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Common Polymorphisms of Growth Hormone: Growth Hormone Receptor Axis in Turkish Children with Short Stature

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What is already known on this topic?

- *A single-nucleotide polymorphism in growth hormone 1 gene (**GH1**) gene, GH1IVS4+90A>T (rs2665802), and loss of exon3 polymorphism of growth hormone receptor gene (**GHR**) gene, exon 3 deleted variant **GHRd3**, were reported to affect growth and height in different populations. **GHRd3** was also reported to affect the response to growth hormone (GH) therapy.*

What this study adds on this topic?

- *We investigated the frequency of both polymorphisms and their correlation to height in Turkish short children. Also, we evaluated the effect of **GHRd3** polymorphism on response to 1-year GH therapy. No significant difference was found for the frequency of A or T allele of GH1IVS4+90A>T polymorphism between idiopathic isolated growth hormone deficiency, idiopathic short stature (ISS) and control groups. The **GHRd3** genotype was significantly lower in the ISS group compared to the control group. There was no effect of **GHRd3**, on response to the first-year GH therapy. This is the first study investigating both of these variants together in Turkish population.*

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ABSTRACT

Objective: A single-nucleotide polymorphism of the growth hormone 1 gene, *GH1*IVS4+90A>T (rs2665802), associated with short stature and a polymorphism of the growth hormone receptor gene, exon 3 deleted variant, associated with increased responsiveness to growth hormone have been reported previously. We aimed to investigate the frequency of both polymorphisms and their correlation to height in Turkish short children. Also, we aimed to evaluate the effect of exon 3 deleted variant on response to 1-year growth hormone therapy.

Materials and Methods: Children with idiopathic isolated growth hormone deficiency (n = 39) and with idiopathic short stature (n = 10) and 50 control subjects were evaluated for anthropometric parameters, annual growth velocity, and annual height gain. Growth hormone receptor gene polymorphisms were analyzed via multiplex polymerase chain reaction; growth hormone 1 gene polymorphism was analyzed via polymerase chain reaction and single-strand conformation polymorphism techniques.

Results: The frequency of genotypes carrying the "A" allele was not significantly higher in the idiopathic isolated growth hormone deficiency group than in the idiopathic short stature and control groups ($P = .03$ for each). The exon 3 deleted variant genotype was significantly lower in the idiopathic short stature group compared to the control group ($P = .01$). There was no effect of exon 3 deleted variant, on response to the first-year growth hormone therapy.

Conclusion: In Turkish population, no correlation was found between the "A" allele of *GH1*IVS4+90A>T polymorphism and idiopathic isolated growth hormone deficiency and short stature, and a significant negative correlation was found between exon 3 deleted variant and idiopathic short stature and short stature. Exon 3 deleted variant has no effect on response to growth hormone treatment.

Keywords: GH1IVS4+90A>T, GHRd3, growth hormone, growth hormone receptor, growth hormone therapy, polymorphism, rs2665802, short stature

INTRODUCTION

Skeletal growth and height attainment are critical processes during transition to adulthood. For normal growth, many hormones, and metabolic and growth factors must interact precisely in the hypothalamic–pituitary–somatotropic axis.[1] While multiple exogenous and endogenous factors such as nutrition, climate, mood, and chronic illnesses affect this process, final height is mainly determined by the genotype.[1]

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Short stature, besides being an indicator of illness, is a physical obstacle and source of stress for the child. Short stature is defined as a condition in which an individual's height is in the third percentile for the mean height of a given age, sex, and population group.[2] Most common cause of short stature is idiopathic short stature (ISS), comprising the 60–80% of the short stature in childhood. Idiopathic short stature defines the children with height below −2 standard deviation (SD) with no identifiable cause and normal growth hormone (GH) responses to GH provocative tests; it is a diagnosis of exclusion. These children are normal size at birth but grow slowly during early childhood so that the average height falls below −2.0 SD by school-age, maintaining a height velocity within the lower normal range. This group of short-stature children may also harbor as yet unidentified mutations.[3] Especially defects in the growth hormone receptor (GHR) and intracellular signaling, and defects in GHR extracellular domain and growth plate may play a role in the etiology of ISS. Rapid development of diagnostic technologies and advances in genetic testing enabled discovery of novel genetic defects in genes associated with GH/IGF-1 (insulin-like growth factor 1) axis in these children.[4]

