

# Effect of growth hormone therapy in children with achondroplasia: growth pattern, hypothalamic–pituitary function, and genotype

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## Abstract

**Objective:** Although there are a few reports on GH therapy in achondroplasia, these were based on a small sample and/or short-term observation. To clarify the effectiveness of GH treatment on short stature in achondroplasia and hypochondroplasia, a long-term treatment study in a larger number of patients was performed.

**Method:** Forty-two children (16 males and 26 females, age 3–14 years) with achondroplasia were examined in this study. Initially, we evaluated hypothalamic–pituitary function and point mutation analysis as previously reported. After the evaluation, the children were treated with GH for more than 2 years; then post-treatment growth velocity and body proportion parameters were determined.

**Results:** The 35 typical variants of our achondroplasia patients showed previously reported point mutation in the fibroblast growth factor receptor 3 gene. The annual height gain during GH therapy was significantly greater than that before therapy ( $3.9 \pm 1.0$  cm/year before treatment vs  $6.5 \pm 1.8$  cm/year for the first year and  $4.6 \pm 1.6$  cm/year for the second year of treatment). The body disproportion had not been aggravated during the treatment period.

**Conclusion:** We conclude that GH might be beneficial in the treatment of short stature in children with achondroplasia in the first 2 years of treatment.

*European Journal of Endocrinology* 138 275–280

## Introduction

Achondroplasia (ACH) is the most common form of chondrodysplasia in humans. This disorder is inherited as an autosomal dominant trait, although the majority of cases are sporadic. Recent studies have revealed point mutations in the transmembrane domain of the fibroblast growth factor receptor 3 (FGFR3) gene in ACH heterozygotes and homozygotes (1, 2).

ACH is characterized by short-limbed dwarfism, macrocephaly with a prominent forehead, and midface hypoplasia. Adult height may be as short as 118–145 cm for men and 112–136 cm for women (3). Such extremely short stature and disproportion causes considerable inconvenience in daily life and places considerable psychological pressure on patients and their families. Although surgical lengthening of lower limbs has been the only established therapy for increasing overall height and improving body proportions, this procedure requires long-term hospitalization and may result in serious complications, such as post-operative infection and fractures or deviation of the bone axis (4).

Recently, there have been some reports describing the beneficial effects of growth hormone (GH) therapy in ACH and hypochondroplasia (HCH) (5–7), although patients with ACH are considered to have normal GH secretion. In fact, GH has been widely used to treat non-GH-deficient forms of short stature, such as Turner's syndrome, skeletal dysplasia, intrauterine growth retardation, chronic illness and idiopathic short stature (8–10). Moreover, a case of impaired GH secretion in ACH has been reported (5). It is well known that most patients with this disease suffer from hydrocephalus. This cerebral hypertension might influence the hypothalamic–pituitary axis. Furthermore, obstructive sleep apnea, which impairs sleep-related GH secretion (11), is often a complication in ACH. These features suggest that some patients with ACH might have hypothalamic–pituitary dysfunction, including abnormal GH secretion, subsequently contributing to short stature.

In this study we evaluated the hypothalamic–pituitary function of patients with ACH and examined the effectiveness of GH administration in ACH diagnosed by mutation analysis (1).

## Subjects and methods

Forty-two children (16 males and 26 females), with typical or mild features of ACH, and whose ages ranged from 3 to 14 years participated in this study at Okayama University Hospital. The diagnosis of ACH was established on the basis of clinical features, such as rhizomelic short stature, large head with a prominent forehead, lumbar lordosis and trident hand, and radiological findings, such as a large skull with a relatively small base, decreased lumbosacral interpedicular distance, a short pelvis with broad iliac wings, and short long-bones with wide and flaring metaphyses (12). The typical form was distinguished from the mild form by a markedly large head, depressed nasal bridge, trident hand and marked lumbar lordosis clinically, and narrow sacrosciatic notches, decreased vertebral interpedicular distance from L-1 to L-5 and a V-shaped appearance of the long-bone metaphyses on radiography. Thirty-five patients were prepubertal and seven were pubertal before GH therapy. Two patients had a family history of ACH. None had a history of significant neurological dysfunction. None had received any pharmacological treatment to accelerate height gain before entry into this study.

