 \times 10^6 \text{ ml}^{-1}$ in PCHH group is shorter than that in the CCHH group. The differences between two groups were statistically significant ($P < 0.05$) at a–d. CCHH: complete congenital hypogonadotropic hypogonadism; PCHH: partial congenital hypogonadotropic hypogonadism.

may produce less GnRH and gonadotropins. Interestingly, mutation of *FGF8* can cause decreased GnRH neurons and peptide concentrations in the hypothalamus.²² In addition, if GnRH is not secreted at a sufficient level, it cannot fully activate function of the gonadal axis. The human *GNRH1* gene encodes a preprohormone, which is ultimately processed to the GnRH decapeptide. *In vitro* studies have shown that mutations of the GnRH decapeptide reduce its binding capacity to the GnRH receptor.^{23,24} Furthermore, decreased expression of the GnRH receptor at the cell surface leads to reduced GnRH signaling. This is supported by the finding that *GNRHR* mutations can cause partial GnRH resistance.⁷ In our study, we found that patients with PCHH had *KAL1*, *FGFR1*, *PROKR2*, *CHD7*, *LHB*, and *NELF* mutations.

We found that a favorable response to gonadotropin therapy for patients with PCHH could be attributable to a larger testicular size. Testicular mass is mainly determined by the seminiferous tubules,^{25,26} and a larger testicular volume is indicative of greater spermatogenesis capacity in normal adult males.²⁶ Testicular mass is also an important predictive factor in estimating spermatogenesis in infertile men,^{27–34} including patients with CHH.^{2,33}

A better response to gonadotropin therapy in patients with PCHH may have other causes. First, *KAL1* and *FGFR1* can directly damage testicular development and spermatogenesis.^{35–37} Some patients with CHH have testicular impairment,³⁷ which cannot be diagnosed unless gonadotropin therapy is initiated. Patients with PCHH have a higher probability of normal testicular function than do patients with CCHH because they have larger testicles after low levels of endogenous gonadotropin stimulation. However, patients with CCHH do not undergo endogenous gonadotropin screening. Second, basal LH and peak LH levels after triptorelin stimulation in the PCHH group were higher than those in the CCHH group. This finding suggested that the hypothalamic–pituitary–gonadal axis of the PCHH group had better reserve function than that in the CCHH group.

In this study, the rate of spermatogenesis in all patients with CHH was 80.3%, which is higher than that (64%–75%) in other studies.^{18,38}

This discrepancy between studies may be attributable to the following factors. First, our participants had a larger basal testicular size than other studies (3.5 ml vs 2.1 ml).¹⁸ Second, we excluded patients with cryptorchidism (56 cases). Cryptorchidism is a poor predictor for spermatogenesis, and patients with cryptorchidism require a longer time to obtain sperm.^{38,39} Third, patients who were included in our study had a longer treatment time than did those in other studies. In our study, patients with poor compliance or incomplete medical data were excluded (228 cases). Our participants underwent gonadotropin therapy for 2 years, whereas patients from other studies underwent treatment for 6–40 months.^{18,38}

There are some limitations to our study. First, although the follow-up time in this study was as long as 24 months, more patients could have produced sperm with a longer follow-up time. Second, HMG mainly acts as FSH and has little effect on LH. Treatment with pure FSH, such as urofollitropin, would have been preferable. Third, inhibin B is closely related to reproductive capacity, but it was not tested in this study because of limitation of research conditions.

In conclusion, diagnosis of PCHH is associated with a higher spermatogenesis success rate, higher sperm concentrations, and shorter therapeutic period for sperm production compared with diagnosis of CCHH.

AUTHOR CONTRIBUTIONS

JFM and XYW designed experiment and revised the article. MH and MN carried out the follow-up reviews and participated in clinical data collection. XW, WLM, QBH, and RZ analyzed data. BQY and YJG wrote the article. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: The genes panel contains 31 known congenital hypogonadotropic hypogonadism pathogenic genes

<i>Genes</i>	<i>Inheritance</i>	<i>Genes</i>	<i>Inheritance</i>	<i>Genes</i>	<i>Inheritance</i>	<i>Genes</i>	<i>Inheritance</i>
<i>ANOS1 (KAL1)</i>	XLR	<i>FGFR1</i>	AD	<i>FGF8</i>	AD	<i>PROKR2</i>	AD
<i>PROK2</i>	AD	<i>NELF</i>	AD	<i>CHD7</i>	AD	<i>KISS1R</i>	AR
<i>KISS1</i>	AR	<i>TAC3</i>	AR	<i>GNRHR</i>	AR	<i>GNRH1</i>	AR
<i>SEMA3A</i>	AD	<i>WDR11</i>	AD	<i>FSHB</i>	AR	<i>LHB</i>	AR
<i>NDN</i>	AD	<i>SOX2</i>	AD	<i>HS6ST1</i>	AD	<i>NROB1 (DAX1)</i>	XLR
<i>HESX1</i>	AD or AR	<i>SOX10</i>	AD	<i>TACR3</i>	AR	<i>FGF17</i>	AD
<i>AXL</i>	AD	<i>IL17RD (SEF)</i>	AD or AR	<i>DUSP6</i>	AD	<i>SPRY4</i>	AD
<i>FLRT3</i>	AD	<i>LEPR</i>	AR	<i>LEP</i>	AR		

AD: autosomal dominant; AR: autosomal recessive; XLR: X-linked recessive; N.K. *KAL1*: Kallmann syndrome 1 gene; *FGFR1*: fibroblast growth factor receptor 1 gene; *PROKR2*: prokineticin receptor 2 gene; *CHD7*: chromodomain helicase DNA binding protein 7; *LHB*: luteinizing hormone beta polypeptide gene; *NELF*: NMDA receptor synaptonuclear signaling and neuronal migration factor gene; *KISS1R*: kisspeptin receptor gene; *GNRHR*: gonadotropin releasing hormone receptor gene; *FGF8*: fibroblast growth factor 8 gene; *PROK2*: prokineticin 2 gene; *TAC3*: tachykinin 3; *WDR11*: WD repeat domain 11 gene; *ANOS1*: anosmin-1 gene; *SEMA3A*: Semaphorin-3A gene; *NDN*:Necdin gene; *HESX1*: Homeobox expressed in ES cells 1 gene; *AXL*: Tyrosine-protein kinase receptor UFO gene; *FLRT3*: Leucine-rich repeat transmembrane protein FLRT3 gene; *SOX2*: Transcription factor SOX-2 gene; *SOX10*: Transcription factor SOX-10 gene; *IL17RD*:Interleukin-17 receptor D gene; *SEF*: Interleukin-17 receptor D; *LEPR*: Leptin receptor gene; *GNRH1*: GnRH-associated peptide 1 gene ; *NROB1*: Nuclear receptor subfamily O group B member 1 gene; *DAX-1*: DSS-AHC critical region on the X chromosome protein 1 gene; *FGF17*:fibroblast growth factor 17 gene; *SPRY4*: Protein sprouty homolog 4 gene; *KISS1R*: KISS1 receptor; *TACR3*