Another cause of short stature is idiopathic isolated growth hormone deficiency (IIGHD). Its incidence is 1/4000 to 1/10 000. It is defined as GH deficiency proven by GH provocation test without any identifiable organic and structural cause.[5] As new genetic testing techniques have developed, novel variants have been discovered in various genes of the growth axis, particularly *GH1* and *GHRHR*, and these variants have been associated with IIGHD.[6,7]

Growth hormone is a multifunctional hormone secreted from the anterior pituitary gland providing skeletal and soft tissue growth in postnatal life. While deletions and point mutations of GH gene cluster or growth hormone 1 gene (*GH1,* OMIM * 139250) cause isolated growth hormone deficiency responding well to GH replacement therapy, single-nucleotide polymorphisms (SNP) of *GH1* were postulated in the etiology of IIGHD and short stature. Growth hormone gene contains many SNPs both in coding and noncoding regions, especially SNPs in the promoter region and intron 4 have been suggested to play a role in the etiology of IIGHD.[8,9] For the first time, Hasegawa et al[4] reported a relationship between IIGHD and *GH1* polymorphisms. With this study, the frequency of "A" allele of a SNP (IVS4+90A>T) (rs2665802) in intron 4 of *GH1* was found to be significantly increased in IIGHD cases.[10] In a second study from Italy, a significant relationship was found between the polymorphism (−57G>T) of the vitamin D-responsive element of the *GH1* promoter and IIGHD.[11] In the same study, "T" allele of IVS4+90A>T polymorphism was prevalent in the IIGHD population unlike the Japanese study.

Most of the activity of GH is mediated by the GHR. Deletion of exon 3 of the growth hormone receptor gene (*GHR,* OMIM #600946) is a common polymorphism in humans. Its frequency has been reported to be as high as 25–32% in different populations.[12-14]

Dos Santos et al[6] investigated the relationship of this polymorphism with short stature in children born with low birth weight (SGA, small for gestational age) and in children with idiopathic short stature. They observed a significant increase in growth acceleration in response to GH therapy in children carrying the exon 3 deleted *GHR* allele (*GHRd3*) compared to non-carriers.[12] In follow-up studies, the relationship between *GHRd3* polymorphism, and GH therapy response and height distribution in different populations was investigated in various studies. While there were studies that reported its positive relationship with GH therapy response and tall stature, some studies reported no effect.[14-20]

The objective of our study is to investigate the allele distribution of these polymorphisms (GH1 IVS4+90A>T, rs2665802, and GHRd3) in Turkish IIGDH and ISS patients and normal subjects, and we aimed to investigate the relationship of these polymorphisms with short stature and height. We also aimed to investigate the effect of GHRd3 polymorphism on response to 1-year GH therapy in IIGHD and ISS patients.

MATERIALS AND METHODS

Patient and Control Groups

This study was conducted between May 2008 and March 2010. Forty-nine children with short stature who were receiving GH therapy for at least 1 year were selected as the patient group (11 from Istanbul University Cerrahpasa Medical School, Department of Pediatric Endocrinology, 29 from Istanbul Bakirköy Maternity and Children's Research and Training Hospital, Department of Pediatric Endocrinology, 9 from Istanbul Goztepe Research and Training Hospital, Department of Pediatric Endocrinology). All children were prepubertal without any identified genetic or organic diseases and SGA birth history. The patients with height below −2 SD, with growth velocity below 0.8 SD or 5 cm/year for at least 1 year, and/or projected target height at least 2 SD or 10 cm below mid-parental height were recruited in the study according to short stature criteria.[21,22] The patients with 2 separate low GH provocative test results were grouped as IIGHD, and the patients with at least 1 normal GH provocative test result were grouped as ISS. Fifty healthy adults with normal height (height ≥25th percentile, ≤97th percentile, ≥−1 SD, ≤+2 SD) were selected as the control group. Adults with height below −1 SD or the 25th percentile were not included in the study so as to draw a clear line between normal and short stature.

