REVIEW

Non-syndromic congenital hypogonadotropic hypogonadism: clinical presentation and genotype–phenotype relationships

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Abstract

Congenital hypogonadotropic hypogonadism (CHH) results from abnormal gonadotropin secretion, and it is characterized by impaired pubertal development. CHH is caused by defective *GNRH* release, or by a gonadotrope cell dysfunction in the pituitary. Identification of genetic abnormalities related to CHH has provided major insights into the pathways critical for the development, maturation, and function of the reproductive axis. Mutations in five genes have been found specifically in Kallmann's syndrome, a disorder in which CHH is related to abnormal *GNRH* neuron ontogenesis and is associated with anosmia or hyposmia.

In combined pituitary hormone deficiency or in complex syndromic CHH in which gonadotropin deficiency is either incidental or only one aspect of a more complex endocrine disorder or a non-endocrine disorder, other mutations affecting *GNRH* and/or gonadotropin secretion have been reported.

Often, the CHH phenotype is tightly linked to an isolated deficiency of gonadotropin secretion. These patients, who have no associated signs or hormone deficiencies independent of the deficiency in gonadotropin and sex steroids, have isolated CHH. In some familial cases, they are due to genetic alterations affecting *GNRH* secretion (mutations in *GNRH1*, *GPR54/KISS1R* and *TAC3* and *TACR3*) or the *GNRH* sensitivity of the gonadotropic cells (*GNRHR*). A minority of patients with Kallmann's syndrome or a syndromic form of CHH may also appear to have isolated CHH, but close clinical, familial, and genetic studies can reorient the diagnosis, which is important for genetic counseling in the context of assisted reproductive medicine.

This review focuses on published cases of isolated CHH, its clinical and endocrine features, genetic causes, and genotype–phenotype relationships.

European Journal of Endocrinology 162 835–851

Introduction

Congenital hypogonadotropic hypogonadism (CHH), also called idiopathic hypogonadotropic hypogonadism by some authors, is a well-known cause of pubertal failure in both boys and girls. CHH is usually due to inadequate secretion of two pituitary gonadotropins, LH and FSH, precluding normal testicular/ovarian endocrine and exocrine functions during periods of physiological activation of the gonadotropic axis. The prevalence of CHH, based on civilian and military hospital series, has been estimated at 1/4000 to 1/10 000 in males (1); it is reported to be between 2 and 5 times less frequent in females (2, 3) (Table 1), but it is probably underestimated in this gender. Isolated or apparently isolated forms (Kallmann's

syndrome with anosmia or hyposmia not spontaneously mentioned by the patient) are usually discovered during adolescence or adulthood, because of incomplete or absent pubertal development. Such patients account for the majority of cases seen by endocrinologists practicing in adolescent and adult healthcare structures (Table 1).

Patients with more complex syndromic forms are rarely seen first by an adult endocrinologist, but they are usually diagnosed by pediatricians during childhood, owing to extragonadal manifestations such as statural growth retardation due to GH deficiency, or multiple anterior pituitary hormone insufficiencies (4, 5), adrenal failure (6), or obesity (7–11) and neurological disorders (12–14), which appear before the age of puberty and are usually predominant.

Table 1 Main characteristics of the 342 patients with congenital hypogonadotropic hypogonadism referred, evaluated, and followed at the Endocrinology and Reproductive Diseases Department at Bicêtre Hospital from 1993 to date (January 2010).

Men=284 (83%)
Women=58 (17%)
Normosmic non-syndromic CHH=176 (51%)
Kallmann's syndrome=139 (41%)
Syndromic=27 (8%)
CHH and adrenal hypoplasia associated with DAX1 mutations=5
CHH multiple pituitary deficiencies associated with PROP1 mutations=4
CHARGE syndrome=2
CHH with cerebellar ataxia (Gordon Holmes syndrome)=2
Bardet Biedl syndrome=2
Prader-Willi syndrome=1
Not yet classified=10

In the last two decades, CHH has gradually been classified on the basis of genetic and pathophysiological features (2, 3, 15–17). Grossly speaking, CHH is now divided into forms secondary to developmental abnormalities (ontogenesis) of the gonadotropin axis, and especially *GNRH* neurons. The paradigm of this form is Kallmann's syndrome, which generally combines CHH and anosmia. This form has recently been the subject of an exhaustive review (17).

The largest CHH patient subgroup is composed of subjects whose entire phenotype (including cryptorchidism) is explained exclusively by pre- and postnatal gonadotropin and sex steroid deficiency, and is almost never associated, as we shall see later, with olfactory disorders or with signs such as those observed in Kallmann's syndrome or in other syndromic forms. These cases of CHH seem to be secondary to isolated functional abnormalities of the neuroendocrine signaling cascade(s) responsible for *GNRH* and/or gonadotropin secretion. There are no known developmental or anatomical disorders of the hypothalamo-pituitary-gonadotropin axis in these patients.

Independently of its cause and mechanism, CHH has also been classified according to the response to pulsatile *GNRH* administration. This approach distinguishes hypothalamic gonadotropin deficiencies due to abnormal hypothalamic *GNRH* secretion from pituitary gonadotropin deficiency due to abnormal *GNRH* responsiveness.

A neuroendocrine classification was proposed a few years ago, based on the nature of abnormal spontaneous LH secretion (2). Here, patients with no pulsatile LH secretion are distinguished from those with a reduced LH pulse frequency or amplitude, and also those with exclusively LH nocturnal secretion (2). Since the discovery of several genetic defects, and with advances in our understanding of the pathophysiological mechanisms underlying CHH, it has emerged that a given LH secretion profile (apulsatile, for example) can be seen in patients with a syndromic form, such as Kallmann's syndrome (18) or isolated CHH with normal olfaction (see below).

The LH secretion profile therefore lacks specificity for classifying CHH due to different mechanisms. In addition, this costly and time-consuming approach has lost much of its diagnostic interest, and is currently reserved for clinical research purposes only.

Clinical presentation

There is no sexual ambiguity in men with CHH, including those most severely affected. These patients have typical external genital organs, albeit sometimes highly hypotrophic. Indeed, masculinization of the urogenital sinus depends on testosterone secretion secondary to fetal Leydig cell stimulation by placental human chorionic gonadotropin (hCG) (19), an extra-pituitary gonadotropin that is secreted normally during gestation in such cases.