The protocol was approved by our Institutional Committee for the Protection of Human Subjects. Written informed consent was obtained from the patients and/or their parents.

### **Hypothalamic–pituitary function before GH therapy**

GH response to provocation tests (insulin-induced hypoglycemia (0.1 IU/kg), L-dopa (10 mg/kg), clonidine (0.1 mg/m<sup>2</sup>), and GH releasing factor (GRF) (1 mg/kg)) and GH secretory pattern during sleep (assessed by averaging ten GH levels obtained at 20-min intervals after sleep induction) were evaluated. Serum GH concentrations were determined by an immunoradiometric assay (Daiichi Radioisotope Co., Tokyo, Japan). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) response to luteinizing hormone-releasing hormone (LHRH) stimuli and thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) stimuli were also evaluated (Dainabot, Tokyo, Japan). Intra-assay variation was less than 2.5% and interassay variation was less than 4.8% for GH assay. The intra-assay variations of LH and FSH were less than 4.2 and 3.3% respectively, and the interassay variations were less than 6.1 and 6.2% respectively.

Less than 10 ng/ml of peak GH levels on GH provocation tests with insulin, L-dopa and clonidine, less than 15 ng/ml of peak GH levels to GH provocation test with GRF, and less than 5 ng/ml of average GH level during sleep were defined as blunted GH secretion, according to the criteria of the Foundation for Growth and Science in Japan.

### **Analysis of genomic DNA by restriction digest**

As described in a previous report (1), genomic DNA, extracted from peripheral lymphocytes by a Blood Amp Kit (Qiagen, Chatsworth, CA, USA), was amplified using the same PCR primer set (1, 13). Each PCR product (10 µl) was incubated with 4–20 U SfcI, Nla III (Biolabs, Beverly, MA, USA) or MspI (TOYOBO, Osaka, Japan) at 37°C from 6 h to overnight. The products were electrophoresed on non-denaturing 4% agarose gels and stained by ethidium bromide.

### **GH treatment**

After evaluation of hypothalamic–pituitary function, all 42 patients were treated with recombinant human GH which was supplied by Novo Nordisk Pharma Ltd (Tokyo, Japan). GH was administered subcutaneously at a dose of 0.5 IU/kg per week (8 patients) or 1.0 IU/kg per week (34 patients), 6 or 7 days per week for at least 2 years. Eight patients were assigned to receive 0.5 IU/kg per week treatment because they met the criteria for the GH treatment as having a GH deficiency according to the Foundation for Growth and Science in Japan.

The children were treated for more than 1 year (all patients for 1 year, 36 patients for 2 years, and 16 patients for 3 years) to determine post-treatment growth velocity and the ratio of arm span to height and of lower limb length (as assessed by the distance between spina iliaca anterior superior and internal malleolus) to height.

Blood count, urinalysis, routine laboratory tests, TSH, thyroxine (T<sub>4</sub>), tri-iodothyronine (T<sub>3</sub>), and serum insulin-like growth factor-I (IGF-I) were determined before and every third month during GH treatment. Serum IGF-I was measured by RIA (Ciba Corning Diagnostica, Tokyo, Japan).

Spinal canal stenosis and the foramen magnum diameter were evaluated by plain X-ray, computed tomography, or magnetic resonance imaging taken before therapy and then yearly during therapy.

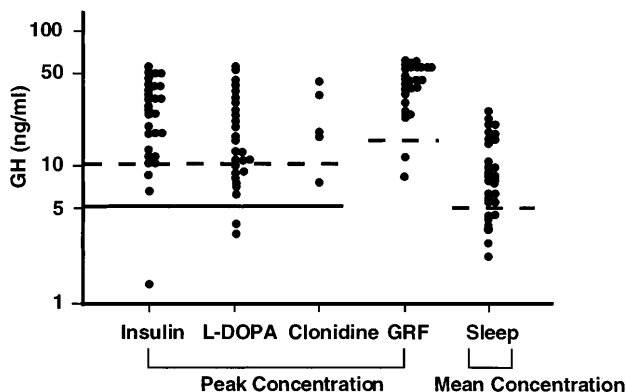
Bone age was not determined because ACH may have altered the radiographic parameter used to assign bone age (6).