DNA Extraction

Genomic DNA was isolated from peripheric blood samples via Invisorb Spin Blood Mini Kit (Invitek-GERMANY, Robert- Rössle-Strasse 10, 13125, Berlin- Buch, Germany).

Analysis of *GH1* IVS4+90A>T Polymorphism

Polymerase chain reaction (PCR) amplification of intron 4 of *GH1* was done with the flanking primers and conditions were reported in the study of Hasegawa et al.[4] generating 542-bp product covering intron 4 and IVS4+90A>T polymorphism (F: 5'-TGACTTTGAGAGCTGTGTTA-3' and R: 5'-AGAAGGACACCTAGTCAGACA-3').[10] Polymerase chain reaction conditions were: 95°C for 7 minutes, followed by 30 cycles of 95°C for 30 seconds, 61.5°C for 30 seconds, and 72°C for 45 seconds, followed by a cycle with a 5-minute extension at 72°C. Polymerase chain reaction products were further cleaved with

HinfI enzyme to generate 433-bp and 99-bp products. Later on, we ran electrophoresis in 20% acrylamide/bis (49 : 1) gel with 10% glycerol after the step of denaturation with denaturing dye (95% formamide with 20 mmol/L EDTA and 10% glycerol) to detect polymorphisms of the *GH1* gene. DNA was visualized for the detection of single-strand conformation polymorphism (SSCP) by silver staining.

Analysis of *GHRd3* Polymorphism

A single multiplex PCR assay designed by Pantel et al[13] was used to identify *GHRd3* and GHR gene with full-length (*GHRfl*) alleles of the *GHR* gene. This assay was performed with 3 primers G1, G2, and G3 (G1: 5'-TGTGCTGGTCTGTTGGTCTG-3', G2: 5'-AGTCGTTCCTGGGACAGAGA-3', G3: 5'-CCTGGATTAACACTTTGCAGACTC-3') (GenbankTM accession number AF155912) and PCR conditions were as follows: initial step of denaturation for 5 minutes at 94°C, followed by 35 cycles of 94°C for 30 seconds, 61.5°C for 30 seconds, and 72°C for 90 seconds, followed by a cycle with a 7-minute extension at 72°C. The products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Statistical Evaluation of the Results

Descriptive statistics were expressed as frequency, percentage, mean, and SD values. Fisher's exact test and X^2 analysis were used for proportional comparisons of genotype frequencies between groups. Non-parametric methods were used because the study groups were small, the number of within-group distributions was low, and the distribution of the measurements did not fit the parametric distribution. The independent Kruskall–Wallis test was used to examine the difference in patient measurements between the study groups, and the Mann–Whitney *U* (post hoc) test was used to determine the groups that made the difference.

The Mann–Whitney *U* test was used to examine the differences in patient measurements for *GH1* and *GHR* polymorphisms in the IGHD and ISS groups. In addition, Mann–Whitney *U* test was used to examine the patient characteristics in the *GH1* and *GHR* polymorphism groups. Also, Mann–Whitney *U* test was used to examine the effect of *GHR* polymorphisms on the response to 1-year GH therapy. Licensed International Business Machines Corporation Statistical Package for Social Science 25.0 package program (Armonk, NY, USA), was used to calculate the statistical significance of the data obtained. The significance of the results was evaluated according to the *P* values obtained. *P* value less than .05 was considered statistically significant. Bonferroni correction was used in the multiple comparisons for the same parameters, and statistically significant *P* value was calculated accordingly.

RESULTS

Patient and Control Groups

Thirty-nine of the children were included in IIGHD group (15 females and 24 males) and 10 patients were included (4 females and 6 males) in ISS group. Fifty healthy adults (36 females and 14 males) with normal stature were selected as the control group. The control group was selected from adults due to the difficulty in forming a control group from children with normal stature due to the existence of many variables such as bone age calculation, growth velocity monitoring, and the effect of puberty if it started.

GH1 polymorphism IVS4+90A>T and *GHR* polymorphism exon3 G*HRd3* were analyzed in both patient and control groups. The relationship between *GHRd3* polymorphism and patients' response to 1-year GH treatment was examined. First-year values of patients who received GH therapy for more than 1 year were taken as basis.

