Congenital Idiopathic Hypogonadotropic Hypogonadism: Evidence of Defects in the Hypothalamus, Pituitary, and Testes

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Context: Idiopathic hypogonadotropic hypogonadism (IHH) with normal smell (normosmic IHH) or anosmia (Kallmann syndrome) is associated with defects in the production or action of GnRH. Accordingly, most IHH patients respond to physiological pulsatile GnRH replacement by normalizing serum LH, FSH, and testosterone (T) levels and achieving gametogenesis; some patients, however, show atypical responses. Interestingly, several IHH-associated genes are expressed in multiple compartments of the hypothalamic-pituitary-gonadal axis.

Objective: The aim of the study was to investigate whether the clinical, biochemical, or genetic characteristics of IHH men with atypical responses to GnRH indicate alternative or additional defects in the hypothalamic-pituitary-gonadal axis.

Subjects: We studied 90 IHH men undergoing long-term pulsatile GnRH treatment over 30 yr.

Design and Setting: We conducted a retrospective study of response to GnRH at a Clinical Research Center.

Interventions: Physiological regimens of pulsatile sc GnRH were administered for at least 12 months. Dose-response studies using iv GnRH pulses assessed the pituitary LH response.

Main Outcome Measures: We measured serum T, LH, FSH, and inhibin B levels, sperm in ejaculate, and determined the sequence of IHH-associated genes.

Results: Twenty-six percent of subjects displayed atypical responses to GnRH: 1) 10 remained hypogonadotropic and hypogonadal, demonstrating pituitary and testicular defects; 2) eight achieved spermatogenesis and normal T but only with hypergonadotropism, indicating impaired testicular responsiveness to gonadotropins; and 3) five remained azoospermic despite achieving adult testicular volumes and normal hormonal profiles, suggesting primary defects in spermatogenesis. Mutations were identified only in KAL1 across groups.

Conclusion: In addition to hypothalamic GnRH deficiency, IHH men can have primary pituitary and/or testicular defects, which are unmasked by GnRH replacement. (*J Clin Endocrinol Metab 95: 3019–3027, 2010*)

Abbreviations: CV, Coefficient of variation; HPG, Hypothalamic-pituitary-gonadal; IB, inhibin B; IHH, idiopathic hypogonadotropic hypogonadism; KS, Kallmann syndrome; nIHH, normosmic IHH; T, testosterone.
diopathic hypogonadotropic hypogonadism (IHH) with normal sense of smell [normosmic IHH (nIHH)] or with anosmia [Kallmann syndrome (KS)] is a rare genetic disorder caused by an isolated defect in the secretion of GnRH by the hypothalamus or, less frequently, by a defect in the action of GnRH on pituitary gonadotropes (1). The pulsatile release of GnRH by the hypothalamus is a key requirement for normal gonadotropin function (2), and exogenous pulsatile GnRH has been used as a therapeutic modality in IHH for more than 25 yr (3, 4). The clinical demonstration that IHH patients are highly responsive to physiological regimens of pulsatile GnRH administration has established that IHH is caused mainly by a hypothalamic defect in GnRH secretion (3). IHH is more prevalent in men (5:1 male-to-female ratio), and it is clinically heterogeneous because it can occur with various nonreproductive phenotypes in addition to anosmia (5–8). As a disease paradigm of GnRH deficiency, IHH facilitates insights into the physiology and pathophysiology of the hypothalamic-pituitary-gonadal (HPG) axis in humans. For example, mutations identified in IHH patients have highlighted genes critical for GnRH biology, puberty, and reproduction (1).