Data in text and tables are expressed as mean ± s.d. Significance of differences between mean values was determined using Wilcoxon signed-ranks test, ANOVA, and Scheffe procedure for multiple comparison.  $P < 0.05$  was considered significant.

## Results

### **Hypothalamic–pituitary function before GH therapy**

All patients showed a normal TSH response to TRH stimulus, and cortisol response to insulin-induced hypoglycemia. Thirty-five patients showed prepubertal



**Figure 1** GH responses to provocation tests. Peak GH concentrations on GH provocative tests with insulin, L-dopa, clonidine and GRF, and mean GH concentrations during sleep are shown. Dotted lines represent the cut-off value for the blunted response (<10 ng/ml for all provocation tests other than GRF test) and the solid line represents the cut-off value for the severe blunted response (<5 ng/ml).

LH and FSH responses to LHRH stimulus; seven patients showed pubertal responses (14). The serum T<sub>4</sub> and T<sub>3</sub> levels were normal in all patients.

Four patients showed blunted responses on two different provocation tests, and one showed a blunted response on three different provocation tests. Five patients showed a combination of a blunted response on one provocation test and low GH concentration during sleep (Fig. 1). As these patients showed significantly lower serum levels of IGF-I compared with age-matched normal children and ACH patients with normal responses to provocative tests, we suspected that 10 of the 42 patients had subnormal GH secretion (SGH). However no patients exhibited severe blunted responses (peak GH value <5 ng/ml) to more than one type of provocation test.

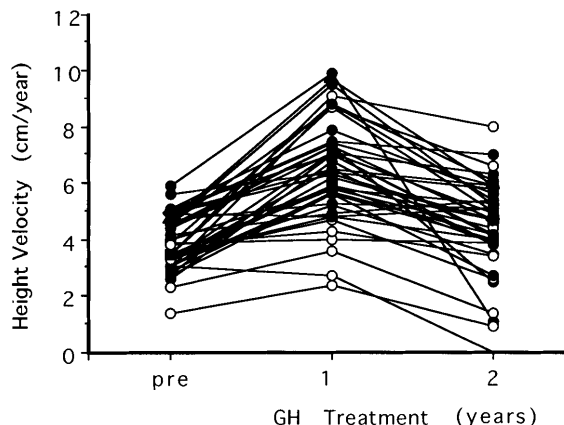
**Analysis of genomic DNA by restriction digest**

In the 35 typical variant forms of ACH, we found SfcI polymorphism in the FGFR3 gene. In all these cases, the 55, 109 and 164 bp fragments that resulted from digestion of the PCR products with SfcI were present. However, there were neither restriction sites for SfcI nor MspI in the seven mild variant forms (1).

There was no significant difference in response to GH between the patient group with mutations in the transmembrane domain and those without mutations. The annual height gains in the patients with and without mutations were 6.3 ± 0.99 cm/year and 7.2 ± 1.02 cm/year (P = 0.439). In the second year of treatment, the respective gains were 4.2 ± 0.9 cm/year and 5.5 ± 1.0 cm/year (P = 0.105).

**GH treatment**

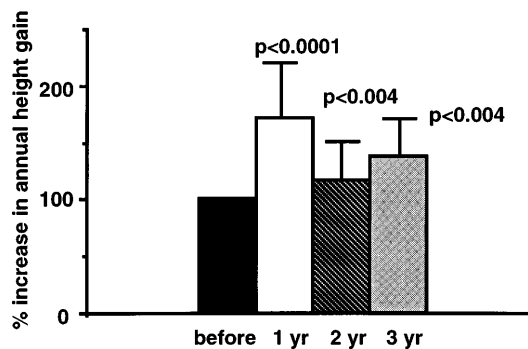
The mean Z-scores for height on admission and for annual height gain before admission in all the patients



