Impact of Polymorphisms of Human β-Adrenergic Receptor Gene on Changes in Height during Growth Hormone Treatment

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Abstract. The aim of this study was to investigate the occurrence of polymorphisms of the β-adrenergic receptor gene in short children and to evaluate the possible influence of the polymorphisms on changes in height and obesity index in response to GH treatment. Of the 75 children enrolled in the study, 40 completed at least 5 years of GH treatment. The genotype distribution of the β2 and 3-adrenergic receptor polymorphisms in the study population did not differ significantly from those reported in non-obese subjects. There were no significant differences in the SD score for height at any given time-point between the group with and without the Trp64Arg mutation of the β3-adrenergic receptor gene. In relation to the Gly16Arg polymorphism of the β2-adrenergic receptor gene, the mean SD score for height increased significantly during GH treatment in children with Arg16Arg and Gly16Arg. In those with Gly16Gly, the score did not show any significant increase during all 5 years of GH treatment. In both the groups with and without the Trp64Arg mutation, the changes in obesity index did not reach statistical significance at any time-point. Only children with Gly16Gly had a significantly higher baseline mean obesity index than those with Gly16Gly. The index also decreased markedly from 21.9% to 5.8% in these children during the first 4 years of GH treatment. Thus, when the impact of the polymorphisms of these two receptor genes was studied simultaneously, it appeared that only the β2-adrenergic receptor polymorphism had an important role to play in modulating the regulation of growth rate and energy expenditure in short children.

Key words: β-Adrenergic receptors, Polymorphism, Short stature, Growth hormone, Obesity


CATECHOLAMINES play an important role in energy expenditure. Genes involved in the regulation of catecholamine functions may therefore be of particular importance in human obesity. Beta-adrenergic receptors have also been implicated in adaptive thermogenesis as they induce expression of the uncoupling protein (UCP)-1 gene in immortalized brown adipose cell lines [1]. Four adrenergic receptor subtypes (β1, β2, β3, α2) activated by catecholamines modulate a large number of biological responses, including adipose tissue lipolysis. While β1, β2 and β3-adrenergic receptors stimulate lipolysis, α2 adrenergic receptors inhibit lipolysis in humans [2, 3]. The genes encoding these receptors thus constitute interesting candidates for study, to explain, at least in part, the genetic predisposition to obesity in humans.

Growth hormone (GH) secretion is known to be decreased in obesity [4]. Even in individuals of normal stature, GH secretion varies to some extent in relation to the body composition [5, 6]. Abdenur et al. reported an inverse correlation between GH secretion and the indices of adiposity in children with idiopathic short stature [7]. However, the reason
why healthy children with a relatively large fat mass require less GH for the maintenance of normal growth than children with less fat is not clear. Fors et al., based on the results of investigation of children with short stature and normal control children, suggested that the serum leptin levels are correlated with the GH secretion and the amount of body fat, and that leptin may be the messenger via which the adipose tissue affects the hypothalamic regulation of GH secretion [8, 9].

Children with GH deficiency usually present with atherogenic risk factors, such as truncal obesity and hypercholesterolemia [10, 11]. Young adults with childhood-onset GH deficiency were reported to have increased intima-media thickness of the carotid artery [12]. These findings prompted us to investigate the occurrence of β-adrenergic receptor polymorphisms in a group of short children with GH deficiency and to evaluate the possible influence of these polymorphisms on the changes in anthropometric parameters in relation to GH treatment.

Subject and Methods

Subjects

A total of 75 children (31 girls and 44 boys), 6 of whom had signs of Turner’s syndrome, were investigated at the Tokyo Women’s Medical University Daini Hospital, Tokyo, Japan. All the children were prepubertal at the start of the GH treatment. Puberty was assessed according to Tanner and Whitehouse [13] for breast and pubic hair development, and according to Zachmann et al. [14] for testicular volume, measured using an orchidometer. When the age at adrenarche and gonadarche differed, the pubertal stage was rated according to the onset of gonadarche: breast development in girls and testicular volume in boys. Height was measured with a portable stadiometer and a digital scale, and expressed as the SD score for chronological age, based on recent Japanese growth references [15]. The severity of obesity was calculated for each child as the obesity index value, which represents the percent deviation from the ideal body weight based on height, age and sex [16]. All the children enrolled in the study were healthy and well nourished. The thyroid, kidney and liver functions were normal during the study, and none of the children had celiac disease.

