Clinical management of congenital hypogonadotropic hypogonadism

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Short Title

Clinical management of CHH

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Precis

A comprehensive review of the clinical evaluation, biochemical and genetic testing, differential diagnosis, and treament of patients with congenital hypogonadotropic hypogonadism

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ABSTRACT

The initiation and maintenance of reproductive capacity in humans is dependent upon pulsatile secretion of the hypothalamic hormone gonadtropin-releasing hormone, GnRH. Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder that results from the failure of the normal episodic GnRH secretion, leading to delayed puberty and infertility. CHH can be associated with an absent sense of smell, also termed Kallmann syndrome, or with other anomalies. CHH is characterized by rich genetic heterogeneity, with mutations in more than 30 genes identified to date acting either alone or in combination. CHH can be challenging to diagnose, particularly in early adolescence where the clinical picture mirrors that of constitutional delay of growth and puberty. Timely diagnosis and treatment will induce puberty, leading to improved sexual, bone, metabolic and psychological health. In most cases, patients require lifelong treatment yet a significant portion of patients (around 10-20%) exhibit a spontaneous recovery of their reproductive function. Finally, fertility can be induced with pulsatile GnRH treatment or gonadotropin regimens in a majority of patients. In summary, this review is a comprehensive synthesis of the current literature available regarding the diagnosis, patient management and genetic foundations of congenital hypogonadotropic hypogonadism relative to normal reproductive development.

1. Introduction

Puberty is one of the most striking postnatal developmental processes in humans. It is accompanied by the acquisition of secondary sexual characteristics, the onset of fertility, the attainment of adult height and imporant psychosocial changes (1). Puberty is initiated by the re-awakening of the hypothalamic-pituitary-gonadal (HPG) axis following a relative quiescence during childhood (2). Pulsatile secretion of gonadotropin-releasing hormone (GnRH) by specialized neurons in the hypothalamus stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary, which in turn stimulate steroidogenesis and gametogenesis in the gonads. Notably, the onset of puberty is preceded by two periods of the hypothalamic-pituitary-gonadal (HPG) axis activity: the fetal life and infancy (minipuberty).

The timing of puberty varies largely in the population, and 50-80% of this variation is genetically determined (3-5). Delayed puberty is defined as a delay of pubertal onset or progression greater than 2SD compared to the population mean (6). Constitutional delay in growth and puberty (CDGP) is the most frequent cause of delayed puberty (2% in the general population), and is related to a transient GnRH deficiency. In CDGP, puberty eventually begins and is completed spontaneously. In contrast, congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disease caused by GnRH deficiency. It is characterized by absent or incomplete puberty with infertility (7). This infertility is medically treatable, and in fact CHH is one of the few treatable cause of infertility in males. When CHH is associated with anosmia, it is termed Kallmann Syndrome (KS).

In this review, we describe the spectrum of clinical presentations in CHH, the diagnostic evaluations including the challenge of differentiating CHH from CDGP, the advances in genetic diagnosis and therapy for CHH, as well as the consequences of a delay in diagnosis. Finally, we discuss the therapeutical options from different perspectives. To achieve these objectives, we will also review the normal physiology of the HPG axis.

2. Fetal development of the hypothalamic-pituitary-gonadal axis

The HPG axis is active in the mid-gestational fetus but quiescent towards term (8). This restraint is removed after birth, leading to a reactivation of the axis and an increase in gonadotropin levels (minipuberty).

The majority of GnRH-secreting neurons are located in the arcuate nucleus and the preoptic area of the hypothalamus (9). GnRH neurons are an unusual neuronal population, as they originate outside the central nervous system in the olfactory placode, and follow a complex migration route to reach their final destination in the hypothalamus (10,11). The complex developmental process of GnRH neurons has unfolded through both murine and human studies. (12-14).

GnRH neurons fate specification occurs from progenitor cells in the olfactory placode at gestational week (GW) 5 in humans, and days 9.5 to 11 in mice (15). Subsequently, the GnRH neurons begin their migration from the nasal placode following the axon guidance of the vomeronasal nerve (VNN) and the olfactory nerve until they cross the nasal mesenchyme and cribriform plate. Thereafter, the GnRH neurons follow the guidance of the VNN ventral branch reaching the forebrain. From here, the GnRH neurons detach from the VNN axons to reach their final destination in the arcuate nucleus and the preoptic area of the hypothalamus. Subsequently, they extend their axons to the median eminence reaching the fenestrated blood-brain capillaries of the hypothalamo-pituitary portal vessels. By day 16 in the mice and around 15 weeks of gestation in human, GnRH is detected in the hypothalamus and the GnRH neuronal system is largely complete (14,16).

Recently, studies of GnRH ontogeny in mice and human using the innovative technique of 3DISCO optical tissue-clearing reveal the detailed dynamics of GnRH neuron ontogeny and migration from

nasal compartment to forebrain. Notably, the number of GnRH neurons in the fetal brain is higher (~10,000) than previously anticipated (14).

LH is detected in the anterior pituitary by GW 9 (17), and is released into the circulation by GW 12 (18-20). The exact timing when pituitary gonadotropin secretion will come under the control of the hypothalamic GnRH is not clear. In anencephalic fetuses without a hypothalamus, pituitary development is normal up to GW 17-18 before it involutes, suggesting that hypothalamic signalling is needed for the maintenance of the gonadotropes from this stage (21). Fetal gonadotropin levels peak at mid-gestation in both sexes. Females generally exhibit high circulating FSH and LH levels in the range of postmenopausal women, which is much higher than in male fetuses (18,19,22-25). Near term, circulating gonadotropin levels decreased. It is thought that this is related to placental estrogens, and gonadal feedback (18-20,24).

The differentiation of the gonads into testicles and ovaries occurs between GW 5-7. It is is a complex process involving a critical role of the *SRY* gene on the Y chromosome for males. During GW 8, the differentiated seminiferous tubules (Sertoli cells) start to produce AMH under the contol of SOX9, which leads to regression of the Mullerian ducts (26). Placental hCG (during the first trimester) and subsequently fetal pituitary LH (from mid-gestation) regulate Leydig cell differentiation to produce testosterone (T) from the fetal Leydig cells (27), which is needed for masculinization of the fetus. Testosterone is needed for the development of the male internal genitalia, while dihydrotestosterone produced by the enzyme 5-α reductase 2 (SRD5A2) induces the formation of the prostate, penis and scrotum. Until mid-gestation, testosterone production is driven by placental HCG rather than by GnRH-induced LH secretion by the fetus. This is consistent with the absence of genital differentiation defects in CHH. However, in the third gestational period penile growth and inguino-scrotal testicular descent occur, mediated in part by testosterone stimulated by GnRH-induced LH secretion (reviewed in (28) and (29)).

In females, the gonads develop into an ovary in the absence of the Y chromosome, however several active signalling pathways need to be present for a normal differentiation of the ovary (30). In addition, the differentiation of internal or external genitalia occurs independantly of the ovaries. In the absence of AMH, the müllerian ducts will develop into fallopian tubes, uterus and a portion of the vagina. In humans, primordial follicles develop in the fetal ovary around GW 15 (31) and are gonadotropin-independent. Steroid production in fetal ovaries is not clear, and appears to be minimal compared to high placental oestrogen production (32).

Fetal reproductive development: Implications for CHH phenotypes

Disruption of the complex ontogeny of the GnRH neurons and olfactory system can lead to GnRH deficiency and, in severe cases, to CHH with or without anosmia. However, during the first trimester of pregnancy, which is critical for sexual differentiation, the GnRH neuronal system is non-functional. Consequently, the differentiation of the genitalia in CHH is normal. In contrast, during late pregnancy GnRH induced LH secretion stimulates further penile growth and testicular descent. Thus, a higher prevalence of micropenis and cryptorchidism is encountered in CHH (reviewed in (29)).

3. Clinical presentation of CHH

3.1 Clinical presentation of CHH during the minipuberty

3.1.1 Normal minipuberty

Within minutes of birth, a brief postnatal LH surge leads to an increase in testosterone levels during the first day of life which then subsides (33).

After the first postnatal week, increased pulsatile GnRH secretion (34) leads to elevated gonadotropins and sex steroid levels in both sexes, with peak levels observed at 1- 3 months of age (minipuberty) (35-39). During this time, FSH levels are higher in girls, and LH levels are predominant in boys (38). In boys,

LH and FSH levels decrease by 6 months of age, however FSH levels remain elevated up to 3-4 years of life in girls (8,38,40).

In boys, T levels start to increase after 1 week postnatally, peak between 1-3 months, and then decline to low prepubertal levels by approximately 6 months (8,38,40). These changes mirror GnRH-induced LH activation. During minipuberty, T levels correlate with penile growth (41), and postnatal T levels have also been associated with male-type behaviour in toddlers (42). In addition, acne, sebaceous gland hypertrophy and increased urinary prostate-specific antigen levels are observed during this period, consistent with androgen bioactivity (39,43). GnRH-induced gonadotropin secretion stimulates the production of inhibin B (a marker of Sertoli cell number and function) (38)) and AMH (44) and the Leydig cell product INSL3 (45). High inhibin B levels remain beyond 6 months of age despite the decrease in gonadotropin secretion (38).

Testicular volume increases during minipuberty. One critical event during this time is the significant proliferation of immature Sertoli cells and spermatogonia induced by FSH, mirroring the increased levels of circulating inhibin B. On average, the Sertoli cell population increases from 260 x 10^6 at birth to 1500 x 10^6 by 3 months of age, and this increase constitutes a critical determinant for future sperm producing capacity in adulthood (46,47). Despite high levels of intragonadal T and the gonadotropin surge, seminiferous tubules do not undergo differentiation and spermatogenesis is not initiated. During this period, androgen receptors (AR) have a very low expression in the Sertoli cells, and therefore they remain immature with high levels of AMH despite increased testosterone during minipuberty (44,48,49).

In girls, elevated gonadotropin levels result in an increase in ovarian follicular development (39,43). Estradiol (E2) levels also start to increase after one week of age (39) and are associated with increased folliculogenesis (43), and then decrease during the second year of life (39). The high circulating E2

levels in girls lead to palpable breast tissue during minipuberty (39,50). The postnatal gonadotropin surge also induces the production of the granulosa cell hormonal peptides inhibin B (38) and AMH (43).

In both sexes, testosterone appears to be a significant modulator of growth during infancy (51) and influences neurobehavioral sexual differentiation (42). Notably, minipuberty appears enhanced in preterm infants and in those born small for gestational age (reviewed in (8)).

The biological significance of minipuberty and its consequences on reproductive capacity are not fully understood. This period may be critical for furture reproductive health, and thus warrants additional investigation. Further, the mechanism that leads to the quiescence of the HPG axis after infancy remains largely unknown.

3.1.2 Minipuberty: Implications for CHH phenotypes

From a diagnostic perspective, minipuberty offers a unique window of opportunity for the early diagnosis of CHH (52). While there are no clear clinical signs of GnRH deficiency in females during minipuberty, a male infant with micropenis and cryptorchidism raises a suspicion of CHH, as these signs may reflect the lack of activation of the HPG axis during fetal and postnatal life. Large retrospective studies on CHH including KS have described a high frequency of cryptorchidism ranging from 30-50% (53,54), consistent with the role of GnRH-induced T secretion during fetal life and minipuberty in testicular descent. Reports on the frequency of micropenis among CHH patients is variable, ranging from 20-40 % in KS patients (55,56). While CHH is reported in 30% of patients with micropenis (57,58), its prevalence among cryptorchid boys is unknown.

3.2 Clinical presentation of CHH during adolescence

3.2.1 Normal puberty

Puberty is characterized by sexual maturation, increased growth velocity, and behavioral changes, and culminates with the acquisition of reproductive capacity.

The precise mechanisms that trigger the initiation of puberty remain unclear and are likely quite complex. Morphologically, dynamic remodelling in GnRH neuron morphology occurs at puberty with > 500 spines associated with increasing synaptic inputs contributing to the sharp increase in GnRH neuron activity (59). Increased excitatory input like glutamate or decreased inhibitory input like aminobutyric acid (GABA) appear to be critical for pubertal onset (60). In addition, recent murine studies have pointed to a critical role of epigenetics in the onset of puberty (61,62). Human genetic studies in CHH have also unraveled key regulators of the GnRH network with the demonstration of loss-of-function of the kisspeptin receptor (*KISSR1*) (63,64) and neurokininB receptor (*TACR3*) (65) resulting in severe GnRH deficiency. From this complex process, it is clear thar the interplay between genetic factors, metabolic cues (e.g. body fat, insulin, leptin, FGF21), the circadian clock, environmental and social cues is critical for the initiation of puberty (66).

Puberty is initiated by the reawakening of the GnRH pulse generator after a relative quiescent period during childhood (67). In the 1970s, it was demonstrated that pulsatile release of GnRH is necessary for the activation of the HPG axis (68). GnRH-induced pulses of LH first occur during the night, but gradually increase to both day and night resulting in gonadal maturation and the completion of puberty (69-71). However, the mechanism that leads to the pulsatility of the GnRH neuronal network is still unknown.

The increase in gonadotropins during early puberty leads to a second wave of proliferation of immature Sertoli cells and spermatogonia under the regulation of FSH prior to seminiferous tubule maturation. This process is associated with an increase in the level of inhibin B, a marker of Sertoli cells function (72). Progressively, GnRH-induced LH stimulates Leydig cell differentiation and their

steroidogenic capabilities leading to testosterone production. The concommitant stimulation of Sertoli cells by FSH and the production of intragonadal testosterone by LH leads to the initiation of spermatogenesis and a sharp increase in testicular volume consisting mainly of maturing germ cells with a related increase in the diameter of seminiferous tubules. During this process, AMH levels start a reciprocal decrease in comparison to testosterone and inhibin B (73). This finding likely reflects changes in androgen receptor expression in immature Sertoli cells, since androgen receptor is present in only 2-15% of Sertoli cells until 4 years of age, whereas its expression can be observed in > 90% of Sertoli cells after the age of eight years (48). Notably, AMH levels begin to decline before any notable increase in testis size can be observed (73,74). In addition, testicular INSL3 secretion increases during the course of puberty with a strong correlation to LH levels (75,76).

In girls, the early stages of follicular growth are primarily driven by intra-ovarian factors. However, pubertal onset is characterized by an increase in gonadotropin levels that are necessary for maturation of the follicules which leads to ovulation (77). GnRH-induced LH stimulates the production of androgens by the theca cells, while increased FSH is needed for the recruitment of ovarian follicles and the aromatisation of androgens to estradiol by the granulosa cells (78). AMH concentrations show only minor fluctuations during female puberty (79), while inhibin B, similar to boys, increases during puberty (80).

Clinically, puberty consists of a series of changes that typically appear in a predictable sequence. However, considerable variation in the timing of pubertal onset exists even among individuals of a given sex and ethnic origin, ranging roughly from 8 to 13 years in girls (81) and 9 to 14 years in boys (82).

In girls, a longitudinal follow-up of 432 girls in the United States (US) between 9.5 and 15.5 years old confirmed that for the majority of Caucasians, the earliest clinically detectable milestone of puberty is

breast development (i.e. thelarche, breast Tanner stage 2) at an average age of ~ 10 years, typically preceding the appearance of pubic hair (i.e. pubarche) by about 4 months (83). In a more recent longitudinal cohort of > 1200 girls also recruited in the US, thelarche occured at ages 8.8, 9.3, 9.7, and 9.7 years for African American, Hispanic, white non-Hispanic, and Asian participants, respectively (84). Almost concomitantly to thelarche, growth velocity begins to accelerate, constituting the growth spurt, another important hallmark of puberty. The latter lasts approximately 2 years and allows for the acquisition of approximately 17-18% of final height (85). Height accrual peaks at a mean age of 11-11.5 years, followed by menarche, occuring approximately 6 months after the peak growth velocity (86). The median time between the onset of puberty and menarche was 2.6 and 2.7 years in US and British cohorts, respectively (86,87). In these studies, only partial correlation was detected between age at onset of puberty and age at menarche, suggesting that both common and unique factors regulate these two milestones. Secondary sexual characteristics development (breast Tanner stage 4 and/or pubic hair stage 5) is completed approximately 1.5 year after the menarche.

In boys, both US (88,89) and European cohorts (82) have highlighted testicular enlargement (volume ≥ 4 ml) as the first clinically detectable sign of puberty, occuring at ~11.5 years, approximately 6-12 months prior to the penis growth (i.e. genital Tanner stage 3) and pubic hair development. Similar to girls, the growth spurt subsequently begins with peak height velocity at age 13.5 years. According to a 7-year longitudinal study of 31 normal boys initially aged 8.6-11.7 years, spermatarche, defined as the presence of spermatozoa in the urine, was detected at a median age of 13.4 years (range, 11.7-15.3 years) (90). This suggests that this is a relatively early pubertal event, often preceding the peak height velocity. Another milestone of male puberty, the age of first ejaculation (conscious) has been less extensively studied, likely due to the potential bias of self-reporting in all available studies. The most recent study of the age of first ejaculation in 1582 boys from Bulgaria showed an average age of 13.27 ± 1.08 years (91). Voice breaking in males is also a distinct event of pubertal development usually occurring during late puberty between Tanner stages G3 and G4 (92). A retrospective analysis of 463

Danish choir boys studied over 10 years showed a median age at voice break of 14.0 years (range 13.9-14.6 years) (93). Complete pubertal development is achieved at an average age of 15.5 years or earlier according to the latest European data (82).

Common hallmarks of puberty in both genders (Figure 1) include bone mass acquisition, body composition changes, and brain development. Bone changes during puberty are detailed in Section 8.5.1. Puberty is accompanied by significant changes in body composition, with distinctly different patterns in girls and boys. In early puberty, the increase in body mass index (BMI) is driven primarily by changes in lean body mass, whereas increases in fat mass are the major contributor in later puberty (94). Gender differences are evident with girls exhibiting a higher proportion of fat mass gain than boys at all stages, with annual increases in BMI largely due to increases in fat mass after the age of 16 years (95). Hormonal changes during puberty also affect the brain by promoting its remodeling and completing the sexual maturation that begins in the prenatal and early postnatal life (96). This has been clearly demonstrated in animal models (97), and is supported by positive correlations between pubertal markers (physical or hormonal) and structural MRI changes in grey and white matter development in humans, even after removing the confounding effect of age (96).

3.2.2 Trends in pubertal onset and progression

It is clear that the average age of menarche has decreased significantly between the 19th and the mid-20th centuries in many countries (98). This secular trend is associated with improved general health, nutrition, and lifestyle. A large Danish comparing puberty in girls in two different periods (1991–93 and 2006–08) demonstrated earlier breast development in the girls born more recently, although the central activation of puberty was not proven (81). This advance in breast development might be due to a higher incidence of obesity or exposure to endocrine disruptors. Similar studies on the age of puberty in boys have also suggested an advanced age of pubertal onset although additional research

is required to confirm this trend. There are racial differences in pubertal onset (99), though this difference is probably decreasing (84).

3.2.3 Delayed puberty

Delayed puberty is defined as pubertal onset occuring at an age of 2 or 2.5 SD later than the population mean. The traditional clinical cut-offs applied are 14 years for boys (testicular volume < 4 ml) and 13 years for girls (absence of breast development) (6). This definition, however, only focuses on the onset of puberty without considering progression of puberty as diagnostic criteria. Recently, the use of a puberty nomogram evaluating not only the pubertal onset but also pubertal progression (in SD/year) led to a more accurate description of normal puberty and its extremes (precocious and delayed puberty) (100) (Figure 2).

The most common cause of delayed puberty in both sexes is the constitutional delay of growth and puberty (CDGP), which is often considered as an extreme variant of normal pubertal timing. In a large series of 232 patients with delayed puberty investigated in a tertiary US referral center, CDGP accounted for 65% of cases in boys and 30% of girls (101) presenting with a delay in puberty. Relatively similar estimates (82% for boys and 56% for girls) were reported in a recent European study encompassing 244 patients who presented with delayed puberty (102). Though its pathophysiology is not fully understood, CDGP has a clear genetic basis, as illustrated by the finding of positive family history in 50-75% of CDGP patients (103).