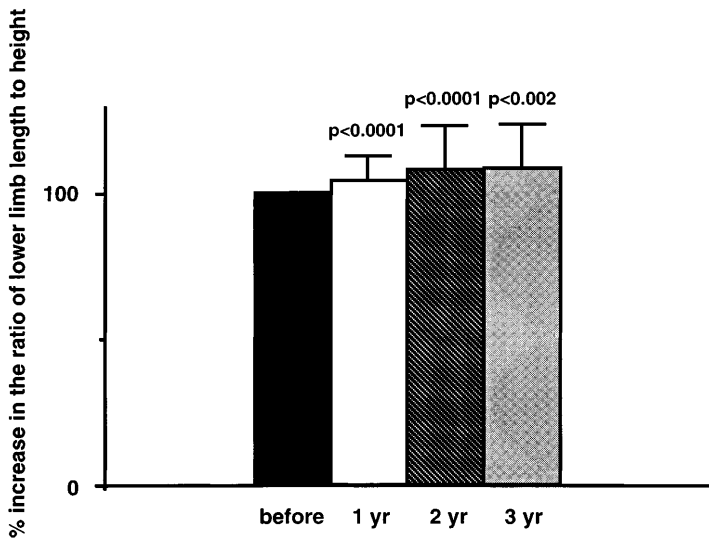
**Figure 2** Individual growth profiles during GH treatment. (○) pubertal patients, (●) prepubertal patients.

were -5.0 ± 1.2 (mean ± s.d.) and -3.1 ± 1.7 respectively. These scores are considered to be extremely low. The height velocity (HV) during the first year of GH treatment was significantly increased compared with that before therapy (6.5 ± 1.8 cm/year vs 3.9 ± 1.0 cm/year, P < 0.0001). At the end of the second year of GH therapy (n = 36), although the velocity was less than that in the first year, it was still significantly greater than that before therapy (4.6 ± 1.6 cm/year, P < 0.002). In the 16 patients who received GH for more than 3 years, the velocity after 3 years of treatment was 5.3 ± 1.0 cm/year; also lower than that in the first year but still significantly greater than before therapy (P < 0.005) (Figs 2 and 3). It is important to notice that the responses to GH after the second year were not uniform. Although most patients exhibited higher velocity during GH therapy, there were some patients who showed lower HV during the second year of therapy than that before treatment.

The ratio of arm span to height was increased during GH therapy, but not to a significant degree. The ratio of



**Figure 3** Annual height gain during GH therapy. This graph indicates percentage increase. P values represent comparison to data before GH treatment. ANOVA and Fisher's *post hoc* test were used for the statistical analyses.



**Figure 4** Ratio of lower limb length to height during GH therapy. The data indicate percentage values of the numbers before GH therapy. *P* values represent comparison to the data before GH treatment. Error bars indicate s.d. ANOVA and Fisher's *post hoc* test were used for the statistical analyses.

lower limb length to height was significantly increased during treatment (Fig. 4). However, the ratio of sitting height to overall height did not show significant change during this study period (Fig. 5).

In 33 patients who did not exhibit secondary sex characteristics during the 3-year course of GH therapy, there was no significant difference in mean HV at the end of each year between the patients with normal ( $n = 23$ ) or subnormal GH secretion ( $n = 10$ ), nor was there any difference between patients treated with 0.5 IU/kg per week GH ( $n = 8$ ) and those treated with 1.0 IU/kg per week GH ( $n = 26$ ) (Table 1).

Nine patients with normal GH secretion who exhibited secondary sex characteristics before or during the 2-year course of GH therapy, received GH at a dose of 1.0 IU/kg per week. The HV of these pubertal patients was significantly lower than that of the 19 prepubertal patients with normal GH secretion who were treated with 1.0 IU/kg per week GH after 1 year of GH therapy. However, there was no significant

difference between the two groups at the end of the second year (Table 2).