By characterizing a large cohort of men with IHH who received long-term exogenous pulsatile GnRH administered in a physiological regimen, we have previously shown that the majority of patients respond well to GnRH replacement by normalizing their serum gonadotropin and testosterone (T) levels, maturing their testes, and achieving spermatogenesis (3). Interestingly, however, a small subset of men deviate from this expected pattern of response to GnRH replacement because they exhibit abnormal hormonal profiles, persistently small testicular volumes, and/or lack of spermatogenic activity (4). Such paradoxical responses suggest that the pathophysiology of hypogonadism in these patients is more complex and may not be limited to an isolated hypothalamic or pituitary defect. However, such atypical response patterns have not been systematically studied to date, and the underlying reasons for them have not been elucidated.

Thus, the aims of the present study were: 1) to categorize the clinical, biochemical, and genetic characteristics of IHH men showing atypical patterns of response to long-term physiological GnRH replacement; and 2) to test the hypothesis that such IHH patients harbor more than one primary defect in the HPG axis.

**Subjects and Methods**

**Study subjects**

Each of the 104 men with IHH who received GnRH therapy at the Reproductive Endocrine Unit of the Massachusetts General Hospital (MGH) between 1979 and 2009 was considered for inclusion in the study. Criteria for the diagnosis of IHH were: 1) age greater than 18 yr; 2) clinical signs or symptoms of hypogonadism; 3) hypogonadal serum T (<100 ng/dl) in the presence of low or normal gonadotropins; 4) normal serum prolactin and ferritin concentrations, and normal thyroid, adrenal, and GH axes as assessed by dynamic testing; and 5) normal radiological imaging of the hypothalamus and pituitary (3, 4). Of the 104 men who had received GnRH, 14 did not adhere to the treatment, developed anti-GnRH antibodies, or did not complete scheduled evaluations, and were therefore excluded from the study. Thus, the study included 90 men with IHH who received long-term pulsatile GnRH. All subjects provided written informed consent before participation. The MGH Human Research Committee approved the study.

**Baseline clinical and biochemical assessment**

A detailed medical and family history was recorded, noting pubertal timing, sexual maturation, gonadal function, sense of smell, and any previous therapy. The physical examination included testicular volume assessment as well as standardized smell testing (8). After discontinuation of any prior treatment (≥4 wk off transdermal T, ≥8 wk off injectable T or gonadotropins), subjects were admitted to the MGH Clinical Research Center for a neuroendocrine evaluation of the HPG axis by overnight frequent blood sampling for serum LH (every 10 min for 12 h), FSH, T, and inhibin B (Iβ) were measured in pools constituted from equal aliquots of the 10-min samples. The endogenous LH secretion pattern was determined using a modification of the Santen and Bardin pulse analysis methodology (9). When LH levels were below the assay detection threshold, half the threshold value (0.8 IU/liter) was used for calculations. The normal ranges of serum LH, FSH, and T levels in adult men have been established using frequent serum sampling (every 10 min for 24 h for LH and FSH; every 20 min for 24 h for T) (10). The normal range of serum Iβ levels in adult men has been established from 81 men with normal history of the timing of puberty and normal physical examination, including normal testicular volume (Crowley, W. F., unpublished observations). A standard seminal fluid analysis was performed in men who could produce an ejaculate (11).

**Pulsatile GnRH therapy**

After baseline evaluation, subjects initiated a regimen of pulsatile GnRH (Salk Institute, La Jolla, CA) administered sc via a portable infusion pump (Ferring Laboratories, Kiel, Germany) at a physiological 2-h interval for at least 12 months as previously described (3, 4). The GnRH dose was initially 5–25 ng/kg per pulse and was progressively increased (maximum dose, ≤800 ng/kg) to achieve and maintain mid-normal serum T levels (300–500 ng/dl). The pituitary gonadotrope responsiveness to GnRH was assessed in the large majority of these men (n = 82) by a GnRH-LH dose-response study in which iv GnRH doses ranging from 2.5 to 800 ng/kg were given randomly as previously described (12); this was done either after achieving normal serum T levels for at least 3 months or after at least 9 months of GnRH administration in subjects whose T did not normalize.
has intraassay CV less than 6% and interassay CV less than 18%.

using a double-antibody ELISA (Serotec, Oxford, UK), which by following the algorithm shown in Fig. 1.

groups according to their clinical and biochemical characteristics were considered “atypical responders” and were classified into sperm were classified as “typical responders.” All other men levels rose into the normal range and who produced mature sperm were classified as “typical responders” and were classified into groups according to their clinical and biochemical characteristics by following the algorithm shown in Fig. 1.