Study protocol

GH deficiency was diagnosed on the basis of a peak GH concentration of less than 10 ng/mL in response to two or more standard provocation tests using insulin, glucagon hypoglycemia or GRH infusion. All subjects including those with Turner syndrome were diagnosed as GH deficiency by these provocation tests. Five of these children had a maximal GH response below 5 ng/mL in all provocation tests. β2- and β3-adrenergic receptor genotypes were determined by restriction fragment length polymorphism analysis. Of the 75 children enrolled in the study, 40 completed at least 5 years of GH treatment. Further analysis was conducted on these children, who were treated for short stature under the aegis of the Foundation for Growth Science in Japan. The mean (SD) chronological age at the start of GH treatment was 7.3 (2.7) years (range, 2.3 to 13.8 years), and the mean (SD) height was −2.63 (0.80) SD scores (SDS; range, −5.06 to −1.51 SDS). Recombinant human GH was administered by daily subcutaneous injection at a dose of 0.5 IU/kg body weight per week. The children were evaluated before, and at 3, 6, 12 and 18 months and 2, 3, 4 and 5 years after the commencement of GH treatment. At each visit, the standing height and body weight were measured. During the 5 years of GH treatment, 9 girls and 4 boys entered puberty at the average age of 12.1 years for girls and 14.6 years for boys.

The protocol for this study was approved by the regional ethics committee. Informed consent was obtained from all of the children and their parents.

Analysis of the polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes by digestion with proteinase K, followed by phenol/chloroform extraction. Trp64Arg of the β3-adrenergic receptor was amplified using the primers 5’-CGCCCAATACGCCA ACAC-3’ and 5’-CCACCAGGACTCCCATCAC-3’. PCR was performed as described by Wieden et al. [17], with denaturation at 95°C for 60 s, annealing at 60°C for 60 s, and extension at 72°C for 120 s for 35 cycles. The PCR products were digested with
10 U of BstNI for 1 h at 37°C. The resultant fragments were analyzed on 2.5% agarose gels.

Amplification of the β2-adrenergic receptor gene sequences was done by PCR in a 40 μl volume of a mixture containing 0.1 U of AmpliTaq gold DNA polymerase, using the following oligonucleotide primers: 5'-CTTTTTGCTGGCACAGCAAT-3' and 5' CCAGTGAGTGAGTAGTTTG-3' for codon 16, 5'-GGCCCATGACCAGATTGCA-3' and 5' GAATGAGGGTCGAGCGTC-3' for codon 27. The annealing temperatures for codon 16 and codon 27 were 56°C and 63°C, respectively. After 30 cycles of amplification, 2 μl aliquots of the PCR products were analyzed on 2% agarose gels to confirm proper amplification. The amplified PCR products were then digested with BsrD1 (codon 16) or Fnu4HI (codon 27). After incubation for 1 h, the digested samples were separated by electrophoresis on 4% agarose gel and visualized by staining with ethidium bromide [18].

Statistical analysis

Results were expressed as means, with the SD indicated in parentheses, unless otherwise stated. Contingency table chi-square tests were used to compare the genotype frequencies in the children of this study and control subjects from a past study [19, 20]. As the distributions of the samples were somewhat skewed, the Wilcoxon rank sum test was applied to examine the significance of the differences between the genotype groups. The differences among three genotype groups were analyzed by one-way analysis of variance. The within-group changes were analyzed by the paired t-test for assessment of the efficacy of GH treatment. Differences were considered significant when the p value was 0.05 or less.

Results

Frequencies of the β2,3-adrenergic receptor genotypes

The results of β2,3-adrenergic receptor genotyping are shown in Table 1. Among the 75 children with short stature, three (4%) had the Trp64Arg mutation in a homozygous form, 27 (36%) were heterozygous for the mutation, and the remaining 45 (60%) did not have any mutation in the β3-AR gene (Trp64Trp homozygote, wild type). No difference in the frequency distribution was observed as compared with that in Japanese non-diabetic subjects studied by Fujisawa et al. [19]. The genotype distribution of the β2-adrenergic receptor Gly16Arg polymorphism in the whole study population (n = 75) was as follows: 20% (n = 15), 52% (n = 39) and 28% (n = 21) had Arg16Arg, Gly16Arg and Gly16Gly, respectively (Arg16 allele frequency: 0.54). The genotype distribution of the β2-adrenergic receptor Gln27Glu polymorphism in the whole study population (n = 75) was as follows: 95% (n = 71), 5% (n = 4) and 0% (n = 0) had Gln16Gln, Gln16Glu and Gln16Glu, respectively (Glu27 allele frequency: 0.03). The frequencies of neither of the aforementioned polymorphisms differed significantly from those reported in non-obese

<table>
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No. of genotypes
Changes in anthropometric measurements and body composition during GH treatment

Standardized height

The mean height SD score improved significantly from $-2.64$ SD to $-1.63$ SD after 5 years of GH treatment in all of the children. To determine if the height SD score was modulated by the Trp64Arg mutation of the $\beta_3$-adrenergic receptor, the children were divided into those with the mutation (Trp/Arg and Arg/Arg, n=14) and those without the mutation (Trp/Trp, n=26, because of the limited number of Arg/Arg subjects). The mean height SD score increased linearly during the 5 years of GH treatment, from $-2.47$ SD to $-1.55$ SD in the group of children with the mutation, and $-2.73$ SD to $-1.67$ SD in the group without the mutation. There were no significant differences at any given time-point between the two groups in terms of the response of the height SD score during the 5 years of GH treatment (Fig. 1a).