CDGP is a diagnosis of exclusion, and other underlying causes of delayed puberty should be actively investigated and ruled out including hypergonadotropic hypogonadism (e.g. Klinefelter syndrome or Turner syndrome), permanent hypogonadotropic hypogonadism (e.g. CHH, tumors, infilitrative diseases) and functional hypogonadotropic hypogonadism (e.g. systemic illness, anorexia nervosa, excessive exercice). In particular, the differential diagnosis between CDGP and CHH in adolescence is

particularly difficult as discussed in detail in Section 7.3. Management options include expectant observation versus short-term sex steroid replacement (6). The latter targets primarily the induction of secondary sexual characteristics in order to alleviate psychosocial distress due to pubertal delay and/or short stature.

3.2.4 Hallmarks of CHH in adolescence

In males

CHH males predominantly seek medical attention in adolescence. The most commonly reported symptoms are absent or minimal virilization, low libido, and erectile dysfunction (104). In the majority of CHH patients, puberty never occurs leading to severely reduced testicular volume (< 4 ml) and the absence of secondary sexual characteristics (i.e. sparse facial and body hair, high pitched voice). In this group (absent puberty), micropenis and/or cryptorchidism are commonly observed. In contrast, a minority of CHH patients exhibit partial GnRH deficiency as evidenced by some spontaneous testicular growth (TV > 4 ml) with little virilization, which subsequently stalls (53). Most CHH patients do not have any ejaculate in the setting of severe hypogonadism. Indeed, testosterone is needed for seminal and prostatic fluids production and optimal ejaculate volume. Thus, the classical clinical presentation of male CHH is absent puberty in 75% of reported patients (105,106), while the remainding exhibit partial puberty.

The majority of CHH patients have eunuchoidal proportions with arm spans typically exceeding height by ≥ 5 cm, reflecting the delayed closure of the epiphysis of long bones in the absence of gonadal steroids. The lack of increased sex steroids levels leads to steady linear growth (107) without a growth spurt, however final height is rarely affected (108). Several studies report that adult height in CHH men exceeds the height of healthy control men (109-111). Other studies show that CHH adolescents, on average, achieve their mid-parental height (108,112). Studying 41 CHH men, a positive correlation was found between the delay of puberty prior to treatment and adult height, such that 6 years or more of

pubertal delay was associated with ~5 cm greater adult height (110). On the other hand, Dickerman et al. reported the growth of 50 adolescents with CHH and found no differences in the achieved normal adult height between boys who were referred before 16 years of age or thereafter (111). Boys in both groups exceeded their predicted final height by 4.9 cm (referred before 16 years of age), and by 6.3 cm (referred after 16 years).

Typical changes of body composition in CHH boys include decreased muscle mass and female body habitus with a gynoid pattern of fat distribution. Mild gynecomastia can be seen in untreated patients due to the imbalance of the testosterone/estradiol ratio. Bone maturation is impaired, with delayed bone age and lower bone density observed relative to peers. The micro-architecture of CHH males has not been assessed, and the risk of fracture is difficult to assess given the lack of large multi-center prospective studies on bone health in CHH.

In females

The most prevalent complaint is primary amenorrhea in nearly 90% of CHH women (113-116). Less than 10% of CHH women had some menstrual bleeding (113,115,117), which in most cases involved one or two episodes of bleeding during adolescence (primary-secondary amenorrhea) before chronic amenorrhea sets in (113-116). Chronic oligomenorrhea has been reported, although at a considerably more rare frequency (118,119).

Several studies have shown that a complete absence of breast development is observed in only a minority of CHH women who have not previously received estrogen replacement therapy (113-115), while different pubertal stages of development occur in the majority of patients. This is inconsistent with a single multicenter retrospective study depicting absent breast development in the majority of affected women (116), however this discrepancy could be related to recruitment bias.

Pubarche also shows great variability, ranging from the complete absence to almost normal pubic hair (113,115). Varying degrees of GnRH defciency may impact ovarian androgen production differently (114) (see below). Further, adrenarche is normal in CHH women leading to the metabolism of androgen precursors (i.e. DHEA, androstenedione) into more active androgens (i.e. testosterone, dihydrotestosterone) thus contributing to pubarche (114,120).

Linear growth and final height in women with CHH have been evaluated in relatively few studies (111,121). The scant published data indicate that the final height in these women is similar to that of the reference population. Thus, these patients are not at risk of small height, unlike hypogonadal women with Turner syndrome (122). In Dickerman's series, the growth of 16 females with CHH was unremarkable (111), whereas a slight mid-childhood deceleration in the growth rate of girls carrying *FGFR1* mutations was recently reported (106).

3.3 Clinical presentation of CHH in adulthood

Although the clinical presentation of CHH in adolescence is more common, some patients do not seek medical attention until adulthood. At this point, low libido, non-existent sexual life and/or infertility, or less commonly bone loss and fractures are the most common complaints. Although male patients usually exhibit prepubertal or small degrees of spontaneous testicular growth, large testicular volume with preserved spermatogenesis is observed in a subset of male CHH patients. This is despite a biochemical profile of low testosterone in the setting (in most cases) of detectable gonadotropins. This condition, not related to a particular genetic form (see below), is called 'fertile eunuch syndrome', and is thought to be due to the presence of low amplitude and/or low frequency or sleep-entrained GnRH pulses only. These pulses are only sufficient to support intra-testicular testosterone, but not enough to achieve a normal circulating testosterone level for full virilization (123). Very rarely, CHH is diagnosed at older age. Recently, Patderska *et al*. described six cases of men who were diagnosed with

CHH after 50 years of age, and who had long-term uncorrected hypogonadism (124). These patients exhibited adverse health events such as osteoporosis (6/6), hypercholestelemia (4/6) and anemia (2/6). Body composition and cardiovascular events were not documented. To the best of our knowledge, there is no report on undiagnosed female patients until age of menopause. Further, data on the natural history of CHH in older men and women is lacking.

In addition, a small subset of patients present with adult onset hypogonadotropic hypogonadism (AHH). These patients report normal pubertal development followed later by the complete inhibition of the HPG axis leading to severe HH. No central nervous system abnormalities or risk factors for functional GnRH deficiencies have been identified (125), and follow-up studies in AHH have shown the absence of recovery (126).

The psychological impact of CHH is often neglected. The absence of sexual hormones and its impact on physical appearance constitutes a major source of psychological distress for hypogonadal males (127). Specifically, CHH can be accompanied by anxiety and depression (128), and these symptoms are frequently underestimated by physicians (129). Low self-esteem and altered body image have also been reported (130) and can prevent adequate psychosexual development (131). Similarly, pschological distress is observed in female CHH patients. A recent online survey suggests a negative perception of CHH women on their health status, with a tendency towards depression (105). This same study suggests that care providers often do not adequately address these issues, and according to patients even have a tendency to dismiss the psychological consequences of their poor pubertal development (105). It is also quite possible that the erroneous perception of their potential infertility (see below) is also a major contributor to their malaise. Further, gender dysphoria (130,132) is reported, though a causal link has not been proven.

3.4 CHH reversal

Although CHH was previously considered as a life-long condition, it is now known that a subset of CHH patients spontaneously recover function of their reproductive axis following treatment (133-135). Reversibility occurs in both male and female CHH patients, and appears to be more common (~10-20%) than previously thought (133-135). Patients with reversal span the range of GnRH deficiency from mild to severe, and many harbor mutations in genes underlying CHH. However, to date there are no clear clinical factors for predicting reversible CHH. Similarly, the genetic signature for reversal remains unclear, although an enrichment of *TAC3*/*TACR3* mutations has been observed in one series of patients (134,136). Importantly, recovery of reproductive axis function may not be permanent, as some patients experience a relapse to a state of GnRH deficiency (134), therefore long-term monitoring of reproductive function is needed. Thus, CHH patients experiencing reversal (i) represent the mild end of the clinical spectrum, (ii) demonstrate the plasticity of the GnRH neuronal system, and (iii) highlight the importance of the effects of environmental (or epigenetic) factors such as sex steroid treatment of the reproductive axis.

3.5 CHH-associated phenotypes

CHH is associated in variable frequency with non-reproductive phenotypes. Among them, anosmia (i.e. lack of sense of smell) is observed in 50% of CHH cases (137), and this co-occurrence is termed Kallmann syndrome (KS). The interconnected link between the GnRH and olfactory systems during early developmental stages explains this association (see above, Section 2) (138).

Other phenotypes are also associated with CHH, although at a lower prevalence. They include mirror movements (synkinesia), unilateral renal agenesis, eye movement disorders, sensori-neural hearing loss, midline brain defects (including absence corpus callosum), cleft lip/palate, dental agenesis, skeletal defects, and cardiovascular defects (7,139) (some of which are illustrated in Figure 3). Three large studies have evaluated the prevalence of these associated phenotypes in CHH, although these studies were retrospective without a systematic evaluation for CHH associated-phenotypes

(137,139,140). A summary of these studies along with the frequency of these phenotypes in the general population can be found in Table 1. The presence of certain additional phenotypes can lead to the diagnosis of syndromic forms of CHH, such as CHARGE syndrome, Waardenburg syndrome, and 4H syndrome. Diagnosis of these syndromes is mainly based on clinical investigation, and the major and minor signs of the most common syndromes are listed in Table 2. Clinical diagnosis of these syndromes may be useful in increasing the diagnostic yield of genetic testing (See sections 6.4 and 6.5)

4. Epidemiology

There is no rigorous epidemiology study on the prevalence of CHH. Two historical studies that examined military records provided some estimation of the prevalence of this disease. One study examined 600,000 Sardinian conscripts at the military checkup, and identified seven cases with normal karyotype presenting bilateral testicular atrophy and anosmia (considered as KS cases), and thus estimated that the prevalence of KS is 1 in 86,000 in that population (141). A second study identified 4 cases of hypogonadotropic hypogonadism among 45,000 French men presenting for military service, and thus determined that the prevalence of CHH is 1 in 10,000 (142). There is no study on the prevalence of female CHH. In the series from the Massachusetts General Hospital of 250 consecutive CHH cases, the male to female ratio is 3.9 to 1. However, this ratio drops to 2.3 to 1 when the familial cases are analyzed separately (123). A recent epidemiological study examining the discharge registers of all five university hospitals in Finland estimated the prevalence of KS is 1:48,000 in Finnish population, with a clear difference between males (1:30,000) and females (1:125,000) (55).

Bias of prevalence in CHH females

The prevalence of CHH/ KS has historically been considered to be skewed towards a male predominance (male/female ratio of 5:1) (137,143). However, recent work suggests that the disease prevalence of in both sexes is in fact more balanced with a sex ratio actually closer to 2:1 (115,116).

Further, analysis of sex ratio for CHH in families with autosomal inheritance demonstrates that the sex ratio is close to being equal (144,145).

Several reasons could help to explain the underdiagnosis of CHH females:

(i) Over the last decade, there has been a refinement of the spectrum of GnRH deficiency in CHH in both males and more recently in females, as the hallmarks of CHH were for a longtime the complete absence of puberty for boys and girls, leading to an underevaluation of the prevalence of CHH in the past (113,115).

(ii) In the 90s, it was thought that X-linked CHH prevail and thus that female CHH were scarce. This schematic view was progressively challenged by the first descriptions of female CHH patients harboring biallelic *GNRHR* mutations where a wide range of pubertal development was described (117,119,146,147). Later, it was shown that variability of the pubertal developmental spectrum was not only restricted to normosmic forms or to a particular genetic cause but was extended to CHH women due to different autosomal genetic causes such as *FGFR1, PROK2 / PROKR2* or *SOX10* (55,118,148-152).

(iii) An ascertainment bias may be present given that women in some countries with mild, non-syndromic forms of CHH are more likely to be treated with contraceptives or hormone replacement therapy (HRT) by their general practitioner or gynecologist, rather than being referred to a reproductive endocrinologist at a tertiary teaching hospital to receive a complete work-up and accurate diagnosis.

5. Diagnosis of CHH

5.1 Clinical diagnosis

5.1.1 Minipuberty

Mini-puberty provides a brief window of opportunity to diagnose CHH. For male infants, micropenis with or without cryptorchidism can be suggestive of CHH. In such cases, hormone testing at 4–12 weeks of life may be used to assist in the diagnosis (52,118,153-158). Typically, GnRH deficiency during minipuberty is evidenced by low serum testosterone, LH and FSH levels (Table 3) based on comparisons with established reference ranges (38,159). However, hormonal testing is not routinely prescribed for male infants with micropenis or cryptorchidism. Further, normative reproductive hormonal data from a large group of controls during minipuberty is lacking. Finally, neonates born from one CHH parent should undergo evaluation of minipuberty with hormonal profiling. The lack of typical clinical features in females caused by the absence of GnRH secretion during minipuberty explains why the diagnosis of neonatal CHH is only rarely made in this group (121,157,160).

5.1.2 Childhood

During childhood, diagnosis of CHH is very challenging as this period is a physiologically hypogonadal period of development, consistent with the relative quiescence of the GnRH pulse generator.

5.1.3 Adolescence and early adulthood

Delayed pubery is the hallmark of a CHH diagnosis in adolscence. Between ages 14-16, CHH is difficult to differentiate from CDGP, a common cause of delayed puberty. Biochemically, both exhibit hypogonadal T or E2 levels and low/normal serum levels of gonadotropins due to GnRH deficiency. Although puberty eventually starts and is completed spontaneously in CDGP, absent puberty (TV < 4 ml) by age 16 is consistent with CHH (100). Similarly, incomplete puberty by age 18 also points to the diagnosis of CHH. However, CHH remains a diagnosis of exclusion. Therefore, a normal imaging of the hypothalamic pituitary area with otherwise normal pituitary function and the absence of risk factors for functional hypogonadotropic hypogonadism is required to confirm the diagnosis (see below).

Once the diagnosis is suspected, it is crucial to assess the onset and severity of GnRH deficiency, as these parameters will be used to tailor treatment. A history of micropenis or cryptorchidism in male CHH patients points to a severe and early onset (prenatal/neonatal form) of CHH (161). The absence of puberty onset by age 16 indicates severe GnRH deficiency, but does not preclude the activity of the HPG axis during minipuberty. Evidence of initial but then stalled pubertal development is consistent with partial CHH, and thus is a less severe form of GnRH deficiency.

5.1.4 Evaluation of CHH-associated phenotypes

It is important to evaluate the presence of CHH-associated phenotypes that may indicate a diagnosis of CHH and have significant utility for genetic counselling. We are detailing here the most common associated phenotypes.

1. History of cryptorchidism with or without micropenis
2. Decreased or absent sense of smell, suggesting Kallmann syndrome, is present in half of the CHH population and should be evaluated using a standardized olfactory test (143). Formal smell testing is especially critical, as 50% of CHH who self-reported a normal sense of smell are in fact hyposmic or anosmic by standardized testing (162); in very young children or in the absence of available olfactometry, MRI imaging may be useful as a surrogate for smell testing if it shows olfactory bulbs hypoplasia or aplasia (see below)
3. Congenital sensori-neural hearing impairment should be systematically evaluated with an audiogram, as hearing loss is usually mild or unilateral, and thus patients may be unaware of their deficit
4. Bimanual synkinesia (mirror movements)
5. Dental agenesis best assessed by panoramic dental X-ray
6. Cleft lip and/or palate, and other midline defects
7. Unilateral renal agenesis or malformation of the urinary tract, both of which should be assessed by renal ultrasound

8. Skeletal anomalies such as scoliosis, polydactyly, clinodactyly, etc

5.2 Biochemical testing

5.2.1 Gonadotropins

Most men and women with CHH have very low circulating gonadotropin levels (53,114,163), with most patients with absent puberty having apulsitile patterns of LH secretion (164). Patients with partial puberty can have low-normal circulating gonadotropins levels, which is inappropriate in the setting of low sex hormones (T or E2) (113,114) (Figure 4).

5.2.2 Estradiol

Females: Circulating estradiol levels in CHH women are usually low or in the lower end of the normal range during the follicular phase using sensitive assays [certain immunoassays or Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid chromatography-Mass Spectrometry (LC-MS)] that allow detection of estradiol concentrations below 10 pg/mL (114,165) (Figure 4). In contrast, the more commonly used immunoassays have a poor sensitivity, and thus are not accurate in this clinical setting (113,116). Insensitive estradiol assays may even result in misdiagnosis or confusion with other causes of anovulation (165).

Males: Two studies investigated the presence of estradiol deficiency in young men with untreated CHH. Trabado *et al*. used a sensitive radioimmunoassay to evaluate 91 untreated CHH men and showed a significant decrease in serum E2 levels when compared to post-pubertal healthy males (166). Serum estradiol levels correlated significantly with serum T, in agreement with the substrate-product relationship between these two sex steroids. Using GCMS, *Giton et al*. confirmed the profound decrease in circulating estradiol in CHH males (120), which was largely corrected after treatment with aromatizable androgens or human chorionic gonadotropin (hCG) (166).

608 **5.2.3 Testosterone**:

609 ***Males:*** The circulating testosterone level in CHH patients are usually low, i.e. less than 3 nmol/L. CHH
610 patients with partial pubertal development and larger testicular volume often also exhibit low T levels
611 similar to those with a complete absence of puberty (53).

612

613 ***Females*****:** Low circulating androgen levels (androstenedione and testosterone) are reported in women
614 with CHH despite normal circulating DHEA sulfate concentrations (114). This relative androgen
615 deficiency is likely subsequent to the inadequate stimulation of theca cells by low circulating LH. This
616 hypothesis has been reinforced by the increase in testosterone levels oberved in CHH women during
617 combined recombinant LH (rLH) plus recombinant FSH (rFSH) stimulation, whereas FSH alone had no
618 effect (114).

619

620 **5.2.4 GnRH test**

621 Pituitary gonadotropin response to a GnRH challenge test has been specifically evaluated in CHH men
622 and women (119).

623 ***Males***: In CHH men, the LH response is highly variable and correlates with the severity of gonadotropin
624 deficiency. However, the latter is already clinically reflected by the degree of testicular atrophy, which
625 questions the adding value of the GnRH stimulation test (117,151,167,168).

626 ***Females***: Pituitary gonadotrope response to GnRH test has only been evaluated in a few case reports
627 (119). In most GnRH deficient women, the peak LH response to GnRH stimulation was blunted relative
628 to normal women (119).

629

630 **5.2.5 Inhibin B**

631 ***Males***: Inhibin B is a hormone secreted by Sertoli Cells. Circulating inhibin B is an marker of Sertoli cell
632 number and function (169,170), and is under the control of FSH (171,172). Healthy seminiferous
633 tubules after puberty also regulate inhibin B production, likely through the regulation of spermatids

(173). Large studies in male CHH patients have evaluated the levels of serum inhbin B (56,163,174). Most CHH men with absent puberty and prepubertal testes +/- micropenis and cryptorchidism exhibit low serum inhibin B levels (< 30-60 pg/ml), indicating a reduced Sertoli cell population. This is consistent with the absence of GnRH-induced FSH stimulation of the seminiferous tubules during minipuberty (see above, Section 4.2.1.) (40,56,168). Higher serum inhibin B levels are encountered in a minority of patients with absent puberty, but in the majority of patients with partial puberty (53) or acquired hypogonadotropic hypogonadism (175) , consistent with a robust activation of the HPG axis during minipuberty. Serum inhibin B levels correlated well with testicular size (53). Low inhibin B levels have been shown to be a negative predictor of fertility (56). Further, a small study demonstrated a good discrimative value of serum inhibin B to differentiate severe CHH from CDGP (see below).

Females: Inihibin B is a marker of the number of antral follicules, and is secreted by the granulosa cells (176). Very few studies have investigated circulating inhibin B levels in CHH females (114). Low Inhibin B concentrations are reported in the range of prepubertal girls (177-179). One study demonstrated the critical role of FSH to stimulate ovarian inhibin B secretion as evidenced by increased inhibin B levels in response to rFSH alone, but no additional change in response to both rFSH and rLH (114).

