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Clinical, Neuroradiological and Molecular Genetic Characteristics of 13 Patients Followed up for Growth Hormone Deficiency

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Abstract

Objective: Growth hormone-releasing hormone (GHRH) and somatostatin are hypothalamic peptides that regulate pulsatile growth hormone (GH) secretion. GHRH binding to its receptor activates signaling, promoting cell proliferation, GH synthesis, and secretion. Mutations in the growth hormone-releasing hormone receptor (*GHRHR*) and growth hormone 1 (*GH1*) genes, which are involved in this pathway, occur as a rare cause of isolated growth hormone deficiency (IGHD). This study aimed to evaluate the clinical features, neuroradiological findings, and molecular genetic test results of 13 patients diagnosed with IGHD, as well as their responses to growth hormone treatment.

Methods: The study included 13 patients from six different consanguineous families who were being followed for isolated growth hormone deficiency. Using next-generation sequencing, biallelic disease-causing variants in the *GHRHR* and *GH1* genes were identified in these patients. Clinical findings, family history, parental consanguinity, and neuroradiological images of the patients were retrospectively obtained from hospital records.

Results: Biallelic variants were identified in the *GHRHR* gene in nine patients and in the *GH1* gene in four patients. The potential impact of these variants on protein structure was assessed using in silico prediction tools, including SIFT, MutationTaster, REVEL, and PolyPhen-2.

Conclusion: Screening for variants in the *GH1* and *GHRHR* genes is recommended for patients with severe growth retardation, short stature. It is important to consider the possibility of multiple affected individuals presenting with similar phenotypes, particularly in regions with a high prevalence of consanguineous marriages. Therefore, comprehensive family screening should be conducted when appropriate.

Keywords: *GH1*, *GHRHR*, Consanguineous marriage, short stature

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Büyüme Hormon Eksikliği ile İzlenen 13 Hastanın Klinik, Nöroradyolojik ve Moleküler Genetik Özellikleri

Öz

Amaç: Hipotalamik peptidler olan büyüme hormonu salgılatıcı hormon (GHRH) ve somatostatin, büyüme hormonunun (GH) pulsatil salınımını düzenleyen temel unsurlardır. GHRH'nin GHRH reseptörüne (GHRH-R) bağlanması, sinyal yolaklarını aktive ederek hücre çoğalması, GH sentezi ve salınımını sağlar. Bu mekanizmada yer alan büyüme hormonu salgılatıcı hormon reseptörü (*GHRHR*) ve büyüme hormon reseptörü 1 (*GH1*) genlerindeki mutasyonlar, izole büyüme hormonu eksikliğinin (İBHE) nadir nedenleri arasında yer alır. Bu çalışmanın amacı, İBHE tanısı konulan 13 hastanın klinik özelliklerini, nöroradyolojik bulgularını ve moleküler genetik analiz sonuçlarını değerlendirmek, ayrıca bu hastaların büyüme hormonu tedavisine verdikleri yanıtı incelemektir.

Yöntemler: Çalışmaya, aralarında akrabalık bulunan 6 farklı aileden, izole büyüme hormonu eksikliği tanısıyla takip edilen ve yeni nesil dizileme yöntemi kullanılarak *GHRHR* ve *GH1* genlerinde biallelik patojenik varyantlar tespit edilen 13 hasta dahil edilmiştir. Hastaların klinik bulguları, aile öyküleri, akrabalık ilişkileri ve nöroradyolojik görüntüleri, retrospektif olarak hastane kayıtlarından elde edilmiştir.

Bulgular: İncelemeler sonucunda, 9 hastada *GHRHR* geninde, 4 hastada ise *GH1* geninde biallelik varyantlar tespit edilmiştir. Varyantların protein yapısı üzerindeki olası etkileri, SIFT, MutationTaster, REVEL ve PolyPhen-2 gibi çeşitli in siliko tahmin araçlarıyla analiz edilmiştir.

Tartışma: Ciddi büyüme geriliği, boy kısalığı ve pozitif aile öyküsü olan hastalarda *GH1* ve *GHRHR* genlerindeki varyantların incelenmesi önerilmektedir. Akraba evliliklerinin sık görüldüğü bölgelerde, benzer fenotiplerle birden fazla etkilenmiş bireyin bulunabileceği göz önünde bulundurulmalı ve aile taraması bu doğrultuda yapılmalıdır.

Anahtar kelimeler: GH1, GHRHR, Akraba evliliği, kısa boy.

