rFSH Pretreatment Followed by rFSH-hCG in Three Males with Congenital Hypogonadotropic Hypogonadism Diagnosed during Childhood due to Extremely Small Testes, Micropenis and/or Bilateral Cryptorchidism

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Abstract

Backgrounds: Congenital male hypogonadotropic hypogonadism (CMHH) is usually treated with hCG-recombinant human FSH combination therapy (hCG-rFSH) to attain fertility. In 25% cases, sufficient spermatogenesis is not observed under the current gonadotropin replacement therapy (GRT) in CMHH like Kallmann syndrome and isolated gonadotropin deficiency. The effectiveness of pretreatment with rFSH monotherapy followed by GRT to improve testicular development and spermatogenesis in adult CMHH and gonadotropin-deficient boys with small testes has been reported by some studies as the physiological treatment for gonadotropin secretion. However, pubertal growth and future spermatogenesis during GRT in CMHH patients diagnosed during childhood because of severe phenotypes such as small testes, micropenis, and cryptorchidism have not been sufficiently evaluated.

Objectives: To mimic the physiological gonadotropin secretion, we introduced a fixed period pretreatment with rFSH monotherapy followed by a low-dose escalation of hCG-rFSH to prevent rapid pubertal progression and observed the patient’s pubertal growth, sexual maturity, and spermatogenesis.

Design: A retrospective clinical study conducted at a university hospital and two pediatric endocrinology outpatient clinics.

Patients: Three Kallmann syndrome patients aged 14.6 - 18 years with CMHH diagnosed during childhood because of their extremely small testes (testicular volume: TV < 1 mL), micropenis and/or bilateral cryptorchidism were included.

Methods and Treatment Protocol: After pretreatment with rFSH (75 IU) daily for 2/4 months, hCG-rFSH was administered based on age-appropriate therapeutic doses.

Results: TV increased two-fold after pretreatment with rFSH monotherapy. TV significantly increased from 0.7 ± 0.3 to 8.3 ± 3.5 mL and serum testosterone elevated to adult levels during hCG-rFSH. Total sperm number per ejaculate in Case 3 was only approximately five sperms by first semen analysis: Cases 1 and 3 experienced emissions and ejaculation. No patient showed rapid bone age progression (ΔCA/BA ratio annually as < 1 in Cases 1 and 2) and pubertal growth was 16.6 ± 4.1 cm and reached almost normal adult height during treatment. The average treatment period was 4.8 ± 0.8 years.

Keywords: Congenital Male Hypogonadotropic Hypogonadism; Kallmann Syndrome; Pretreatment with rFSH Monotherapy; Cryptorchidism; Extremely Small Testes; Micropenis

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Abbreviations

Introduction
Congenital male hypogonadotropic hypogonadism (CMHH), a rare treatable disorder of male infertility, is treated with long-term pulsatile GnRH administration or more commonly by subcutaneous human chorionic gonadotropin (hCG)-human recombinant follicle stimulating hormone (rFSH) combination therapy (hCG-rFSH) to induce virilization and attain fertility. Unfortunately, in approximately 25% of these cases, sufficient spermatogenesis is not successfully observed under the current hCG-rFSH protocol such as Kallmann syndrome (KS) and isolated gonadotropin deficiency [1,2]. Predictive factors for poor fertility prognosis include cryptorchidism, prepubertal testicular volume (TV) (< 4 mL) and low serum levels of inhibin B due to gonadotropin deficiency between the middle and late fetal stage [2]. The proliferation of Sertoli cells (SCs) in response to increased follicle stimulating hormone (FSH) levels of mini-puberty in the first months of life is important in testicular development [3]. FSH is continuously secreted in early childhood [4] and increases TV approximately 3-fold from neonatal age to the onset of puberty exclusively with SCs and early germ-cell proliferation [5]. In early puberty, GnRH secretion is reactivated, stimulating both luteinizing hormone (LH) and FSH, which induce both Leydig cells and SCs proliferate and increase testosterone production from the mature Leydig cells. These physiological processes demonstrate that FSH secretion is important in testicular development not only in puberty but also in prepubertal age. Dwyer, et al. [6] reported the effectiveness of rFSH monotherapy followed by hCG-rFSH improve spermatogenesis in adult patients with CMHH and small testes. In addition, Raivio, et al. [7] reported that despite the small TV, the results of > 80% MHH boys aged 9.9 - 17.7 years) primed with rFSH displayed sperm suggested a beneficial effect of rFSH priming on testicular function in future fertility.