Assessment of response to physiological GnRH replacement

The hormonal response of the HPG axis was assessed after 2, 6, 12, 18, and/or 24 months of pulsatile GnRH replacement by measurement of serum T, I<sub>B</sub>, LH, and FSH levels; assessment of testicular volume; and analysis of seminal fluid in men who could produce an ejaculate. Subjects whose serum T, LH, and FSH levels rose into the normal range and who produced mature sperm were classified as “typical responders.” Other men were considered “atypical responders” and were classified into groups according to their clinical and biochemical characteristics by following the algorithm shown in Fig. 1.

Hormone measurements

Because this study spanned a 30-yr period, two immunoassay systems were used to measure LH and FSH, as previously described (4). Serum LH and FSH were measured with microparticle enzyme immunoassays using an AxSYM instrument (Abbott Laboratories, Chicago, IL); intra- and interassay coefficients of variation (CVs) were less than 7.5%. The assay sensitivity for both LH and FSH was 1.6 IU/liter based on a human menopausal gonadotropin standard, which is equivalent to 0.34 mIU/ml LH or 0.66 mIU/ml FSH based on pituitary standards. The Second International Reference Preparation was used as the human menopausal gonadotropin reference standard for consistency with previous reports (4, 8). Serum T concentrations were measured using the DPC Coat-A-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA), which has intra- and interassay CVs of less than 10%. I<sub>B</sub> was measured using a double-antibody ELISA (Serotec, Oxford, UK), which has intraassay CV less than 6% and interassay CV less than 18%.

Data representation and statistical comparisons

The serum T, LH, FSH, and I<sub>B</sub> levels of IHH men who receiving long-term GnRH replacement were categorized using an algorithm based on the following criteria. First, did the serum levels of T normalize? Second, did the serum levels of LH and FSH normalize? Lastly, did mature sperm develop in the ejaculate? (Fig. 1). According to these criteria, the patients were classified into four categories. Sixty-seven of 90 patients displayed the expected response to GnRH replacement; these men achieved normal serum T (270–1100 ng/dl), LH (4.2–17 IU/liter), and FSH (1.8–14 IU/liter) within 12 months and had sperm in their ejaculate within 18 months of treatment. Subjects in this group were therefore called “typ-
ical responders” and are represented as a gray shaded area in Figs. 2 and 3. The study cohort included two pairs of brothers, both typical responders; thus, the frequency of typical responders among unrelated patients was 65 of 88 (74%). In the remaining 23 patients (26%), three distinct patterns of abnormal response to long-term GnRH were identified (Figs. 1 and 2): 1) 10 men remained hypogonadotropic and hypogonadal, with low-normal LH/FSH, serum T less than 200 ng/dl, and no sperm, despite GnRH doses up to 800 ng/kg (group 1, green lines in Fig. 2); 2) eight men achieved normal serum T and produced sperm, but did so with high LH (>17 IU/liter) and FSH (>14 IU/liter) levels (group 2, red lines in Fig. 2); 3) five men remained azoospermic after at least 21 months despite achieving normal serum T, LH, and FSH (group 3, blue lines in Fig. 2). Table 1 shows the phenotypic and genetic characteristics of subjects with atypical responses to GnRH replacement. Although typical responders exhibited mutations in almost all IHH genes screened, atypical responders displayed mutations exclusively in KAL1 (Table 2).