IVS4+90A>T polymorphism could not be studied in 1 patient due to failure of SSCP gel run in the IIGHD group; evaluations for this polymorphism were made for 48 patients.

Since the pre-treatment annual growth velocities of 3 patients in the IIGHD group could not be learned, the differences between the pre- and post-treatment annual growth velocities for these patients could not be calculated. These parameters were evaluated for 46 patients. The features and comparison of the patient and control groups are given in Table 1.

There was no significant difference between 2 patient groups regarding parameters of age, pre- and post-treatment height standard deviation scores (SDS) for first-year GH therapy, height SDS difference, pre- and post-treatment growth velocity (cm/year and SDS) for first-year GH therapy, and the growth velocity difference (cm/year and SDS) (Table 1).

Distribution of *GH1*IVS4+90A>T Polymorphism

In the IIGHD group, 16 of 38 patients had homozygous TT genotype, 20 had heterozygous TA genotype, and 2 had AA genotype; while homozygous TT genotype was detected in 8 of 10 patients of ISS group and heterozygous TA genotype in 2 of them. Homozygous TT genotype was detected in 33 and heterozygous TA genotype was detected in 17 of 50 subjects of the control group. No homozygous AA genotype was found in ISS and control groups (Table 2.) Different group combinations were compared; one comparison between all 3 groups and 3 pairwise comparisons between each 2 groups were made. We corrected the statistically significant *P* value with Bonferroni correction for 3 post hoc tests; we calculated the statistically significant *P* value as .05/3 = .0167 (Table 2).

No significant difference was found between any groups regarding distribution of any of the 3 genotypes ($P = .06$). Since the "AA" genotype was not observed in every group, the genotypes carrying the "A" allele were taken together and compared with the "TT" genotype; again no significant difference was found for genotype frequencies between the 3 groups ($P = .03$) (Table 2). The frequencies of "A" and "T" alleles were also calculated; the frequencies of A allele were 31.6%, 10%, and 17% in IIGHD, ISS, and control groups, respectively. The frequencies of T allele were 68.4%, 90%, and 83% in IIGHD, ISS, and control groups, respectively, as shown in Table. 3.

No significant difference was found in the pairwise comparison of the groups for "TT," "TA," and "AA" distribution ($P = .05$ for IIGHD and ISS, $P = .07$ for ISS and control, $P = .12$ for IIGHD and control), and no significant difference was found for pairwise comparison of any groups for the frequency of the "TT" genotype compared to those carrying the "A" allele, "TA+AA" ($P = .03$ for IIGHD and ISS, $P = .06$ for

Table 1. Characteristics of Patient and Control Groups and Comparison of Data

Parameters	IIGHD Group (n = 39)	ISS Group (n = 10)	*P*	Control Group (n = 50)	*P*
	Mean ± SD	Mean ± SD		Mean ± SD	
Age (years)	8.17 ± 2.41 (1.79-13.41)	10,16 ± 2.77 (8-15.15)	.04	30.88 ± 7.9 (21-49)	.01*
Pre-treatment height SDS	−3.20 ± 0.89 (−1.448/−5.851)	−2.94 ± 0.88 (−1.652/−3.954)	.13	0.41 ± 0.75 (2.708/−0.783)	.01*
Height SDS at the end of 1-year GH therapy	−2.72 ± 0.76	−2,32 ± 1.67	.47		
Pre- and post-treatment height SDS difference	0.48 ± 0.36	0.63 ± 1.06	.68		
Growth velocity during 1-year GH therapy (cm/year)	7.4 ± 1.42	6.81 ± 1.59	.31		
Pre-treatment growth velocity (cm/year)**	4.69 ± 1.78	4.74 ± 0.8	.90		
Growth velocity difference (cm/year)**	2.75 ± 1.94	2.06 ± 1.72	.29		
Pre-treatment growth velocity SDS**	−1.47 ± 1.56	−1.11 ± 0.52	0.25		
Growth velocity SDS during 1-year GH therapy	2.1 ± 1.84	2.08 ± 2.23	.98		
Growth velocity SDS difference**	3.55 ± 2.44	3.19 ± 2.19	.67		