CHH is less often diagnosed in boys before the age of puberty, usually based on unilateral or bilateral cryptorchidism or micropenis during the neonatal period. These cases can be confirmed by hormonal investigations if done before the age of 6 months – the only period of childhood during which testosterone and gonadotropin deficiencies can be documented (20–23). Indeed, given the 'physiological' gonadotropin deficiency characteristic of childhood after the sixth month of life, the diagnosis can only be suspected on the basis of cryptorchidism and/or micropenis, sometimes associated with signs pointing to a particular cause. For example, Kallmann's syndrome may be suspected in a prepubertal patient with anosmia or mirror movements (17), especially when there is already a positive family history. In the latter cases, the diagnosis can be confirmed by genetic analysis, which is far easier when a mutation has already been identified in the family. However, in this syndrome, and particularly in autosomal forms, care must be taken when predicting the likely gonadotropin phenotype in adulthood, in view of the phenotypic variability of a given mutation, even within the same family (17, 18, 24, 25).

In most cases, the possibility of CHH is raised when pubertal development is incomplete or absent after the age of 13 years in girls and 14 years in boys (26). The diagnosis is highly probable if the pubertal delay is associated with cryptorchidism or micropenis, or if it persists after the age of 18 years. When the diagnosis is made later in life, the patient may have pubic hair due to testosterone production by peripheral (i.e. extragonadal) conversion of androgens, which are normally secreted by the patient's adrenal glands (27). This may lead the inexperienced physician to believe, falsely, that puberty is beginning, whereas physical examination will show a prepubertal testicular volume or at least very hypotrophic testes.

Patients with isolated CHH can usually be distinguished from adolescents with constitutional late

puberty by virtue of their growth pattern, as the former have normal height for chronological age (28), while the latter tend to be short (29). Associated signs pointing to a syndromic form are very helpful for distinguishing between the two situations, which is otherwise difficult. Indeed, it is easy to diagnose severe CHH in a patient with cryptorchidism or micropenis, whereas partial or intermediate forms are more difficult to distinguish from constitutional late puberty on clinical grounds alone.

Gynecomastia can be seen in patients with untreated CHH. However, it usually occurs when the patient is treated with either hCG or supraphysiological doses of testosterone, in which case, testosterone aromatization may lead to a decrease in the testosterone:estradiol ratio. Given that adrenal androgen secretion is independent of the gonadotropin axis and therefore occurs normally in patients with isolated CHH, it is theoretically also possible that peripheral aromatization of adrenal androgens may also contribute to gynecomastia.

Partial congenital gonadotropin deficiency seems to affect only a minority of male patients. It is characterized by a certain degree of virilization, gynecomastia, and a testicular volume above 4 ml or even close to normal (18, 30) (see below). However, with developments in molecular genetics, familial genetic studies and sequencing of relatives now allow very partial forms to be diagnosed in subjects who have not spontaneously consulted a physician, yet who have moderate clinical and endocrine abnormalities (18, 30).

All patients with CHH have normal statural growth during childhood and, despite the absence of the pubertal growth spurt, statural retardation is very rarely a presenting symptom (31). On the contrary, the absence of long-bone epiphyseal closure explains these patients' frequent eunuchoid aspect and relative tallness. Retarded bone maturation, osteopenia, and osteoporosis are frequent when the gonadotropin deficiency is discovered in adulthood (31–34).

The so-called reversible forms were identified nearly 30 years ago in several case reports (35–41), mainly in male patients with Kallmann's syndrome. They were initially identified on clinical grounds, and were later characterized in detail, both clinically and hormonally, in a UK series published by Quinton *et al.* in 1999 (42) and, more recently, by Raivio *et al.* (43). Their existence was demonstrated in patients with mutations in *KAL1*, *FGFR1*, the *GNRH* receptor (*GNRHR*) gene, and *PROKR2* (43–46). These reversible forms are associated with very late activation of pulsatile gonadotropin secretion (42, 43), pointing to late activation of the *GNRH* pulse generator and/or gonadotropic cells, allowing gonadotropin secretion to improve with time. This clinical variant should be suspected if testicular volume increases in the absence of endocrine therapy or during testosterone administration (35–46).

When these reversible forms occur before 20 years of age in a subject with normal olfaction and no

identifiable mutations, they are difficult to distinguish from constitutional late puberty.

In women, CHH is revealed by primary amenorrhea in over 90% of the cases (47). Breast development can be highly variable; it is often present and sometimes almost normal (30, 47) (see below). Similarly, pubic hair may be absent, sparse, or even normal. These partial forms, which are frequent in women, probably contribute to explaining the underestimated prevalence of this condition in females. In a very mild form, so far described in only a minority of women, CHH can be revealed by isolated chronic anovulation, whereas estradiol secretion is adequate for endometrial development; this can be shown by the onset of bleeding after progestin administration, as well as by the existence of menstruation or even oligomenorrhea. These attenuated forms have also been described in women having conceived spontaneously (see below).

One difficulty in women with sporadic CHH, normal olfaction, and no identified mutation is the differential diagnosis of functional hypothalamic amenorrhea (48). In women referred for primary amenorrhea and with a hormonal profile suggesting HH but without anosmia or hyposmia or identified genetic anomalies, the diagnosis of CHH must only be considered with care, after ruling out underweight, eating disorders, excessive physical activity, and chronic underlying conditions (48). These patients must be followed up in order to re-assess the function of their gonadotropin axis and ovulatory mechanics with respect to their body composition. When body weight or body mass index is at the lower limit of normal, body fat measurement can also be useful to screen for functional hypothalamic amenorrhea in this setting.

Investigations

In males, the diagnosis of hypogonadotropic hypogonadism is based on low plasma total testosterone levels, usually associated with a concurrent decline in LH and FSH (49). The diagnosis is very easy in patients with complete gonadotropin deficiency (which is usually the case), when the total testosterone is below 1 ng/ml (3.467 nmol/l) or even 0.5 ng/ml in the majority of the affected patients (49). The concentration of inhibin B, another testicular hormone, is also low in patients with gonadotropin deficiency (18, 30, 50–53). In the setting of CHH, this points to functional Sertolian cell deficiency, which depends on FSH deficiency (51). However, inhibin B is not a sufficiently sensitive marker for positive diagnosis of hypogonadism, because it can be normal in partial forms; however, in the same way as testicular volume, with which it correlates closely, inhibin B provides useful information on the degree of gonadotropin impairment (18, 30, 50–53).

Maintenance of circulating anti-Müllerian hormone concentrations at prepubertal levels (51, 52, 54, 55) is

another characteristic of men with CHH, and reflects the absence of pubertal testicular maturation, which is induced by intratesticular testosterone (54, 55). However, given the complex regulation of this Sertolian hormone – in opposite directions by testicular testosterone and FSH (51, 55) – its use for diagnostic purposes is delicate and is still being evaluated.