5.2.6 Anti-Mullerian Hormone (AMH):

Males: Circulating AMH levels in male CHH patients have been studied during the neonatal period and in adulthood (before and after gonadotropin or testosterone treatment) (155,174,180). During minipuberty, CHH infants have low AMH, which can be normalized by rFSH and rLH treatment (155,161). Untreated CHH adults have high AMH levels, similar to the prepubertal levels in normal boys, indicating the immaturity of Sertoli cell population (174). rFSH treatment in previously untreated CHH patients will induce the proliferation of immature Sertoli cells, and thus increase AMH levels, while subsequent hCG treatment will increase intratesticular T levels and dramatically inhibit AMH (174).

Females: Circulating AMH concentrations were significantly lower in women with CHH than in healthy women (Figure 4)(114). This work showed the relative dependency of circulating AMH levels on pituitary gonadotropins. However, almost two-thirds of these patients had serum AMH levels within the normal range. The subgroup of CHH women with the lowest ovarian volume and antral follicular count were also those with lower FSH levels and significantly lower AMH levels. Thus, low AMH should not be considered a poor fertility prognosis, as fertility can be efficiently restored in these patients by both pulsatile GnRH and gonadotropin administration. Both treatments will induce an increase in AMH levels.

5.2.7 Other pituitary hormones

In the evaluation of CHH, it is important to rule out other pituitary defects by performing an exploration of the complete pituitary axis (e.g. to rule out hyperprolactinemia) (181) (See also Section 6). A baseline profile including measurments of prolactin, free T4, TSH, morning cortisol and IGF1 should be performed. In case of suspected pituitary insufficiency, appropriate dynamic challenge tests should be performed (181).

5.3 Radiological testing

Pelvic ultrasound

Studies on uterine morphologies in CHH women are limited (113,114,182). Pelvic or transvaginal ultrasound (when appropriate) demonstrated variable uterine hypoplasia (113-115,182) which correlated with the severity of estradiol deficiency (182) and endometrial atrophy (183). Ovarian volume (OV) in CHH females was evaluated in two recent studies, which showed a significant reduction in mean OV compared to healthy adult women of similar age (113,114). Notably, the decrease in OV is greater in KS than in normosmic CHH, consistent with a more severe GnRH deficiency in KS (113). The only study that quantified the number of ovarian antral follicles (AF) showed a significant decrease in

686 the average number of AF compared to normal, age-matched women, consistent with the low levels
687 of AMH (114). Thus, a combined decrease in OV and AF count is a phenotypic characteristic of CHH
688 women, and is often mistakenly considered by many infertility doctors as an indication of a poor
689 fertility prognosis. However, OV and AF respond favorably to gonadotropin stimulation in female CHH
690 (see below).
691
692 **Testicular ultrasound**
693 The measurement of testicular size is important to determine the severity of GnRH deficiency, as well
694 as to track the progress of testicular maturation during fertility treatment. While an orchidometer is
695 often used in clinical practice, testicular ultrasound (US) has the advantage to assess not only size but
696 also testicular localization. A study measuring 151 testes in 76 adults concluded that both methods
697 were equally accurate in the hands of an experienced clinician (184). More recently, a study comparing
698 testicular US and two types of orchidometers (Prader and Rochester) in 65 males (age 7-24 years) with
699 varicocele found a strong correlation between the results of the two different methods (185). As
700 expected, both orchidometers overestimated testicular volume by approximately 6 cc in comparison
701 to ultrasound, likely due to the interference of surrounding soft tissues. However, when assessing the
702 ability to distinguish asymmetry between the two testes, the sensitivity of orchidometers in detecting
703 a size difference of 10-25% was relatively low. Thus, ultrasound has the added value during baseline
704 evaluation to simultaneously assess testicular size in detail and rule out renal malformations during a
705 single evaluation. However, subsequent evaluations can be conducted reliably with an orchidometer.
706
707 **Brain MRI** is performed at baseline to exclude hypothalamic-pituitary lesions, and to assess defects in
708 the olfactory bulbs, corpus callosum, semilunar canals, cerebellum (156,186) and midline (187). KS
709 patients will typically exhibit unilateral or bilateral olfactory bulb agenesis, olfactory tract agenesis
710 and/or gyrus malformation associated with their anosmia/hyposmia (188). However, a few KS patients
711 present normal olfactory structures despite clinically confirmed anosmia. Further, an anomaly of the

semicircular canals is an important finding, as it could indicate the need for additional testing to explore diagnosis of CHARGE syndrome in these patients (189).

Bone density and microarchitecture: CHH work-up should include the measurement of bone mass via dual-energy X-ray absorptiometry (DXA) to assess bone mineral density (BMD) (160). Bone quality can be evaluated by processing a trabecular bone score (TBS) or by performing a high-resolution peripheral quantitative computed tomorgraphy (HR-pQCT). The latter provides a more detailed assessment of bone microarchitecture at peripheral sites (distal radius, tibia) (190). On the other hand,TBS is a textural index that evaluates pixel grey-level variations in the lumbar spine DXA image, providing an indirect index of trabecular microarchitecture. This index is readily available from the DXA scan (191). Bone work-up should be done at baseline and repeated at least two years after HRT to assess the beneficial effect of sex steroids on bone mass and guide subsequent monitoring. The use of FRAX, a clinical algorithm for assessment of fracture risk, has not been validated in this particular population (192).

5.4 Other tests

5.4.1 Olfaction

Olfactory function represents a hallmark in the clinical assessment of CHH, as approximately 50% of patients have a defect in the sense of smell and are defined as having Kallmann syndrome, also known as “olfacto-genital dysplasia” (193). Olfactory function is generally quite easy to test using semi-quantitative methods such as the UPSIT score (162) or the Sniffin’ Sticks (194,195) tests which gives age- and gender-matched scores relative to a reference population. Alternatively, smell function may also be assessed using volatile-stimulated chemosensory evoked potentials (196), however this is less practical in a clinical setting. Partial or subtle olfactory impairment may be seen in some patients (hyposmia or microsmia) raising the question of a continuum rather than a binary classification

(162,197). While a self-report of anosmia is sensitive and specific, the self-reporting of a normal sense of smell is unreliable (162). Therefore formal smell testing should be pursued for all CHH patients.

5.4.2 Hearing

The prevalence of hearing loss in CHH is reported to be between 5-15% (Table 1). Nevertheless, there are no large studies with systematic evaluations of hearing in CHH patients, as an audiogram is seldom performed during baseline evaluation. Hearing defects range from unilateral, mild hearing loss to complete bilateral sensorineural deafness, however conductive hearing loss is seldom encountered (143). Notably, the association of CHH with hearing loss points to specific genetic mutations (i.e. *CHD7, SOX10, IL17RD*) (140,160) and can guide for specific genetic testing (see Genetic Testing below).

5.4.3 Spermiogram

Spermiogram is defined as the quantitative and qualitative analysis of semen in order to assess male fertility potential (198). Among the primary parameters, ejaculate volume (which is testosterone dependent) as well as sperm motility and morphology are the most critical. The latest WHO criteria for interpretation of semen analysis were published in 2010 (199) based on semen samples from over 4500 men in 14 countries and defined the lower reference limits for the following parameters: 1.5 ml for semen volume, 15 million per ml for sperm count, 40% for total motility and 4% for normal morphology. Most CHH patients at baseline (particularly those with severe hypogonadism) exhibit severe erectile dysfunction and an absence of ejaculate, rendering a spermiogram impossible. However, with fertility treatment the majority of CHH males will develop sperm in their ejaculate. Interestingly, the concentration of sperm needed for fertilization in CHH patients is much lower compared to the WHO guidelines (200). In conclusion, spermiogram is indicated at baseline (when possible) and serially after the initiation of fertility treatment.

6. Genetics of CHH

6.1 Genetic determinants of pubertal timing

The timing of puberty varies widely in the general population and is influenced by genetic, environmental, and epigenetic factors (3). The studies of pubertal timing in families and twins provide evidence that 50–80% of this variation is caused by genetic factors (3-5). Recent genome-wide association studies (GWAS) in large populations shed light on the genetic determinants underlying the heritability of pubertal timing. By studying ~ 370,000 women of European ancestry, Day *et al*. reported ~ 400 independent loci robustly associated with the age at menarche (201). The individual effect size of each locus ranges from 1 week to 1 year, however the cumulative effect of all identified genetic signals only explains 7.4% of population variance in age at menarche. Similar results are seen in GWAS on pubertal timing in males using age at voice breaking as proxy for pubertal timing. A large number of the identified loci are implicated in BMI, height and epigenetic regulation consistent with the critical links between energy balance, growth and development, and reproduction. Further, a subset of loci implicated in the timing of puberty are located in imprinted regions, (e.g. *MKRN3* and *DLK1*) which exhibit significant effects when paternally inherited (201). Notably, a few menarche loci are enriched in or near genes that underlie CHH (e.g. *FGF8*, GNRH1, *KAL1*, *KISS1*, *NR0B1*, *TACR3*, etc.) or central precocious puberty (*MKRN3*). In conclusion, puberty timing is a highly polygenic trait, likely involving many individual genetic signals. Further studies on larger cohorts with well-studied phenotypes are needed to uncover genetic players and determine the contribution of gene-environmental interactions.

6.2 Genes involved in CHH

CHH is a complex and heterogeneous genetic disorder with approximately 60-70% of cases initially appearing sporadic (137). In familial cases, X-linked, autosomal dominant, and autosomal recessive patterns of inheritance have been observed. The first genetic breakthrough in CHH came in 1989 with the identification of an Xp22.3 deletion in a fetus with KS (138). Subsequent genetic studies identified mutations in *ANOS1* (formerly known as *KAL1*) within the previously reported Xp22.3 deletion as the

first gene underlying the X-linked form of KS (202,203). Since this initial discovery, mutations in more than 30 loci have been identifed via cytogenetic, candidate gene studies, linkage analysis (64,204,205), pathway analysis (206), and next-generation sequencing strategies (207,208). Mutations in these CHH genes act either alone or in combination (144,160) to result in CHH with or without anosmia. Genes underlying CHH are classified according to their function in the neuroendocrine control of reproduction: (i) GnRH fate specification; (ii) GnRH neuron migration/olfactory axon guidance; (iii) GnRH neuron homeostasis; and (iv) gonadotrope defects (e.g. *GNRH1* and *GNRHR*) (209) (Figure 5).

In this review, we discuss the most commonly mutated genes involved in CHH, as well as those which have a critical role in GnRH ontogeny and biology. The remaining genes associated with CHH are listed in Figure 5.

***ANOS1* (Anosmin 1)**

ANOS1 (OMIM 300836), encodes anosmin-1 and mutations in this gene are inherited in an X-linked fashion. Thus, males with a hemizygous mutation in this gene are affected, while heterozygous females are non-expressing carriers, apart from a few exceptional cases (113). Anosmin-1 is an extracellular matrix protein critical for the guidance of GnRH neurons towards the olfactory bulb during development (210). *ANOS1* mutations are seen almost exclusively in KS patients (frequency 5-6%) (206,211), and often result in severe GnRH deficiency. Synkinesia, unilateral renal agenesis, sensori-neural hearing loss are also described in KS patients harboring *ANOS1* mutations (137,143,212). Most mutations result in frameshifts and premature stop codons, although a smaller number of missense amino acid substitutions have been described (213-215). Hemizygous mutations in *ANOS1* are typically highly penetrant, and oligogenicity involving this gene has not been reported.

***FGFR1/FGF8* (Fibroblast growth factor receptor 1/fibroblast growth factor 8)**

FGFR1 (fibroblast growth factor 1, OMIM 136350) is the most frequently mutated gene in CHH patients across multiple studies ranging from 8-10% of CHH probands (206,211,216,217). It is inherited in a predominantly autosomal dominant fashion, may exhibit reduced penetrance, and can be inherited along with mutations in one or more other CHH genes (i.e. oligogenicity) (206,211,215,218). Studies in murine models showed that Fgfr1 is involved in both GnRH neurogenesis (219), olfactory bulb development (220) and GnRH neuron homeostasis (221,222).

Pathogenic *FGFR1* mutations have been reported in patients with varying degrees of GnRH deficiency. Patients harboring *FGFR1* mutations can have a spectrum of olfactory defects (from normal to anosmia) and a variable frequency of CHH-associated phenotypes such as cleft lip/palate, scoliosis, dental agenesis, and skeletal defects (149,168,216,223,224). Of particular interest is the association of CHH with split-hand foot malformation (225,226) wherein *FGFR1* mutations were detected in > 80 % of the patients (225).

In 2008, FGF8, a critical ligand for FGFR1 for fate specification of GnRH neurons, was identified through human genetics and murine models of CHH. Rare mutations with minor allele frequencies (MAF) of less than 1% in *FGF8* (OMIM 600483) were described in CHH patients with variable penetrance (227,228). CHH-associated phenotypes similar to those seen in patients with mutations in *FGFR1* were described in patients harboring *FGF8* mutations.

To expand the FGFR1/FGF8 pathway, genes within the tightly controled ”FGF8 synexpression group” (genes showing similar spatiotemporal expression patterns and developmental regulation as FGF8) were screened in CHH patients (206). Mutations in five genes within this group were identified in CHH patients, including *FGF17* (fibroblast growth factor 17, OMIM 603725), *IL17RD* (interleukin 17 receptor D, OMIM 606807), *DUSP6* (dual specificity phosphatase 6 OMIM 602748), *SPRY4* (sprouty homolog 4 (*Drosophila*), OMIM 607984), and *FLRT3* (fibronectin leucine rich transmembrane protein 3, OMIM

604808). Heterozygous mutations in these genes combined with mutations in other genes within the FGF pathway (*FGFR1*, *FGF8, ANOS1* and *HS6ST1*) were present in 23% of the CHH patients (206). Mutations were primarily found in KS patients, consistent with the role of FGF8 signaling in olfactory placode development and GnRH neuron fate specification (227,229). Oligogenic inheritance was observed, as well as reduced penetrance and variable expressivity (206).

***FGF21/KLB/FGFR1* signalling pathway**

Recently, heterozygous mutations in *KLB* (β-Klotho) have been described in CHH patients. KLB is an obligate co-receptor with FGFR1 for FGF21 (fibroblast growth factor 21, OMIM 609436), and is expressed during the post-natal period in the hypothalamus. Although no mutations were found for *FGF21*, 4% of CHH patients were found to harbor mutations in *KLB*, with the majority of these patients exhibiting a variety of metabolic defects including obesity, insulin resistance, and dyslipidemia (222). These results combined with additional murine studies suggest that the FGF21/KLB/FGFR1 pathway may be a link betwen reproduction and metabolism. Mutations in *KLB* exhibited incomplete penetrance, variable expressivity, and oligogenic involvement, however autosomal dominant inheritance was the primary mode of transmission (222).

***CHD7* (Chromodomain helicase DNA binding protein 7)**

CHD7 (OMIM 608892) encodes an important chromatin modulator and transcription regulation in stem cells of the olfactory placode and neural crest (230-232). Heterozygous *CHD7* mutations occur with a prevalence of approximately 6% in CHH patients (189,233-235). Mutations in *CHD7* are found in both KS and normosmic CHH (186,189,233-236). Notably, *CHD7* is the primary gene underlying CHARGE syndrome (coloboma, heart defects, choanal atresia, retardation of growth and development, genital hypoplasia, ear anomalies)—a developmental disorder exhibiting both clinical and genetic overlaps with CHH. *CHD7* mutations occur in approximately 60% of CHARGE syndrome patients, and

are primarily *de novo* and protein truncating variants (i.e. frameshift or nonsense) (237). In contrast, *CHD7* mutations in CHH patients are primarily inherited missense mutations (189,235,236,238). A recent work investigated CHARGE-associated features in a cohort of CHH patents harboring pathogenic *CHD7* mutations. Indeed, careful evaluation of these CHH patients allowed the reclassification of 3 out of 5 patients as having CHARGE or atypical CHARGE syndrome (189).

***PROK2* (Prokineticin 2) and *PROKR2* (Prokineticin receptor 2)**

PROKR2 (OMIM 607123) and *PROK2* (OMIM 607002) encode prokineticin receptor 2 and its cognate ligand prokineticin 2. PROK2 functions as a chemo-attractant during GnRH neuron migration and is also involved in GnRH secretion (239). Combined, mutations in *PROK2* and *PROKR2* are present in 4-7% of CHH patients (151,211) and can be found as heterozygous, compound heterozygous, or in homozygous states (151,240,241). More often, patients harbor heterozygous *PROKR2* mutations, and a dominant negative effect of some of these mutations has been demonstrated (242,243). Incomplete penetrance and/or oligogenicity is possible, given that even dominant negative effects do not fully account for the CHH patient phenotypes (241,243,244). Originally described only in KS patients (245), subsequent studies have reported mutations in normosmic CHH patients as well (246). Associated phenotypes in CHH patients harboring mutations these 2 genes include sensorineural hearing loss, scoliosis, and sleep disorders (140,245).

***SOX10* (SRY [sex determining region Y]-box 10)**

Heterozygous mutations in *SOX10* (OMIM 602229) were first described in Waardenburg syndrome type 4c, which is characterized by deafness, pigmentary abnormalities, and Hirschprung disease (247). SOX10 is a member of the SOX transcription factor family, and is involved in an array of multi-organ developmental processes. Notably, anosmia with absent olfactory bulbs is almost always present in patients with Waardenburg syndrome. This led to the discovery of heterozygous *SOX10* mutations in 3% of patients with only KS (152). Notably, in KS patients with hearing loss, the prevelance of *SOX10*

mutations rises to 30% (152,248). Additionally, other clinical features may exist in KS patients with mutations in *SOX10* that overlap with Waardenburg syndrome, specifically pigmentary abnormalities (i.e. abnormal iris pigmentation and isolated patches of white hair) (248-250) (Figure 3). Reduced penetrance, oligogenicity and variable expressivity have been observed (152,215,248,250-252).

***SEMA3A* (semaphorin 3A) & *PLXNA1* (plexin A1)**

A murine knockout model for Sema3A (semaphorin 3A) showed significant defects in both olfactory bulb development and GnRH neuron migration (253)—features strikingly similar to KS in humans. Shortly thereafter, mutations of *SEMA3A* in 6% of KS patients were reported, consistent with the murine model (254,255). Class 3 semaphorins often signal through interactions with the group of transmembrane receptors called plexins (and specifically through plexin A1). Further, a mouse knockout for PlexinA1 also exhibited a developmental phenotype similar to KS in humans (256). Thus, it is not surprising that heterozygous missense mutations in *PLXNA1* (plexin A1, OMIM 601055) were identified in 6% of KS probands (256). Mutations in either *SEMA3A* or *PLXNA1* were also involved in oligogenic inheritance. Additionally, a report of heterozygous mutations in *SEMA3E* (semaphorin 3E, OMIM 608166), a gene involved in GnRH neuron development, has been reported in two KS brothers (257). However, confirmation of mutations in *SEMA3E* in a larger cohort of CHH or KS patients has not yet been performed. Taken together, the biology of the semaphorin pathway combined with the reports of mutations in KS patients strongly supports the importance of this family of genes in the pathophysiology of CHH.

***GNRH1* (Gonodotropin releasing hormone 1) & *GNRHR* (gonadotropin releasing hormone receptor)**

Homozygous and compound heterozygous mutations in *GNRHR* (OMIM 138850) were first reported over 30 years ago in patients with normosmic CHH (117), (146,258). It was over a decade later before autosomal recessive mutations in *GNRH1* (OMIM 152760) were discovered (259,260), due to the relative rarity of patients harboring mutations in this gene. Combined, mutations in GNRHR and

GNRH1 are present in approximately 3-5%) of patients with CHH (145,211), and are found exclusively in patients with normosmic CHH. Mutations in *GNRH1* and *GNRHR* are inherited almost exclusively in an autosomal recessive mode and are highly penetrant (144,145). However, infrequent instances of oligogenic coupling with mutations in other CHH genes (primarily *FGFR1*) have been reported (206,215,218). Typically, patients harboring mutations in these two genes do not present with CHH-associated phenotypes.