**Group 1: GnRH deficiency, pituitary resistance, and testicular failure—a “triple defect”**

In this subset of IHH men (n = 10), pulsatile GnRH administration increased LH and FSH into the lower range of levels seen in typical responders (P > 0.05, for difference from typical responders; Fig. 2), consistent with GnRH deficiency. Despite the increase in LH and FSH levels, T levels remained hypogonadal, IGF levels did not normalize (P < 0.05 compared with typical responders), and the patients remained azoospermic, suggesting an additional testicular defect. Although very high doses of GnRH (up to 800 ng/kg per pulse) were administered, LH levels remained inappropriately low for the frankly hypogonadal T levels, suggesting an additional partial defect at the level of the pituitary. Indeed, the pituitary sensitivity of these men to GnRH, quantified as the LH response to a log order of GnRH doses, was dramatically lower than that of all other investigated subjects (P < 0.001; Fig. 3). Notably, men in this group had either KS or nIHH and exhibited large phenotypic variability in clinical indices reflecting the onset and severity of GnRH deficiency, namely prior pubertal development, testicular volume, and incidence of microp phalus and cryptorchidism. The majority also exhibited various additional nonreproductive phenotypes (Table 1). Genetic defects were identified in two of the four familial cases. A missense change in the KAL1 gene (p.F517L) was found in a patient with KS and severe hypogonadism with
Bilateral cryptorchidism, microphallus, and infantile testes (Table 1, patient 1). A patient with KS was found to have partial tetrasomy of chromosome 22q11.2 [47, XY, idic(22)(q11.21q11.1)] on karyotype analysis, which is characteristic of cat eye syndrome (Table 1, patient 4). This patient lacked many of the syndrome’s common features, such as iris coloboma, anal atresia, congenital heart defects, and renal malformations. Although this was a familial case of KS, both parents had normal karyotype, which indicates that this chromosomal aberration, albeit de novo, cannot by itself account for the disease in this family. nIHH with hypothalamic, pituitary, and testicular defects has been associated with mutations in DAX1 (NROB1), which cause X-linked congenital adrenal hypoplasia manifested as adrenal insufficiency in infancy or childhood (13, 14). A DAX1 rare sequence variant (p.T418M) identified in one patient (Table 1, patient 3) is unlikely to account for his phenotype because he had KS rather than nIHH and no clinical evidence of adrenal insufficiency; moreover, this amino acid is not conserved among vertebrates, and the mouse Dax1 homolog actually has methionine at the equivalent position. No mutations were identified in the other screened IHH genes in group 1.

### Group 2: GnRH deficiency and testicular resistance—a “dual defect”

In this subset of IHH men (n = 8), pulsatile GnRH administration increased T into the range seen in typical responders (P > 0.05 for difference from typical responders; Fig. 2) and stimulated spermatogenesis, consistent with the diagnosis of GnRH deficiency and testicular resistance. The patients were characterized by bilateral cryptorchidism, microphallus, and infantile testes (Table 1, patient 1). A patient with KS was found to have partial tetrasomy of chromosome 22q11.2 [47, XY, idic(22)(q11.21q11.1)] on karyotype analysis, which is characteristic of cat eye syndrome (Table 1, patient 4). This patient lacked many of the syndrome’s common features, such as iris coloboma, anal atresia, congenital heart defects, and renal malformations. Although this was a familial case of KS, both parents had normal karyotype, which indicates that this chromosomal aberration, albeit de novo, cannot by itself account for the disease in this family. nIHH with hypothalamic, pituitary, and testicular defects has been associated with mutations in DAX1 (NROB1), which cause X-linked congenital adrenal hypoplasia manifested as adrenal insufficiency in infancy or childhood (13, 14). A DAX1 rare sequence variant (p.T418M) identified in one patient (Table 1, patient 3) is unlikely to account for his phenotype because he had KS rather than nIHH and no clinical evidence of adrenal insufficiency; moreover, this amino acid is not conserved among vertebrates, and the mouse Dax1 homolog actually has methionine at the equivalent position. No mutations were identified in the other screened IHH genes in group 1.