Although widely used, the practical value of the GNRH test is increasingly being called into question because of its mediocre cost-effectiveness. Indeed, the GNRH test provides no extra diagnostic information relative to baseline gonadotropin levels evaluated with modern assays. In addition, it cannot show whether the gonadotropin deficiency is hypothalamic or pituitary in origin: the results can be negative in profound hypothalamic gonadotropin deficiency and positive in partial pituitary deficiency, both in patients with CHH (18, 30) (see below) and in those with acquired postpubertal HH (56). In congenital gonadotropin deficiencies, the response to GNRH test is highly variable and depends on the severity of the deficiency, which is often reflected by the degree of testicular atrophy (18, 30, 57, 58) in men and by breast development in women. Thus, in men with complete forms and a testicular volume below 2 ml, the response is often very weak or absent, while in men with partial deficiencies and testicular volume above 6 ml, the response can be positive, or even supranormal in the case of LH. In sum, the GNRH test serves more to confirm the severity of congenital gonadotropin deficiency, which is often already clinically perceptible, than to make a positive diagnosis.

Plasma estradiol concentrations are often low in women with CHH (3, 30, 47) (see below), sometimes being near the detection limit. They seem to correlate with breast development: in the absence of breast development, circulating estradiol concentrations are very low (usually undetectable), while estradiol is detectable with a sensitive assay when breast development exceeds stage B2 (J Young unpublished observations). A similar relationship exists between pubertal development and gonadotropin concentrations: the latter are often very low or undetectable in the absence of breast development, while in patients with stage B3 or B4 breast development, they can reach values close to those observed in the early follicular phase of women with normal cycles (Young J *et al.* manuscript in preparation).

Before making a firm diagnosis of isolated congenital gonadotropin deficiency, all antepituitary functions must be investigated in order not to miss hyperprolactinemia, global anterior pituitary insufficiency, or an associated endocrine disorder that may be part of syndromic forms of CHH (4–11).

In the absence of cryptorchidism and anosmia, primary juvenile hemochromatosis may mimic CHH (59). It is therefore useful to rule out iron overload, given the therapeutic implications in this disorder. Primary juvenile hemochromatosis can be ruled out by

measuring serum iron and the transferrin saturation coefficient.

Magnetic resonance imaging (MRI) of the brain and olfactory bulbs is useful in CHH. Although the findings are nearly always normal in isolated normosmic CHH (60) (Young J, unpublished results), MRI can rule out an expansive, infiltrative, or malformative disorder of the hypothalamo–pituitary region. MRI can also be used to analyze the olfactory bulbs and furrows in a search for signs of Kallmann's syndrome (unilateral or bilateral aplasia or hypoplasia, and effacement of the furrows), for instance, when semi-quantitative olfactometry is not available (61, 62). MRI is also useful for examining other anatomical structures such as the corpus callosum and cerebellum, which are occasionally abnormal in Kallmann's syndrome (61, 62) and abnormal in the Gordon Holmes syndrome (63) respectively.

Renal ultrasound examination is especially interesting in Kallmann's syndrome, as it can reveal renal malformation or agenesis, particularly in patients with a *KAL1* anomaly (2, 3, 64, 65), whereas it is practically always normal in isolated CHH (J Young unpublished data). Ultrasound examination of the testicles and internal genital organs is very useful to determine testicular volume precisely, and to assess the integrity of the seminal vesicles and vas deferens, which, if abnormal, can complicate infertility treatment.

In women, pelvic sonography is useful for determining the size of the uterus (66), which reflects estrogen impregnation, as well as endometrial thickness, ovary size, and the number and size of ovarian follicles (66–68), which may correlate with the severity of gonadotropin deficiency.

Isolated non-syndromic CHH

Before classifying patients in this category, a detailed physical examination and family history are necessary to rule out a mild syndromic form (69). The work-up must systematically include a semi-quantitative assessment of olfaction (olfactometry) to detect hyposmia which, contrary to complete loss of smell, is difficult to detect by simple interview. The patient should also question his or her family, if possible, to identify a previously unrecognized olfactory defect. This is important, because through the widespread use of systematic surveys and mutation screening of affected families, mutation-bearing relatives may be identified who were initially considered asymptomatic or as having apparently isolated CHH, but who in fact have partial or complete olfactory deficiency (18, 24, 25, 30). Close examination of the outer ear and hearing is also useful to rule out a mild CHARGE syndrome that may be mistaken for non-syndromic CHH or Kallmann's syndrome (69) (see below).

CHH due to pituitary GNRH resistance and loss-of-function mutations of the GNRHR gene

GNRHR gene defects were the first identified cause of non-syndromic CHH (70, 71). Systematic analysis of the gene coding for GNRHR in patients with isolated CHH managed in our department led to the discovery in 1997 of mutations responsible for the loss of GNRHR function in a man and his non-consanguineous sister, both of whom had partial CHH with normal olfaction (70). The two patients were compound heterozygotes, while the parents and another sister were heterozygous and had a normal phenotype, indicating recessive autosomal transmission of the disorder. The Glu106Arg mutation located in the first extracellular loop partially inhibited GNRH binding to its receptor. In contrast, the Arg262Glu mutation located in the C-terminal part of the third intracellular loop did not modify GNRH affinity, but affected signal transduction (70).

Since this first description, many familial and some sporadic cases of gonadotropin deficiency and GNRHR mutations have been reported (71–91) in subjects of both sexes with complete or partial gonadotropin deficiencies (Fig. 1).

The relatively large number of patients now identified allows genotype–phenotype relationships to be established between the degree of GNRHR signal transduction inhibition and the partial or complete nature of CHH and the positive or negative response to acute or prolonged pulsatile administration of exogenous GNRH (70–91). However, conflicts between the results of receptor function studies *in vitro* and the clinical phenotype have also been described in unrelated

subjects or even within a given family having the same mutation and different degrees of gonadotropin deficiency (73, 90). The most consistent characteristic of patients with GNRHR mutations is their pituitary resistance to pulsatile GNRH administration when the phenotype is severe and their spontaneous LH secretion is non-pulsatile (73, 90). This hormonal profile distinguishes these patients from those with mutations of *GPR54/KISS1R*, *TAC3*, or *TACR3*, whose pituitary, even in severe forms, seems to be highly sensitive to pulsatile exogenous GNRH, as will be discussed below. In CHH patients with GNRHR mutations and partial gonadotropin deficiency, LH release may be spontaneously pulsatile, but may show a below-normal pulse amplitude (70, 73). Similarly, a high dose of GNRH may elicit a response in these partial forms, with an increase in the LH pulse amplitude (73). Pregnancy has even been obtained after pulsatile administration of GNRH in one such case (77). In the female case reported in our princeps publication (70), two ovulations and two successive pregnancies were even obtained after clomifen citrate administration (J Young, unpublished results). This response raises the possibility that the patient's gonadotropic cells were stimulated by the increased endogenous GNRH secretion induced by clomifen, despite her partial receptor dysfunction.

GNRHR mutations seem to be the most frequent cause of familial CHH with normal olfaction, apparently representing nearly 40% of cases in some series (79, 92–94). They are more rarely found in sporadic cases, affecting fewer than 5% of such patients (79, 92–94).