The purposes of CMHH treatment diagnosed during childhood are to improve psychosocial problem due to delayed puberty, to mimic natural puberty, to maximize statural growth and to attain fertility. However, the treatment methods have not beenestablished yet on a global scale. Thus, we previously proposed a new treatment protocol using preemptive rFSH therapy prior to age-appropriate low-dose administration of GRT in CMHH patients during pubertal age [1].

In this report, pretreatment with rFSH monotherapy followed by hCG-rFSH based on a proposed new protocol [1] was applied to patients with CMHH diagnosed at an early stage of childhood because of extremely small testes, micropenis and/or bilateral cryptorchidism and their pubertal growth and gonadal function were followed up on.

Patients and Methods
The data of three patients [age range, 14.6 - 18 years [at the start of rFSH monotherapy] CMHH patients diagnosed during childhood with prepubertal testes [TV 1 mL or less], micropenis and/or cryptorchidism] were retrospectively collected from the clinical records between 1998 and 2019 at the University of Tokyo, Hikari Clinic, and Tanaka Growth Clinic. The study was approved by the University of Tokyo Hospital Ethics Committee and two clinics possessed research permits. Informed consent of patients and/or their guardians was obtained. All patients were diagnosed as having KS by clinical history such as anosmia or hyposmia, low serum testosterone levels (< 130 ng/dL < 4.5 nmol/L) with undetectable serum gonadotropin levels (LH < 0.5 IU/L and FSH < 2.0 IU/L). Puberty was assessed based on the Tanner stage [8]. TV was measured using by Prader orchidometer. Serum gonadotropin levels were measured as described on LIS

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medience (https://www.medience.co.jp). Unfortunately, no laboratory in Japan can measure inhibin B levels. Semen analysis was performed by visual inspection using a hemocytometer (Case 3). The lower reference limit for total sperm number is $39 \times 10^6$ spermatozoa per ejaculate as per the World Health Organization (WHO) criteria [9]. Olfactory acuity was evaluated by Alinamin test [10] in Case 3.

**Treatment protocol**

All patients were treated with rFSH (Gonal-F, follitropin alfa, Merck Serono, Geneva, Switzerland) according to the treatment protocol for CMHH with small TV [1]. Following rFSH (75 IU) administration daily for 2 (Cases 1 and 2) [1] or 4 months (Case 3) [6], hCG (human chorionic gonadotrophin, ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) and rFSH were administered based on age-appropriate therapeutic doses [1]. Dwyer, et al. [6] used 4 months of rFSH pretreatment, but the inhibin B levels after 2 months of rFSH treatment reached the highest levels, which were similar to the normal range. Through direct personal communication with Pitteloud (CHUV, corresponding author of Dwyer, et al. [6]), we confirmed that a span of 2 months of rFSH pretreatment is sufficient. The patients visited the outpatient clinic at 3-month intervals during the course of treatment. Only the patient in Case 3 was administered low-dose testosterone therapy for 3.8 years to induce virilization before pretreatment with rFSH monotherapy. All patients did not undergo prior GnRH or gonadotropin therapy.

**Results**

Table 1 shows the patients' clinical and genetic information (diagnosis, cryptorchidism, micropenis, olfactory function, bone age progression, pubertal height gain, adult height, pubertal development, serum hormone values, treatment, rare variants and associated phenotype). Figure 1 shows the treatment course and pubertal maturation (TV, Tanner pubic hair [P] and genital stages [G]) of the cases: Case 1, 2 and 3).