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with a hypothalamic defect. Strikingly, however, their normalization of T was achieved only via hypergonadotropism, i.e. supraphysiological levels of LH and FSH (Fig. 2). Because the pituitary sensitivity of these subjects to GnRH was not significantly different from typical responders (P > 0.05; Fig. 2), their hormonal profile (Fig. 2) indicates reduced testicular responsiveness to gonadotropins. Specifically, the finding of increased LH levels in the presence of normal T indicates that the responsiveness of the Leydig cell to LH is compromised but ultimately capable of achieving normal production rates of T. Moreover, the fact that FSH levels were very high in the setting of significantly low I$_{18}$ levels (P < 0.05 compared with typical responders; Fig. 2) indicates some degree of seminiferous tubule dysfunction, which ultimately leads to reduced feedback at the pituitary level. Taken together, these findings suggest a global defect in testicular function in these men, which, nevertheless, is only partial such that it does not preclude normal spermatogenesis. The alternative explanation that both the LH and FSH molecules are somehow defective, such that they fail to fully activate signaling, cannot be eliminated on the basis of these data but is highly unlikely because it would postulate defects of both gonadotropin species. The clinical presentation of hypogonadism in this group was rather severe because each subject had a history of cryptorchidism, microphallus, and/or absent puberty (Table 1). Notably, all but one man in this group had KS. Associated nonreproductive phenotypes were also common and included synkinesia as well as skeletal and midline abnormalities. Three of the seven familial cases in group 2 (Table 1, patients 11, 14, and 15) had frameshift mutations in the $KAL1$ gene predicted to lead to truncated protein products. The other screened IHH genes were not found to harbor mutations in this group.

**Group 3: GnRH deficiency with azoospermia—a “dual defect”**

This class of atypical responders (n = 5) achieved serum T, LH, FSH, and I$_{18}$ levels that were statistically identical to typical responders ($P > 0.05$; Fig. 2). The normalization of the hormonal profile by pulsatile GnRH indicates that these subjects have GnRH deficiency. The normal hormonal profile also predicted normal pituitary sensitivity to GnRH, and this was confirmed experimentally in a subset of these patients ($P > 0.05$ compared with typical responders; Fig. 3). Although these men achieved adult testicular volumes, they remained azoospermic after long-term GnRH administration (21 months to 10.5 yr). Semen samples from all subjects were alkaline (pH > 7.2) with normal ejaculate volume, making obstruction an unlikely cause of their azoospermia. Azoospermia in the presence of adult testicular volumes and normal hormonal profiles strongly indicates that, in addition to their hypothalamic defect, these men had a defect in spermatogenesis, which, given their normal I$_{18}$ levels, most likely affects the final steps of the process (15). These patients were heterogeneous in terms of their sense of smell, degree of pubertal development, testicular volume and descent, and history of microphallus. The single familial case in group 3 had a nonsense mutation in the $KAL1$ gene (Table 1, patient 20); no other mutations were identified in IHH-associated genes in this group.

**Discussion**

**Physiological pulsatile GnRH replacement demonstrates a hypothalamic defect in IHH subjects**

The large majority (74%) of IHH men receiving physiological pulsatile GnRH replacement in the present study achieved adult reproductive hormone levels, significant testicular growth, and development of sperm in the ejaculate. These outcomes are in agreement with the presence of a hypothalamic defect in IHH (3, 4). The concept that IHH is primarily a hypothalamic disorder caused by GnRH deficiency has been validated by the discovery of loss-of-function mutations in genes controlling the ontogeny, migration, survival, and functionality of GnRH neurons (1). Although as many as 26% of the IHH men did not respond to long-term pulsatile GnRH in a typical pattern, GnRH replacement nevertheless succeeded in stimulating LH and FSH secretion in all groups of atypical responders, albeit to different degrees. This pituitary response dem-