Next, we divided the study population according to the $\beta_2$-adrenergic receptor Gly16Arg polymorphism. Concerning the $\beta_2$-adrenergic receptor polymorphism, children with Arg16Arg and Arg16Gly tended to experience a greater increase in height SD score, although statistical analysis was not possible due to the small number of children with Gly16Gly. In the children with Arg16Arg (n=12), the mean height SD score increased significantly from $-2.30$ to $-1.59$ during the first 3 years; thereafter, however, it remained constant. In those with Gly16Arg (n=20), the score increased significantly from $-2.73$ to $-1.38$ during the 5 years of GH treatment. In the children with Gly16Gly (n=8), the mean height SD score did not show any significant increase even at the end of 5 years of GH treatment. Thus, the mean SD score for height increased significantly at each evaluation point in the children with Arg16Arg and Gly16Arg after 1 year of GH treatment (Fig. 1b).

Obesity index

The mean obesity index value in the children enrolled in the study decreased from 9.2% to 7.8% (not statistically significant) during the 5 years of GH treatment. An additional analysis was performed to analyze the change in body composition in relation to the presence of the $\beta_3$-adrenergic receptor Trp64Arg polymorphism. The baseline mean obesity index value was more than 8% above the average in both the groups with and without the mutation, although the difference did not attain statistical significance. The mean obesity index value decreased during the first 4 years of GH treatment, but increased again thereafter in both the groups. The differences in the changes of the obesity index value from the baseline value between the two groups were not statistically significant at any time-point during the 5 years of GH treatment (Fig. 2a).

To further evaluate the relative importance of the $\beta_2$-adrenergic receptor polymorphism at codon 16, we conducted a stratified analysis for the three genotype groups. At baseline, the children with Gly16Gly had a significantly higher mean obesity index value than those with Gly16Arg. The mean obesity index...
Time from start of GH treatment (years)

Fig. 2. Changes in obesity index values during GH treatment in short children. Closed circle denote significant differences (P < 0.05) between genotype groups at the baseline. The within-group changes were analyzed by the paired t-test for assessment of the efficacy of GH treatment. Asterisks denote significant differences (P < 0.05) vs. values at the time of initiation of GH treatment.

a. β3-adrenergic receptor polymorphism (● Trp64Trp, ■ Trp64Arg & Arg64Arg)
b. β2-adrenergic receptor polymorphism (● Arg16Arg, ■ Arg16Gly, ▲ Gly16Gly)

value decreased in Arg16Arg subject from 13.1% to 9.0% after the first 2 years of GH treatment and increased thereafter. In contrast, it did not change appreciably in Gly16Arg subjects even at the end of 5 years of treatment and decreased markedly from 21.9% to 5.8% in Gly16Gly subjects at the end of the first 4 years of GH treatment (Fig. 2b).

Discussion

The present study revealed two significant findings. First, the β2-adrenergic receptor Gly16Arg genotype was associated with significant changes in the height SD score during 5 years of GH treatment, and second, this polymorphism was related to the baseline obesity index value measured before the commencement of GH treatment.

The frequency of the mutated allele of β3-adrenergic receptor Trp64Arg in Japanese was higher than those reported in other populations except for Pima Indians [21]. The high frequency of the mutated allele in the Japanese whose BMI (22.8 ± 3.9 kg/m2) was lower than those of several other ethnic groups suggests that the mutation itself appears not to be a major determinant of obesity in explaining the racial difference. The frequency of the β2-adrenergic receptor Glu27 allele in non-obese Japanese subjects of both genders was much lower as compared with that found in French or Swedish subjects, and this may explain the racial differences observed between Japanese and European subjects. In the present study, although the mean height SD score of the children was −2.64 at the start of GH treatment, the frequencies of the β3-adrenergic receptor Trp64 Arg mutation and β2-adrenergic receptor Gly16Arg and Glu27Glu mutations in these children were similar to those reported in the literature for normal Japanese subjects [19, 20]. These cross-sectional data show that the β2,3-adrenergic receptor polymorphism is not associated with short stature in Japanese children.