INTRODUCTION

Growth is an essential process that starts at fertilization and progresses through various stages of development. Weight and height are key indicators of physical growth, which is typically divided into four distinct stages: the fetal period, infancy, childhood, and adolescence[1]. Growth hormone deficiency (GHD) is a rare but significant reason for reduced height in childhood[2]. Growth hormone (GH), produced by the anterior pituitary gland, plays a crucial role in childhood growth. Any disruption or disorder in the secretion of this hormone results in reduced stature. GHD is characterized by short stature resulting from alterations that affect the production and secretion of GH[3]. Two types of growth hormone deficiency (GHD) are recognized: combined pituitary hormone deficiency (CPHD) and isolated growth hormone deficiency (IGHD). The disease is observed around 1 in every 4,000 to 10,000 live births[1,4]. Structural abnormalities in the hypothalamic-pituitary region or genetic factors, such as familial IGHD, may lead to IGHD. Although most cases are sporadic, 3% to 30% of patients have more than one affected individual within the same family, suggesting a potential underlying inherited cause. IGHD is classified into four familial forms: autosomal dominant (Type II), autosomal recessive (Types IA and IB), and X-linked (Type III)[5]. However, in the latest classification, biallelic variants in the *GH1* gene have been divided into two subgroups as autosomal recessively inherited types IA and IB (IGHD IA; OMIM #262400, IGHD IB; OMIM #612781), while the autosomal dominantly inherited form, which occurs due to heterozygous variants in the *GH1* gene, has been described as type II (IGHD II; OMIM #173100). The form resulting from biallelic variants in the *GHRH* receptor gene (GHRHR) has been updated as IGHD type IV (IGHD IV; OMIM #618157). Although familial IGHD is primarily

caused by pathogenic variants in the *GHRHR* and *GH1* genes, it can also, in rare cases, result from heterozygous variants in the *SOX3* or HESX1 genes[6]. Individuals affected by pathogenic variants in the *GH1* exhibit different phenotypic characteristics compared to those with the disorder resulting from variants in the *GHRHR* gene. These distinguishing features include a prominent forehead, flat nasal bridge, small face, and microphallus in males. In most patients diagnosed with sporadic IGHD, no mutations have been described. However, previous studies emphasize that in individuals with a height standard deviation (SD) score below -4.5, which is defined as severe growth retardation, the prevalence of deletions and point mutations in the *GH1* gene may be as high as 20%, although this rate varies depending on ethnicity and family history.

Mutations in the *GHRHR* and *GH1* genes are frequently linked to a normal or hypoplastic anterior pituitary gland and a eutopic (correctly positioned) posterior pituitary gland, as demonstrated on cranial magnetic resonance (MRI) scans. Conversely, a deletion has been documented in the *GH1* gen in a patient presenting with an ectopic posterior pituitary[7].

Our objective is to present the neuroradiological and clinical findings of 13 patients with severe short stature diagnosed with familial IGHD by identifying biallelic pathogenic variants in the *GHRHR* and *GH1* genes. We aim to evaluate their outcomes following GH treatment and contribute to the literature by introducing these variants into the molecular etiology of the condition.

METHODS

13 patients between the ages of 0-18 who were diagnosed with GHD and presented to the pediatric genetics and/or pediatric endocrinology outpatient clinic with short stature and dysmorphic facial findings between March 2016 and December 2023 were involved. Anthropometric measurements, detailed dysmorphological examination results, and radiological findings of the patients were obtained retrospectively from the hospital records. All patients underwent all standard tests for short stature typically conducted in the pediatric endocrinology outpatient clinic. These tests included a biochemical profile, erythrocyte sedimentation rate, complete blood count, liver and kidney function assessments, celiac disease screening, cortisol measurements, thyroid function tests, serum insulin-like growth factor binding protein-3 (IGFBP-3), and analysis of Insulin-like growth factor 1 (IGF-1) levels. GHD was diagnosed by performing provocative GH tests (clonidine and L-dopa stimulation tests). The diagnosis was confirmed by genetic tests performed on the patients.

Patients with abnormal routine screening test results for syndromic and disproportionate short stature or skeletal dysplasia, who did not have a confirmed molecular diagnosis, were excluded from the study.

All patients underwent an MRI scan to evaluate the pituitary gland before recombinant growth hormone (rGH) was administered.