**TABLE 2. Atypical responders are a genetically distinct class**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Atypical responders (n = 21)</th>
<th>Typical responders (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NELF</td>
<td>p.T480A c.1165-14_22del$^b$</td>
<td></td>
</tr>
<tr>
<td>PROK2</td>
<td>p.V331M</td>
<td>p.R164Q</td>
</tr>
</tbody>
</table>

Two patients (a and b) were found to have two mutations each.
onstrates that GnRH deficiency is present, at least to some extent, in these patients. Taken together with the absence of mutations in the GnRH receptor, these observations strongly indicate that both typical and atypical responders have a consistent central feature of GnRH deficiency. Thus, a hypothalamic defect is the common denominator of this large IHH cohort as previously suggested (3), and this is true regardless of the outcome of GnRH treatment.

A subset of IHH men have pituitary resistance to GnRH

In group 1 (10% of the study cohort), serum LH and FSH levels increased into the normal adult range with long-term GnRH treatment but remained inappropriately low for the persistently hypogonadal levels of T. Moreover, the GnRH-LH dose-response curve of group 1 is markedly shifted to the right (Fig. 3). Taken together with the normal pituitary gland imaging and normal function of the other endocrine cells of the anterior pituitary, these findings strongly indicate that the pituitary gonadotropes are refractory to GnRH action. Pituitary resistance to GnRH is the hallmark of a subset of nIHH patients (~5%) who have mutations in the gene that encodes the GnRH receptor (GNRHR) (14–16). In those subjects, only pulsatile GnRH administration at pharmacological doses can overcome the pituitary block and elicit normal gonadotropin secretion and gonadal stimulation (16). Mice lacking Gnrhr have sexual immaturity but apparently normal GnRH neuron ontology (17), indicating that complete pituitary resistance to GnRH is likely sufficient to cause hypogonadotropic hypogonadism. Although very high doses of pulsatile iv GnRH failed to properly stimulate LH secretion in group 1, none of these subjects harbored GNRHR mutations, indicating a novel form of pituitary resistance. Moreover, four of the 10 subjects had KS, in which GnRH neuron migration is presumably impaired (18), further supporting the notion that these men have an additional hypothalamic or other central nervous system developmental defect.

The genetic basis of pituitary resistance to GnRH in group 1 is unknown. One patient had a missense mutation in KAL1; another had partial tetrasomy of chromosome 22 characteristic of cat eye syndrome (OMIM no. 115470), which has been previously associated with IHH in rare cases (19–21), although this particular patient lacked many of the syndrome’s common features. Genes regulating GnRH neuron ontology like FGFR1 and FGFR1 are also critical for pituitary development in vertebrates (22–25), suggesting that the hypothalamic and pituitary defects in IHH patients. Mutations in novel genes either downstream of the GnRH receptor or in other pathways central to gonadotrope development and function could contribute to this form of IHH.

An alternative explanation for the reduced efficacy of GnRH treatment in group 1 is that the exogenously administered hormone may have reduced bioavailability in these subjects. GnRH has a half-life of minutes (26) and is degraded by specific peptidases (27, 28). It is therefore conceivable that exogenously administered GnRH has decreased bioavailability in group 1 patients due to enhanced proteolysis. Although this theoretical possibility cannot be excluded by the present study, the fact that there is no known paradigm for increased catabolism of a hypothalamic hormone makes this scenario rather unlikely. Additionally, reduced responsiveness to GnRH was observed not only during long-term sc administration of physiological doses (Fig. 2), but also during iv administration of supraphysiological pulses (Fig. 3), such that a putative pharmacokinetic factor should theoretically play a lesser role.