***TAC3* (tachykinin 3) & *TACR3* (tachykinin receptor 3)**

***KISS1* (kisspeptin 1) & *KISS1R* (kisspeptin 1 receptor)**

TAC3 (OMIM 162330) and *TACR3* (OMIM 162332) encode neurokinin B and its receptor, respectively, while *KISS1* (OMIM 603286) and *KISS1R* (OMIM 604161) encode kisspeptin and kisspeptin receptor, respectively. Both neurokinin B and kisspeptin are expressed in a distinct subset of hypothalamic neurons called KNDy neurons (Kisspeptin, Neurokinin B and Dynorphin neurons) (261). KNDy neurons are upstream of GnRH neurons, and regulate the activity of GnRH neurons (261). Biallelic mutations in *KISS1R* were reported in 2003 (63,64) in CHH patients, and opened the field of exploration of the kisspeptin pathway in the pathogenesis of CHH. Subsequently, mutations in *KISS1* were also discovered in CHH patients (262) confirming the role of this pathway in GnRH neuron regulation. Using SNP arrays in combination with homozygosity mapping in consanguineaous families, homozygous mutations *TAC3* and *TACR3* were identified in CHH patients without associated phenotypes (263). Combined mutations in these two receptor-ligand pairs account for approximately 2-4% of CHH patients (64,65,145,264,265). Autosomal recessive mutations in these genes are highly penetrant, and oligogenicity is seldom seen.

6.3 Oligogenicity in CHH

Historically, CHH was originally thought to be a monogenic disorder, however reports of patients with more than one mutation in known CHH genes challenged this concept (218,245). This is consistent with

reduced penetrance and variable expressivity within and between families harboring identical mutations in the known CHH genes. The concept of oligogenic inheritance (a synergistic effect between mutations in 2 or more genes) was first reported for retinitis pigmentosa in the mid-1990s (266), and Bardet-Biedl syndrome (267). The first systematic evaluation of oligenicity in CHH patients by screening eight genes was conducted in 2010 and identified oligogenicity in 2.5% of CHH patients (211). Subsequent studies evaluating larger sets of CHH-related genes demonstrated an even larger degrees of oligogenicity ranging from 7% (17 genes screened) (206) to 15% (25 genes screened) (215).

It is often difficult to assess the combined effect of two mutants on a single patient, however Falardeau *et al*. reported a family with a male CHH proband and his father with CDGP (227). Genetic testing revealed digenic mutations in *FGFR1* (p.R250Q) and *FGF8* (p.K71E, *de novo*) in the proband, while only the *FGFR1* mutation was detected in the father (227). The functional assay investigating the downstream mitogen-activated protein kinase (MAPK) FGFR1 signalling showed a synergistic effect of the combination of these two mutations, consistent with the clinical picture of this family (227). Further, the double heterozygous mice (Fgfr1+/- x Fgf8+/-) showed a significantly larger reduction in the total number of GnRH neurons compared to single heterozygous Fgfr1+/- or Fgf8+/- mice (268). Altogether, these data suggest that oligogenic mode of inheritance plays an important part in CHH, and can partially explain the observed variable expressivity and incomplete penetrance.

It is important to note, however, that the exact functional mechanisms or biology underlying most of the oligogenic mutations reported in patients (206,215,245) has not been elucidated, despite the statistical enrichment of oligogenicity in the CHH population (211,215).

The advent of high-throughput sequencing has significantly enhanced the ability to detect multiple rare variants in individual patients. However, the assessment of a single variant's pathogenicity and the synergistic effects between variants remains challenging.

970

971 **6.4 Molecular testing and counseling**

972 Genetic counseling is useful to inform patients about the underlying genetics of CHH and to counsel
973 patients who want fertility treatment on the recurrent disease risk in their offsprings. A detailed family
974 history, including questions about pubertal development, infertility, and CHH-associated phenotypes
975 (e.g. anosmia, cleft lip/palate, missing teeth, digit defects) should be performed. Approximately 30%
976 of CHH probands have family members exhibiting CHH that extends beyond first degree relatives
977 (Figure 6, Pedigree 1). Further, isolated anosmia or delayed puberty within family members can be
978 considered as familial cases (137,139,150).

979

980 Given the complex nature of CHH genetics including oligogenic inheritance and a lack of definitive
981 clinical signatures to aid in gene selection, it is valuable to minimize cost and maximize diagnostic yield
982 for both diagnostic testing and genetic counseling. Testing for a CHH gene panel or whole exome
983 sequencing (WES) is currently an accepted method and results in a 30-50% diagnostic yield (144).
984 Notably, whole genome sequencing (WGS) is a promising technology, as it allows the detection of
985 exonic mutations missed by WES, and intronic or regulatory region mutations as well as genomic
986 insertions/deletions (269,270). This is a critical added value for WGS, as multiple micro-, partial-, and
987 full-gene deletions have been reported in the known CHH genes (63,254,271-273).

988

989 With the relative ease and decreasing cost of identifying variants in CHH genes using high-throughput
990 sequencing, the major challenge to date is the evaluation of pathogenicity for these variants. Variants
991 in highly penetrant CHH genes inherited in an X-linked manner (*ANOS1*) or those primarily inherited in
992 an autosomal recessive mode (*GNRHR/GNRH1, TAC3/TACR3, KISS1/KISS1R*) are evidently pathogenic.
993 Pedigree 2 (Figure 6) shows a clear autosomal inheritance of homozygous mutations in *TACR3* in the
994 proband. Importantly, in instances of homozygous mutations in families with no evidence of
995 consanguineous relations, the testing of parental DNA is critical. In the absence of parental DNA,

996 deletions of one allele resulting in the appearance of homozygosity cannot be ruled out by Sanger, and
997 therefore must be followed up with additional testing such as CGH array (144,254,274,275), multiplex
998 ligation-dependent probe amplification (MLPA) or fluorescent in situ hybridization (FISH) for
999 clarification and accurate genetic counseling. However, most CHH-associated variants in these genes
1000 are heterozygous, missense, and often inherited from asymptomatic parents.
1001
1002 Recently, novel standards and guidelines for evaluating genetic variants have been developed by the
1003 American College of Medical Genetics (ACMG) (276) and are especially useful in the clinical practice.
1004 The pathogenicity is determined based on 28 criteria, which integrate data from population studies,
1005 computational and predictive algorithms, functional assays, segregation analysis and others. Of special
1006 note, the presence of *de novo* mutations and the segregation of inherited variants are particularly
1007 informative to both pathogenic determination and genetic counseling. Therefore, collection of DNA
1008 samples from parents and other family members is especially critical in CHH, even when the case
1009 appears to be sporadic. This is especially important as *de novo* mutations in CHH genes have been
1010 reported (144,157,186,215,248,277,278). Pedigree 3 in Figure 6 clearly demonstrates the importance
1011 of evaluating parental DNA to demonstrate the *de novo* nature of the detected *FGFR1* mutation. This
1012 is of critical importance in genetic counseling regarding future offspring, however it is important to
1013 note that gonadal mosaicism, although rare, may be present in either parent.
1014
1015 Pedigree 4 (Figure 6) shows an example of the incomplete penetrance that can be observed in CHH
1016 patients. Specifically, while the proband and his sister harbor a pathogenic mutation in *FGFR1* that
1017 results in Kallmann syndrome, the mother carrying the same mutation exhibits no evident
1018 reproductive defect. In this instance, mutations in yet-to-be-discovered CHH genes, epigenetic effects,
1019 or environmental factors may contribute to the variable phenotypes observed. Similarly, the R250Q
1020 *FGFR1* mutation in Pedigree 5 shows variable expressivity as the father with the mutation only exhibits
1021 delayed puberty. However, when this mutation is combined with a *de novo FGF8* K100E mutation in

the proband, the full CHH phenotype is present. Indeed, functional studies on these two mutants demonstrated a synergistic effect, thus providing *in vitro* support for a model of oligogenicity (227).

In summary, the genetics of CHH is complex given the large number of loci discovered to date, with each accounting for only a small proprotion of patients. Further, variable expressivity, reduced penetrance (observed both within and across families, even those carrying the same mutation), and oligogenic inheritance further complicate the genetic counseling of individual patients. Therefore, it is advisable to (i) employ high-throughput sequencing (i.e. targeted panels, or preferrably whole exome or genome sequencing) to increase diagnostic yield (ii) collect detailed clinical information on both reproductive and associated phenotypes in the extended family, and (iii) test the proband, parents, and family members to aid in the proper ACMG classification of detected variants.

6.5 Clinical and Genetic overlap in CHH-associated syndromes

Progress in discovering genetic causes of disease have identified genetic overlaps of syndromes, and this is true for CHH. Several associated phenotypes have been described in CHH including anosmia, defining KS. The association of split hand-foot malformation (SHFM) and CHH (with or without anosmia) increased considerably the odds of carrying an *FGFR1* mutation (225). Interestingly, Hartsfield syndrome has holoprosencephaly and SHFM as the major phenotypes, but can often include cleft palate and olfactory bulbs agenesis as well (279). Several reports identified mutations in *FGFR1* in patients with Hartsfield syndrome (279,280). Thus, Hartsfield syndrome has a significant clinical overlap with KS that can be in a large part due to the genetic overlap. Similarly, reports show significant clinical and genetic overlap between KS and Waardenburg syndrome (See above and Table 2), with mutations in *SOX10* being present in both disorders (152,281,282).

Over the last few years, the importance of defining overlapping syndromes is becoming clinically relevant. Indeed, refining these associations of phenotypes (for example in CHH) greatly enhances the diagnostic yield of targeted gene screening. While *FGFR1* mutations occurs in approximately 10% of CHH patients, it is present in 87% of patients with both CHH and SHFM (225). Similarly, while SOX10 mutations underlie 4% of KS, mutations in this genes are found in 30% of patients with KS and hearing loss (160). These advances challenge the traditional phenotypic classification of syndromes.

So why do we have overlapping syndromes? In the case of *FGFR1*, it is a typical pleiotropic gene, meaning that it is involved in multiple developmental processes. *FGFR1* has a role in GnRH fate specification (227), olfactory bulb development (220), limb development (283), ear (284), kidney development (285,286) etc. As such, it is not surprising that decreased FGFR1 signaling underlies several syndromes that share a phenotypic overlap. The nature of the individual genetic mutations as well as potential oligogenic interactions may indeed underlie the phenotypic spectrum observed between these overlapping syndromes.

7. Differential diagnosis of CHH

7.1 Structural causes

Structural causes affecting the hypothalamic-pituitary axis may lead to central hypogonadism. These causes can be classified into tumors (pituitary adenomas, craniopharyngeomas and other central nervous system tumors), irradiation, surgery, apoplexy or infiltrative diseases (i.e. haemochromatosis, sarcoidosis and histiocytosis). Less commonly, head trauma or subarachnoidal haemorrhage can be associated with central hypogonadotropic hypogonadism (287-289). Most patients with structural causes have multiple pituitary hormone deficiencies in addition to central hypogonadism (288). In early adolescence, brain MRI is indicated in patients with delayed puberty and hypogonadotropic

hypogonadism when there is a break in growth spurt, pituitary hormone deficiency (including diabetes insipidus), hyperprolactinemia, and when there are symptoms of mass effect (headache, visual impairment, or visual field defect). In late adolescence or adulthood, brain MRI is indicated in patients with isolated severe HH (T < 5 nmol/L, high suspicions of CHH) and in patients with combined pituitary hormone deficiency, hyperprolactinemia or symptoms suggestive of mass effect (287,288,290).

A particular condition is hereditary hemochromatosis because unlike the above-mentioned conditions it can often provoque isolated HH with no additional pituitary deficiencies. The etiological hemochromatosis form that causes the most challenging diagnostic issues is juvenile hemochromatosis, linked with Hemojuvelin mutations because it can be revealed, as CHH, by absent or delayed puberty (291). Hemochromatosis can be confirmed by serum measurement of iron, ferritin and transferrin saturation coefficient (292).

7.2 Genetic causes : Combined pituitary hormone deficiency (CPHD)

CPHD is a rare congenital disorder characterized by impaired production of pituitary hormones affecting at least two anterior pituitary hormone lineages with variable clinical manifestations. CPHD may manifest as (i) isolated pituitary hormone deficiencies, (ii) a component of other syndromes (i.e. septo-optic dysplasia which combines CPHD with hypoplasia of the optic nerve or midline defects), or (iii) pituitary stalk interruption syndrome with ectopic posterior pituitary gland (293). To differentiate CPHD from CHH, biochemical assessment of pituitary function with measurements of IGF1, morning cortisol, TSH, and free T4 and prolactin is needed in addition to evaluating specific clinical manifestations of selective anterior pituitary hormone deficiency. Even subtle indications of insufficiency for one of the pituitary hormones warrants further testing with appropriate dynamic challenge tests and brain MRI (181).

7.3 Transient GnRH deficiency: constitutional delay of growth and puberty (CDGP)

During early adolescence, distinguishing CHH from CDGP is extremely challenging, as a delay in puberty is a hallmark of both diseases and hypogonadotropic hypogonadism is present in both. While GnRH deficiency is permanent in most cases of CHH, CDGP is a state of transient GnRH deficiency where puberty eventually begins and is completed without hormonal treatment (6). In addition, CDGP is a common cause of delayed puberty, whereas CHH is considerably more rare. Differentiating CHH from CDGP is crucial in order to allow an early diagnosis of CHH, avoid delay regarding hormonal replacement, and alleviate the psychological burden associated with delayed sexual maturation (160). We will review some features that may assist in this differential diagnosis, noting that while individual indicators may not provide a definitive resolution a combination of multiple indicators and clinical observation will strengthen arguments for or against a particular diagnosis (Figure 7):

Growth velocity was recently suggested to help differentiating the different etiologies of delay puberty (6), but was subsequently shown to offer no additional diagnostic value in separating between CDGP and CHH (102,108).

Testicular size may discriminate boys with CHH from those with CDGP. In a retrospective study of 174 boys with delayed puberty at age 14-15 years, TV (< 1 ml, measured clinically) showed a 100% sensitivity and 91% specificity to distinguish CHH from CDGP (294).

The presence of cryptorchidism and/or micropenis strongly argues in favor of CHH, reflecting the absence of sexual hormones during minipuberty (6,102). In a series of 174 boys referred to a tertiary center for evaluation of delayed puberty, cryptorchidism was present in 36% boys with CHH and only in 2% of boys with CDGP (108).

CHH-associated phenotypes argue against a diagnosis of CDGP. Most notably, anosmia unrelated to facial trauma, surgery or chemical exposure favors a diagnosis of KS. The presence of anosmia or other

CHH-associated phenotypes may favor a diagnosis of CHH, but must also be weighed against their frequency in the general population (Table 1).

A positive family history of CDGP cannot rule out CHH, as CHH families are often enriched with family members with CDGP (295). Additionally, autosomal dominant inheritance is seen in both CHH and CDGP (103).

Biochemical evaluation: To date, no biochemical marker can fully differentiate CHH from CDGP (296) in early adolescence. When GnRH stimulation test results in a flat gonadotropin response, CHH is probable. However, a normal gonadotropin response cannot distinguish between partial CHH and CDGP. Inhibin B levels may be a useful diagnostic adjunct, with low values suggesting severe GnRH deficiency (Varimo, 2017). Nevertheless, some overlap persists between partial CHH, CDGP and healthy controls (297,298), and there is no consensus on the optimal cut-off value, thereby highlighting the need for larger prospective studies. Further, AMH and INSL3 do not improve accuracy for differential diagnosis.

Genetic testing is a promising prospect, however evidence as to whether CHH and CDGP exhibit common or distinct genetic backgrounds remains unclear. Mutations in *IGSF10* have been reported in both CDGP and CHH families (299). A shared genetic basis is also partly supported by a previous work identifying putative pathogenic mutations of known CHH genes in 14% of CDGP probands (300), which was significantly higher than in controls. Further, meta-analysis of GWAS studies including 370,000 women on the age of menarche revealed more than 400 loci associated with the timing of puberty, several of which overlap with known CHH genes, such as *TACR3, GNRHR*, etc (201). Nevertheless, a recent study using whole exome sequencing in two cohorts of CHH and CDGP probands suggested distinct genetic architecture (215) with CDGP resembling the control population in terms of both the frequency of pathogenic variants in known CHH genes and the presence of oligogenicity. Confirmation

of these results with larger studies is needed and could lead to a broader use of genetic testing to complement clinical and biochemical data for diagnosis of CHH in adolescence.

7.4 Transient GnRH deficiency: Functional hypogonadotropic hypogonadism

Similar to CDGP (see above), functional hypogonadotropic hypogonadism (FHH) is difficult to differentiate from CHH. FHH (frequently termed as functional hypothalamic amenorrhea [FHA] in females) is a reversible form of GnRH deficiency, usually induced by stressors such as caloric deficits, psychological distress and/or excessive exercise (301,302). In adolescents, the frequency of FHH is rising (3-5% of the population among young woman, (303)) and can manifest as primary amenorrhea (304), further complicating its distinction from CHH. Interestingly, there is a genetic susceptibility in the inhibition of the HPG axis in the presence of predisposing factors. A shared genetic basis of CHH and FHA in women has been described (305).

For both genders, malnutrition due to an organic disorder such as coeliac disease, inflammatory bowel disease (Crohn, ulcerative colitis) or other chronic inflammatory and infectious states should be ruled out as the primary cause underlying a patient's hypogonadtropic hypogonadism before rendering a diagnosis of CHH.

7.5 Hypogonadotropic hypogonadism associated with metabolic defects

Currently, metabolic syndrome, obesity or diabetes are the most common disorders associated with adult-onset hypogonadotropic hypogonadism (306). Contrary to CHH, this disorder is characterized by mild GnRH deficiency most commonly occuring after puberty (306). It is thought that hypothalamic inflammation is one of the causative factors that alters the function of gonadotropin-releasing hormone (GnRH) neurons and/or pituitary gonadotroph cells (307). Notably, with the increasing incidence of childhood obesity, HH linked with metabolic syndrome is also on the rise in early

adolescence, especially in boys. It is characterized by delayed puberty and will be an increasing part of the differential diagnosis of CHH (308-310).

8. Treatment of CHH

With appropriate hormonal replacement therapy, CHH patients can develop secondary sexual characteristics, maintain normal sex hormone levels, reproductive life, and achieve fertility. There exist several regimens of treatment with different administrative routes. The choice of treatment depends on the therapeutic goal, the timing of treatment, and the personal preference of each individual patient. The advantages and disadvantages of different treatment regimens are summarized in Table 4 & 5.

8.1 Neonatal treatment of CHH

To date, hormonal therapy during the neonatal period is only applied in male patients exhibiting micropenis/cryptorchidism and hypogonadotropic hypogonadism (29,118,154,155,158,311). An equivalent therapy is not proposed in female patients, as the consequences of severe prenatal GnRH deficiency in females is not clear. Further, female infants with severe prenatal GnRH deficiency do not exhibit detectable alterations in internal or external genital development, thus increasing the difficulty for detection and early diagnosis.

In male infants with severe GnRH deficiency, the main goals of hormonal treatment during the neonatal period or early childhood are to increase the penile size and to stimulate testicular growth. Early reports in 1999 and in 2000 have described the benefit of early androgen therapy in boys with either CHH or CPHD (153,311). Testosterone treatment can increase penile size and stimulate scrotal

development. However, the changes in testicular volume and Sertoli hormone levels (inhibin B and AMH levels) were not reported.

In 2002, Main *et al.* reported the effect of subcutaneous injections of rLH and rFSH during the first year of life in a CHH infant born with micropenis (154). This treatment led to a growth in penile length (1.6 to 2.4 cm), and a 170% increase in testicular volume accompanied by an increase in inhibin B levels. Similarly, Bougnères *et al*. reported the use of gonadotropin infusion in two neonates—one diagnosed with CHH and the other with CPHD (155). In this study, rLH and rFSH were administrated subcutaneously via a pump for 6 months. This treatment not only corrected the micropenis in both patients (8 to 30 mm and 12 to 48 mm, respectively), but also induced testicular growth (0.57 to 2.1 ml and 0.45 ml to 2.1, respectively). Serum LH and FSH levels increased to normal or supranormal levels, leading to an endogenous secretion of T, INB and AMH. Similarly, Sarfati *et al.* reported another case with a perinatal diagnosis of KS based on presence of an *ANOS1* (*KAL1)* mutation, the detection of renal agenesis during fetal life, and the presence of micropenis at birth (118). The combined gonadotropins infusion from 1 to 7 months of age induced the normalization of testicular size (0.33 to 2.3 ml) and penis length (15 to 38 mm). Recently, Lambert & Bougneres reported the effect of combined rLH and rFSH injections in a series of eight male infants with either CHH or CPHD (158). All patients presented with either cryptorchidism or high scrotal testis at the time of diagnosis, and were treated with gonadotropin infusion. Apart from the increase in both penile length and testicular size, the authors observed complete testicular descent in 6 out of 8 cases. However, the effect of combined gonadotropin treatment on cryptorchidism in CHH infants will need to be formally assessed by randomized controlled trials. Further, the effect of such treatment on cryptorchid males without hypogonadism remains unknown.