Figure 1 Congenital hypogonadotropic hypogonadism: the clinical spectrum in males and females with GNRH receptor mutations and genotype–phenotype relationships. *Members of the same kindred. **Members of the same kindred. The pubertal status of a homozygous Cys279Tyr-affected man is unknown (Ref. (79)).

In most early reports, the *GNRHR* defects consisted of point mutations leading to amino acid substitutions (91–95) and to receptor dysfunction, seemingly affecting either ligand binding or signal transduction specifically, without altering *GNRH* binding to its receptor (91, 95). Rarer mutations lead to frameshifts or premature stop codons, resulting in a truncated protein (78, 82), but no true *GNRHR1* deletions have so far been described (94).

GNRHR1 point mutations are distributed throughout the coding sequence of the receptor, including the NH₂ terminus, transmembrane domains 2–7, extracellular loops 1 and 2, and intracellular loop 3 (92, 93, 95, 96). Two mutational hotspots have been identified, encompassing the Gln106Arg and Arg262Gln mutations. Six homozygous and ten compound heterozygous combinations of *GNRHR* mutants have been described in patients with partial or complete CHH. *In vitro* expression of 17 *GNRHR* mutants in heterologous cell systems showed that some mutants are completely non-functional (Glu90Lys, Ala129Asp, Arg139His, Ser168Arg, Ala171Thr, Cys200Tyr, Ser217Arg, Leu266Arg, Cys279Tyr, Pro320Leu, and Leu314X-stop), whereas others retain a variable degree of function (Asn10Lys, Asn10Lys_Gln11Lys, Thr32Ile, Gln106Arg, Arg262Gln, and Tyr284Cys) (92, 93, 95, 96).

Concerning the functional impairment of *GNRHR1*, although earlier *in vitro* expression studies suggested that these mutations altered only specific functions of the molecule (ligand binding, receptor activation, or interaction with coupled effectors), several findings made in Michael Conn's laboratory have challenged this simplistic view and indicated that *GNRHR* protein misfolding and resulting misrouting are mechanisms that, by themselves, may lead to loss of human *GNRHR* (h*GNRHR*) function. In interesting reviews, this group summarized the experimental data that led them to this conclusion, and explained how these studies revealed previously unsuspected features of the mutant h*GNRHR*. Furthermore, the same group provided data that challenged the classical genotype–phenotype correlation studies involving separate expression of mutant h*GNRHR* constructs, and which suggested that the phenotype and degree of loss of function of a given compound heterozygous combination were mainly dictated by the less severely affected allele. Indeed, they showed that this assumption does not take into consideration the potential dominant negative effect of a given h*GNRHR1* mutant on the other (95, 96).

CHH in patients carrying *GPR54/KISS1R* mutations

In 2003, two groups independently identified loss-of-function mutations in *GPR54/KISS1R* in patients with familial and sporadic forms of normosmic CHH,

and also discovered the reproductive role of this receptor and its putative ligands, the kisspeptins, encoded by the *KISS1* gene (97, 98). This finding was a major breakthrough in modern neuroendocrinology, and placed the spotlight on the roles of this receptor and its ligands in physiological control of both hypothalamic *GNRH* and pituitary gonadotropin secretion. These aspects have been addressed in a number of recent reviews (99–101), and we will therefore focus on the clinical and endocrinological features of patients who harbor these mutations, and particularly on common and/or specific characteristics.

The first reports of *GPR54/KISS1R* mutations in normosmic CHH were published by de Roux *et al.* in September 2003 (97). The patients were members of a consanguineous French family with eight children, five of whom had CHH. The index case was a 20-year-old man referred for late puberty. He had typical signs of hypogonadism, with small intrascrotal testes (4 ml), sparse pubic hair, a penis of 7 cm, and a bone age of 15.0 years. He had a normal sense of smell and no associated abnormalities. Three brothers were reported to have similar clinical features, but few details were provided in this princeps paper. The 16-year-old sister had partial hypogonadism with partial breast development, and had experienced a single episode of uterine bleeding. Hormonal evaluation showed low plasma testosterone in all the affected males and low plasma estradiol in the affected female, associated in every case with low plasma gonadotropin levels and with a blunted to 'normal' gonadotropin response in the *GNRH* test (97). Sequencing studies of the affected siblings showed a homozygous deletion of 155 bp spanning the splice acceptor site of the intron 4–exon 5 junction and part of exon 5. The deletion was absent, or present on only one allele, in unaffected family members, pointing to autosomal recessive inheritance.

In the second paper, published only a month later, Seminara *et al.* (98) described a large consanguineous Saudi Arabian family in which three marriages between first cousins had produced six affected and thirteen unaffected offspring. All affected individuals were found to carry a homozygous single-nucleotide mutation (443T>C) in exon 3, resulting in the replacement of the leucine at position 148 by a serine (Leu148Ser) and provoking *GPR54* loss of function *in vitro* (98, 102). Initial clinical and hormonal findings in this pedigree had in fact been reported first by Bo-Abbas (103) and then by Pallais (104). Clinical and endocrinological evaluation of the affected individuals revealed low sex steroid levels and low gonadotropin levels, confirming the diagnosis of CHH. Some of the patients subsequently underwent fertility therapy. Interestingly, the four affected males who received exogenous gonadotropins experienced testicular maturation, with the appearance of sperm in the ejaculate and subsequent fertility; this, in principle, ruled out associated primary testicular

dysfunction (104). In the same way, a female patient received pulsatile *GNRH* therapy, leading to ovulation and conception (104).

The same group published a detailed description of another male patient harboring the compound heterozygous loss-of-function mutations Arg331X and X399Arg in *GPR54/KISSR* (98). This patient (designated 03406 in several papers) presented at 17 10/12 years of age for late puberty, and had typical physical signs of complete hypogonadism (no facial hair, sparse axillary and pubic hair, hypotrophic (1.2 ml) intrascrotal testes, and no microphallus) and a normal sense of smell. Accordingly, the plasma testosterone level was very low, as were his gonadotropin levels, but the latter responded significantly to *GNRH* challenge. Testicular biopsy showed azoospermia with germinal hypoplasia and early atrophy (98). This individual had markedly higher sensitivity to exogenous pulsatile *GNRH* than a cohort of CHH patients receiving similar therapy, as well as an increase in testicular volume and spermatogenesis during long-term *GNRH* therapy. His wife became pregnant after one-and-a-half years of this treatment, when his testicular volume had reached 12 ml; subsequent semen analysis showed a sperm count of 7 million/ml (104).