Age and pre-treatment height SDS are given as mean ± standard deviation; minimum and maximum values are given in parenthesis.
All other values are given as mean ± standard deviation.
SDS, standard deviation score; GH, growth hormone; F, female; M, male; IIGHD, idiopathic isolated growth hormone deficiency; ISS, idiopathic short stature.
Kruskall–Wallis test was used for the comparison of 3 groups. Mann–Whitney *U* test was applied for the comparison of two study groups.
**These parameters were calculated for 36 IIGHD patients.
*$P < .05$ was considered as significant.

ISS and control, $P = .03$ for IIGHD and control) as shown in Table 2.

Distribution of *GHRd3* Polymorphism

In the IIGHD group, 22 of 39 patients had homozygous fl/fl genotype, 13 of them had heterozygous fl/d3, and 4 of them had homozygous d3/d3 genotype. While no homozygous d3/d3 genotype was found in the ISS group, 9 patients had a homozygous fl/fl genotype and 1 patient had a heterozygous fl/d3 genotype. Of the 50 cases in the control group, 21 had homozygous fl/fl genotype, 26 had heterozygous fl/d3 genotype, and 6 had homozygous d3/d3 genotype. When we compare the frequencies of fl/fl, fl/d3, d3/d3 separately, no significant difference was found between 3 study groups ($P = .57$). Due to the low frequency of d3/d3 genotype, all groups were compared in terms of the distribution of homozygous fl/fl and d3 allele carrying genotypes, fl/d3+d3/d3. Different group combinations were compared; 1 comparison between all 3 groups and 3 pairwise comparisons between each 2 groups were made. We corrected the statistically significant *P* value with Bonferroni correction for 3 post hoc tests; we calculated the statistically significant *P* value as .05/3 = .0167 (Table 2). No significant difference was found between the 3 groups for this comparison ($P = .02$) as shown in Table 2.

Table 2. Comparison of *GH1* and *GHR* Genotypes Distribution in Patient and Control Groups

		Group			*P* for All Groups	*P* for IIGHD and ISS	*P* for ISS and Control	*P* for IIGHD and Control
		IIGHD (n = 39)	ISS (n = 10)	Control (n = 50)				
Genotype		n (%)	n (%)	n (%)				
GH1 genotype**	TT	16 (42.1)	8 (80)	33 (66)	.06	.05	.07	.12
	TA	20 (52.6)	2 (20)	17 (34)				
	AA	2 (5.3)	0 (0)	0 (0)				
	TT	16 (42.1)	8 (80)	33 (66)	.03	.03	.06	.03
	TA+AA	22 (57.9)	2 (20)	17 (34)				
GHR genotype	fl/fl	22 (56.4)	9 (90)	21 (42)	.57	.31	.39	.53
	fl/d3	13 (33.3)	1 (10)	26 (52)				
	d3/d3	4 (10.3)	0 (0)	3 (6)				
	fl/fl	22 (56.4)	9 (90)	21 (42)	.02	.02	.01*	.08
	fl/d3+d3/d3	17 (43.6)	1 (10)	29 (58)				

IIGHD, idiopathic isolated growth hormone deficiency; ISS, idiopathic short stature; n, number. Percentages are given in parenthesis.
Chi-square and Fisher's exact tests were applied for the comparison of 3 genotypes of both *GH1* and *GHR* polymorphisms, Chi-square test was applied for the comparison of TA with TT+AA and fl/fl with fl/d3+d3/d3genotypes.
***GH1* genotypes were calculated for 38 IIGHD patients
*Statistically significant *P* value < .0167 using Bonferroni correction.

Table 3. *GH1* and *GHR* Allele Frequencies of Patient and Control Groups

	IIGHD Group	ISS Group	Control Group
GH1	%	%	%
T	68.4	90	83
A	31.6	10	17
GHR			
fl	73	95	68
d3	27	5	32

IIGHD, idiopathic isolated growth hormone deficiency; ISS, idiopathic short stature; GHR, growth hormone receptor.