**Case 1**

The patient is a 23-year-old man who was diagnosed as having CMHH at 4 years because of cryptorchidism and micropenis and he was performed orchiopexy. He visited the clinic at the age of 18 with delayed puberty and hyposmia. rFSH monotherapy (75 IU/day) was administered daily for 2 months, then switched to hCG-rFSH (hCG therapeutic dose was 1000 IU once weekly and rFSH therapeutic dose 75 IU was once weekly). rFSH therapy was changed to 75 IU twice weekly and continuously administered same dose and frequency for 5 years. After 10 months, the hCG therapeutic dose was increased to 1500 IU twice weekly. TV was less than 1 mL and the testes were soft upon palpation at the start of treatment. TV increased two-fold after 2-month priming with rFSH monotherapy. TV gradually increased from 1 mL to 8 mL during the 5-year hCG-rFSH treatment period. Testosterone reached adult level within 1 year (Figure 1). Nocturnal emission and ejaculation of semen were confirmed in the fifth year of treatment. He ejaculated semen several times, but the semen test has not been performed yet. The pace of progression of Tanner pubic hair (P) and genital stages (G) was not slow, but the development of TV was slower than that in healthy Japanese boys during the 5-year hCG-rFSH treatment period (Figure 1) [11,12]. During treatment, bone age progression was close to normal ($\Delta$CA/$\Delta$BA was 0.9~1), and pubertal growth was 12.8 cm/3 years. His adult height was 182.4 cm (Table 1).

He exhibited both PROKR2 (OMIM 607123) and LHB (OMIM 152780) heterozygous missense mutations (Table 1). Minor allele frequency (MAF) of PROKR2 p.Trp178Ser, (c.533G>C), heterozygous mutation (rs201835496) from ExAC database is 0.0002. SIFT/Polyphen-2 of computational algorithms predicted that this mutation might be deleterious/probably damaging for structure and function of protein, respectively. Similarly, MAF of LHB p.Arg88Trp (c.262C>T) heterozygous mutation (rs146251380) from ExAC database is 0.0003. SIFT and Mutation Taster predicted that mutation might be deleterious (affected), but Polyphen-2 estimated it as benign.

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Case 2

The patient is an 18-year-old man diagnosed as having KS at 14.4 years because of micropenis and bilateral small testes (TV < 0.5 mL), delayed puberty and anosmia. rFSH monotherapy (75 IU/day) was administered daily for 2 months, then switched to hCG-rFSH (hCG therapeutic dose was 1000 IU once weekly and rFSH therapeutic dose was 75 IU twice weekly). After 2 years, the hCG therapeutic dose was increased to 1500 IU twice weekly. rFSH therapeutic dose (75 IU) was continuously administered twice weekly for 3 years. TV was < 0.5 mL at the start of treatment and increased two-fold after 2-month priming with rFSH monotherapy. TV gradually increased to 12 mL during the hCG-rFSH treatment period for 3 years. Testosterone reached adult level within 1 year. The pace of progression of Tanner pubic hair (P) and genital stages (G) was not slow, but the development of TV was slower than that in healthy Japanese boys [11,12] (Figure 1). The semen test has not been performed yet. After three years of treatment, his height reached to the average height of Japanese men at 18 years, from 153 cm to 169 cm, and pubertal height gain during hCG-rFSH was 16 cm/3 years (168.9 cm is average height at the age of men who are 18 years based on the Ministry of Internal Affairs and Communications "National Health and Nutrition Survey in 2019"). During treatment, bone age progression was slower than normal (ΔBA/ΔCA was 0.1~0.76) (Table 1). No significant mutations were identified in responsible genes for hypogonadotropic hypogonadism on next-generation ecome sequencing.