Collectively, these studies suggest that combined gonadotropin therapy in male CHH patients during the neonatal period can have a beneficial effect on both testicular endocrine function and genital

development. This treatment may be superior to androgen therapy, as it stimulates Sertoli cell proliferation and the growth of seminiferous tubules, as evidenced by the marked increase in TV (161).

It is possible that the normalization of penis size in the neonate will lead to a normal adult penis size during subsequent pubertal virilization with exogenous testosterone or hCG, thus preventing the feeling of inadequacy often reported by CHH males with micropenis. In parallel, the increase in testicular size, which correlates with the increase in Sertoli cell mass, could lead to a better outcomes in terms of sperm output during fertility induction in adolescence or adulthood (29). Taken together, these data imply that combined gonadotropin therapy in males during the neonate period may attenuate the psychological effects of micropenis later in adolescence, and potentially improve sexuality and fertility in adulthood. However, there is no data to support such a treatment in female neonates.

8.2 Pubertal induction

8.2.1 Induction of female secondary sexual characteristics

The literature focusing on the induction of puberty in teenagers (and adult women) with CHH is limited. However, the therapeutic objectives are well-defined (160,312,313): to achieve breast development; to ensure external and internal genital organ maturity and other aspects of appearance consistent with femininity; and to promote psychosexual development with respect to emotional life and sexuality (105). In addition, puberty induction also increases uterine size, which is important for future pregnancy. Finally, optimizing growth in order to achieve a final height close to the predicted parental mean target is important, along with acquiring normal bone mineral density (313,314).

Most therapeutic regimens inducing feminization in CHH are not evidenced-based. Instead, they arise from expert opinions (160,313,315-317) partly due to the paucity of patients (314,317-320). Further, regimens have often mirrored Turner syndrome treatment (321). Thus, a dogmatic attitude is to be

avoided. We propose that the choice of treatment integrates the patient's opinion, while maintaining a favorable risk-benefit balance.

In practice, administering estradiol (orally or transdermally) to CHH girls induces feminization, however available protocols vary widely (318,319). As transdermal estrogen in adulthood is associated with a good efficacy profile and reduced cardiovascular events, it is reasonable to prioritize this formulation for pubertal induction (314).

Transdermal estradiol administration is often started at low doses (for instance 0.05–0.07 µg/kg nocturnally, from 11 years), with the goal of mimicking estradiol levels during early puberty. In older CHH girls when breast development is a priority, transdermal estradiol is started at 0.08–0.12 µg/kg (313,314,322). The estradiol dosage should then be increased gradually over 12–24 months. After maximizing breast development and/or after the break-through bleeding, cyclic progestagen is added. In the majority of CHH females, estroprogestin therapy is effective to induce harmonious development of the breasts and genitals. In turn, this increases the patient's sense of femininity, thus potentially contributing to a satisfactory emotional and sexual life (105). Estrogen treatment also increases uterine size (115), and estroprogestin therapy induces monthly withdrawal bleeding. However, this treatment does not restore ovulation. Finally, estrogen therapy induces a growth spurt and increases bone density in the majority of CHH female adolescent and older women (323). The treatment options are summarized in Table 4.

8.2.2 Induction of male secondary sexual characteristics

Therapeutic goals in the adolescent CHH male are also well defined: to induce virilization; to reach optimal adult height; to acquire normal bone mass and body composition; to achieve normal psychosocial development; and gain fertility. However, available treatment regimens may not always

cover all of these aspects. The hormonal treatment options for the induction of puberty in male CHH are presented in Table 5.

As with CHH girls, there is a paucity of literature and a lack of randomized studies comparing different treatment modalities, with only one randomized study including few CHH (324). Difficulties also arise from studies aggregating heterogeneous cohorts of CHH patients in terms of clinical presentation (i.e. degree of spontaneous puberty) and genetics.

Early treatment is crucial and usually involves an injectable testosterone ester such as testosterone enanthate (104,313,325). Pediatric endocrinologists treating younger patients (from 12 years of age) typically begin treatment with low-dose testosterone (for example, 50 mg of testosterone enanthate monthly) and gradually increase to full adult dose (250 mg every 2-4 weeks) over the course of 18–24 months. For CHH patients seeking treatment in later adolescence or early adulthood, a higher dose of testosterone can be used to induce rapid virilization. Initial testosterone doses (such as 100 mg testosterone enanthate monthly) can be quickly increased to 250 mg IM monthly. Such regimens induce secondary sexual characteristics and maximize final height (313,326). Side effects for T treatment include erythrocytosis, premature closure of the epiphysis (if doses are too high during the first year of treatment), and occasional pain and erythema at the injection site. Of note, testosterone treatment does not stimulate testicular growth or spermatogenesis (104,325), since intragonadal T production is needed to stimulate spermatogenesis. In contrast, increased testicular growth during testosterone treatment indicates CHH reversal and requires treatment withdrawal followed by hormone profiling (135).

Induction of testicular maturation

Gonadotropins are used for fertility treatments in adult CHH patients, but can also be used to induce pubertal maturation in adolescent CHH males. An additional advantage of gonadotropin treatment

compared to testosterone treatment is the stimulation of testicular growth and spermatogenesis. Therefore, gonadotropin treatment may offer important psychological reassurance in adolescents and enhance self-confidence. Varying treatment protocols including hCG alone or in combination with FSH have been used to induce puberty in boys (327-332). In a retrospective analysis of CHH boys, Bistrizer *et al.* showed a comparable virilizing effect of monthly testosterone injections and weekly hCG injections (5000 IU/week), but testicular growth was significantly larger in boys treated with hCG (327). A concern for high dose of hCG treatment is its potentially deleterious effect on germ cells with increased apoptosis, and thus negative consequences for future fertility (333). However, the deleterious effect of hCG has not been demonstrated in CHH males with cryptorchidism. Rohayem *et al*. studied a relatively large group of adolescents with delayed puberty before they reach full virilization, of which the majority had complete absence of puberty at baseline (n = 34) (334). The adolescents received low dose hCG (250-500 IU twice weekly) with increasing increments of 250-500 IU every 6 months, and rFSH was added once serum T achieved targeted pubertal level (5.2 nmol/L). This treatment led to a substantial increase in TV (bi-testicular volumes: 5 ± 5 to 34 ± 3 ml) and induction of spermatogenesis in 91% of patients.

Pretreatment with FSH in adolescents

The rationale behind priming with FSH alone in patients with severe GnRH deficient is that the mass of Sertoli cells is a predictor of future sperm output. FSH induces proliferation of immature Sertoli cells prior to seminiferous tubules maturation in rats (335) , *Macaca mulatta* (336), and probably also in humans ((337). Conversely, adult men with biallelic inactivating *FSHR* mutations exhibit small testicular size and variable degrees of spermatogenesis failure (338). In addition, it has been suggested that CHH patients with absent puberty +/- micropenis and cryptorchidism likely have a suboptimal Sertoli cell complement due to lack of minipuberty as evidenced by low inhibin B levels and could thus benefit from pre-treatment with FSH. A study of 14 gonadotropin-deficient boys treated with rFSH priming showed significant increases in inhibin B and TV in the absence of an increase in intragonadal T

production consistent with proliferation of Sertoli cells (339). A subsequent study (see below) showed similar results in adolescents and young adults (340). Thus, pretreatment with FSH prior to testicular maturation appears to compensate for the suboptimal Sertoli cell proliferation during late fetal life and minipuberty, and thus could be beneficial in adolescent males for future fertility. However, this treatment is intensive, requires frequent injections and close follow-up, and might not be optimal for all adolescent CHH patients. A large multicenter study to evaluate the benefits of pre-treatment with FSH in severe cases of adolescent and adult CHH is warranted.

8.3 Hypogonadism treatment in adults

8.3.1 Females

Estroprogestin (E-P) treatment is required in adult hypogonadal CHH females for maintaining bone health, increasing the sense of femininity, improving emotional and sexual life, and promoting general well-being. Estradiol can be given either orally (at a dose of 1–2 mg) or transdermally (50 µg daily by patch or 1–2 pumps of 0.06% gel daily) with a cyclic progestin regimen (e.g. micronized progesterone 200 mg or dydrogesterone 10 mg, daily during the last 14 days of the cycle) to avoid endometrial hyperplasia. The treatment should be maintained at least until the natural age of menopause. E-P treatment induces monthly withdrawal bleeding but does not restore ovulation. It is important to note that oral contraceptive pills are not the optimal treatment for CHH women due to the following arguments: (i) CHH females do not need contraception; and (ii) the effect of ethinylestradiol in bone health is less established than the effect of 17β-estradiol. In addition, there is no evidence of increased risk for thromboembolic events in CHH females on E-P substitution.

8.3.2 Males

Long-term androgen treatment is required in male CHH patients to maintain normal serum T levels, libido, sexual function, bone density and general well-being. The different regimens of T replacement therapy are summarized in Table 5.

Testosterone can be given as an injectable formulation (aromatizable androgen such as enanthate, cypionate or undecanoate) or transdermal application (163,325,341). The maintenance dose of testosterone is usually 250 mg of T enanthate IM every 2-4 weeks or 50-80 mg of testosterone gel daily (Table 5). The surveillance of trough serum T levels is important, as there exists considerable variation regarding the metabolism of exogenous testosterone products among CHH patients (136). For testosterone injections, the frequency of injections should be assessed according to the trough serum testosterone measurement, targeting a level of 10-14 nmol/l. For patients treated with testosterone gel, the target for random serum T level is between 15 and 20 nmol/l. The advantage of T gel is its pharmacokinetics with a more stable T concentration within the normal adult range, and the lack of minimally invasive injections. However, patients on T gel should avoid skin contact with others (partners or children) as there are known risks for hyperandrogenism in women or for precocious puberty in children. Whatever the treatment used, CHH men are challenged to adhere to long-term treatment and poor adherence may contribute to adverse effects on bone, sexual and psychological health (129).

8.4 Fertility treatment

8.4.1 Induction of fertility in females CHH

Infertility in women with CHH is caused by impaired pituitary secretion of both gonadotropins, LH and FSH, leading to an impaired ovarian stimulation. Specifically, GnRH deficiency leads to an impairment in follicular terminal growth and maturation resulting in chronic anovulation. However, there is no evidence of a decreased follicular reserve (114). This point must be emphasized to patients and their families as soon as the diagnosis is made. Indeed, the combination of small ovaries, decreased antral follicular count, and low circulating AMH concentrations observed in women with CHH could wrongly suggest an alteration in ovarian reserve and a poor fertility prognosis (114). In contrast, these patients

should be informed that ovulation induction will lead to a fairly good outcome in terms of fertility in the absence of a male factor of infertility or significantly advanced age (> 35 years) (114,115,342-344).

Before considering ovulation induction, sono-hysterosalpingography or traditional hysterosalpingography must be performed in order to evaluate both the integrity and the permeability of the uterine cavity and fallopian tubes (345). Further, an associated male infertility factor should be ruled out by obtaining a semen analysis (344). Couples should be advised on the optimal timing of sexual intercourse during the ovulation induction, as this first-line therapy does not require *in vitro* fertilization (114,115,342,343).

The goal of ovulation induction therapy in female patients with CHH is to obtain a mono-ovulation to avoid multiple pregnancies. Ovulation can be achieved either with pulsatile GnRH therapy or stimulation with gonadotropins. The latter includes either extractive or recombinant (r) FSH treatment followed by hCG or rLH to trigger ovulation (346). The therapeutic choice will depend on the expertise of each center and the local availability of the different medical therapeutics.

Pulsatile GnRH treatment

Pulsatile GnRH therapy via a pump was first proposed by Leyendecker *et al*. to induce ovulation in women with different causes of hypogonadotropic amenorrhea (WHO I, anovulation) (347-349). Given its remarkable efficiency in acquired forms of HH, pulsatile GnRH was successfully applied to CHH women (350) and other causes of acquired HH (351-353). Both subcutaneous and intravenous routes for GnRH administration are appropriate to restore fertility (351,354). Pulsatile GnRH restores the physiological secretion of pituitary gonadotropins, which in turn induces ovulation in CHH patients (259,264,355-357). The major advantage of pulsatile GnRH therapy compared to gonadotropin treatment is the decreased risk of multiple pregnancy or ovarian overstimulation (351,352,357). Consequently, it requires less monitoring and surveillance during treatment. Therefore, pulsatile GnRH

1405 treatment should be considered the first-line of therapy in CHH females, given that it is the most
1406 physiological regimen and results in fewer side effects.
1407
1408 Physiologically, GnRH pulse intervals vary throughout the menstrual cycle, as evidenced by LH pulse
1409 studies in a large series of women with regular menses (358). Based on this study, the frequency of
1410 GnRH pulses is set for every 90 minutes during the early follicular phase of treatment, and
1411 subsequently accelerated to every 60 minutes during the mid and late follicular phase. After ovulation,
1412 the frequency is reduced to every 90 minutes. Finally, during the late luteal phase, there is a further
1413 decrease to every 4 hours that will favor FSH secretion over LH. However, pulsatile GnRH at a constant
1414 frequency of 90 minutes also induces maturation of ovarian follicles, an LH surge and ovulation (359).
1415
1416 The dosage of GnRH required to restore normal ovulation has been well studied in females with CHH
1417 or functional hypothalamic amenorrhea. Intravenous doses of 75 ng per kg per pulse are considered a
1418 physiological dose to induce adequate pituitary gonadotropin secretion and ovarian stimulation (360).
1419 In 30% of CHH females, additional pituitary resistance is present, requiring increased GnRH doses and
1420 longer stimulation (356). Once ovulation is achieved, the corpus luteum must be stimulated to produce
1421 progesterone, which is mandatory for embryo implantation. The pulsatile GnRH pump is able to
1422 maintain endogenous pulsatile LH secretion sufficient to ensure progesterone release by the corpus
1423 luteum until the endogenous secretion of hCG from the placenta begins (357,361). Another treatment
1424 option for luteal support is hCG (1500 IU every 3 days for 3 times). Injections of hCG are less costly and
1425 well tolerated. The success rate of ovulation induction is excellent in CHH females, reaching 90%
1426 ovulation per cycle, and 27.6% conception per ovulatory cycle. The number of cycles needed to obtain
1427 a pregnancy is quite variable, ranging from one to six cycles (354,357). Multiple pregnancy rate is
1428 slightly higher than the general population at 5-8% (360), but much lower than with gonadotropin
1429 therapy. Notably, pulsatile GnRH pump can be effective even in the presence of GnRH resistance, such
1430 as in women with CHH who harbor partial loss-of-function mutations in *GNRHR* (355,356).

When administered subcutaneously, higher doses (15 mcg per pulse) are needed, and typically the frequency of pulses are kept at one every 90 minutes. The success rate is slightly lower at 70% of ovulation rate per cycle (362). However, the subcutaneous administration has no risk of phlebitis, and is more convenient.

GnRH pulse treatment is discontinued when pregnancy occurs, and adverse effects in early pregnancy have not been reported (363). After several unsuccessful cycles of GnRH stimulation, gonadotropin therapy should be proposed (see below) (342,343) to bypass a potential pituitary resistance associated or not with loss-of-function *GNRHR* mutations. (146,356).

Gonadotropin treatment

In CHH women, ovulation can also be achieved with FSH treatment followed by hCG or rLH to trigger ovulation. Women with severe GnRH deficiency have very low gonadotropin levels, thus requiring both FSH and LH during the follicular phase. LH stimulates the ovarian theca cells to produce androgen substrates allowing sufficient secretion of estradiol by the maturing follicles (114,183,342,364). Estradiol is necessary for optimal endometrial thickness and cervical mucus production, which in turn are needed for sperm transit and embryo implantation (114). Typically, subcutaneous hMG (human menopausal gonadotropins, FSH + hCG) doses of 75–150 IU per day are sufficient to induce ovulation. Usually, a dominant follicle (>18 mm) will mature in approximately 12 days. The starting dose of hMG is often increased or decreased depending on the ovarian response, as assessed by repeated serum estradiol measurements or by using ultrasonography to count and measure maturing follicles every other day. This regimen minimizes the risk of multiple pregnancies and ovarian hyperstimulation syndrome. After ovulation, progesterone production can be stimulated by repeated hCG injections, or direct administration of progesterone during the postovulatory phase until the end of the luteal phase.

***In vitro* fertilization**

If conception fails after repeated successful ovulation induction in CHH females, *in vitro* fertilization may be an alternative (365,366).

8.4.2. Induction of fertility in CHH males

CHH is one of the few medically treatable causes of male infertility (344). Likely due to very small testicular size in the majority of patients, many doctors will simply assume that the patient is irremediably infertile. However, fertility treatments in CHH males have very good outcomes. Fertility induction can be accomplished either by long-term pulsatile GnRH therapy or with combined gonadotropin therapy.

Pulsatile GnRH treatment

Pulsatile GnRH treatment is a logical approach in patients with CHH seeking fertility. Physiological GnRH secretion is episodic, and therefore GnRH treatment requires intravenous or subcutaneous GnRH administration in a pulsatile manner via mini-infusion pump (367). This therapy will stimulate gonadotropin secretion and in turn intragonadal testosterone production, resulting in the initiation and maintenance of spermatogenesis as evidenced by increased testicular volume and sperm output by 12 months of treatment on average. The common initial dose is 25 ng/kg per pulse every 2 hours, with a subsequent titration to normalize serum testosterone to the adult normal range (56,368-370). Response to treatment varies according to degree of GnRH deficiency with normalization of TV and successful induction of spermatogenesis for all patients with partial puberty. On the contrary, TV and sperm counts are lower in patients with absent puberty and 18% of these patients remained azoospermic despite 12-24 months of pulsatile GnRH treatment (56). A systematic literature review on this issue is listed in Table 6.

Gonadotropin treatment

Gonadotropin treatment (hCG alone or combined with rFSH) is another treatment option for fertility induction in male CHH patients. While intramuscular (IM) injections were prescribed in the past, subcutaneous gonadotropin injections are currently preferred, and various formulations are used. Typical doses vary from 500 to 2,500 UI 2-3 times a week for hCG, and from 75 UI to 225 UI 2-3 times a week for FSH preparations, namely hMG, highly purified urinary FSH (uFSH) or recombinant FSH (rFSH). The dosage of hCG is adjusted based on trough serum T, and rFSH dosage is titrated based on serum FSH levels and sperm counts.

Fertility outcomes in CHH men

From the early 1970s to 2017, a series of forty papers were published that address fertility and spermatogenesis in CHH patients, and included more than one thousand CHH patients (Table 6). More than 80% of the patients reported in the literature have been treated by combined gonadotropin therapy. Although the GnRH pump is an effective therapy to induce spermatogenesis in the absence of pituitary defect, the significant use of gonadotropins may indicate that GnRH therapy is not available in several countries around the world, including the US where it has been largely used only in a research setting. Further, this therapy is expensive and likely less comfortable than gonadotropin injections given the long period (1-3 years) needed to mature the testes.

The systematic review of published studies demonstrated the effectiveness of both pulsatile GnRH and gonadotropin therapy to induce spermatogenesis and fertility in men with CHH (371-373), however no clear superiority of GnRH versus gonadotropins was observed. Similarly, none of the available FSH preparations appear to differ in terms of sperm output.

