Large height gain by growth hormone therapy in combination with GnRH analog in two pubertal sibs with a GH-releasing hormone receptor mutation

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Submitted
Abstract

Context: Patients with GHRH receptor (GHRH-R) mutations present with familial isolated GH deficiency, which untreated leads to a severely compromised adult height. Few data are available about the efficacy of treatment with GH in combination with a gonadotropin-releasing hormone (GnRH) analog (GnRHa) in adolescence.

Objective: To describe the evolution of growth and skeletal age of a brother and sister of Moroccan descent with a homozygous GHRH-R mutation who presented at an advanced age (16 and 14.9 years, respectively) and pubertal stage (Tanner stage G4 and B3, respectively) with a height of -5.1 SDS and -7.3 SDS on treatment with a combination of GH and GnRHa for 2.5 and 3 years followed by GH alone.

Methods: GH was given in a dosage of 0.7 mg/m²/day (25 μg/kg/day) sc and triptorelin in a dosage of 3.75 mg/4 weeks im. Height and pubertal stage were measured three-monthly, bone age yearly.

Results: Combined GH and GnRHa treatment resulted in a height gain of 24 cm and 28.2 cm respectively, compared to the initial predicted adult height by the method of Bayley-Pinneau. Adult height was within the population range and well within the target range.

Conclusions: Our patients demonstrate that, in case of isolated GH deficiency caused by a GHRH-R mutation, combined treatment of GH and GnRHa can be very effective in increasing final height, even at an advanced bone age and pubertal stage.
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Introduction

Growth Hormone Releasing Hormone (GHRH) and somatostatin are hypothalamic peptides that regulate the pulsatile GH secretion. GHRH stimulates GH synthesis and release, while somatostatin inhibits GH release (1). Binding of GHRH to the GHRH receptor (GHRH-R) activates the signaling cascade, resulting in cellular proliferation, GH synthesis, and secretion (2).

Several mutations in the GHRH-R gene have been identified, all showing an autosomal recessive inheritance pattern. Clinically, patients with homozygous GHRH-R mutations present with familial isolated GH deficiency (GHD), including proportionate short stature, variable anterior pituitary hypoplasia, low IGF-I and IGFBP-3 levels, subnormal stimulated GH levels and good response to exogenous GH (2-5).

In 2001, we described the GH secretion in two patients with a novel homozygous single base pair transition (G->C) at the splice donor site of intron VII of the GHRH-R gene (6). At the time of diagnosis, they were 14.9 and 16 years old and had reached an advanced stage of puberty. Both patients were treated with GH in combination with a gonadotropin-releasing hormone (GnRH) analog (GnRHa). We demonstrate that this combination can be highly effective in increasing final height, even at this advanced age and maturational stage.

Methods

Auxological measurements

Height, body mass index (BMI) and target height were expressed as standard deviation score (SDS) based on Dutch references for children of Moroccan descent (7). Sitting height/height ratio and head circumference were expressed as standard SDS based on Dutch references (8, 9). Target height was calculated from the parents heights corrected for sex and secular trend: [height of father and mother +/- 13]/2 + 4.5) (8).
Biochemical measurements

Biochemical assays have been described previously (6). An arginine test was performed with 0.5 gr/kg arginine in 30 min.

Radiographic measurements

Bone age and predicted adult height was assessed by scoring the radius, ulna and short bones (RUS), using the Tanner and Whitehouse-2 (TW-2) method (10). In addition, bone age was assessed with the Greulich and Pyle method (11), and predicted adult height according to the tables of Bayley and Pinneau (12).

Patients

The patients were the third and fourth son and daughter of a Moroccan family with 6 children. The parents were not aware of consanguinity. The other four children were healthy. Father’s height was 170 cm and mother’s height was 156.4 cm, resulting in a target height of 174.2 cm (0 SDS) for the brother and 161.2 cm (0 SDS) for the sister. The boy was referred to our hospital because of short stature at the age of 16 yr. Three months before, he and his sister had immigrated from Morocco. No previous growth data were available. His medical history was uneventful and he had no other complaints. Auxological, biochemical, and radiological features are summarized in Table 1. Total 24-h GH production was greatly diminished, as has been described earlier in detail (6). The maximal rise of TSH after 200 μg TRH was 5.7 mU/liter after 20 min, which was considered a suboptimal response. A normal increase of prolactin was observed (from 4.5 to 15 μg/liter). Although computed tomography at the age of 16 yr showed a partial empty sella, a magnetic resonance imaging scan at the age of 25 yr showed no abnormalities of the pituitary and hypothalamus.