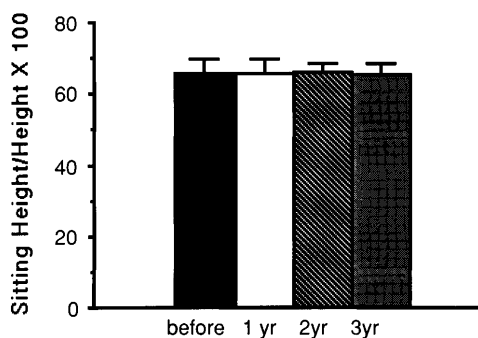
The serum IGF-I level increased significantly after GH therapy ( $359 \pm 196$  ng/ml first year,  $P < 0.0001$ , and  $383 \pm 223$  second year,  $P < 0.0001$ , vs  $191 \pm 173$  before treatment). There were no significant changes in thyroid function tests or routine laboratory data during the GH treatment. Nor was there any noticeable change in spinal cord compression or narrowing of the foramen magnum. No adverse effects were observed during the treatment period.

### Hypothalamic-pituitary function after GH therapy

Thirty-six patients who received GH treatment for more than 2 years showed normal TSH response to TRH stimulus and a normal cortisol response to insulin-induced hypoglycemia at the end of the second year of therapy. Two of the 31 patients who had showed prepubertal LH and FSH responses to LHRH stimulus before GH therapy demonstrated pubertal responses after 2 years of therapy. These patients were a 10-year-old male whose testicular volume was 3 ml and a female whose breast development was Tanner stage 2 (15).

### Discussion

As observed by Shiang *et al.* (1) and Rousseau *et al.* (2), the vast majority of studies, including this and other examinations of Japanese ACH patients, have revealed SfcI sites, with MspI sites observed in the remainder. No patients were identified with Nla III site mutation, in this study. Further, in the mild form, we could find neither SfcI nor MspI sites, and we should therefore classify these mild forms as HCH, as proposed by Rousseau *et al.* (2). There was no demonstrable



**Figure 5** Ratio of sitting height to height during GH therapy. The values remain constant during the treatment. Error bars indicate s.d. ANOVA and Fisher's *post hoc* test were used for the statistical analyses.

**Table 1** Mean height velocity ( $\pm$  s.d.) in prepubertal achondroplasia patients during GH therapy.

GH secretion	Group	GH dosage (IU/kg per week)	Number	Before (cm/year)	Year 1 (cm/year)	Year 2 (cm/year)
Normal	A	1.0	20	4.1 $\pm$ 1.0	7.4 $\pm$ 1.5	4.5 $\pm$ 1.3
	B	0.5	3	4.3 $\pm$ 0.9	6.8 $\pm$ 0.5	6.0 $\pm$ 2.3
Subnormal	C	1.0	5	3.9 $\pm$ 0.8	6.8 $\pm$ 1.6	6.0 $\pm$ 0.9
	D	0.5	5	3.7 $\pm$ 0.8	5.9 $\pm$ 0.7	4.5 $\pm$ 0.6

Statistical analyses were performed by Wilcoxon signed-ranks test, but there were no significant differences between groups A and B, A and C, C and D, and B and D.

significant difference in the response to the GH treatment of patients with ACH or HCH. This result may probably be due to the small number of patients with HCH.

GH administration increased HV in a large number of ACH patients with no adverse effects. However, many of the authors of the Committee on Genetics (16) so far conclude that GH cannot be considered to be effective in increasing stature without description of data. There are several reports on GH therapy in ACH or HCH (5–7, 17, 18), although most involved either only small numbers of patients or short-term observation. An initial growth acceleration and waning thereafter was observed in almost all cases published so far. In our study also, HV accelerated during the first year of GH treatment, but declined during subsequent years. Nevertheless, the HV during the second and third year of GH treatment was still greater than that before therapy in the majority of patients. As shown in Fig. 2, however, there were a few patients who showed reduced growth in the second year as compared with the pretreatment period. The precise mechanisms of this low responsiveness are still unclear, although genotype difference might offer a clue. Although we could not prove a significant difference between the patients with recognizable ACH mutations and those without them, some HCH patients have been reported to respond well to GH therapy (19) and it may be that HCH is not a single entity of disorder (20). This point should be clarified by determining all the types of mutation which lead to HCH.

