BRIEF REPORT

Antipituitary Antibodies against Gonadotropin-Secreting Cells in Adult Male Patients with Apparently Idiopathic Hypogonadotropic Hypogonadism

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Context: Hypogonadotropic hypogonadism (HH) can occur at any stage of life as an isolated congenital or acquired abnormality or within a more generalized pituitary or hypothalamic impairment. However, the defect in patients with idiopathic HH is still unknown.

Objective: The aim of this study was to investigate the prevalence of antipituitary antibodies (APA) in a group of HH patients with or without Kallmann’s syndrome and to characterize their pituitary target.

Design: We conducted a cross-sectional cohort study.

Setting: The study was performed at the Endocrinology Unit of the Second University of Naples.

Patients: Twenty-one HH patients with normal sense of smell (group 1), 10 patients with Kallmann’s syndrome (group 2), 13 patients with idiopathic HH associated with other pituitary hormone deficiencies (group 3), and 50 normal controls were studied.

Main Outcome Measures: APA were evaluated in patients and in controls by indirect immunofluorescence. Moreover, a magnetic resonance imaging (MRI) of the hypothalamic-pituitary region was performed in all three groups of patients.

Results: APA were detected at high titer in eight out of 21 patients in group 1 (38%) and in five of 13 in group 3 (38.4%), and at low titers in two out of 10 in group 2 (20%) and in three of 50 controls (6%). In patients of group 1, APA immunostained selectively gonadotropin-secreting cells, whereas in those of group 3, they immunostained other pituitary hormone-secreting cells also. None of patients in group 1 showed alterations on MRI, whereas all patients in group 2 showed aplasia/hypoplasia of the olfactory bulbs/tracts and/or of olfactory sulci. Among the five APA-positive patients in group 3, three had normal MRI, one had findings of empty sella, and one had findings of autoimmune hypophysitis.

Conclusions: Our results suggest that some apparently idiopathic cases of HH, both isolated and associated with other pituitary impairment, can be caused by an early autoimmune process involving the gonadotrophs at pituitary level. Future longitudinal studies are needed to clarify the natural history of this process and the possible effect of early corticosteroid therapy. (J Clin Endocrinol Metab 92: 604–607, 2007)

HYPOGONADOTROPIC HYPOGONADISM (HH) can occur at any stage of life as an isolated congenital or acquired abnormality or within a more generalized pituitary or hypothalamic defect (1). Kallmann’s syndrome (KS) is a congenital form of HH, usually associated with anosmia or hyposmia in which alterations of several specific genes prevent the migration of the GnRH cells and the olfactory nerve from their common origin by the olfactory placode early in fetal development (1–2). Although the genetic alterations responsible for KS have been almost completely elucidated (1, 3–5), the defect in patients with normosmic idiopathic HH (nIHH) is still not well known (6). So far, studies on the possible involvement of autoimmunity in these patients are lacking. To investigate whether autoimmune mechanisms could play a role in the gonadotropin deficiency of patients with idiopathic HH, we planned this study aimed to: 1) evaluate the presence of antipituitary antibodies (APA) in patients with isolated idiopathic HH or idiopathic HH associated with other pituitary hormone deficiencies; 2) identify in possible APA-positive patients whether gonadotrophs or other pituitary hormone-producing cells are targeted by these antibodies.

Patients and Methods

A cohort of 31 males with isolated HH (age range, 19–38 yr) were studied. The diagnosis of isolated HH included: 1) a serum morning testosterone level less than 3.5 nmol/liter associated with low gonadotropin levels; 2) normal thyroid, adrenal, GH, and prolactin (PRL) secretions. Twenty-one of these patients with a normal sense of smell were defined as nIHH (group 1), and the remaining 10 patients with...
anosmia/hyposmia were classified as KS (group 2). All patients in group 1 had normal hypothalamic-pituitary findings on magnetic resonance imaging (MRI), whereas those in group 2 had aplasia/hyposplasia of the olfactory bulbs/tracts and/or of the olfactory sulci (7).