In the paper by Lanfranco *et al.* (105), the *KISS1R*-mutated proband, who had CHH and bilateral cryptorchidism, was the only child of a consanguineous German family. At the age of 31, as he wished to have children, pulsatile *GNRH* therapy was started to stimulate spermatogenesis. Testosterone levels assessed several months later were normal, while gonadotropin levels were markedly increased (LH: 11.5 IU/l; FSH 24.9 IU/l), indicating associated testicular damage, possibly due to the cryptorchidism. At the same time, ultrasound scan showed two hypotrophic intrascrotal testes (right: 3 ml; left 5.3 ml). At the age of 33, after 2 years of pulsatile *GNRH* therapy, semen analysis showed persistent oligoasthenoazoospermia, but he nonetheless fathered a healthy son by IVF. *GPR54/KISS1R* sequencing showed a homozygous insertion of a cytosine after nucleotide position 1001 (1001_1002insC), resulting in a frameshift in the open reading frame, starting from amino acid 301 and including the seventh transmembrane domain, thereby elongating *GPR54* protein from 398 to 441 amino acids. The functional consequences of the mutation, although likely to be deleterious, were not tested *in vitro*.

The proband reported by Semple *et al.* (106), a man of mixed Turkish-Cypriot and Afro-Caribbean ancestry, was born at term after an uneventful pregnancy. He was noted to have a microphallus and undescended testes at birth, and serum gonadotropins, assayed during the 'window of opportunity' at an age of 2 months, were almost undetectable (LH: 0.5 mIU/ml, FSH: 0.5 mIU/ml). His pituitary function was otherwise normal. He was found to be a compound heterozygote for two missense mutations in *GPR54*, namely cysteine 223 to arginine

(C223R) in the fifth transmembrane domain and arginine 297 to leucine (R297L) in the third extracellular loop. In cells stably expressing *GPR54*, the C223R mutant was found to exhibit profoundly impaired signaling, whereas the R297L variant was associated with mild signaling impairment (106).

Finally, Tenenbaum-Rakover *et al.* (107) identified five patients with CHH belonging to two unrelated consanguineous Arab-Muslim families from Syria and Israel. All the affected subjects were homozygous for another *GPR54/KISS1R* missense mutation that replaced a leucine with a proline at residue 102 (L102P), and completely abolished *GPR54* signaling. The proband of the first family was a female born to first-cousin parents. She initially presented with primary amenorrhea and partial pubertal development (Tanner stage B4). Pretreatment evaluation of the hypothalamo-pituitary-gonadal axis confirmed HH, and pelvic sonography revealed a small uterus and small ovaries, each bearing fewer than five follicles <10 mm in diameter. Importantly, the proband was subsequently treated with exogenous pulsatile *GNRH*, which led to ovulation and to two normal pregnancies (107).

In the second family, the proband was born to consanguineous parents. Micropenis and cryptorchidism were noted at birth. He had 11 siblings, including two sisters with primary amenorrhea but partial breast development (Tanner stage B3), well-developed pubic hair, and typical hormonal features of CHH, at 16 and 17 years of age. In this second family, displaying a variable degree of hypogonadism, *GNRH* tests also showed intrafamilial variability in LH and FSH responses, with a blunted LH response in the male proband but a large increase in LH in his sisters. The authors of this paper reported that hormone studies of the proband revealed persistent pulsatile LH secretion with a normal frequency but a very low amplitude, suggesting that *GPR54* inactivation impaired but did not prevent the neuroendocrine onset of puberty.

Although the number of *GPR54/KISS1R*-mutated patients reported to date is relatively small (fewer than 20), certain shared clinical and endocrinological characteristics are worthy of interest.

As in patients harboring *GNRH* mutations, none of the patients carrying *GPR54/KISS1R* mutations were reported to have anosmia or other associated signs noted in Kallmann's syndrome or other syndromic forms of CHH.

When treated with *GNRH* or gonadotropins, all the patients with *KISS1R* mutations responded, strongly indicating that loss-of-function mutations in *GPR54/KISS1R* do not diminish the sensitivity of gonadotropic cells to *GNRH* or the sensitivity of the gonads to gonadotropins. Thus, in humans as in mice, *GPR54/KISS1R* loss of function mainly appears to affect hypothalamic *GNRH* secretion, with no discernible direct effect on the pituitary or gonads. Accordingly, the similar hypothalamic *GNRH* concentrations

observed in *gpr54*^{-/-} mice and their wild-type counterparts indicate that these neurons migrate normally during development, and that biosynthesis of the decapeptide by *GNRH* neurons is preserved (98, 100). Thus, the *GPR54/KISS1R* system appears to be involved mainly in hypothalamic *GNRH* release. This was supported by direct evidence, obtained in functional studies of rats (*ex vivo*) and sheep (*in vivo*), showing that kisspeptins can stimulate *GNRH* secretion in the hypothalamus: kisspeptin-10 was shown to elicit *GNRH* release by rat hypothalamic explants (99–101) (and references therein), while intracerebral kisspeptin injection to sheep induced *GNRH* release into cerebrospinal fluid. Together, these data strongly suggested that the primary site of kisspeptin action in the control of the gonadotropic axis was hypothalamic *GNRH* neurons. Further support for this view came from expression and functional studies showing that in rats, 77% of *GNRH* neurons co-express *GPR54/KISS1R* mRNA, and that kisspeptin efficiently induces *c-fos* expression (an early marker of activation) in >85% of *GNRH* neurons (99–101) (and references therein). In addition, the ability of kisspeptin to stimulate *GNRH* neurons was confirmed by electrophysiological recordings *in situ*, which showed that kisspeptin was capable of evoking long-lasting depolarization responses in >90% of this neuronal population in the adult mouse hypothalamus (99–101) (and references therein). In sum, compelling evidence from different animal models demonstrates that kisspeptins are able to directly activate hypothalamic *GNRH* neurons, and that this is a major mechanism underlying their potent inducing effect on gonadotropin release.

In view of the published data summarized above, showing the remarkable pituitary sensitivity to *GNRH* in patients with *GPR54/KISS1R* mutations, pulsatile administration of this decapeptide is logical and effective, even at relatively low doses, and even in subjects with severe CHH. This responsiveness contrasts with that of patients with *GNRHR* mutations, who, with the exception of those with moderate forms, are resistant to the decapeptide.

Another interesting finding in men with CHH and *GPR54/KISS1R* mutations is the relatively high frequency of patients with cryptorchidism or micropenis. Cryptorchidism is considered to reflect gonadotropin deficiency in the antenatal period (22). The gonadotropin deficiency would therefore appear to be present in these patients well before the age of puberty. This is strengthened by the findings of Semple *et al.* (106), also outlined above, who confirmed the existence of gonadotropin deficiency in one such patient assessed during the neonatal period. In sum, these data indicate that activation of Kiss/*GPR54* signaling in the hypothalamus is not specifically necessary to trigger puberty as stated initially (98), but is involved in all phases of physiological activation of the gonadotropin axis.