Case 3

The patient was an 18-year-old man diagnosed as having KS. At the age of 2 years and 7 months, he was suspected to have CMHH because of micropenis and bilateral cryptorchidism and underwent orchiopexy. Anosmia was evaluated by Alinamin test [10] and ANOS1 (KAL1) (OMIM 300836) R423X hemizygous mutation was identified at the age of 9 years. He was diagnosed with CMHH due to low response in LHRH stimulation test (peak LH 0.8 IU/L and peak FSH 4.2 IU/L) and HCG stimulation test (peak testosterone 22 ng/dL, 0.76 nmol/L) at the age of 12.7 years. Low-dose testosterone was subsequently administered to induce virilization. At the age of 16.5 years, treatment was changed to rFSH monotherapy (75 IU/day) for 4 months by referring to Dwyer’s protocol [6] and TV was doubled from 1 to 2 mL after 4 months of starting rFSH monotherapy. From the age of 17 years to date, he was switched to hCG-rFSH (the hCG therapeutic dose was 5000 IU twice weekly and rFSH therapeutic dose 75 IU was twice weekly). After 4 months of switching to hCG-rFSH, his testosterone reached adult level. TV gradually increased to 5 mL but the pace of testicular development was slower than that in healthy Japanese boys [11,12] during the 2-years of hCG-rFSH treatment (Figure 1). Nocturnal emission and ejaculation of semen were sometimes recognized from the age of 17.5 years. Several sperm (four to five per ejaculate) was confirmed by first semen analysis at the age of 18 years. Tanner pubic hair (P), genital stages (G) and bone age were evaluated at the start of hCG-rFSH (16.5 years old) only. His current height (169 cm) reached the average height of Japanese men at 18 years. He gained 21 cm pubertal growth during the 5.5-year treatment period including testosterone low-dose administration to induce virilization (Table 1).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
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<tbody>
<tr>
<td>Diagnosis</td>
<td>KS</td>
<td>KS</td>
<td>KS</td>
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<tr>
<td>Cryptorchidism</td>
<td>Bilateral</td>
<td>No</td>
<td>Bilateral</td>
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<tr>
<td>Micropenis</td>
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<td>Yes</td>
<td>Yes</td>
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<td>Olfactory function</td>
<td>Hyposmia</td>
<td>Anosmia</td>
<td>Anosmia</td>
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<td><strong>Growth</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CA at the start of rFSH monotherapy (year)</td>
<td>18</td>
<td>14.6</td>
<td>16.5</td>
</tr>
<tr>
<td>BA at the start of rFSH monotherapy (year)</td>
<td>12.8</td>
<td>13</td>
<td>14.8</td>
</tr>
<tr>
<td>ΔBA/ΔCA of first treatment year</td>
<td>0.9</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td>ΔBA/ΔCA of second treatment year</td>
<td>1</td>
<td>0.5</td>
<td>Adult bone age</td>
</tr>
<tr>
<td>ΔBA/ΔCA of third treatment year</td>
<td>0.9</td>
<td>0.76</td>
<td>Adult bone age</td>
</tr>
<tr>
<td>Treatment period from the start of rFSH monotherapy to present (year)</td>
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<td>3.8</td>
<td>1.6</td>
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<td>Height at the start of hormone replacement therapy (cm)</td>
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<td>148</td>
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<tr>
<td>Adult height (cm)</td>
<td>182.4</td>
<td>169</td>
<td>169</td>
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<tr>
<td>Pubertal height gain (cm)</td>
<td>12.8</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Time taken to reach adult height (year)</td>
<td>3 years</td>
<td>3.8 years</td>
<td>5.5 years*4</td>
</tr>
<tr>
<td>Pubertal development</td>
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<td></td>
<td></td>
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<tr>
<td>Testicular volume at start of treatment (mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
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</table>

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Current testicular volume (mL) | 8 | 12 | 5
---|---|---|---
Treatment period to emission and ejaculation (year) | 5.1 | (-) | 1.6
Confirmation of spermatogenesis by semen analysis (per ejaculate) | NE | NE | Four to five
Serum hormone value at start of treatment*1 | | | |
LH (IU/L) | 0.14 | 0.13 | < 0.1
FSH (IU/L) | 0.63 | 0.62 | 0.5
Testosterone (ng/dL) (nmol/L) | 11 (0.38) | < 3 (< 0.10) | 11 (0.38)