There was no significant difference in the pairwise comparison of the genotype distributions of study groups ($P = .31$, .39, and .53) as shown in Table 2. When the patient groups were compared with the control group 1 by 1, there was no significant difference between the IIGHD group and the control group, and between ISS and IIGHD groups ($P = .08$ and $P = .02$, respectively) as shown in Table 2. Besides, there was a significant difference between the ISS group and control group ($P = .01$). Thus, the frequency of fl/fl genotype was significantly high in the ISS group compared to the control group.

The frequencies of "d3" and "fl" alleles were also calculated; the frequencies of "d3" allele were 27 %, 5%, and 32% in IIGHD, ISS, and control groups, respectively. The frequencies of "fl" allele were 73%, 95%, and 68% in IIGHD, ISS, and control groups, respectively, as shown in Table. 3

GHRd3 and Response to GH Therapy

Patient groups were evaluated separately and together for *GHRd3* polymorphism distribution and for response to GH treatment. Since the number of patients in the ISS group was low, statistical significance alone could not be obtained in this group. When the relationship between *GHRd3* allele carriage and response to 1-year GH treatment was evaluated, no significant differences were found in growth velocity in any patient group (shown in Table 4.). Similarly, no significant relationships were found between *GHRd3* carriage state and pre- and post-treatment height SDSs. When the IIGHD group was evaluated alone, no significant relationship was found between *GHRd3* polymorphism and any parameter.

DISCUSSION

We have found that there was no significant difference for the frequency of *GH1*IVS4+90A>T polymorphism between study groups and the control group. The frequency of "d3" allele carrying genotypes of *GHRd3* polymorphism fl/d3+d3/d3 were significantly low in the ISS group compared to the control group, while there was no significant difference between ISS and IIGHD groups, and between IIGHD and control groups. We have not observed any effect of *GHRd3* on response to first-year GH therapy.

The *GH1* gene contains quite a lot number of polymorphisms.[23-25] In particular, they are abundant in the promoter region, nearly 30 times more compared to the rest of the gene.[9,24-26] Horan et al[9] identified 40 types of haplotype of this multivariable proximal promoter region in the British population. Millar et al[26] defined 24 more polymorphisms in West Africans in the entire *GH1*. For the first time, Hasegawa et al[10] showed the relationship of *GH1* gene polymorphisms with GH levels and height. They found the distribution of some polymorphisms varied between Japanese IIGHD patients and short and normal height adults with normal GH secretion. "A" allele frequency of GH1IVS4+90A>T (rs2665802), one of these SNPs in intron 4, was significantly higher in IIGHD patients than other groups. In different studies conducted in Italy, Spain, and Benin, the same SNP was found to be common in the normal populations.[11,26,27] In all of them, the more common allele was the "T" allele, with a frequency of 57.8% in Japanese, 51.5% in Italians, 54.9% in Hispanics, and 96.9% in Beninese.[10,11,26,27] In the study of Esteban et al.[27] there was no relationship between this SNP and height while in the study of Giordano et al.[11] unlike the Japanese study, the proportion of genotypes carrying the "T" allele instead of the "A" allele was found to be higher in the IIGHD group compared to normal controls. In a recent study from

Table 4. Relationship of GHR Genotype and Response to 1-Year GH Therapy

	IIGHD Group+ISS Group (49 Cases, 19 F : 30 M)		
GHR Genotype	**fl/fl (n = 31, 12 F : 19 M)**	**fl/d3+d3/d3 (n = 18, 7 F : 11 M)**	
	Mean ± SD	**Mean ± SD**	***P***
Age (year)	8.63 ± 2.37	8.47 ± 2.98	.32
Pre-treatment height SDS	−3.1 ± 0.84	−3.24 ± 0.99	.13
Height SDS at the end of 1-year GH therapy	−2.58 ± 1.1	−2.73 ± 0.82	.47
Pre- and post-treatment height SDS difference	0.51 ± 0.62	0.51 ± 0.47	.74
Growth velocity during 1-year GH therapy (cm/year)	7.07 ± 1.09	7.62 ± 1.93	.73
Pre-treatment growth velocity (cm/year)**	4.87 ± 1.32	4.4 ± 2.06	.15
Growth velocity difference (cm/year)**	2.26 ± 1.57	3.25 ± 2.3	.22
Pre-treatment growth velocity SDS**	−1.11 ± 1.32	−1.91 ± 1.46	.89
Growth velocity SDS during 1-year GH therapy	1.84 ± 1.81	2.54 ± 2.02	.76
Growth velocity SDS difference**	3 ± 2.07	4.35 ± 2.71	.13