Pubertal development fails in all individuals with homozygous or compound heterozygous *GPR54/KISS1R* mutations, while heterozygous family members have normal reproductive phenotypes, indicating monogenic recessive inheritance.

Like *GNRHR* mutations, *KISS1R* mutations seem to be more frequent in familial probands than in non-familial probands. However, documented mutations are currently much more common in *GNRHR* than in *KISS1R*, and additional studies of patients with *GPR54/KISS1R* mutations will be necessary to determine the full spectrum of phenotypes that can result from mutations in the latter gene, and also to evaluate their precise prevalence in the population of patients with normosmic non-syndromic CHH.

Neurokinin B and its receptor NK3R, encoded by *TAC3* and *TACR3*, two new actors involved in the neuroendocrine control of gonadotropic function

December 2009 saw the publication of an important paper describing mutations affecting two unexpected genes in familial forms of normosmic CHH (108). Topaloglu *et al.* identified a key role for neurokinin B in the human reproductive axis by means of genome-wide single-nucleotide polymorphism (SNP) analysis of nine inbred Turkish families with CHH. Males and females with severe congenital gonadotropin deficiency belonging to these consanguineous Turkish families had homozygous missense mutations in *TAC3* gene leading to a loss of function of neurokinin B, a tachykinin family neuropeptide, or of its seven-transmembrane-domain receptor NK3R encoded by *TACR3* (Table 2).

In three of these families, the authors found homozygous mutations (encoding Gly93Asp and Pro353Ser) in the transmembrane domain of NK3R, and, in one family, a Met90Thr substitution located in the canonical tachykinin motif of mature neurokinin B (108).

Pedigree analysis also revealed that heterozygous carriers of the Met90Thr substitution were unaffected, indicating autosomal recessive CHH and, therefore, the absence of haploinsufficiency or a dominant negative effect of the mutations. Functional analysis demonstrated that both ligand and receptor mutations resulted in impaired receptor signaling *in vitro* when expressed in a heterologous cell line.

In a more recent paper (109), the same group provided further evidence for the involvement of neurokinin B and its receptor in the regulation of human reproductive function. They sequenced the *TACR3* gene in a family in which three siblings had nCHH, and found that all the affected siblings were homozygous for the His148Leu mutation, located in the first extracellular loop of the NKB receptor. The His148Leu mutant receptor exhibited profoundly

Table 2 Clinical characteristics in published congenital hypogonadotropic hypogonadism patients with *TACR3* or *TAC3* mutations (adapted from Refs (108) and (109)).

Patient number	1	2	3	4	5	6	7	8	9	10	11
Family NR Mutation	I/ <i>TACR3</i> Gly93Asp/ Gly93Asp	I/ <i>TACR3</i> Gly93Asp/ M/24	III/ <i>TACR3</i> Pro353Ser/ Pro353Ser	II/ <i>TACR3</i> Pro353Ser/ F/15.3	III/ <i>TACR3</i> Pro353Ser/ F/16.2	III/ <i>TACR3</i> Pro353Ser/ F/14	IV/ <i>TACR3</i> His148Leu/ F/26	IV/ <i>TACR3</i> His148Leu/ M/25	IV/ <i>TACR3</i> His148Leu/ M/14.5	V/ <i>TAC3</i> Met90Thr/ Met90Thr	V/ <i>TAC3</i> Met90Thr/ F/17.5
Sex/age	F/20	M/24	M/12.8	F/15.3	F/16.2	F/14	F/26	M/25	M/14.5	F/21.5	F/17.5
Reproductive phenotype at diagnosis	Primary amenorrhea, absent breast development	Micropenis, mean TV: 1 ml	Micropenis, cryptorchidism at birth	Absent breast development	Primary amenorrhea, absent breast development	Primary amenorrhea, absent breast development	Primary amenorrhea, absent breast development	Micropenis, in early infancy	Micropenis, cryptorchidism at birth TV: 1 ml	Primary amenorrhea, absent breast development	Primary amenorrhea, absent breast development
Reproductive phenotype in parents/heterozygous kindred	Normal/normal		Normal/normal or status uncertain as prepubertal		Normal/normal or status uncertain as prepubertal			Normal/normal		Normal/normal	
FSH (IU/l)	3.23→12.78	3.03→11.53	1.15→6.37	4.9→16.3	2.06→7.99	2.0→8.48	0.9→11	5.3→ND	0.7→2.9	0.05→2.2	0.41→2.2
basal→stimulated											
LH (IU/l)	0.1→2.86	0.2→3.24	0.1→2.25	0.5→6.22	0.26→2.27	0.1→0.9	0.1→4.0	2.7→ND	0.1→0.5	0.06→2.53	0.1→3.84
basal→stimulated											
Other anterior pituitary dysfunction	No	No	No	No	No	No	No	No	No	No	No
Anosmia or associated signs	No	No	No	No	No	No	No	No	No	Mild mental retardation	Mild mental retardation

TV, testicular volume.

impaired signaling in response to NKB in a heterologous expression system using calcium flux as the functional readout.

The precise mechanism whereby neurokinin B and NK3R exert their effects on the central neuroendocrine control of reproduction remains to be established. The authors suggested that it might involve an effect on hypothalamic *GNRH* release (108–114), with NK3R being expressed on rodent *GNRH*-expressing neurons (113). In addition, axons of neurons expressing neurokinin B are closely anatomically apposed to those of *GNRH* neurons within the median eminence of the hypothalamus (113, 114), and NKB-immunoreactive varicosities have been reported to be in direct contact with *GNRH*-immunoreactive axons (113, 114). Finally, NKB expression is highest in the arcuate nucleus, where it colocalizes with estrogen receptor- α and dynorphin 23, both of which are involved in progesterone feedback in response to *GNRH* secretion (114).

Neuroendocrinological studies are underway worldwide to unravel the intimate mechanisms by which these two new factors influence *GNRH* secretion (114).

In a recent study, we identified three unrelated patients with the same homozygous substitution in the *TAC3* intron 3 splice acceptor site (c.209-1G>C) and three siblings with a homozygous mutation in the *TACR3* intron 2 splice acceptor site (c.738-1G>A). We showed that these two mutations invalidate neurokinin B and its receptor NK3R respectively (115). Interestingly, like Topaloglu *et al.*, we observed in several patients a dissociation between very low LH levels and normal or near-normal FSH levels, with this gonadotropin responding excessively to *GNRH* challenge. This particular hormonal profile points to specific neuroendocrine impairment in patients with altered neurokinin B signaling. Finally, pulsatile *GNRH* administration normalized circulating sex steroid levels and LH release in these patients, and restored fertility in a woman (115). Taken together, these findings demonstrate the hypothalamic origin of the gonadotropin deficiency in these genetic forms of normosmic non-syndromic CHH. Therefore, both neurokinin B and NK3R play a crucial role in human hypothalamic *GNRH* release.