Moreover, 13 males with HH associated with other pituitary hormone deficiencies (group 3) were also studied. In particular, three of them had also GH deficiency, and 10 of them had panhypopituitarism. MRI revealed empty sella in three patients and findings suggestive of lymphocytic adeno-hypophysitis (LYH) in another patient. The 23 patients with HH at prepubertal onset had been treated with human chorionic gonadotropin (HCG) since they were 18 yr old; the 21 patients with HH at postpubertal onset were treated with gonadotropins and/or testosterone. In patients of group 3, other pituitary hormone deficiencies were corrected with appropriate replacement therapies. All patients gave their informed consent to the study, which was approved by the local ethical committee.

Finally, 50 normal age-matched males were also studied to detect APA.

**Statistical analysis**

Data are expressed as mean ± sd, unless otherwise specified. Non-parametric analysis was used because of the non-Gaussian distribution of the data. Differences between the frequencies were evaluated by χ² test. Differences among the groups were evaluated by the Kruskal-Wallis and Mann-Whitney U tests. A value of P < 0.05 was considered statistically significant.

**Immunological evaluation**

APA were evaluated in all three groups of patients and in normal controls by an indirect immunofluorescence method on cryostat sections of young baboon pituitary gland supplied by Bio System Italia S.r.l. (San Martino Buon Albergo VR, Italy), as previously described (8). In particular, fluorescein isothiocyanate (FITC) conjugated goat antihuman IgG sera were used to detect the presence of APA, then positive serum samples were tested with FITC goat antihuman IgG, IgM, IgA sera separately. Even if APA were thought to be positive starting at dilution 1:2 and they are expressed as end-point dilution titer, taking into account our previous experience (8, 9) and the usually low sensitivity of immunofluorescence, we considered an arbitrary cutoff of 1:8 to determine the positive samples. In APA-positive sera, antibodies against single five pituitary hormone-producing cells were determined by a four-layer double immunofluorescence technique, as previously described (10).

In particular, the same cryostat section, in a first immunostaining step, was tested against patient serum and then FITC goat antihuman IgG sera; and, in a second immunostaining step, against rabbit sera anti-GH, -ACTH, -TSH, -PRL, -LH, and/or -FSH separately, followed by rhodamine goat sera antirabbit IgG. The different color of anti-Ig conjugated against the human serum and against the animal serum, green (FITC) and red (rhodamine), respectively, allowed direct assessment of whether the patient’s serum and the animal’s serum stain the same or different pituitary cells. Finally, the sera positive for APA were preabsorbed with increasing excess (10⁻¹⁰ m, 10⁻⁸ m, 10⁻⁶ m) of FSH and LH (follitropin α, Gonal f and lutropin, Luveris, respectively; kindly supplied by Serono, Rome, Italy) for 18 h at 4 C and then retested to exclude a possible interference of antibodies against gonadotropins.

**Results**

The behavior of APA in the three groups of patients and in normal controls is depicted in Fig. 1A. APA were detected at high titers (ranging from 1:32 to 1:128) in eight of 21 patients in group 1 (38%) and in five of 13 in group 3 (38.4%), whereas they were found at low titers (1:2–1:4) in two of 10 patients in group 2 (20%) and in three of the 50 controls (6%).

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**Fig. 1.** A, Behavior of APA in 21 patients with nIHH (group 1), in 10 patients with KS (group 2), in 13 patients with nHH associated with other pituitary hormone deficiencies (group 3), and in 50 controls. Black circles indicate the significantly positive patients; we considered an arbitrary cutoff of 1:8 able to distinguish the true positive samples. B, Immunofluorescence in cryostat section of young baboon anterior pituitary gland tested against serum of patients with isolated nIHH in a primary step adding FITC goat sera anti-human Ig (left panel) and in a second immunostaining step adding rabbit antiserum against gonadotropins followed by rhodamine goat sera antirabbit IgG (right panel). The overlapping color in the same cells, green in the left panel and red in right panel, indicates that cells immunostained by APA are the gonadotrophs.
Regarding the five APA-positive patients in group 3, MRI was normal in three, but it revealed empty sella syndrome in one and findings suggestive of LYH in another patient.