The overall success rate in term of sperm output was variable across studies (64 to 95% success), with sperm counts ranging from zero to several hundred million/ml. It is well established that even low sperm concentrations in CHH men are sufficient to impregnate partners (200). The weighted average

median time to achieve sperm production was slightly over a year (Table 6). Pregnancy was successfully achieved in 175 CHH patients' partners (Table 6), and successful pregnancies were reported in 16 to 57% of CHH patients desiring fertility. Conversely, 192 patients were not able to produce sperm despite long-term gonadotropin treatment (median 24 months), corresponding to 12-40% depending upon the study. In patients with azoospermia after treatment or poor sperm quality, more invasive treatments such as testicular sperm extraction were proposed followed by intracytoplasmic spermatozoid injection (ICSI) (374), however the outcomes are not clearly outlined in these studies.

The major limitations of most studies are (i) the often small population size, (ii) the inclusion of all types of patients with hypogonadotropic hypogonadism (i.e. severe, partial, or adult onset HH, which are known to have different outcome in terms of fertility); (iii) the inclusion in some studies of cryptorchid men with variable dates of surgery postnatally that could also impact prognosis; (iv) the absence of studies taking into account the genetic mutations as a predictor for treatment outcome; and (v) the absence of prospective randomized studies comparing head-to-head gonadotropin treatment to pulsatile GnRH therapy.

Despite these limitations, there are some lessons to be learned: (i) sperm counts may improve but rarely normalize in CHH patients based on WHO criteria; (ii) low sperm concentration does not always preclude fertility in men with CHH; and (iii) several predictive factors have been identified in this population:

Testicular volume. TV is an indicator of the degree of GnRH deficiency and is a positive predictor of sperm output (56). When we consider the entire population of CHH treated for infertility (n=994), the average testicular size was 3.5 mL at baseline and increased to 8.6 mL by the last visit. However, the spectrum of TV at baseline varies widely within and across studies. Thus, it is not surprising that studies including patients with milder forms of GnRH deficiency had the best sperm output (Table 6). In

contrast, studies in which the majority of CHH men exhibited prepubertal testes tended to have the poorest results. These patients usually lack the beneficial stimulatory effects of gonadotrope activation during the minipuberty. Based on these results, a randomized study including pre-treatment with rFSH prior to GnRH was performed (See below, (340)).

Cryptorchidism. The presence of unilateral or bilateral undescended testes reflects the severity of gonadotrope axis deficiency, and is thus one of the main features of antenatal-onset GnRH deficiency. Cryptorchidism is recognized as a negative predictor of sperm output, and patients with bilateral cryptorchidism have lower sperm counts than those with the unilateral variant or those without cryptorchidism. Also, cryptorchid patients require a longer time to attain spermatogenesis (56). Despite >1,000 CHH men included in the various studies focusing on spermatogenesis/fertility, only 19.4% had cryptorchidism. Further, in 42% of studies no patients with cryptorchidism were included. Furthermore, 30% of studies explicitly excluded cryptorchidism because of an expected poorer spermatogenesis prognosis. A number of factors may be involved in the cryptorchidism-related germ cell depletion, including apoptosis of germ cells in a testis that remains too long in the abdomen (375). In this setting, a surgical correction should be recommended as early as 6 months to 1 year of age (376).

Prior exposure to androgens. A single study considered prior androgen therapy to be associated with a poorer prognosis (377), but this result was not reproduced in subsequent studies (56,378-381). Thus, the impact of prior androgen treatment on fertility remains controversial.

Pretreatment with FSH

As detailed above, the fertility outcome with GnRH or classical gonadotropin therapy is suboptimal. In 2013, a randomized study explored the addition of rFSH pre-treatment to standard GnRH pulsatile therapy in young adults with severe GnRH deficiency (TV <4mL) and no prior gonadotropin therapy

(340). Patients with cryptorchidism were excluded in this study. After 4 months of rFSH alone, rFSH increased inhibin B levels into the normal range and significantly doubled mean testicular volume from 1 to 2 mL in the absence of increased intragonadal T. Further, histological findings demonstrated increase in the diameter of the seminiferous tubules compared to baseline without any sign of maturation, as well as enhanced proliferation of immature Sertoli cells and spermatogonia (340). Following 2 years of pulsatile GnRH, both groups (with and without rFSH pre-treatment) had normalized serum T levels and exhibited significant testicular growth. All patients in the pre-treatment group developed sperm in their ejaculate (versus 4 out of 6 in the GnRH-only group) and showed trends toward higher maximal sperm counts, although this did not reach statistical significance. Thus, larger prospective multicenter studies are needed to support the superiority of pre-treatment with FSH prior to classical treatment (GnRH or hCG+FSH) on improving fertility outcomes in patients with severe GnRH deficiency, with and without cryptorchidism.

8.5 Management of adverse health events related to CHH

8.5.1 Bone loss and fracture

The recent mixed longitudinal study employing 2014 healthy children has significantly improved our understanding of skeletal development; McCormack et al. showed that (i) at age 7 years, healthy children had obtained only 29.6%-38.1% of maximal observed whole body mineral content (BMC); (ii) during puberty, a significant gain in BMC occurred, (iii) the mean age at peak rate of whole BMC aquisition was 14.0 years in boys, and 12.1-12.4 years in girls (382) which was, on average, 0.6-1.2 years after the peak height velocity, and (iv) another 6.9% to 10.7% of maximal observed BMC was gained after linear growth had ceased (382). The relative roles of androgens and estrogens in bone metabolism in bone health was recently investigated in adult men with an elegant model, in which endogenous sex steroids were suppressed with goserelin acetate and the patients were subsequently treated with increasing doses of testosterone only, or in combination with aromatase inhibitor anastrozole to suppress conversion of testosterone to estradiol (383). The results from this study

demonstrated that bone resorption increased markedly once estradiol levels were low even if serum testosterone was substantially elevated (383). Estradiol deficiency, generated in this model, primarily affected the cortical bone, and cut-offs of <10 pg/ml for E2 and < 200 ng/dl (6.9 nmol/l) for testosterone (with intact aromatization) were suggested undesirable for bone health (383).

Consistent with these data, low BMD is present in the majority of CHH patients with variable degree of bone defects. Bone remodeling is mostly low as suggested by the only study that performed histomorphometric analysis of iliac crest bone biopsies of CHH patients with low bone mass (384). Data on bone remodeling markers are inconclusive and do not always correlate with BMD (385). Evidence on fracture incidence is scarce with some reports of incidental vertebral fractures but no comparison of prevalence against controls (385,386).

Given the importance of pubertal surge of sex steroids for peak bone mass, it is not surprising that bone deficits have been recognized in patients with CHH (384,385,387). Nevertheless, important variability exists regarding the degree of bone involvement in CHH patients, as illustrated by a recent report of older never-treated CHH patients with near-normal BMD and no significant difference compared with patients treated by HRT (388). In particular, the prevalence of low BMD in a cohort of untreated CHH patients has never been explored.

HRT is the first-line treatment for CHH-associated bone loss, with antiresorptive drugs (bisphosphonates, denosumab) as second-line therapeutic choices (389). HRT also maintains lean mass. Given the male gender predominance of CHH, the effect of gonadal steroid replacement has been principally studied in males receiving testosterone and/or gonadotropins. Testosterone increases BMD in CHH (384,390) and mixed hypogonadal cohorts (391-394). Increased levels of bone formation markers such as P1NP, usually observed early in the course of treatment, possibly reflect the anabolic

effects of androgens (395,396). It remains unclear whether testosterone replacement fully reverses the bone phenotype (391) or only partially improves BMD (390).

Age at onset of HRT might be a crucial prognostic factor for the therapeutic response. In the first study exploring the link between CHH and bone, Finkelstein *et al* described bone densities measured by computed tomography in 21 men with isolated GnRH deficiency, of whom 15 initially had fused epiphyses and 6 had open epiphyses. The majority of patients had received prior androgen treatment. After bringing testosterone levels to within the normal range, the younger group increased both cortical and trabecular bone densities, whereas those with initially fused epiphyses displayed only an increase in cortical bone density (384). The authors hypothesized that this difference reflects the physiological bone accretion that occurs during normal sexual maturation. Significantly, an inverse correlation between age at initiation of hormonal replacement and bone outcomes is found in some studies, further supporting this hypothesis (390,393,394). These data imply, thus, that there is a critical period of skeletal response to sex steroids, which would further stress the importance of timely diagnosis of CHH. Nevertheless, another study focusing on older CHH patients (median age of 56 years) revealed substantial bone response to testosterone replacement despite delayed diagnosis and onset of HRT (124).

Therapeutic adherence may also explain the variability observed. Highlighting the importance of compliance to HRT, Laitinen *et al* demonstrated that prolonged cessations in HRT (more than 5 years in total) were associated with decreased bone mineral density in the lumbar spine, hip, femoral neck and whole body, although no difference was observed in fracture prevalence (385).

It should be noted that some genes involved in CHH may also have direct implications on bone health, which may confound the results reported from the small series of CHH men. Specific genetic causes that may directly affect bone include *FGF8*, *FGFR1* and *SEMA3A* (397,398).

Despite the importance of estrogen for male skeleton, measurement of estradiol is not routinely performed in CHH patients with bone defects. This attitude is based on the fact that standard testosterone treatment is aromatizable and corrects low estrogen levels (166). However, this should be considered in cases with suboptimal response to HRT and after excluding more frequent causes such as inadequate compliance.

As in other causes of secondary osteoporosis, adequate calcium intake (> 1000 mg/day) should be assured. Vitamin D deficiency is prevalent in CHH population (386) and should also be corrected. Targeting levels > 30 µg/l (= 75 nmol/l) is reasonable in the presence of low BMD. A small retrospective study suggested that the central hypogonadism as seen in CHH might lead to worse bone outcomes as compared to primary hypogonadism independently of gonadal steroids levels (399). The authors postulated that this link is mediated by more severe vitamin D deficiency in CHH due to decreased LH-dependent vitamin D 25-hydroxylation in the testes. Nevertheless, no difference in vitamin D levels was detected in a larger cohort of CHH patients in comparison with age- and BMI-matched controls (400). Further studies addressing this issue should focus on removing the bias of seasonal variation of vitamin D.

8.5.2 Metabolic defects

Metabolic defects are present in CHH patients and are commonly thought to be secondary to sex steroid deficiency (307,401). The prevalence of overweight and obesity in CHH patients is between 40-50% according to a recent nationwide Italian cohort of patients (116), similar to the general Italian population (402). However, the prevalance of metabolic syndrome is increased in CHH in comparison to the general population, as illustrated by another study (403). The latter compared 332 young CHH patients without prior androgen treatment versus 395 age- and BMI-matched controls and revealed

significantly increased prevalence of all components of metabolic syndrome (waist circumference, arterial blood pressure, fasting glucose, HOMA-IR, serum triglyceride levels).

The mechanism underlying this association has been more extensively studied in men. Testosterone stimulates glucose uptake in insuline-responsive tissues including skeletal muscles, cardiomyocytes and adipocytes (404-406) by increasing translocation of glucose transporter type 4 (GLUT4). In addition, androgens directly increase muscle mass and inhibit the visceral fat deposition, and act on the liver to promote lipid oxydation (307,407,408). Further, the relationship between testosterone and metabolic dysfunction is bi-directional with extreme obesity being accompanied by hypogonadotropic hypogonadism, a phenotype reversed after weight loss (409).

It is thus not surprising that sex steroid deficiency leads to unfavorable changes in body composition, glucose metabolism and inflammation status. Several studies have shown an improvement of metabolic profile when testosterone is started in CHH and in a lesser degree in men with late-onset hypogonadism, in hypogonadal men with diabetes (307). Testosterone therapy in CHH leads to an improvement in insulin sensitivity (410,411), a reduction in high-sensitivity C-reactive protein levels (410) and LDL cholesterol (412), as well as increased lean mass and decreased visceral adiposity (411). Further, short-term withdrawal of testosterone therapy in male CHH patients causes mild insulin resistance and increased fasting glucose levels (401). Similarly to testosterone, gonadotropin replacement therapy resulting in T production is accompanied by increased lean mass, reduced body fat and waist-to-hip ratio, increased insulin sensitivity and reduced triglycerides levels (413). Surprisingly, a large retrospective study of 208 male CHH patients in Turkey reported an increase in waist circumference, blood pressure and lower triglycerides levels following testosterone replacement (403). However, the patients included in this cohort were healthy with normal weight (mean BMI 21.9 kg/m^2) and absence of insulin resistance (mean HOMA-IR 2.04) at baseline.

It is possible that genetic determinants predispose certain CHH patients to metabolic distrurbances. As discussed above, leptin deficiency or resistance leads to defective signaling of different metabolic cues to the hypothalamus, which normally regulate both energy homeostasis and reproductive capacity (414). Recently, the FGF21/KLB/FGFR1 pathway was also highlighted as an important player underlying the link between reproduction and metabolism (222). In this study, the majority of CHH probands harboring *KLB* mutations (9/13) exhibited some degree of metabolic defect (i.e. overweight, insulin resistance, and/or dyslipidemia), consistent with the potential role of this pathway in metabolic health.

9. Conclusions

Despite a relatively straight-forward diagnostic criteria, the phenotypic spectrum of CHH is broad. This includes a significant proportion of reversal cases, an overlap with common reproductive disorders such as CDGP and FHH, and the presence of CHH as a component of more complex entities such as CHARGE and Waardenburg syndromes. Timely diagnosis is required, yet, the clinical presentation and biochemical profile are often not fully informative in early adolescence as the presentation of CHH closely resembles that of CDGP. One possible opportunity for earlier diagnosis is during minipuberty, but currently the importance of evaluating minipuberty is not known, and large normative datasets are lacking. The advance of biochemical testing with minimal blood samples (e.g. blood dry-spots) offers the potential to assess the HPG axis function in neonates in normal and disease states.

In terms of genetics, the discovery of genes involved in GnRH ontogeny have helped to elucidate the pathophysiology of the disease and have assisted in rendering an accurate diagnosis. The advent of high-throughput sequencing technologies have significantly increased the identification of rare variants. However, this results in a specific challenge to classify for pathogenicity, especially in the

context of the oligogenicity seen in CHH. Large, multi-national studies are required to define CHH genetic risks associated with the spectrum of rare variants.

Finally, regarding the treatment of CHH, we have successful therapies to induce secondary sexual characteristics. However, we do not know the added value of starting gonadotropins in the neonatal period or adolescence to mature the gonads instead of waiting for adulthood in terms of improving future reproductive capacity and increasing self-esteem in male CHH patients.

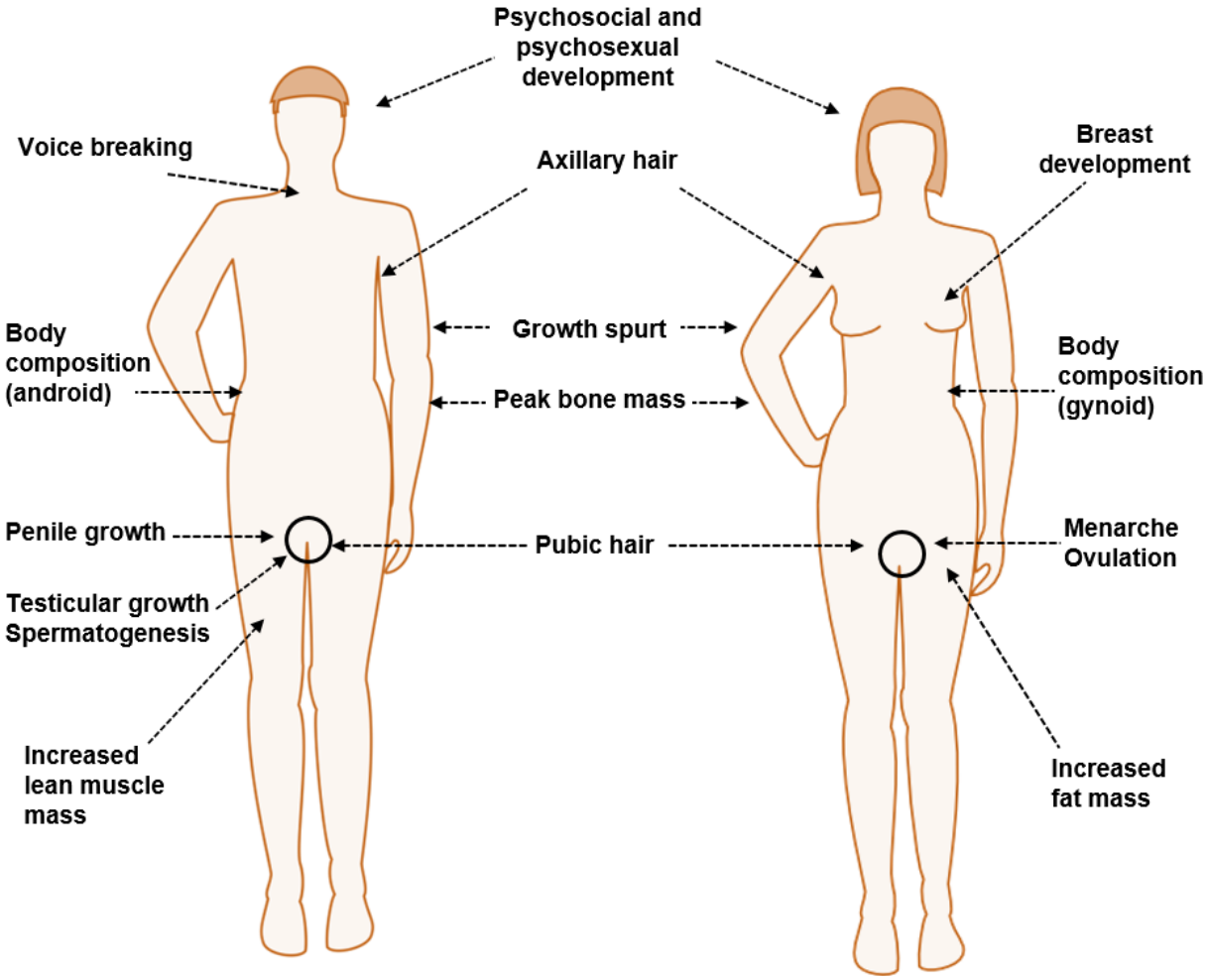

Figure 1. Pubertal Hallmarks in males and females. Graphic representation of major clinical changes during puberty in males and females. Common changes are shown in the middle while sex-specific changes are demonstrated in the lateral side of each figure.