***GNRH1* mutations as a cause of CHH in humans**

GNRH is crucial for regulating reproduction in mammals. It is synthesized by hypothalamic neurons, and released from nerve endings into the portal circulation. After binding to the membrane *GNRH* type 1 receptor, it stimulates gonadotropic cells of the anterior pituitary to synthesize and release LH and FSH. These two peptides then travel through the general circulation to the gonads, where they stimulate the synthesis and secretion of sex steroid hormones, and gametogenesis.

The discovery in 1977 of a hypogonadal mouse lacking *GNRH* (the hpg mouse) and the clear role of a *gnrh1* deletion, demonstrated in 1986, suggested that mutations in *GNRH1* might cause human CHH. However, proofs that *GNRH1* mutations are involved in CHH pathogenesis were only published in 2009.

In 1977, Bruce Cattanaach and colleagues discovered a natural mouse mutant in which adults of both sexes exhibited complete hypogonadism (116). They also demonstrated that the phenotype was related to a deficiency in hypothalamic *GNRH*, and to the resulting reduction in pituitary LH and FSH contents and circulating gonadotropin levels (116) (Fig. 2). Evidence of a causal relationship between hypogonadotropic hypogonadism and *GNRH* deficiency in the hpg mouse was provided later by several groups who showed that when placed in the third ventricle, grafts of normal fetal preoptic tissue (from the region containing the majority of *GNRH* neurons in rodents) reversed the endocrine deficiencies of both male and female hpg mice (117, 118).

Peter Seeburg's group demonstrated in 1986 that the hereditary autosomal recessive hypogonadotropic hypogonadism of hpg mice is caused by a deletion of 33.5 kb encompassing the distal half of *gnrh1* (119). The partially deleted gene, lacking the two exons that encode most of the GAP, was transcriptionally active, as shown by *in situ* hybridization of hypothalamic tissue sections, but the *GNRH* peptide was undetectable by immunocytochemistry or RIA with antibodies against

Figure 2 Relationship between normosmic non-syndromic congenital hypogonadotropic hypogonadisms, Kallmann's syndrome (KS), and other syndromic causes. Milder forms of the complex syndromic causes, for instance, *CHARGE* syndrome, can mimic KS or non-syndromic normosmic forms.

different sections of the *GNRH* precursor (119). The same group, using transgenic techniques, restored the normal *GNRH* gene in hpg mice and observed full recovery of pituitary and gonadal functions (120), thus demonstrating the crucial role of *gnrh1* in hypothalamic control of rodent reproduction.

Analysis of the hpg mouse *gnrh1* gene revealed a large deletion, leaving only the promoter region, the first two exons encoding the signal peptide, the *GNRH* decapeptide itself, and the first 11 amino acid residues of GAP. However, as this mutation left intact the region encoding the *GNRH* decapeptide, the reason why the hpg mouse was hypogonadal remained elusive. One hypothesis was that this gene truncation led to a non-functional *GNRH* precursor mRNA molecule and, therefore, to an inability of *GNRH* neurons to produce and secrete *GNRH*. The intimate mechanism of this mystery was finally explained by the group led by K Kim, who analyzed *GNRH* pre-mRNA splicing (121). These authors proposed that the genomic deletion of the two last exons, which contain exonic splicing enhancers, led to the accumulation of the first-intron-retained transcripts, which can be transported to the cytoplasm and strongly inhibit the translation of the retained first-intron transcripts into *GNRH* peptides, given the suppressing effect of intron 1 retention on the translational activity of the downstream open reading frame, finally resulting in a lack of functional *GNRH* and hypogonadism in the hpg mouse (121).

Using a candidate-gene approach, we spent many years screening the subgroup of our CHH patients with non-syndromic normosmic disease in order to identify mutations in the *GNRH1* gene. Among nearly 150 probands analyzed, we identified a homozygous *GNRH1* frameshift mutation in January 2008. The mutation involved insertion of an adenine at nucleotide position 18 (c.18-19insA), in the sequence encoding the N-terminal region of the signal peptide containing protein precursor of *GNRH* (prepro-*GNRH*), in two siblings of Romanian origin (122). The index case was a young man who was referred at 18 years of age for pubertal failure. He had typical signs of complete hypogonadism, with small intrascrotal testes, no pubic hair, and micropallus, but a normal sense of smell. His affected normosmic sister, who was evaluated at 17 years of age, also had complete hypogonadism, with no breast development, no pubic hair, no menarche, and a small uterus and two small ovaries on pelvic sonography (122). Both siblings had very low levels of gonadal steroids and serum gonadotropins, a blunted response to *GNRH* challenge, normal to low levels of prolactin, and otherwise normal pituitary function. In the affected sister, who had a non-pulsatile baseline LH secretory pattern, we also had the opportunity to study the pituitary response to exogenous pulsatile *GNRH* in order to distinguish between hypothalamic and pituitary gonadotropin deficiencies. As reported by Charlton *et al.* in hpg mice (123), we found that

endogenous LH pulsatility was restored by exogenous *GNRH*, demonstrating the hypothalamic origin of the gonadotropin deficiency and the apparently normal responsiveness of pituitary gonadotropic cells to *GNRH*. Pulsatile *GNRH* administration also restored the patient's ovarian function, as indicated by increased circulating levels of estradiol and inhibin B and the recruitment of a single dominant follicle seen on sonography. Both index subjects were homozygous for the mutation, whereas their unaffected parents and sister were heterozygous and had a normal reproductive phenotype. The disorder was thus transmitted, as in hpg mice, as an autosomal recessive trait. These observations suggested that in both humans and mice, one copy each of the *GNRH1* and *gnrh1* genes is sufficient for normal *GNRH* secretion and for normal function of the gonadotropic axis, thus ruling out haploinsufficiency.

The homozygous c.18-19insA *GNRH1* mutation that we detected in both affected individuals resulted in a frameshift that, if translated, would result in an aberrant peptide starting at amino acid 7 of the *GNRH* precursor (122). This aberrant peptide, with a truncated peptide-signal sequence devoid of its hydrophobic core, completely lacked the normal *GNRH* decapeptide sequence, strongly suggesting its pathological nature, and had a total length of 42 amino acids instead of the 92 amino acids of the normal prepro-*GNRH* peptide (122). In addition, our *in vitro* experiments clearly demonstrated the deleterious nature of the homozygous mutation: the transfected *GNRH1* mutant could not sustain *GNRH* secretion by AtT20 cells (which possess proconvertases), while the same cells were able to produce and secrete mature *GNRH* when transfected with the expression vector of the wild-type *GNRH1* gene (122).