GH treatment was started at the age of 16.2 yr (Genotropin, Pharmacia, Stockholm, presently Pfizer, US) at a dosage of 0.7 mg/m²/day (25 μg/kg/day). After six weeks of GH treatment, a low level of total thyroxine was found (56 nmol/liter (normal value 70-160 nmol/liter)) with a normal T3 and TSH level. Substitution with levothyroxine was initiated. Retesting 6 years later revealed a normal TSH response to
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Table 1. Auxological, biochemical and radiological characteristics of the patients. Tanner stages: PH: pubic hair, G: genital, B: breast.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Brother</th>
<th>Sister</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At start of GH therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>16</td>
<td>14.9</td>
</tr>
<tr>
<td>Height [cm (SDS)]</td>
<td>135.2 (-5.1)</td>
<td>116.9 (-7.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>26.5</td>
<td>23</td>
</tr>
<tr>
<td>BMI [kg/m² (SDS)]</td>
<td>14.5 (-2.9)</td>
<td>16.8 (-1.8)</td>
</tr>
<tr>
<td>Head circumference [cm (SDS)]</td>
<td>49.7 (-3.8)</td>
<td>50.3 (-2.7)</td>
</tr>
<tr>
<td>Sitting height/height [ratio (SDS)]</td>
<td>0.52 (0.2)</td>
<td>0.51 (-0.4)</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>PH4, G4</td>
<td>PH1, B3</td>
</tr>
<tr>
<td>IGF-I [ng/ml (SDS)]</td>
<td>14.5 (-7.0)</td>
<td>26.8 (-6.4)</td>
</tr>
<tr>
<td>GH maximum in exercise test (mU/liter)</td>
<td>2.8</td>
<td>0.5</td>
</tr>
<tr>
<td>GH maximum in arginine test (mU/liter)</td>
<td>5.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Bone age - Greulich and Pyle (yr)</td>
<td>14</td>
<td>11.6</td>
</tr>
<tr>
<td>Predicted adult height - Bayley-Pinneau (cm)</td>
<td>144.2</td>
<td>127.4</td>
</tr>
<tr>
<td>Bone age - TW-2 (yr)</td>
<td>14.6</td>
<td>12.1</td>
</tr>
<tr>
<td>Predicted adult height - TW-2 (cm)</td>
<td>148.6</td>
<td>120.3</td>
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<tr>
<td><strong>At final height</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>21.3</td>
<td>20.5</td>
</tr>
<tr>
<td>Height [cm (SDS)]</td>
<td>168.2 (-1)</td>
<td>155.6 (-1)</td>
</tr>
<tr>
<td>Target height [cm (SDS)]</td>
<td>174.2 (0)</td>
<td>161.2 (0)</td>
</tr>
</tbody>
</table>

TRH (6). Administration of 100 μg GnRH, showed a maximal increase of LH to 10.1 U/liter, which was considered as a pubertal response. Plasma testosterone was 32.4 nmol/liter (923 ng/dl). To prevent further progression of puberty and bone maturation, GnRH agonist (GnRHa) treatment was initiated one month after the start of GH (Decapeptyl CR, 3.75 mg every 4 wk im). GnRHa treatment resulted in prepubertal testosterone levels within one month and decreased the testicular volumes from 15 ml to 6 ml after 9 months of treatment. GnRHa was discontinued at the age of 18.7 yr (total duration of treatment 2.4 yr). Subsequently, the GH dose was increased to 1 mg/m²/day (34 μg/kg/day). Adult height was reached at
Figure 1A. Growth chart for Dutch children of Moroccan descent of the boy with a GHRH-R mutation. The squares represent the bone age (TW-2 RUS).

Figure 1B. Growth chart for Dutch children of Moroccan descent of the girl with a GHRH-R mutation. The squares represent the bone age (TW-2 RUS).
the age of 21.3 yr and was 168.2 cm (~ 1.0 SDS), which was well within his target range (Fig. 1A). After reaching adult height he has received GH replacement at a dosage of 0.4 mg/day sc.

The girl was referred to our hospital at the same time as her brother, at the age of 14.9 years. Her medical history was also uneventful. Her auxological, biochemical, and radiological characteristics are summarized in Table 1. At physical examination she had a doll like face and increased abdominal fat. No dysmorphic features were noticed. Menarche had occurred, but it was uncertain at what age. She had regular menstrual cycles. Total 24-h GH production was greatly diminished, as was earlier described in detail (6). The maximal rise of TSH after 200 μg TRH was from 0.8 to 6.7 mU/liter after 20 min, which was considered as a subnormal response. Prolactin increased from 3.6 to 21 μg/liter (normal). Computed tomography at the age of 15 yr showed an empty sella, which was confirmed on the magnetic resonance imaging scan at 23 yr of age.