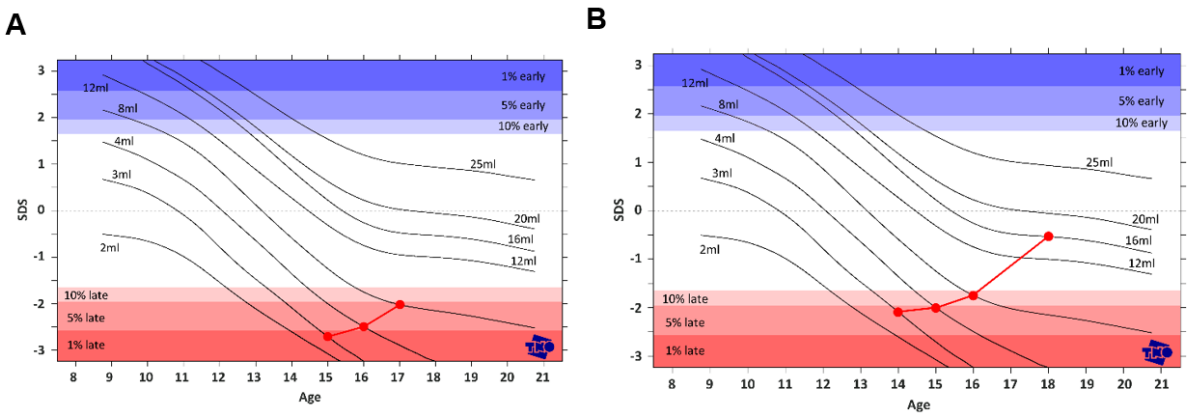

Figure 2. Pubertal progression in two male patients with delayed puberty. Testicular volume (TV) was plotted on the age-matched puberty normogram. **(A)** Patient 1 was diagnosed with delayed puberty at age 14 (TV 3mL) and completed pubertal development at age 18 (TV 16mL) confirming CDGP diagnosis. **(B)** Patient 2 was diagnosed at age 15 (TV 3mL) and discharged at age 17 (TV 8mL), despite the fact that his progression is still abnormal (< -2SD) using the pubertal nomogram. Thus the differential diagnosis between CDGP and partial CHH is still unclear. Pubertal nomogram obtained with agreement from Dr. Van Buuren from http://vps.stefvanbuuren.nl/puberty/

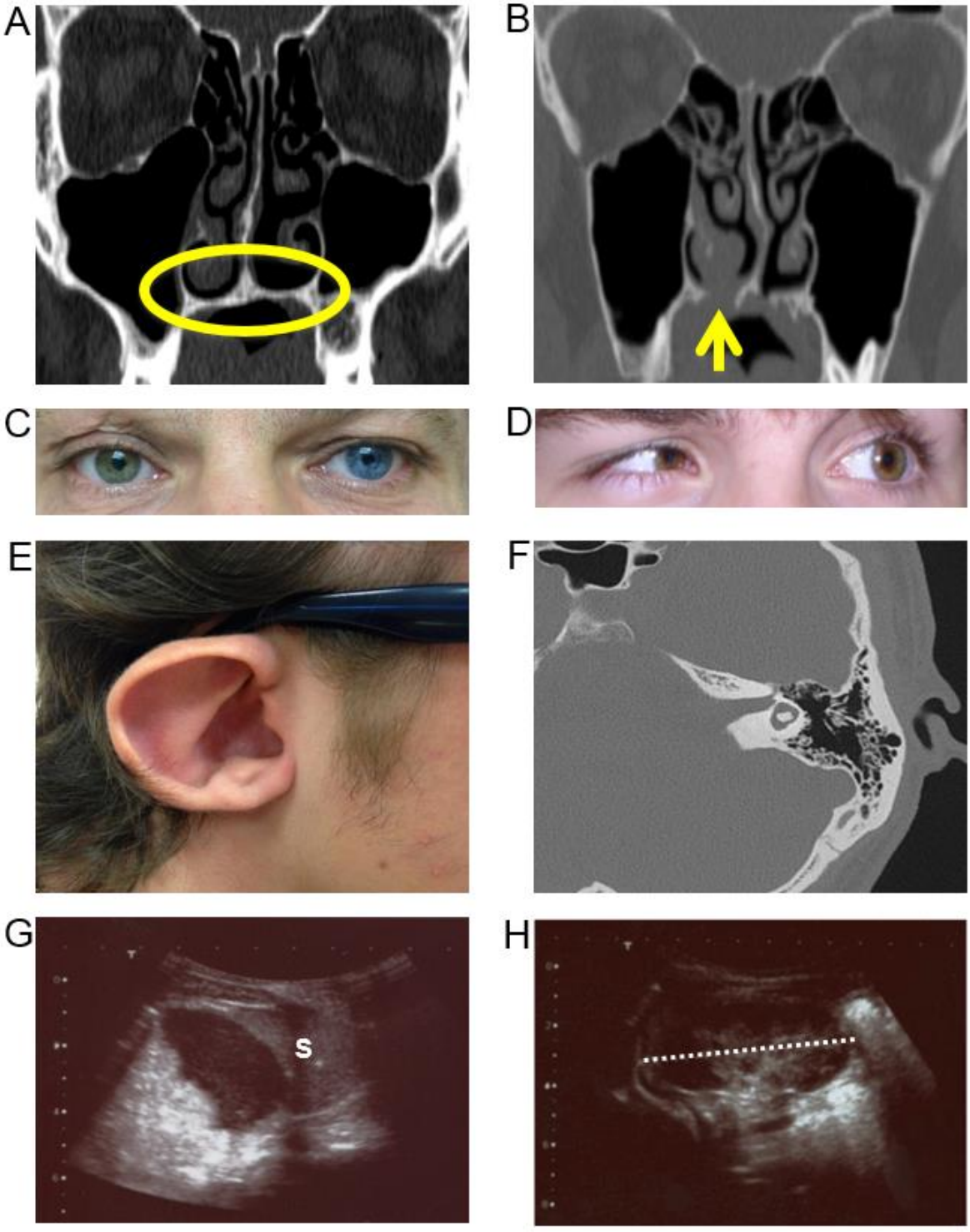

Figure 3: Non-reproductive, non-olfactory signs associated with Kallmann syndrome. (A) Coronal CT scan showing the normal palatine bone in a normal subject (yellow circle). **(B)** Cleft palate (yellow arrow) in a patient with Kallmann syndrome carrying a heterozygous *FGFR1* mutation (adapted from Maione et al., JCEM 2013). **(C)** Iris depigmentation of left eye in a patient with *SOX10* mutation. **(D)** oculomotor nerve palsy suggesting left VI cranial nerve damage in a teenager with Kallmann syndrome and a heterozygous *CHD7* mutation (adapted from Marcos et al., JCEM 2014). **(E)** Ear pavilion abnormality suggesting CHARGE syndrome in a male CHH patient initially referred for Kallmann syndrome. **(F)** Inner ear CT scan showing patient's semicircular canals hypoplasia in a male patient with Kallmann syndrome and deafness resulting from a heterozygous *SOX10* mutation (adapted from Maione et al., Clin Endo 2016 and Pingault et al., Am J Hum Genet 2013). **(G)** Post-natal kidney ultrasound, left posterior fossa view showing absent left kidney in a male neonate with an *ANOS1* mutation (s=spleen). **(H)** Right kidney ultrasound in same patient revealing compensatory hypertrophy (dotted line indicates kidney length of 65 mm) (adapted from Sarfati et al., Orphanet J Rare Dis 2015)

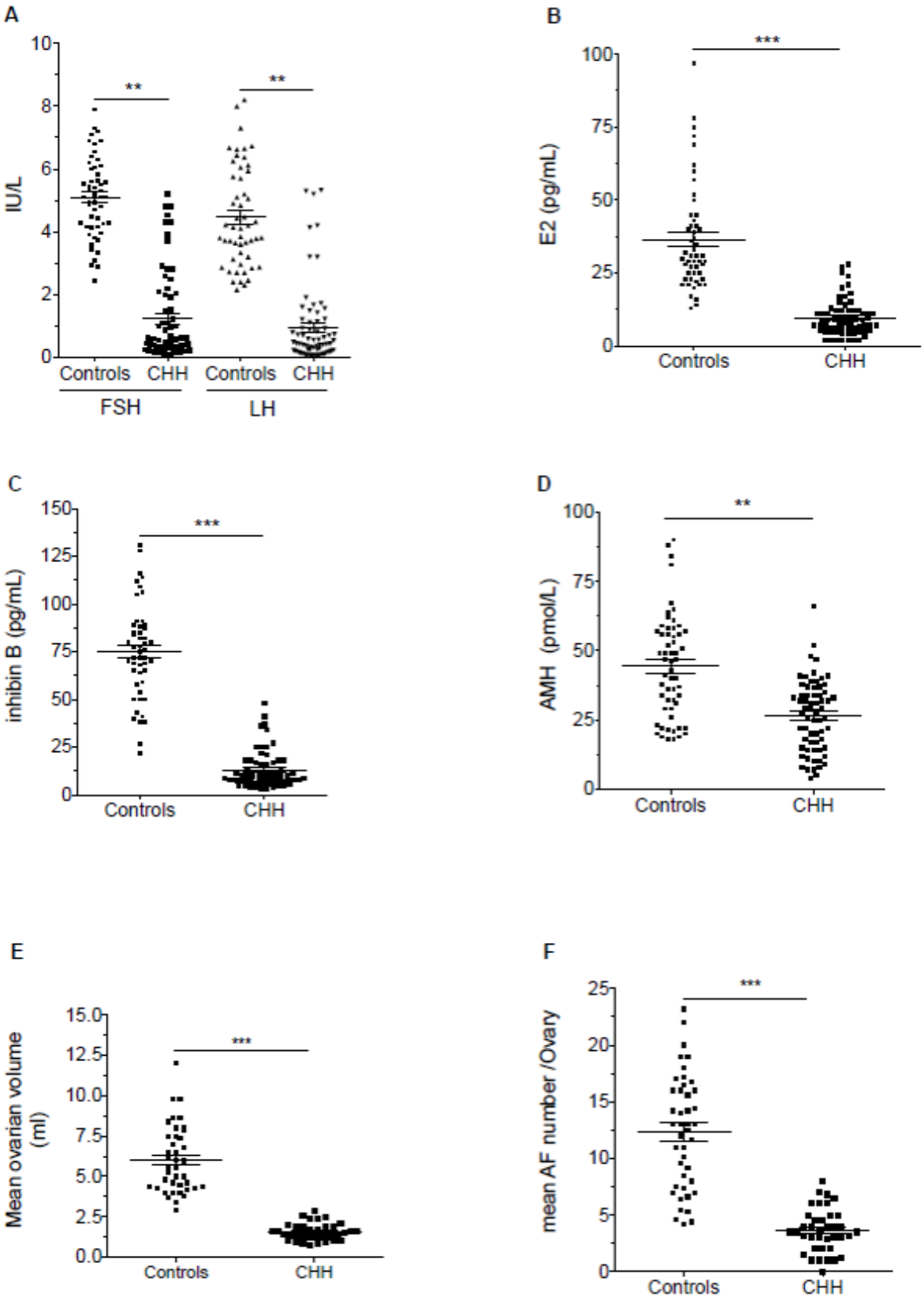

Figure 4. Horomone levels and ultrasound features in female CHH patients compared with healthy controls. Serum FSH and LH (panel A), estradiol (E2) (panel B) and serum ovarian peptides inhibin B (panel C), and AMH (panel D) levels in in untreated women with CHH (n = 68, aged from 18 to 34 years) and age-matched healthy young women (Controls, n = 52). Mean ovarian volume (panel E), total mean antral follicle (AF) number /ovary (panel F) in untreated women with CHH (n = 39) and in healthy women (n = 41). Adapted from Bry-Gauillard H et al., JCEM, 2017.

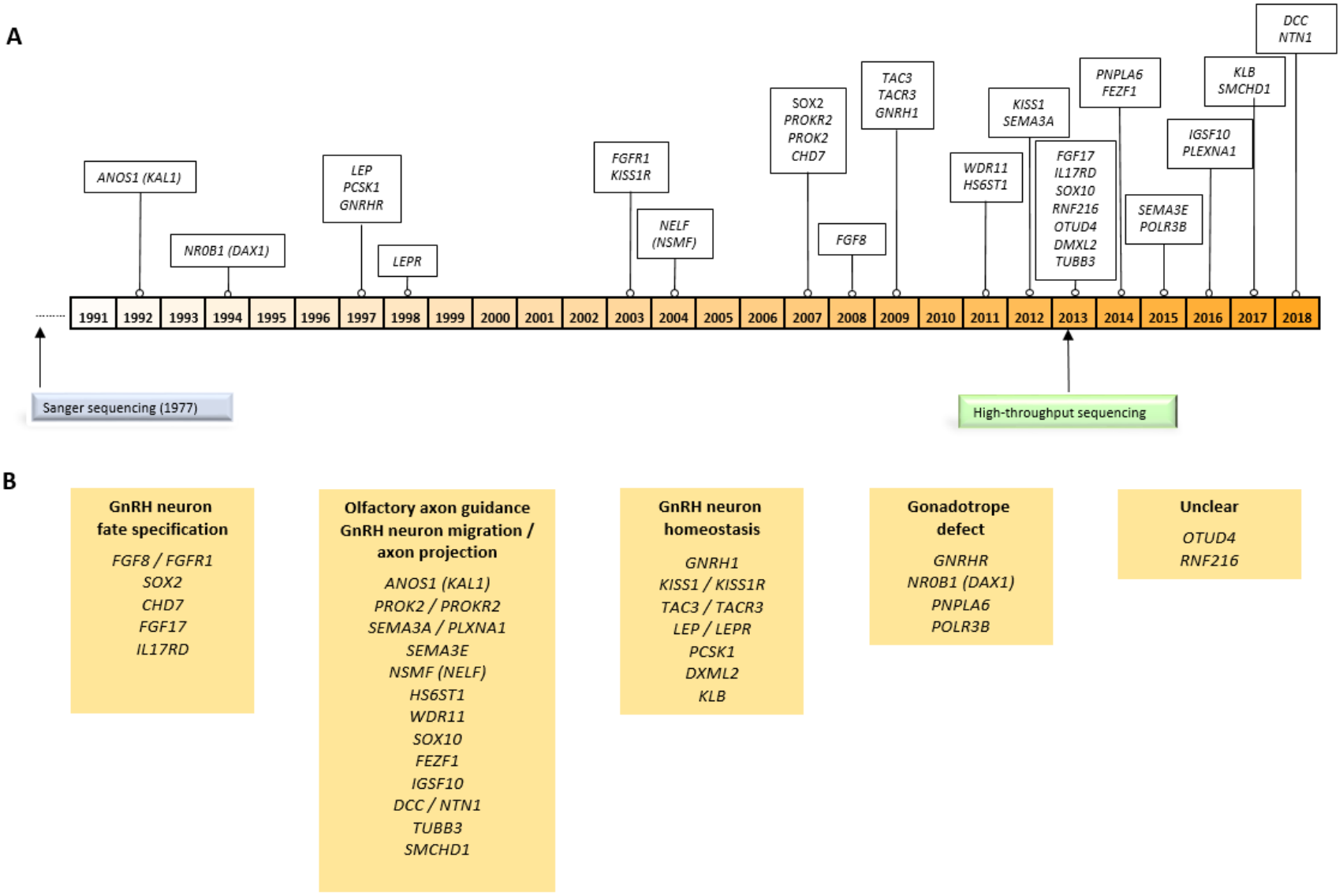

Figure 5. Genetics in CHH. (A) Timeline of gene discovery in CHH and CHH-overlapping syndrome. **(B)** Biological involvement of CHH genes in GnRH neuronal system. CHH, congenital hypogonadotropic hypogonadism.

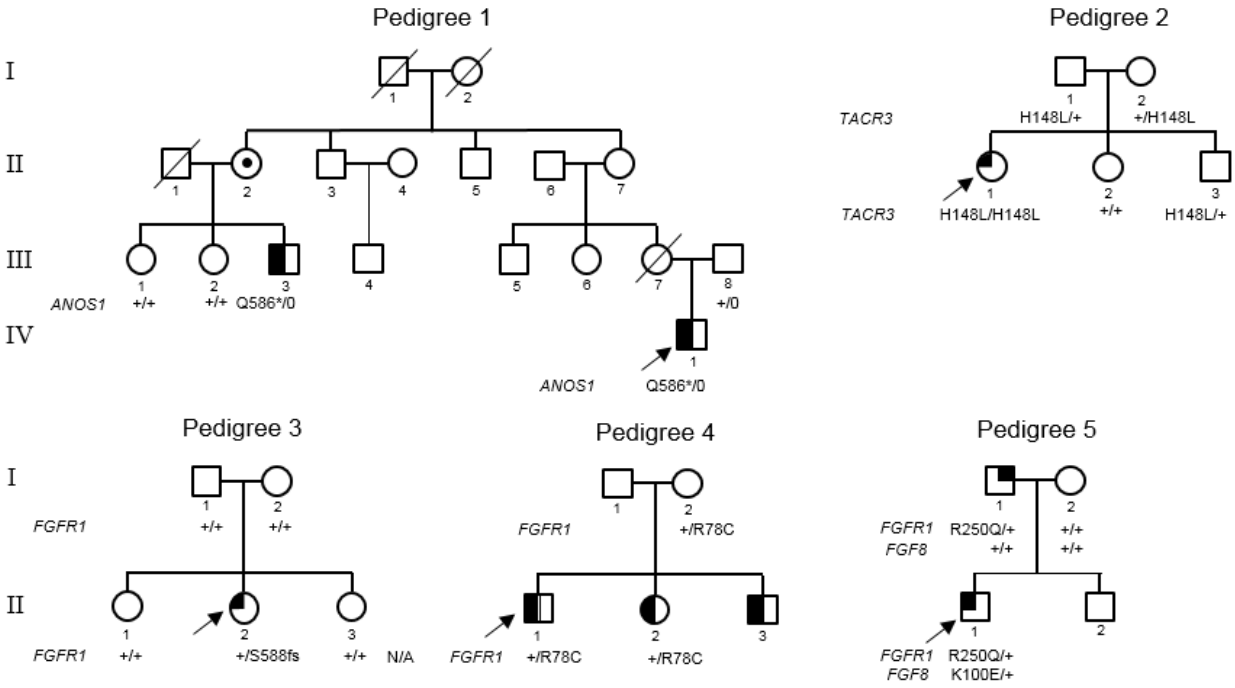

Figure 6: Pedigrees and gene mutations in CHH and KS patients. All gene variants listed are rare (minor allele frequency <0.5%) and predicted to be damaging by standard protein prediction algorithms (SIFT and/or PolyPhen2). All variants are classified as pathogenic or likely pathogenic according ACMG recommendations (Richards 2015 PMID: 25741868). **Pedigree 1**: X-Linked Kallmann syndrome caused by *ANOS1* mutation; **Pedigree 2**: autosomal recessive mode of inheritance; **Pedigree 3**: autosomal dominant with variable expressivity; **Pedigree 4**: *de novo* mutations; **Pedigree 5**: oligogenic mutation with de novo mutation in *FGF8*. Circles denote females; and squares denote males; arrows mark probands. A diagonal slash through a symbol means the subject is deceased. Regarding the gene mutations, + represents wild-type (reference) sequence, and a 0 is present in hemizygous male subjects for genes on the X chromosome. NA, not available.

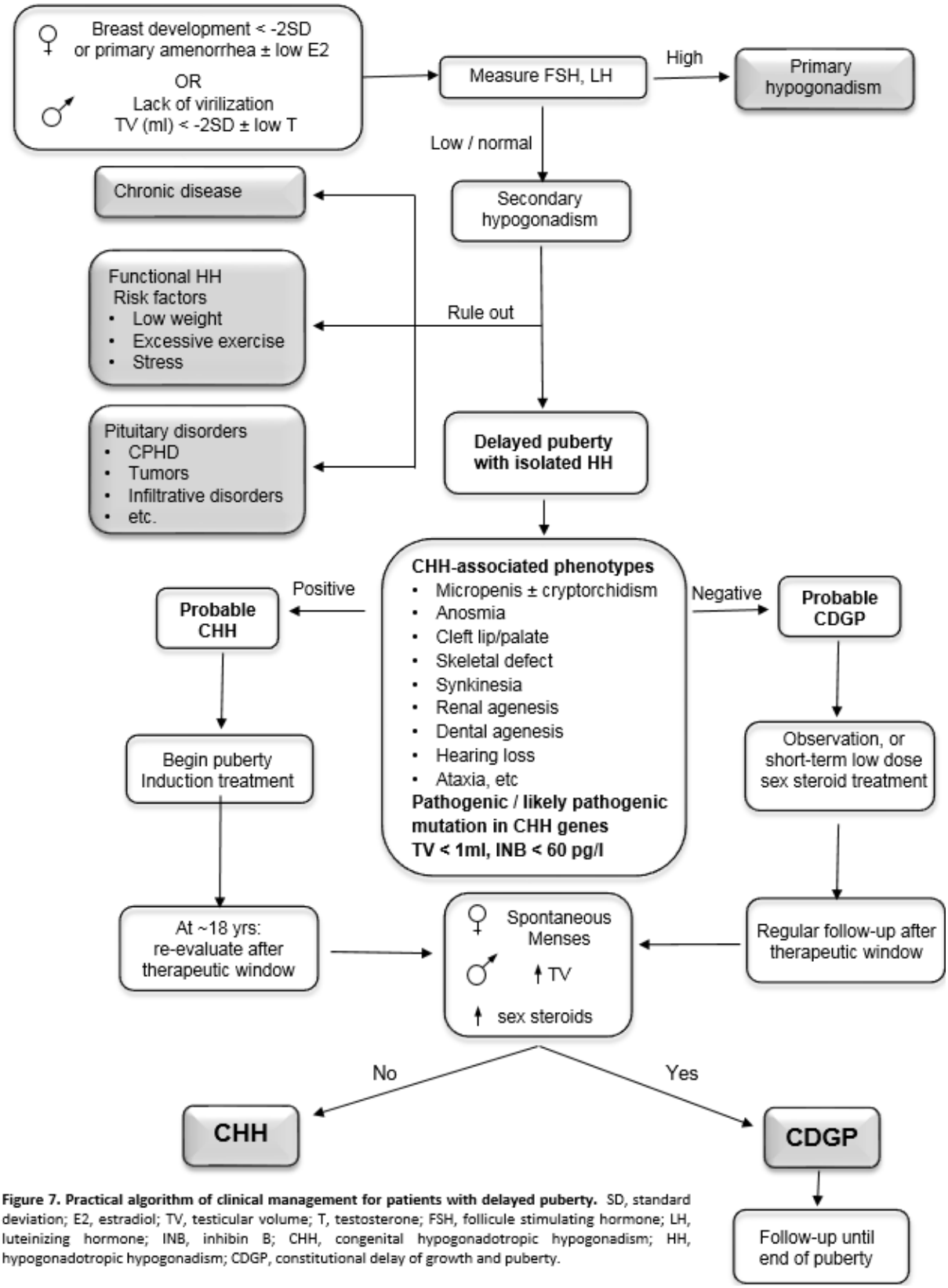

Figure 7. Practical algorithm of clinical management for patients with delayed puberty. SD, standard deviation; E2, estradiol; TV, testicular volume; T, testosterone; FSH, follicule stimulating hormone; LH, luteinizing hormone; INB, inhibin B; CHH, congenital hypogonadotropic hypogonadism; HH, hypogonadotropic hypogonadism; CDGP, constitutional delay of growth and puberty.