It is noteworthy that these two CHH patients had normal to low plasma prolactin levels, despite homozygous frameshift mutations putatively leading to the translation of an aberrant peptide also completely lacking the GAP sequence (122). Prolactin levels in both patients remained normal despite long-term sex hormone replacement therapy, ruling out the possibility that their normal to low prolactin levels were merely related to their sex steroid deficiency (124, 125) and demonstrating that, in physiological conditions, GAP or putative processed GAPs do not act on the human pituitary gland to regulate prolactin secretion, contrary to Seeburg's group assertion in *Nature* in 1985 (126). In this respect, it is interesting to go back to Cattanach's first publication describing the hpg mouse (116) and the very low pituitary prolactin content in both males and females. This important finding, which strongly argued against an inhibitory role of GAP in prolactin secretion, was omitted from Nikolics' paper (126).

After the initial demonstration that *GNRH1* mutations could cause CHH, a second homozygous frameshift *GNRH1* mutation (G29GfsX12) was identified. It was predicted to disrupt the three C-terminal

amino acids of the *GNRH* decapeptide, and to produce a premature stop codon. The latter mutation was found in a prepubertal boy originating from Armenia who was evaluated at an age of 8 years 8 months for cryptorchidism and microphallus (127). This patient, with possible CHH and a normal sense of smell, had not entered puberty at the age of 13 years 6 months, but was subsequently virilized by testosterone enanthate treatment. Unfortunately, no further clinical or hormonal data have since been provided to establish categorically the diagnosis of CHH (2). Interestingly, the boy's parents, who were most likely heterozygous carriers, had normal pubertal timing, as in the family we described, further supporting the autosomal recessive nature of this genetic form of CHH. The latter paper also mentioned heterozygous variants identified in four patients with CHH (127), consisting of a non-synonymous missense mutation in the eighth amino acid of the *GNRH* decapeptide, a nonsense mutation causing premature termination of the GAP sequence, and two sequence variants causing non-synonymous amino acid substitutions, one in the signal peptide and the other in GAP. However, the contribution of these heterozygous *GNRH1* variants to the pathogenesis of normosmic CHH remains to be established.

Normosmic, non-syndromic CHH, Kallmann's syndrome, and complex syndromic causes of CHH: are they related/how are they related?

As indicated above, the CHH phenotype associated with mutations in *GNRHR1*, *GPR54/KISS1R*, *GNRH1*, *TAC3*, and *TACR3* is limited to the consequences of the resulting gonadotropin and sex steroid deficiencies. These patients do not have associated clinical manifestations such as those seen in Kallmann's syndrome or in more complex syndromic forms of CHH such as the CHARGE syndrome (Fig. 2). However, there have been several reports over the years of families in which some members have Kallmann's syndrome, while others have normal or apparently normal olfaction (not always assessed by semi-quantitative olfactometry) (2, 3, 18, 24, 30, 128–130). Since the discovery of five genetic specific causes of Kallmann's syndrome, it has been established that this dissociation between the two cardinal signs of Kallmann's syndrome is mainly seen in patients with autosomal forms, while in X-linked forms with documented *KAL1* mutations, the two cardinal signs are almost always present together (17, 18, 25).

A recent study indicated that *FGFR1* mutations were present in 7% of 134 'normosmic' patients (130), which is close to the frequency (10%) of such mutations in patients with Kallmann's syndrome. However, only in a minority of these 134 patients, sense of smell was assessed, semi-quantitatively, raising the possibility that some of them were hyposmic and implying that the

FGFR1 mutation frequency in subjects with true normosmic CHH might be lower than reported by these authors.

In the same way, Kim *et al.* (131) performed a mutation screening of the *CHD7* gene in 197 patients with CHH with and apparently without olfactory abnormalities. These authors identified seven heterozygous mutations in three sporadic Kallmann's syndrome and four sporadic normosmic CHH patients. Therefore, it was proposed that these conditions could be considered mild allelic variants of CHARGE syndrome. However, as stated above, Jongmans *et al.* (69) identified *CHD7* mutations only in Kallmann's syndrome patients who had additional phenotypic features of CHARGE syndrome. This study therefore indicated that patients diagnosed with Kallmann's syndrome should be screened rigorously for clinical features consistent with CHARGE syndrome, and when deafness, heart abnormalities, dysmorphic ears, and/or hypoplasia or aplasia of the semicircular canals are found, *CHD7* sequencing should be recommended.

The need to distinguish between real normosmic non-syndromic CHH and apparently normosmic Kallmann's syndrome or CHARGE syndrome is not purely academic, as it also has important implications for patient management. Indeed, the mode of transmission seems to be constantly autosomal recessive in normosmic non-syndromic CHH with identified mutations. Thus, in the absence of consanguinity, normosmic non-syndromic CHH candidates for assisted reproduction can be reassured that the risk of having an affected child is very low. In contrast, given the autosomal dominant transmission of Kallmann's syndrome due to *FGFR1* mutations, or CHARGE syndrome associated with *CHD7* mutations, the risk of transmission is 50%. Moreover, the variable phenotypic expression, even among the members of the same family, makes it difficult to predict the severity of the syndrome or the risk of associated disorders in these patients' future children. For this reason, the phenotype of patients with apparently non-syndromic CHH must be thoroughly documented, including a meticulous search for telltale signs of a mild syndromic form.

Digenic inheritance

Normosmic CHH and Kallmann's syndrome have longtime been considered as monogenic disorders with Mendelian inheritance pattern. However, in 2006, Dode *et al.* published a first case of possible digenic inheritance in Kallmann's syndrome (24). Indeed, they described a patient who was heterozygous for the p.L173R loss-of-function mutation in *PROKR2* that also carried a missense mutation, p.S396L, in *KAL1* exon 8. Since then, digenic inheritance has been shown in other Kallmann's syndrome or normosmic CHH patients who bore mutations in both *PROKR2* and *PROK2*

or in *FGFR1* and *NELF* or *GNRHR* (132) as well as in *PROKR2* and *GNRH1*, *GNRHR*, or *KISS1R* (30, 127, 129). Defects in different genes could act synergistically to induce the CHH or the Kallmann's syndrome phenotype, or to modify the severity of the *GNRH* deficiency, partially explaining the phenotypic variability observed within and across families with CHH and Kallmann's syndrome.

Thus, we must probably reconsider our current simplistic approach in studying the genetics of CHH or Kallmann's syndrome and not only consider the mode of inheritance as dominant or recessive all the more, since oligogenicity has implications for genetic counseling also.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported in part by grants from University Paris Sud 11: Grant 'Bonus Qualité Recherche'; INSERM- Agence Nationale de la Recherche: Grant 'ANR KalGenopath', and Programme Hospitalier de Recherche Clinique Assistance Publique Hôpitaux de Paris (PHRC): Grant 'Hypo-Proteo'.

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Received 27 February 2010

Accepted 4 March 2010