Atypical responders have additional gonadal defects

In most men with congenital GnRH deficiency, physiological regimens of GnRH result in gonadotropin-induced T secretion and spermatogenesis, demonstrating that the gonads, although immature, retain their potential for functionality with appropriate stimulation (3). In addition to hypothalamic and pituitary defects, patients with atypical responses to GnRH showed clinical and biochemical evidence of defects in testicular function. These defects affect the Leydig cells and/or seminiferous tubules and range from resistance to gonadotropins to defective spermatogenesis. The variability of the gonadal responsiveness to gonadotropins in IHH patients has been appreciated for many years (29, 30). Developmental hypoandrogenism secondary to GnRH deficiency is an obvious cause of testicular dysfunction in IHH. Transient activation of the HPG axis in early infancy promotes Leydig cell proliferation and differentiation, Sertoli and germ cell proliferation, and testicular enlargement (31); this “mini-puberty of infancy” is typically absent in IHH subjects. Thus, the functionality of the testes in IHH may be reduced due to GnRH deficiency in infancy (31) and further compromised by germ cell apoptosis and Leydig cell dysfunction resulting from associated cryptorchidism (32).

Among patients with typical responses to GnRH in the present study, 20% had a history of unilateral or bilateral cryptorchidism. Similarly, two of the 10 patients in group 1, and one of the five patients in group 3 were cryptorchid, indicating that cryptorchidism by itself cannot account for the atypical responses of all subjects in these groups. Even in group 2, where as many as 60% of patients were cryptorchid, other etiologies must be sought for the testicular resistance in the remaining 40% of patients. Although
later descent of the testes in these noncryptorchid patients cannot be excluded as a contributing factor to testicular damage, it is noteworthy that two such patients harbored mutations in KAL1. KAL1 is expressed in the human mesonephric duct and tubules (33) as well as in human germ cells (http://symatlas.gnf.org/), suggesting that its lack might also contribute to testicular defects in these men.

In group 3, azoospermia persisted despite long-term GnRH therapy. The fact that these subjects achieved adult testicular volumes and had normal hormonal profiles including normal serum T and IGF strongly indicates that progression significantly (15). It is thus reasonable to conclude that the lack of GnRH and its consequences are not the cause of defective spermatogenesis in this group. Rather, a primary testicular defect leading to arrest of spermatogenesis likely exists in addition to the hypothalamic GnRH deficiency. Unfortunately, testicular biopsies were not available to confirm this conclusion, a limitation of this retrospective study.

Interestingly, IHH-associated genes operating in the hypothalamus are also expressed in the germ line, such as FGF8 in embryonic prespermatogonia (34), FGFR1 in adult spermatogonia (35), and PROK2 in primary spermatocytes (36). In addition, genes encoding GnRH and its receptor(s) are also expressed in the gonads, where they may have autocrine or paracrine regulatory functions (37). However, men with atypical responses to GnRH did not harbor mutations in any of these genes. Therefore, the co-occurrence of primary hypothalamic and testicular defects in a subset of IHH men might be a clue for the existence of yet unknown IHH-associated genes that function in both the hypothalamus and testes.

In conclusion, pulsatile GnRH is a unique biological probe for interrogating the pathophysiology of the HPG axis. Because IHH is predominantly a hypothalamic disorder caused by GnRH deficiency, atypical clinical responses to physiological GnRH are unusual. A prior history of partial sexual maturation, levels of IGF of at least 60 pg/ml, and absence of cryptorchidism predict a favorable outcome to physiological GnRH treatment (4). Nevertheless, patients who do not meet these criteria may still respond favorably. In patients who achieve normal T and spermatogenesis in response to GnRH, no modification of treatment is required. In patients with persistent hypogonadotropism, a trial of gonadotropin treatment is indicated; in those with persistent azoospermia despite normal hormonal profiles, obstructive lesions should be excluded. Albeit unusual, paradoxical responses are highly informative because they unmask defects at the level of the pituitary and/or testes. Although the exact genetic and pathogenic mechanisms of pituitary and testicular defects in IHH patients with atypical responses to GnRH are yet unknown, they are unlikely to be exclusively secondary consequences of GnRH deficiency. Rather, multiorgan primary defects in IHH men may have shared genetic origins in signaling pathways that control reproduction by operating in the hypothalamus, pituitary, and/or testes.

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