Table 1. The prevalence of main non-reproductive phenotypes in CHH versus general population.

Phenotypes	All CHH		KS		General population
	Waldstreicher et al. (n = 106) (27)	Quinton et al. (n = 215) (29)	Quinton et al. (n = 112) (29)	Costa-Barbosa et al. (n = 219) (30)	
Anosmia / hyposmia	55%	52%	100%	100%	0.01%
Mirror movement	NA	20%	31%	19%	0.0001%
Unilateral renal agenesis	NA	10%	15%	8%	0.05%
Eye movement disorders	3%	20%	27%	NA	0.02 - 0.0002%
Hearing loss	6%	5% [a]	8% [a]	15%	0.02%
Cleft lip/palate	7%	5%	4%	6%	0.1% (31)
Dental agenesis	NA	NA	NA	14%	4 - 7% (32)
Syndactyly, polydactyly, camptodactyly	NA	NA	NA	5%	0.03 - 0.1% (33) 0.2 - 1.3% (34) 1% (35)
Scoliosis	NA	NA	NA	13%	0.05 - 0.1% (36)

NA: not assessed; [a] only sensorineural hearing loss is included. Prevalence in the general population: anosmia data from NIH Genetic and Rare Disease Information Center (https://rarediseases.info.nih.gov/, accessed in January 2018); for mirror movement, eye movement disorders and hearing loss, data were obtained from NIH Genetics Home Reference (https://ghr.nlm.nih.gov, accessed in January 2018); unilateral renal agenesis data is from Orphanet (http://www.orpha.net/consor/cgi-bin/index.php, accessed in January 2018).

Table 2. Complex syndromes and phenotypic overlap with CHH

Syndrome	Genetic overlap	Major signs	Minor signs
CHARGE syndrome	*CHD7, SEMA3E*	coloboma, choanal atresia, semi-circular canal dysplasia	**hypothalamic-pituitary defect**, **sensorineural hearing loss**, ear malformation, mental retardation, congenital heart defect
Waardenburg syndrome	*SOX10*	**sensorineural hearing loss**, abnormal pigmentation	**hypogonadotropic hypogonadism**, **anosmia with OB aplasia/hypoplasia,** facial dysmorphism, megacolon, semi-circular canal dysplasia, congenital heart defect
Hartsfield syndrome	*FGFR1*	**split-hand/foot malformation,** holoprosencephaly	**anosmia**, **hypothalamic-pituitary defect**, **syndactyly,** facial dysmorphism
Adrenal hypoplasia congenita	*DAX1*	**hypogonadotropic hypogonadism,** adrenal hypoplasia	-
Bardet–Biedl syndrome	-	**hypogonadotropic hypogonadism**, **polydactyly**, **renal anomalies**, rod-cone dystrophy, obesity, learning difficulties	**anosmia**, **dental anomalies**, **brachydactyly, syndactyly**, developmental delay, diabetes mellitus, congenital heart disease, ataxia
Gordon Holmes syndrome	*PNPLA6, RNF216, OTUD4*	**hypogonadotropic hypogonadism**, cerebellar ataxia	-
Congenital obesity	*LEP, LEPR, PCSK1*	**hypogonadotropic hypogonadism,** obesity	-
4H syndrome	*POLR3B*	**hypogonadotropic hypogonadism, hypodontia,** hypomyelination	-
TUBB3 E410K syndrome	*TUBB3*	CFEOM, **hypogonadotropic hypogonadism, anosmia**	intellectual disability, facial weakness, trachomalacia, vocal cord paralysis, later-onset cyclic vomiting, progressive peripheral neuropathy
Bosma arhinia microphtalmia syndrome	*SMCHD1**	arhinia, **hypogonadotropic hypogonadism**	anophthalmia, **coloboma,** cataract, nasolacrimal duct atresia, **choanal atresia, cleft palate**

Phenotypes which overlap between these syndromes and CHH are highlighted in bold. OB, olfactory bulb; CFEOM: congenital fibrosis of the extraocular muscles. *Mutation in *SMCHD1* are only identified in CHH patients with associated phenotype of Bosma arhinia microphtalmia syndrome.

Table 3. Clinical and biochemical characteristics of neonatal CHH males reported in literature

Case No.	Clinical signs		Hormonal testing				Diagnosis	Neonatal treatment	References
	Neonatal signs	Family history	Age (months)	T (nmol/L)	LH (IU/L)	FSH (IU/L)			
1	micropenis	hyposmia	4	n.d.	n.d.	0.18	CHH	hCG, T	Main et al., 2000
2	ascending testis	CPHD	3.5	n.d.	0.07	0.18	CPHD	T	
3	micropenis	none	0-7.9	n.d.	n.d.	0.05-0.17	CHH	rFSH + rLH, T	Main et al., 2002
4	micropenis	n.r.	2	0.03	0.19	0.19	CPHD	rFSH + rLH	Bougnères et al., 2008
5	micropenis	n.r.	3.5	0.06	0.03	0.12	CHH	rFSH + rLH	
6	micropenis, cryptorchidism, CLP, SHFM	CHH, CLP	2	n.d.	n.d.	0.4	CHH	rFSH + rLH	Villanueva et al., 2014
7	micropenis	KS	1	0.1	0.04	0.18	KS	rFSH + rLH	Sarfati et al., 2015
8	micropenis, cryptorchidism	none	3	0.3	n.d.	n.d.	CHH	T	Xu et al., 2017
9	micropenis, cryptorchidism	n.r.	6	0.2	0	0.4	CPHD	rFSH + rLH	Lambert et al., 2016
10	micropenis, cryptorchidism	n.r.	4.5	0.2	0.4	1	CHH	rFSH + rLH	
11	micropenis, cryptorchidism	n.r.	2.5	0.1	0.1	0.8	CHH	rFSH + rLH	
12	cryptorchidism	n.r.	5	0.1	n.d.	0.3	CHH	rFSH + rLH	
13	micropenis, cryptorchidism	n.r.	0.25	0.2	n.d.	0.21	CHH	rFSH + rLH	

T, testosterone; LH, luteinizing hormone; FSH, follicle stimulating hormone; n.r., not reported; CLP, cleft lip palate; hCG, human chorionic gonadotropin; rFSH, recombinant FSH; rLH, recominant LH; n.d., not detectable.

Table 4. Medical treatment for puberty induction, hypogonadism and infertility in female CHH patients.

Treatment	Dosing & administration	Advantage	Disadvantages
Induction of puberty in girls			
17β-oestradiol (tablets)	Initial dose: 5 µg/kg daily P.O. ↑ 5 µg/kg increments every 6-12 months Up to 1-2 mg daily	Natural oestrogen	Less referable than transdermal route
17β-oestradiol (patch)	Inital dose: 0.05 - 0.07 µg/kg , only nocturnal ↑ to 0.08 - 0.12 µg/kg every 6 months Up to 50 - 100 µg/24 h	Natural oestrogen No hepatic passage (decrease thromboembolic risk)	Small dose patch not available, need to cut the patch of 25ug/24h
Progesterone	Added after full breast development or break-through bleeding, during the last 14 days of menstrual cycle		
Treatment of hypogonadism in adult females			
Estroprogestin therapy (tablets)	17β-oestradiol 1 or 2 mg Progestin: during the last 14 days of the months micronized progestin 200mg P.O. daily, or dydrogesterone 10 mg P.O. daily	Mimic the physiological hormone changes	
Estroprogesin therapy (patch or gel)	17β-oestradiol patch 50-100 ug/24h daily, OR 17β-oestradiol gel 7.5 - 15 mg daily Progestin: during the last 14 days of the months micronized progestin 200mg P.O. daily, or dydrogesterone 10 mg P.O. daily	Mimic the physiological hormone changes	
Treatment of fertility in adult females			
Pulsatile GnRH	**I.V. pump**: 75 ng/kg per pulse every 90 min Dose adaped based on response, up to 500 ng/kg per pulse **S.C. pump**: 15 µg per pulse every 90 min Dose adaped based on response, up to 30 µg per pulse Luteal phase: continue GnRH pump, OR	Most physiological treatment Possibility to adjust pulse frequency in I.V. pump High success rate Less risk in multiple pregnancy	Not available in many countries Require centers with expertise Risk of phlebitis for I.V. treatment (rare) Pituitary resistance (rare)
Gonadotropins	hMG (FSH + LH) 75 - 150 IU S.C. daily, dose adapted based on follicular growth Induction of ovulation by hCG 6500 IU S.C. injection Luteal phase: hCG 1500 U every 3 days for 3 times Progesterone 200mg intravaginal daily	Available around the world Self-injection	More expensive Higher risk of overstimulation Requires close monitoring of E2 & US Higher risk of multiple pregnancy

Table 5. Medical treatment for puberty induction, hypogonadism and infertility in male CHH patients.

Treatment	Dosing & administration	Advantage	Disadvantages
Induction of puberty in boys			
Testosterone enanthate (TE)	Initial dose: 50 mg I.M. monthly ↑ 50 mg increments every 6 - 12 months, Up to 250 mg monthly	Standard care with long clinical experience Aromatizable to E2: promote bone maturation	Premature epiphyseal closure (high dose) Could inhibit TV & spermatogenesis Impact on future fertility: unknown
Gonadotropin	hCG: initial dose 250 IU twice weekly, S.C. ↑ 250 - 500 IU increments every 6 months Up to 1500 IU 3 times weekly rFSH: dose 75-150 IU 3 times weekly, S.C.	Stimulate TV growth & spermatogenesis Pre-FSH treatment can be beneficial in patients with TV < 4ml or history crytorchidism	Not standard treatment Need good compliance in adolescent patients Need studies in larger cohorts
Hypogonadism treatment in adult males			
Testosterone enanthate (TE)	250 mg I.M. every 2-4 weeks Interval adjusted based on trough T	Cost effective Available around the world Self injection	Relative frequent I.M. injection S.C. route is under investigation (Kaminetskyet al., Sex Med, 2015)
Testosterone undecanoate (TU)	1000 mg I.M. every 10-14 weeks Interval adjusted based on trough T	Cost effective Infrequent injection	Interval of treatment is highly variable, follow-up of trough T is important Injections by nurses
Testosterone gel	50-80 mg transdermal daily	Non invasive Self-administrated	Risk of transmission by skin contact
Treatment of infertility in adult males			
Pulsatile GnRH	S.C. pump: 25 ng/kg per pulse every 120 min Dose adapted based on serum T Up to 600 ng/kg per pulse	Most physiological treatment	Not available in many countries Require centers with expertise Pituitary resistance (rare)
Gonadotropin	hCG: dose 500-1500 IU 3 times weekly, S.C. Dose adjusted based on trough T rFSH: dose 75-150 IU 3 times weekly, S.C. Dose adjusted based on serum FSH, sperm count	Available around the world For patients with absent puberty (TV < 4ml): pre-rFSH treatment increases fertilty prognosis	Relative expensive for rFSH Frequent injections

Table 6. Fertility outcomes in male patients with congenital hypogonadotropic hypogonadism: summary of 44 published studies.

Study #	Male CHH patients included (n)	nCHH (n)	KS (n)	CHH/KS with cryptorchidism (n)	Median Basal TV (mL)	Median Maximal TV (mL)	Median Max. Sperm Count (10^6/mL)	Median TTS (months)	Therapy failure (persistent azoospermia) (n)	Therapies used	Pregnancies* (n)	Reference
Combined gonadotropin therapy												
1	10	8	2	NA	NA	NA	NA	16	1	hMG+hCG	4	Gayral et al., 1975
2	36	25	11	NA	NA	NA	5.1	5	9	hPG	12	Bremner et al., 1981
3	15	7	8	7	NA	NA	8.5	10	5	hMG+hCG	8	Finkel et al., 1985
4	13	7	6	4	2.4	6.9	1.3	11.5	1	hMG+hCG	2 (7)	Ley et al., 1985
5	13	13	0	0	1.2	3.1	3.0	NA	2	hMG+hCG	3	Okuyama et al., 1986
6	24	17	7	Excluded	6.8	13.9	16.7	7.6	not included	hMG+hCG	22	Burris et al., 1988
7	8	NA	NA	NA	2.1	9	1.0	24	2	hMG + hCG	1 (8)	Liu et al., 1988[+]
8	18	9	9	5	2.5	8	4	12	9	hMG+hCG	1	Schopohl et al., 1991[++]
9	16	8	8	4	3.5	13.3	6.0	23.1	2	hMG+hCG	NA	Saal et al., 1991
10	18	NA	NA	Excluded	3.7	14.9	2.6	34.2	4	hCG+hMG	7 (10)	Vicari et al., 1992
11	10	4	6	Excluded	4	12	18.5	24	NA	hMG+hCG	3 (4)	Schaison et al., 1993
12	7	6	1	6	1.2	9	10.0	11.8	1	hMG+hCG	3	Jones et al., 1993
13	9	7	2	0	2.2	8	8.0	14	2	hMG+hCG	4	Kung et al., 1994
14	26	12	14	13	1.5	3.8	2.2	12.2	12	hMG+hCG	3	Kirk et al., 1994
15	35	19	16	Excluded	4.3	11.1	14.0	5	12	uFSH+hCG	1 (4)	Burgués, et al., 1997
16	27	16	11	Excluded	3.6	10.5	16.5	9	3	uFSH+hCG	2 (10)	Eur Metrodin Group, 1998
17	18	9	9	8	4.4	15.3	1.2	6	2	hMG+hCG	3	Buchter et al., 1998[+++]
18	10	8	2	Excluded	3.5	9.6	5.0	6.6	2	rhFSH+hCG	2	Liu et al., 1999
19	26	17	9	Excluded	2	12	1.5	9	4	rhFSH+hCG	4 (7)	Bouloux et al., 2002
20	20	13	7	5	8	NA	5	5.5	NA	rhFSH / uFSH+hCG	NC	Liu et al., 2002
21	9	8	1	4	3	7.5	5.1	16.8	NA	hMG+hCG	NA	Depenbush et al., 2002
22	26	11	15	11	5.7	12	5.0	7	10	rhFSH+hCG	NA	Bouloux et al., 2003
23	23	18	5	9	1.6	4.85	1.0	52	7	hMG+hCG	NA	Miyagawa et al., 2005

24	4	4	0	0	4.1	6.8	2.05	12	0	hMG +hCG	3	Zorn et al., 2005
25	4	2	2	2	1	5.5	3.0	10	1	rhFSH+hCG	NA	Raivio et al., 2007
26	25	16	9	Excluded	NA	14	5.2	5.1	1	rhFSH+hCG	5 (30)	Matsumoto et al., 2009
27	77	48	29	Excluded	3.4	11.7	8.2	18	13	rhFSH+hCG	14 (51)	Warne et al., 2009
28	51	34	17	12	6.5	NA	8.0	23	NA	rhFSH / uFSH+hCG	38	Liu et al., 2009
29	10	9	1	0	NA	9	7.0	9.8	1	hMG / rhFSH+hCG	4	Oldereid et al., 2010
30	31	22	9	Excluded	3.8	9	22.8	12	NA	rhFSH / uFSH +hCG	10 (22)	Sinisi et al., 2010
31	19	8	11	9	4.5	10.2	7.1	11	1	hMG+hCG	5 (11)	Rohayem et al., 2016
32	223	112	111	40	2.1	8.1	11.7	15	80	hMG+hCG	17	Liu et al., 2017
33	38	18	20	19	2.5	16.5	15.0	55	3	rhFSH+hCG	0	Rohayem et al., 2017
Sub-Total	**899**	**515**	**358**	**158**					**190**		**181***	
Pulsatile GnRH therapy												
34	5	3	2	NA	3	4.5	4.1	3	3	GnRH	1	Hoffman et al., 1982
35	10	6	4	NA	NA	NA	4.2	12	1	GnRH	3	Morris et al., 1984
36	30	NA	NA	0	5	18	68	5	1	GnRH	18 (30)	Shargil, 1987
37	5	NA	NA	NA	2.4	11.5	0.1	24	3	GnRH	2 (5)	Liu et al., 1988[+]
38	10	8	2	1	4	14	19.2	12	0	GnRH	NA	Aulitzky et al., 1988
39	18	10	8	4	2	10	4.7	5	4	GnRH	1	Schopohl et al., 1991[++]
40	28	17	11	13	2	12	2	10.7	7	GnRH	3	Delemarre-Van de Waal 1993
41	6	4	2	3	6.8	14.9	1.6	4	1	GnRH	3	Buchter et al., 1998[+++]
42	52	26	26	21	3.3	12	15.0	24	9	GnRH	NA	Pitteloud et al., 2002
43	35	12	23	9	2.3	9	NA	12	9	GnRH	NA	Gong et al., 2015
44	20	9	11	4	2.9	10.8	14.2	15.6	NA	GnRH	5 (14)	Mao et al., 2017 (28051040)
Sub-Total	**219**	**95**	**89**	**55**					**38**		**36***	
Total, n	**1118**	**610**	**447**	**213**					**228**		**217***	
Mean					**3.4**	**9.8**	**7.59**	**15.3**				
Weighted mean**					**3.51**	**10.8**	**9.83**	**15.2**				

CHH: congenital hypogonadotropic hypogonadism; n= number of male CHH patients; nCHH: CHH without reported Kallmann syndrome features; KS: patients with Kallmann syndrome;

TV: mean testicular volume; TTS: time to induce sperm appearance in ejaculate (months);

Therapies used: FSH-preparations used in combination with chorionic gonadotropin (hCG): hMG: human menopausal gonadotropin (FSH+LH); uFSH: urinary highly purified FSH; rhFSH: recombinant human FSH; hPG: human pituitary gonadotropin (mixture of FSH and LH); GnRH: gonadorelin (pulsatile administration via a pump).

Excluded: CHH/KS patients with cryptorchidism excluded from the study design.

NA: not available; NC: non calculable

Data are reported as number or medians, as appropriate.

Please note that, because of the wide range of some parameters (notably sperm count, which may range from 0.01 to >300 million/mL), we chose to show medians instead of means.

*Pregnancies obtained (number in parentheses indicate the total number of patients treated who wished paternity).

** To underline the importance of the populations' size, we also calculate the weighted means of these medians (i.e. we calculated weighted means of 8,489.9 10^6/mL (patients x sperm count), and 10,341.4 mL (patients x testicular volumes)). The weighted means are then divided on the total number of patients whom these numbers refer (respectively 864 for the sperm count and 956 for the testicular volumes).

+, ++ and +++: data from the same study.

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