APA-positive and APA-negative patients of group 1 and group 3 were compared with respect to their characteristics and to those of patients in group 2 (Table 1). In particular, the prevalence of associated autoimmune diseases and their prevalence in parents or in first-degree relatives was significantly higher in APA-positive patients of both groups 1 and 3 with respect to APA-negative patients of the same two groups and to patients of group 2 (Table 1). Immunofluorescence pattern was characterized by an intracytoplasmatic staining in some pituitary cells in group 1 patients and in several pituitary cells in group 3 patients. In particular, by four-layer double immunofluorescence method, APA in group 1 patients immunostained selectively ganadotrophs (Fig. 1B) and only rarely some PRL-producing cells, whereas in group 3 patients, these antibodies immunostained, in addition to gonadotrophs, GH-secreting cells in one patient with HH associated with GH deficiency and GH-, ACTH-, and TSH-secreting cells in four of those with panhypopituitarism. No variation of immunostaining was observed when the sera of APA-positive patients were preabsorbed with LH or FSH until the highest concentration (10^-4 m).

**Discussion**

The genetic alterations responsible for KS have been almost completely elucidated (1–5) but the defect in patients with idiopathic HH without KS is still not well known, especially in those at postpubertal onset (11). Antibodies to pituitary-secreting cells, particularly PRL-secreting cells, were first detected by Bottazzo et al. (12) in some patients with autoimmune mono- or polyendocrinopathies. Subsequently, APA to gonadotropins or to gonadotrophs were detected in some patients with acquired hypogonadism (13), in some women with Sheehan’s syndrome (14), and in children with cryptorchidism (15), suggesting a possible negative influence of these antibodies on the pituitary-gonadal axis.

The first remarkable point emerging from our study is that the prevalence of APA at high titer (≥1:18) was 38% in patients with isolated nIHH (group 1) and 38.4% in patients with HH associated with other pituitary dysfunctions (group 3), whereas neither patients with KS (group 2) nor controls resulted positive with titer ≥1.8, which, as previously specified, we considered an arbitrary cutoff to determine the positive patients. Thus, after the results of our previous papers (8–10), we suggest that APA, also in patients with idiopathic HH, only when present at high titer, could be considered a good marker of autoimmune pituitary involvement.

The second important point emerging from our study is that, in retesting the APA-positive sera of patients with nIHH using a double indirect immunofluorescence method, we demonstrated that the target of these antibodies in such patients are the gonadotrophs; moreover, the lack of variations of immunostaining when APA-positive sera were preabsorbed with LH and FSH indicates that these antibodies are directed against some intracytoplasmatic antigens but not against the gonadotropins as themselves. These results suggest that an autoimmune process involving the gonadotrophs may be the underlying cause of hormonal impairment in several patients with nIHH positive for APA, whereas in those negative for APA, the gonadotropin deficiency may be caused by other mechanisms, which probably include genetic abnormalities. Concerning this, none of eight positive patients in group 1 had specific genetic alterations.

In positive patients in whom HH was associated with other pituitary hormone deficiencies, APA also immunostained GH-secreting cells in one of the patients with associated GH impairment and all five pituitary hormone-secreting cells in the four with panhypopituitarism. This could be due to the different duration of the autoimmune process or to the existence of putative antigens selectively expressed by gonadotrophs in the cases of isolated idiopathic HH and antigens shared with other pituitary hormone-secreting cells when HH is associated with other pituitary hormone failure.

All APA-positive patients with HH without anosmia, isolated or associated with other hormone pituitary deficiencies, showed normal findings on MRI except for one patient with empty sella syndrome and another patient with characteristics suggestive of LYH. Even if the pituitary biopsy is the gold diagnostic standard for LYH, which may be also suggested by some particular findings on MRI (16–18), in some