GHR, growth hormone receptor gene; SD, standard deviation; SDS, standard deviation score; GH, growth hormone; F, female; M, male; IIGHD, idiopathic isolated growth hormone deficiency; ISS, idiopathic short stature.
Mann–Whitney *U* test was applied; fl/d3 and d3/d3 were combined.
**These parameters were calculated for 46 patients.
*$P < .05$ was considered significant.

Egypt conducted with IGHD and ISS patients, genotypes with "A" allele were significantly high in shorter children. They found significant relationship between "A" allele and low GH levels in IGHD and with short stature in both groups. They suggested to use this SNP as a biomarker for short stature.[28] In our study, we found the frequency of "T" allele 83 % in normal controls, while no "AA" genotype was found. But, unlike the Japanese and Egyptian series, the "A" allele was not significantly higher in the IIGHD group compared to the normal control and ISS groups. This variable relationship of this SNP with height in different populations may be due to a couple of reasons: this SNP alone may have no effect on GH levels but may be in linkage disequilibrium with other effective polymorphisms; it may have an insufficient effect requiring the effect of another regulatory region in which again it is in linkage disequilibrium; the variants in linkage disequilibrium with this SNP may vary in different nations causing different allele frequencies.

To find the frequency of this SNP in the normal Turkish population, it would be helpful to first investigate the frequency of the polymorphism in a large cohort with normal height distribution. Also, if the "A" allele of this polymorphism is associated with low GH levels, it may be useful to investigate its association with GH secretion in larger patient groups rather than the normal population.

Exon 3 deletion polymorphism of the *GHR* gene (*GHRd3*) is common in many populations. This is assumed to be an evolutionary adaptation. This thought arises from the data obtained from various studies showing that the *GHRd3* allele responds better to GH than the *GHRfl*.[12,14,15,18,29,30] Also in a recent study, Resendez et al[31] showed that in addition to its effect on growth, its evolutionary preference is due to GHRd3's association with protection from edematous severe acute malnutrition primarily in males. Dos Santos et al[12] examined *GHR* transcription activity at various GH concentrations in vitro; they showed that cells carrying the *GHRd3* allele at all GH doses responded better than *GHRfl* carrying ones. Due to this GH efficacy increasing feature, it is possible to establish a relationship between its prevalence in various populations and the average height.

In the comparison of different studies, the frequencies of the *GHRd3* allele were lower in the Japanese and in the Korean populations (22% and 19%, respectively) than that of the European populations.[29,32] Its frequency was significantly high in Western African people, whose average height is higher than that of the Caucasian populations; 70% of the studied population had the d3/d3+fl/d3 genotype, while this frequency was 47% in the British control group.[26] Researchers suggested that this high frequency in Africans may be due to an adaptative response to environmental nutrient deficiency and chronic starvation.[26] In our study, the frequency of GHRd3 allele in the control group was found to be 32%, lower than Europeans and Africans, and higher than Japanese and Koreans, like the average height of our population. In another study from Turkey, *GHRd3* allele frequency was found to be 45.5% in 477 healthy adults with normal stature giving a similar depiction.[20]