<table>
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<th>Treatment</th>
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<th>4 months daily</th>
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<td>No</td>
<td>Testosterone</td>
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<td>Treatment after rFSH monotherapy</td>
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<td>hCG + rFSH</td>
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<td>Rare variant</td>
<td>PROKR2: p.Trp178Ser, (c.533G&gt;C); LHB: p.Arg88Trp (c.262C&gt;T)</td>
<td>None</td>
<td>ANOS1 (KAL1): p.R423X (c.1417C&gt;T)</td>
<td></td>
</tr>
</tbody>
</table>

| Associated phenotype | Osteoporosis | None | None |

Table 1: Clinical information of CMHH patients diagnosed during childhood with severe phenotypes.

*1Age-and sex-matched Japanese reference data are shown in the text. *2Height at the start of rFSH monotherapy. *3Height at the start of testosterone replacement therapy. *4Time taken to reach adult height from the start of testosterone replacement therapy.

CMHH: Congenital Male Hypogonadotropic Hypogonadism; NE: Not Examined; hCG: Human Chorionic Gonadotropin; rFSH: Recombinant Follicle Stimulating Hormone; BA: Bone Age; CA: Chronological Age; KS: Kallmann Syndrome; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; PROKR2: Prokineticin Receptor 2; LHB: Luteinizing Hormone Subunit Beta; ANOS1: Anosmin 1; KAL1: Kallmann Syndrome 1.

Figure 1: Clinical treatment course and changes in testicular volume in CMHH patients diagnosed during childhood with severe phenotypes. The left vertical axis is the testosterone value, and the right vertical axis is the FSH value. The horizontal axis shows the treatment period. The dotted line is the FSH value. The straight line is the testosterone value. Gray ovals indicate change in testicular volume. T (mg) is administration of testosterone (Enarmon depot, Testosterone Enanthate, ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) in Case 3. P is Tanner pubic hair and G is genital stages.
Discussion

In this report, we described that rFSH monotherapy administration before hCG-rFSH combination therapy according to a proposed new protocol [1] in CMHH diagnosed during childhood because of severe phenotypes (extremely small testes, micropenis and cryptorchidism) was an effective treatment for testicular development, normalization of adult testosterone level, and pubertal growth and maturation without rapid progression. In particular, TV significantly increased from an initial volume of TV before treatment to 8.33 ± 3.51 mL during treatment. Only Case 3 showed 4-5 sperms in the ejaculate by the first semen analysis, whereas Cases 1 and 3 experienced nocturnal emission and ejaculation.

rFSH monotherapy followed by GRT was successful in inducing testicular development and attainment of fertility have been reported in adult CMHH and gonadotropin-deficient boys with small testes (< 4 mL) [6,7,13], but, one boy with KS patient with both bilateral cryptorchidism and micropenis displayed azoospermia [7]. Corresponding to the results, compared with CMHH patients with small testes and without cryptorchidism, CMHH patients with both small testes and cryptorchidism showed longer median time to sperm appearance in semen, lower rate of spermatogenesis and lower mean sperm concentration by conventional GRT [14]. Moreover, only 36% of MHH with small initial TV (< 4 mL) exhibited spermatogenesis in the ejaculate even after long-term gonadotropin therapy [15]. Therefore, pre-pubertal small testes (< 4 mL) and cryptorchidism with CMHH patients are predictive risk factors for insufficient sperm production and subsequent infertility in adulthood [2,14,16,17] because of cryptorchidism is associated with impairment of germ cell maturation [16,17]. Thus, to minimize germ cell loss as fertility index is adversely affected in boys with cryptorchidism beyond a year, orchidopexy should be performed before 1 year of age [16-18]. However, cases 1 and 3 experienced orchiopexy due to bilateral cryptorchidism at the age of 4 and at the age of 2 years and 7 months, it is speculated that they have negative prognostic factors for future fertility because of delayed orchidopexy over the age of 1 year and extremely small testes.