At the age of 15 yr GH treatment was started (Genotropin, 0.8 mg/m²/day = 28 μg/kg/day sc) and one month after the start of GH a GnRHa was added (Decapeptyl CR 3.75 mg, every 4 wk im). Prior to treatment a GnRH test showed a pubertal response with a LH peak of 17.3 mU/liter 30 min after 100 μg GnRH iv. Plasma estradiol was 64 nmol/liter. On GnRHa treatment plasma estradiol was < 40 pmol/liter and menstrual cycles stopped. Total thyroxin levels decreased after 6 weeks of GH treatment (total thyroxine 61 nmol/liter), whereas T3 and TSH levels were normal, for which levothyroxine replacement therapy was initiated. Retesting at adult age showed a normal TSH response to TRH and levothyroxine was discontinued (6). The GnRHa was stopped after 3 yr of treatment at the age of 17.1 yr. At that moment bone age was 12.9 yr according to TW-2 and 12 yr according to Greulich and Pyle. Subsequently, the GH dose was increased to 1.3 mg/m²/day (30 μg/kg/day). Puberty progressed normally after discontinuation of the GnRHa. At 20.5 yr she had Tanner stage B5 and regular menstrual cycles. Her final height was 155.6 cm (-1 SDS), which was well within the target range (Fig. 1B). After reaching final height, GH injections were discontinued for 4 yr, but after retesting GH treatment was reinstituted in a dosage of 0.4 mg/day. During uneventful pregnancies, at the age of 26 and 27 yr, GH was discontinued after the first trimester. She delivered two healthy babies, a girl and a boy, who are growing normally.
Results

Result of GH and GnRHa treatment
Treatment with the combination of GH and GnRHa during 2.5 yr in the male sib resulted in a height gain of 24 and 19.6 cm according to the pretreatment predicted adult height determined by Bayley/Pinneau and TW-2, respectively. In the female sib a height gain of 28.2 and 35.3 cm was established after 3 yr of combination treatment compared with the pretreatment predicted adult height determined by Bayley/Pinneau and TW-2, respectively.

Discussion

Mutations in the GHRH-R gene account for approximately 10% of the patients with familial isolated GHD. In both our patients a homozygous G-> C transition at the splice donor site of intron VII was found, altering the first basepair of the intervening intronic sequence after exon 7. This mutation interrupts the signal transduction of GHRH, causes GHD and consequently severe postnatal growth retardation. Our patients were diagnosed at the ages of 16 and 14.9 years, respectively, and they both showed signs of advanced puberty. We hypothesized that GH treatment alone at this advanced stage of puberty would accelerate bone maturation progressively and thereby limit the effect on final height, while administration of GnRHa might delay the process of epiphyseal fusion by inhibiting the secretion of gonadotropins and gonadal sex steroids. Our cases illustrate that severely GH deficient adolescents can greatly benefit from the combination GH and GnRHa treatment, even at an advanced age, bone age and pubertal stage.

The large height gain on GH and GnRHa treatment as we experienced in our patients is far beyond the average additional effect of GnRHa in group analyses (approximately 7 cm) (13-17). Also in individual patients with GH deficiency we are not aware of reports on such extremely good result. We speculate that, in our cases, who have a severe GH deficiency caused by a well-defined genetic disorder, the intact GH –IGF-I axis downstream the level of the GHRH-R resulted in maximal GH sensitivity. This is supported by the significant rise of IGF-I seen in the IGF-I generation test in patients with a GHRH-R mutation (18) and the good growth
response to GH therapy in those patients (2-5, 18). The good sensitivity allows optimal catch-up growth, while the absence of estrogen restricts epiphyseal maturation. The only report on GH and GnRHa therapy in a boy with a GHRH-R mutation also showed a positive effect on linear growth, but this was limited to a few centimeters (19).

The TSH response to TRH was suboptimal in our patients, suggesting mild secondary hypothyroidism. A blunted TSH response was also reported in other patients with a GHRH-R mutation (18). One could hypothesize that hypoplasia of the pituitary is one of the contributing factors, however the pituitary size was normal in the brother. Another possible explanation for the blunted TSH response is that GHRH is necessary for maximal TSH production, although the normal TRH test at adult age argues against this. The low T₄ levels after the start of GH therapy were probably the result of the positive effect of GH on 5’deiodinase activity, the enzyme converting T₄ into T₃ (20). In general, treatment is not necessary as T₄ levels recover spontaneously, but in our cases levothyroxine treatment was started to create optimal conditions for growth.

In conclusion, this report shows that in patients with isolated GH deficiency due to a GHRH-R mutation, combined treatment of GH and GnRHα can have a great effect on adult height, even if started at an advanced bone age and pubertal stage.
References