In this study, we calculated the severity of obesity (%) in each subject as the obesity index value. Kuromaru et al. reported, based on a study of female children, that in parallel with the decrease in obesity index value, the amount of body fat as measured with bioelectrical impedance analyzer also decreased during the first 3 years of GH treatment [22]. This observation may explain the superior value of the obesity index value as an indicator of the amount of body fat in children with GH deficiency.

To our knowledge, no data have been published yet on the relation of β2,3-adrenergic receptor polymorphism to the growth rate during GH treatment. An interesting and important finding of this study is the differences in the response to GH treatment among the children with various genotypes of the β2-adrenergic receptor. These differences were also associated with differences in the baseline obesity index value among these subjects. It is noteworthy that only β2-adrenergic receptor Gly16Gly subjects showed significant changes in both the height SD score and obesity index value during GH treatment. Table 1 shows the combined analyses of β2,3-adre-
nergic receptor polymorphisms. The two polymorphisms were not in linkage disequilibrium in our sample; that is, the Trp64 allele of the β3-adrenergic receptor was not associated with the Arg16 allele of the β2-adrenergic receptor. Taken together, the results of the study indicate that β2-adrenergic receptor polymorphisms influence the changes in height and amount of body fat with GH treatment in short children. The Glu27 allele was detected in 0 of 15 subjects homozygous for Arg16 allele, whereas 3 of 21 subjects homozygous for Gly16 allele carried the variant allele indicating that the Glu27 allele was in linkage disequilibrium to the Gly16 allele. Although the Gly16 variant and the Glu27 variant were in linkage disequilibrium, the incidence of Glu27 was much lower than that of Gly16 and those samples were too small to estimate statistically.

Lipolysis of fat cells can be stimulated by catecholamines, via the mediation of subtypes of β-adrenergic receptors, and inhibited via the α2-adrenergic receptor. Human adipose cells express not only the β3-adrenergic receptor, but also significant amounts of the β2-adrenergic receptor. Yang and MccElligott [23] reported that the β2-adrenergic receptor is the predominant lipolytic receptor in human white adipose tissue and that treatment of obese animals with selective beta2-agonists promotes a decrease in the fat mass and increase in muscle mass [24]. It should be noted that β2-adrenergic receptors are expressed not only in adipose tissue but also in a variety of other tissues, including the arteries in skeletal muscles and pancreatic beta-cells which affect insulin sensitivity and secretion. There is also the possibility that the association of the polymorphism with obesity is attributable to the distribution of the β2-adrenergic receptor in tissues other than the adipose tissue. Our longitudinal data on the obesity index value in subjects with the Gly16 allele subjects were consistent with the finding that adipocytes from subjects homozygous for the Gly16 allele showed fivefold higher sensitivity to terbutaline, a β2-adrenergic receptor-selective agonist, than those from subjects homozygous for the Arg16 allele [18].

GH has many effects on metabolism in addition to promoting growth [25, 26]. The main goal of GH treatment is the improvement of final height in children with GH deficiency. The role of GH in the regulation of protein, carbohydrate, and lipid metabolism has largely been ignored in children. Fors et al. based on the results of investigation of a group of children with short stature and normal control children, reported a significant negative correlation between the amount of body fat and GH secretion and a significant negative correlation between the serum leptin levels and GH secretion [8]. As reported by us previously, while there was a significant correlation between the change in the total body fat amount and change in the serum leptin levels during the first 12 months of GH treatment, the leptin concentration per unit fat mass did not change [9]. In this study, we detected significant genetic differences in the responses of the obesity index value and height SD score to GH treatment for short stature. The genetic differences in the response to GH could be due to the effect of leptin on body fat. Impaired β2-adrenergic receptor activity may not promote the height SD gain through the fluid factors such as leptin and PAI-1 that were produced in adipose tissue. Further studies focusing on the role of leptin and catecholamines are needed to elucidate the influence of β2-adrenergic receptor polymorphism on the amount of body fat and height.

In short children, we detected significant genetic differences in the responses of the height SD score and the obesity index value to GH treatment. In light of these data, the analysis of β2-adrenergic receptor polymorphism may be of particular interest for the estimation of the effects of GH in both children and adults.

In conclusion, the results of this study show that while the height SD score increased significantly in response to GH treatment among children with the β2-adrenergic receptor Arg16Arg and Gly16Arg alleles, no significant change in the height SD score was observed in children with the β2-adrenergic receptor Gly16Gly allele, and that the β3-adrenergic receptor polymorphism exhibits no association with these parameters. Thus, when the impact of these two polymorphisms was studied simultaneously, only the β2-adrenergic receptor polymorphism was found to have an important role in modulating the regulation of the growth rate and energy expenditure in short children.

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References