**TABLE 1.** Clinical, hormonal, and immunological characteristics of 44 patients with HH; 21 patients with nIHH (group 1), 10 with KS (group 2), and 13 with nIHH associated with other pituitary hormone deficiencies (group 3)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th></th>
<th></th>
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<th>Group 2</th>
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<th>Group 3</th>
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<tbody>
<tr>
<td></td>
<td>APA positive (n = 8)</td>
<td>APA negative (n = 13)</td>
<td>P†</td>
<td>APA positive (n = 10)</td>
<td>APA negative (n = 8)</td>
<td>P‡</td>
<td>APA positive (n = 5)</td>
<td>APA negative (n = 8)</td>
<td>P§</td>
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<td>Age at evaluation (yr)</td>
<td>28.75 ± 5.04</td>
<td>27.23 ± 5.33</td>
<td>NS</td>
<td>22.40 ± 2.07</td>
<td>27.40 ± 3.21</td>
<td>29 ± 4.78</td>
<td>NS</td>
<td>4.78 ± NS</td>
<td>4.78 ± NS</td>
<td>&lt;0.001</td>
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<tr>
<td>Testosterone (nmol/liter)</td>
<td>0.93 ± 0.19</td>
<td>0.95 ± 0.20</td>
<td>NS</td>
<td>0.80 ± 0.07</td>
<td>0.84 ± 0.15</td>
<td>0.95 ± 0.23</td>
<td>NS</td>
<td>0.94 ± 0.21</td>
<td>0.89 ± 0.11</td>
<td>&lt;0.001</td>
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<tr>
<td>LH (IU/liter)</td>
<td>0.90 ± 0.23</td>
<td>0.74 ± 0.28</td>
<td>NS</td>
<td>0.80 ± 0.15</td>
<td>0.90 ± 0.19</td>
<td>0.78 ± 0.19</td>
<td>NS</td>
<td>0.89 ± 0.21</td>
<td>0.90 ± 0.21</td>
<td>&lt;0.001</td>
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<tr>
<td>FSH (IU/liter)</td>
<td>0.88 ± 0.26</td>
<td>0.85 ± 0.23</td>
<td>NS</td>
<td>0.81 ± 0.137</td>
<td>0.94 ± 0.21</td>
<td>0.89 ± 0.11</td>
<td>NS</td>
<td>0.89 ± 0.21</td>
<td>0.90 ± 0.21</td>
<td>&lt;0.001</td>
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<tr>
<td>Associated autoimmune diseases (no.)</td>
<td>7†</td>
<td>1†</td>
<td>&lt;0.001</td>
<td>0</td>
<td>5†</td>
<td>0</td>
<td>&lt;0.001</td>
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<tr>
<td>Family history of autoimmune diseases (no.)</td>
<td>7†</td>
<td>1†</td>
<td>&lt;0.001</td>
<td>1</td>
<td>5†</td>
<td>0</td>
<td>&lt;0.001</td>
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NS, Not significant.
† APA-positive vs. APA-negative patients in group 1.
‡ APA-positive vs. APA-negative patients in group 3.
§ APA-positive vs. patients in group 2 and APA-negative patients in group 3.
§§ APA-positive vs. patients in group 3.
△ P < 0.001 vs. patients in group 2 and APA-negative patients in group 3.
△△ P = 0.001 vs. patients in group 2 and APA-negative patients in group 3.
cases of biopsy-proven LYH with slight pituitary lymphocytic infiltration, MRI findings can be absent (19). With this in mind, it could be useful to search for APA in clinical practice for the diagnosis of these particular forms of apparently idiopathic HH in which pituitary biopsy is not agreed to and morphological findings on MRI are inconclusive. Interestingly, our APA-positive HH patients showed high prevalence of associated autoimmune diseases, in particular, chronic autoimmune thyroiditis, suggesting that these cases of normosmic HH (nHH) may be included in autoimmune polyendocrine syndrome, in particular, in type III autoimmune polyendocrine syndrome.

In conclusion, our results suggest that some cases of apparently idiopathic HH may be caused by an autoimmune pituitary process involving selectively the gonadotrophs in isolated nHH and also other pituitary hormone-secreting cells in nHH associated with more generalized pituitary dysfunctions.

Thus, we recommend searching for APA in patients with apparently idiopathic HH, especially in those at onset postpubertally and with an MRI negative for pituitary tumor or lesion. Future longitudinal studies are needed to clarify the natural history of this process and the possible effect of early corticosteroid therapy, according to what has been suggested for other autoimmune diseases (17–18, 20).

Acknowledgments

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References


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