Kobori, et al. investigated testicular development and sperm acquisition rate (%) in Japanese adults with CMHH after conventional hCG-rFSH [19]. The sperm acquisition rate (%) after hCG-rFSH was 85.7% (17/20) in adult patients with CMHH [19]. The TV (mL) before treatment was R: 4.37 ± 2.34 (2 - 10) and L: 4.33 ± 2.35 (1 - 20) and TV after treatment was R: 8.48 ± 4.57 (1 - 20) and L: 8.50 ± 4.19 (2 - 19) in adult patients with CMHH [19]. Although TV before treatment in CMHH patients diagnosed during childhood was extremely smaller than that of adult CMHH patients, TV after treatment in CMHH patients diagnosed during childhood increased to almost the same volume as that in adult MHH patients. The result indicates that rFSH pretreatment may be effective for developing TV in CMHH diagnosed during childhood with severe phenotypes. However, the sperm acquisition rate (%) after conventional hCG-rFSH combination therapy was 55.5% (5/9) in CMHH patients diagnosed during childhood [1]. Moreover, the mean duration to achieve the appearance of sperm in the ejaculates was 10.7 months with hCG-rFSH therapy in adult Japanese MHH (of diverse etiologies) [19]. In contrast, in spite of 2 - 5 years of GRT, sperm has not been confirmed except for Case 3 in this report by semen analysis. CMHH with cryptorchidism often require extended courses of treatment [2,14,15,20,21]. It is suggested that reproductive function of CMHH patients diagnosed during childhood with severe phenotypes may be more severely impaired than that of adult CMHH patients because small testes and histories of cryptorchidism are negative determinants of fertility outcome [14,15,22-25].

TV before treatment in adult patients is larger than that of our patients (≤ 1.0 ml) and severe phenotype (extremely small testes, micropenis, and cryptorchidism) leads to diagnosis during childhood. Adult patients with CMHH are mainly treated by Urologist, but CMHH patients diagnosed during childhood are mainly treated by Pediatric Endocrinologist. Both CMHH patients may not be the same entity. Thus, hCG-rFSH combination therapy may be effective to attain fertility in adult patients with CMHH but insufficient in CMHH diagnosed during childhood. Because CMHH diagnosed during childhood exhibits the most severe phenotypes (small testes 1 mL or less, micropenis, and cryptorchidism) due to fetal and infantile gonadotropin deficiency that impair inguinoscrotal testicular descent at the middle to late fetal stage and testicular development from early childhood following mini-puberty in infancy to pubertal age [3]. As described above, FSH continues to be secreted from birth to adulthood for testicular development [4] and induces SCs and early germ-cell proliferation in
immature testes [5]. Therefore, introducing rFSH priming before hCG-rFSH therapy is necessary for CMHH diagnosed during childhood with severe phenotypes to compensate for the loss of mini-puberty.

The proposed new treatment protocols [1] for CMHH patients diagnosed during childhood recommend that age-appropriate low-dose administration is started based on growth and puberty observed during adolescence without undesired rapid pubertal progression and to improve psychosocial problem associated with delayed puberty [1,2]. Under the treatment we have proposed [1], the rapid progression of pubertal maturation and bone age have not been observed in any cases and the height of all patients almost reached the average adult height of Japanese men during treatment. Similar to our previously reported data [1], the earlier low-dose hormone replacement therapy was started, the better it was in pubertal growth, as seen in Case 3; the age at the start of treatment was 12 years old. It is expected to induce the maturation of physiological secondary characteristics over a treatment period of 4 to 5 years if patients start at a timing closer to the onset of puberty, because therapeutic dose are increased gradually every 6 months to mimic natural puberty, depending on age at the start treatment [1,26,27]. In our study, the average treatment period of approximately 5 years has passed since the start of treatment and the therapeutic dose is still being increased to full adult dosing in Case 2 who has not yet been evaluated sperm production by semen analysis, but TV well developed from < 1 mL to 12 mL. Since he has reached near adult height and his bone age has advanced to adult bone age, the therapeutic dose will be adjusted to appropriate full adult dosing to attain fertility according to our treatment protocols [1].