Based on the relationship of this polymorphism with the height distribution in normal populations, it can be predicted that the frequency of the normal population will show a significant difference from that of short patient groups. Upon evaluation of our results, while a significant difference was found between the ISS group and the control group ($P = .01$), there was no significant difference between the IIGHD and the control group for *GHRd3* genotype frequencies ($P = .08$). This may be due to the probable etiologies of short stature in 2 patient groups. In IIGHD group, the major problem is low GH levels, while in ISS group, the reason is the reduced answer to GH in receptor or in post-receptor level. The presence of homozygous fl/fl genotype in 9 of 10 ISS patients in our study supports the argument that *GHRd3* polymorphism is effective on height gain. Similarly, in the study of Audi et al.[8] the frequency of the GHRd3 allele was significantly lower in short SGA patients compared to normal adults. In the same study, when they grouped the normal adults according to the height and examined the distribution of allele frequency, the mean height of the homozygous d3/d3 genotype group was found to be taller than the other groups.[8] This relationship was not demonstrated in most other studies.[12,17,29,33] The researchers have postulated that the *GHRd3* polymorphism does not make a difference in physiological conditions, it only potentiates the effect of GH therapy.[12] However, it is more reasonable to expect this polymorphism, which shows a clear relationship with the height differences between the different nations, to exhibit a more pronounced relationship with the height phenotype under physiological conditions, as in our study.

Various studies have been conducted on the effect of GHRd3 polymorphism on GH therapy response. For the first time, Dos Santos et al.[12] investigated the relationship between the GHR genotype and treatment response in ISS and SGA children receiving GH treatment for at least 2 years, and they found positive relationship between the growth acceleration and the *GHRd3* allele for both years. In the following period, many studies have been conducted on the effect of *GHRd3* polymorphism on the GH therapy response in various patient groups (ISS, IGHD, GHD, ISS, Turner Syndrome, SGA).[9,10,11-13,18,19,24-27] In addition to studies showing that it has an effect[14,15,18,29,30], there are also studies arguing otherwise.[8,16,17,34-38] In a review of 15 studies examining the relationship between GH treatment and *GHRd3* polymorphism, an average of 0.5 cm/year growth velocity difference was observed in allele carriers during the first year of GH treatment in various patient groups, and this effect was more pronounced at lower doses and at older ages.[19] In contrast, in a multicenter study in Turkey, *GHRd3* polymorphism effect was not observed in the response to GH therapy in GHD patients and Turner Syndrome.[39] In our study, we observed no effect of *GHRd3* polymorphism on GH therapy response in either of patient groups. These conflicting results may be attributed to different parameters used in each study. Another reason may be other genetic or hormonal variables that regulate the bioactivity of GH treatment and its effect on the receptor. All of the studies reported are short-term studies; longer-duration studies should be conducted to examine the effect of GHRd3 polymorphism on the long-term effect of GH therapy and the final height. In addition, it is necessary to investigate the presence of other polymorphisms or variables that may affect the response to GH therapy.

Our limitations in this study were the low number of the study groups and the short term of follow-up for the patient groups.

Testing larger cohorts for longer periods would give better results.

There is still limited information available about genetic variants affecting height in the normal population and short people. With the advancement of high-throughput genetic sequencing technology such as whole-exome sequencing or whole-genome sequencing and screening larger populations for polymorphic variants, it will be possible to obtain more data on genetic variants that affect height in the near future.

In conclusion, the *GH1 g*ene intron 4 polymorphism+90A>T is commonly observed in our normal population, while genotypes carrying the "A" allele were not significantly different in the IIGHD group with low GH levels. The frequency of *GHRd3* allele in our control group was higher than the Eastern Asians and lower than the Europeans and Africans, similar to our population's average height. Accordingly, we have concluded that this polymorphism is one of the factors affecting the height distribution in the general population. In contrast, *GHRd3* has no effect in response to GH treatment. This is the first study in which these two polymorphisms were investigated together in the Turkish population.

Data Sharing Statement

The authors state that individual participant data that underlie the results reported in this article after deidentification (text, tables, figures, and appendices) and study protocol will be available for sharing for the time period beginning 9 months and ending 36 months following article publication. The data will be shared with investigators whose proposed use of the data has been approved by an independent review committee ("learned intermediary") identified for this purpose, for individual participant data meta-analysis. Proposals may be submitted up to 36 months following article publication. Proposals should be directed to mdegulec@gmail.com. To gain access, data requestors will need to sign a data access agreement.

Ethics Committee Approval: This study was approved by the local Ethics Committee of İstanbul University Cerrahpasa Medical Faculty (no. 10251/2008).

Informed Consent: The legal guardians of all participants in patient groups and all participants in control group provided their written informed consent for participation to the study.

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