The HV during the first year of GH treatment of patients treated with GH at 0.5 IU/kg per week did

not differ from those treated at 1.0 IU/kg per week. Moreover, the HV during the first year of treatment in SGH patients did not differ from that of those with normal GH secretion. This may indicate that GH secretion in ACH or HCH is normal, with some patients having somewhat blunted responses. After 1 year, however, the HV of pubertal patients was significantly lower than in patients during the prepubertal stage.

Further studies are therefore essential to establish the parameters of the most effective GH administration regimen, such as the duration of administration, dose, timing of the start of therapy, and so on.

Another important observation was that GH did not promote further disproportion. The ratio of lower limb length to height was significantly increased after GH therapy. However, a more reliable parameter in body proportion – the ratio of sitting height to height – did not change significantly during the study period. Short limbs present problems for ACH patients both psychologically and in daily life, and it is thus very meaningful that GH did not aggravate this disproportion with the increment in annual height gain.

Membranous ossification is generally considered normal in ACH, and the inner border of the foramen magnum grows by membranous ossification (6). If GH promotes membranous ossification, both spinal cord compression and narrowing of the foramen magnum could theoretically be worsened. However, there was no evidence of this after GH therapy. Furthermore, there is a report that by the age of 3–4 years, the foramen magnum has essentially reached its adult size in ACH (21). Thus, our study suggests that GH treatment might not induce neurological complications in patients over 3–4 years and could be safe.

During the 2-year course of GH therapy, two patients developed secondary sex characteristics and showed pubertal LH and FSH responses to the LHRH stimulus. The clinical features of these patients at puberty were reasonable in consideration of their ages, and were not considered to be at all excessive. We cannot guess the extent to which puberty progressed by LH and FSH responses to the LHRH stimulus, although we can judge whether the patient is prepubertal or pubertal by those responses (14). In this study, it appeared that GH did not cause sexual maturation to progress excessively.

The use of age-, sex- and genotype-matched controls was not a practicable option in this study, and we could

**Table 2** Mean height velocity ( $\pm$  s.d.) in prepubertal and pubertal achondroplasia patients treated with 1 IU/kg per week GH during therapy.

	Number	Before (cm/year)	Year 1 (cm/year)	Year 2 (cm/year)
Prepubertal	19	4.1 $\pm$ 1.0	7.4 $\pm$ 1.5	4.5 $\pm$ 1.3
Pubertal	9	3.4 $\pm$ 1.1	4.7 $\pm$ 2.1	3.4 $\pm$ 2.0
<i>P</i> values <sup>a</sup>		NS	< 0.005	NS

<sup>a</sup>Growth promoting effects were compared between prepubertal and pubertal patients by the Wilcoxon signed-ranks test. NS = not significant.

not report on the adult height of the patients. A further follow-up study is thus needed to determine the adult height, but this will probably have to wait for at least 5 years. To date, we do not have any longitudinal growth curve for Japanese ACH, and its establishment should be another future project to clarify the effectiveness of this GH treatment. The comparison of growth curves during the treatment to this natural longitudinal growth curve may offer a conventional control. A cross-over study might be another way to evaluate treatment effectiveness; in fact a limited number of patients who were not included in this study were assigned to a 3-year cross-over study, consisting of 1 year of treatment followed by 1 year of resting and another 1 year of treatment. The patients during the resting period showed similar growth velocities to those in the pretreatment period and regained a high growth velocity upon commencement of the second year of treatment (T Kubo, H Tanaka & Y Seino, unpublished observations).

In conclusion, GH therapy is beneficial in the treatment of short stature in children with ACH and HCH before surgical lengthening of the lower limbs. However it is still premature to conclude that GH treatment will improve adult height.

## Acknowledgements

This study was supported in part by grants from the Ministry of Health Welfare and the Ministry of Education of Japan and by grants from the Research Society for Metabolic Bone Diseases.

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Received 1 October 1997

Accepted 20 October 1997