It is noteworthy that Case 1 had digenic/oligogenicity mutations in two congenital HH responsible genes (PROKR2 and LHB mutations) (Table 1). Cole LW., et al. [28] previously reported that PROKR2 W178S mutant allele decreased intracellular calcium mobilization and exhibited decreased MAPK signaling and receptor expression. According to the guidelines of the American College of Medical Genetics and Genomics (ACMG) [29,30], well-established in vitro functional studies can confirm the damaging effect on the gene or gene product. Taken together with those functional analysis, population data and computational algorithms, PROKR2 W178S mutation will be strongly classified as pathogenic considered mutations. However, the heterozygous PROKR2 mutations have also been found in apparently unaffected individuals and the frequency of PROKR2 homozygous mutations were rare, which suggests that a PROKR2 mutation is digenic/oligogenic mode of inheritance of the disease in heterozygous patients [31]. Furthermore, Case 1 had an LHB R88W heterozygous mutation, which MAF is extremely low frequency and supported a deleterious effect on the LHB gene by two computational evidences. Studies on large CHH cohorts mentioned that at least 20% of cases are oligogenic [2,32]. Thus, in Case 1, the oligogenicity of PROKR2 and LHB mutations might cause severe HH at an early stage and slower response to treatment for spermatogenesis and testicular development. However, during long-term treatment, as he has recently experienced frequent emission and ejaculation, he may be able to confirm his spermatogenesis by semen analysis in the near future. Case 3 had an ANOS1 hemizygous nonsense mutation that is indicated a very strong pathogenic mutation by the guidelines of the ACMG [29,30]. Costa-Barbosa., et al. reported that male KS subjects with an ANOS1 mutation displayed extremely lower TVs than all-non ANOS1 probands [33] and 50% of patients with ANOS1 mutations exhibited poor response to GnRH therapy. However, Case 3 and a boy of CMHH patient (13) with ANOS1 nonsense mutations displayed sperms after rFSH priming therapy followed by rFSH-hCG. It is likely that KS patients with ANOS1 mutations might benefit from rFSH pretreatment.

Although we recommended rFSH priming before gonadotropin therapy, they are still at investigational levels reality; therefore, further research is necessary, particularly at the pediatric level, to establish an MHH treatment protocol in the future. Boehm., et al. also exhibited that an international, multicenter randomized trial is needed to determine the optimal approach to fertility treatment in men with CHH and severe GnRH deficiency (with or without cryptorchidism) [2].

Conclusion

rFSH monotherapy prior to age-appropriate hCG-rFSH combination therapy according to a proposed new protocol in CMHH patients diagnosed during childhood with severe phenotypes is an effective treatment for testicular development, normalization of testosterone levels, pubertal growth without rapid bone age progression, and pubertal maturation. However, compared with adult CMHH patients treated by conventional rFSH-hCG, CMHH patients diagnosed during childhood exhibited comparable testicular enlargement after rFSH

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pretreatment with hCG-rFSH, but had a lower sperm acquisition rate and required a longer time of treatment to spermatogenesis because of their severe phenotypes suggestive of negative prognostic factors for future fertility such as extremely small testes, micropenis, and/or cryptorchidism. Thus, they need to adjusted appropriate full adult therapeutic dosing and long-term treatment to attain fertility. Moreover, reproductive dysfunction in CMHH patients diagnosed during childhood might be caused by gene-mutations, such as digenic/oligogenic PROKR2, ANOS1 mutations. This CMHH treatment protocol is still at investigational levels globally and further research is necessary in an international multicenter trial.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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