A targeted next-generation sequencing (NGS) panel (TruSight One Sequencing Panel by Illumina) was performed following DNA isolation from the patients' peripheral blood samples. For target enrichment, the Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA) was utilized following the manufacturer's instructions. Paired-end sequencing of all samples was performed on the Illumina NextSeq platform (Illumina Inc., San Diego, CA). The NGS data analysis was conducted using the Integrative Genomics Viewer (IGV) and Illumina VariantStudio software.

The effects of the identified variants on protein structure were assessed using in silico prediction tools, including MutationTaster,

REVEL, PolyPhen-2, and SIFT. American College of Medical Genetics and Genomics (ACMG) guideline was used to classify the pathogenicity of the variants 8.

This study was performed in compliance with the principles established in the 2008 Declaration of Helsinki, and ethical clearance was granted by the Local Ethics Committee (approval date/number: May 24, 2024/74).

FINDINGS

All patients exhibited severe short stature and growth retardation, with height and weight deviation SD scores below -2.5. Among the 13 patients enrolled in the study, 8 (61.5%) were male and 5 (38.5%) were female. A positive family history was observed in four patients (4/13, 31%), and parental consanguinity was reported in all six families (6/6, 100%). Major dysmorphic findings (prominent and high forehead, triangular face, deep-set eyes) were observed in 4 patients (F1-1, F1-2, F2-1, F3-1) (Figure 1). The results of erythrocyte sedimentation rate, complete blood count, blood biochemistry, kidney and liver function tests, celiac disease screening, and thyroid function levels were found to be normal, serum IGFBP-3 levels and the serum IGF-1 were found to be remarkably below the normal range in all affected individuals (Table 1).

The mean age of the patients at the time of admission was 3.84± 2.47 years, the mean height SD value was -5.6 ± 1.4, the weight SD value was -4.54 ± 1.56, while the mean age at initiation of recombinant GH (rGH) treatment was 5.12 ± 2.55 years and the height SD value was -5.97 ± 1.31. The mean height velocity (year/cm) before rGH treatment was 3.6±0.85. rGH treatment was administered to 11 patients (11/13, 84.6%) at a dose of 0.03 mg/kg/day. After rGH treatment, the mean annual height growth and first-year height SD values were found as 13.36 ± 1.75 cm and -4.43 ± 1.41, respectively. All affected individuals showed a positive response to rGH treatment, with an improvement in post-treatment height SD values compared to pre-treatment values. The weight, height and GH values of the patients at the time of first admission, the rGH treatment dose they received, and the height SD values before and after rGH treatment are summarized in Table I and Table II respectively.

Figure 1. Homozygous c.59G>A, p.Trp20* nonsense variant in the *GH1* gene was detected in twin siblings (A1). Among the dysmorphic features, a triangular face, high and prominent forehead, depressed nasal bridge and root, and deep-set eyes are noteworthy.

Table I: Patients' initial presentation, follow-ups before and after rGH treatment, and radiological imaging findings.

Patient	Gender	Chronological age (year)	Bone age (year)	Height (cm)	Height SD	Weight (kg)	Weight SD	IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	Age at rGH treatment (year)	Height at rGH treatment (cm)	Height SD at rGH treatment	Height at first year after the treatment (cm)	Height SD at first year after the treatment	Annual height velocity (cm/year)	Cranial MRI	Pituitary gland MRI
F1-1	F	2.42	1.5	63.5	-7.1	6.3	-5.58	<7	180,7	3.25	70	-6.56	86	-4.11	16	N	N
F1-2	M	2.42	1.5	63.5	-7.3	7	-6,25	<7	145	3.25	72	-6,55	88	-4.08	16	N	hypoplasia
F2-1	M	1.70	0.5	62	-6.2	5.6	-6.59	9.47	283.9	2.33	65	-7.19	76.2	-6.89	11.2	N	N
F3-1	M	0.70	0.3	58	-4.8	6.5	-2.41	<7	143	-	-	-	-	-	-	-	-
F4-1	M	7.90	3.5	97	-5.6	12	-5.89	<15	50	8.9	101	-5.6	113	-4.12	12	N	hypoplasia
F4-2	M	8.00	4.5	98.8	-5.9	13.8	-5.74	10.3	358	9.1	100	-5.9	111.3	-4,64	11.3	N	hypoplasia
F4-3	F	4.16	1.5	83.2	-4.56	10.6	-3.45	<7	410	4.8	87.4	-4.63	96	-3.58	12	N	N
F5-1	M	2.75	1.5	82	-3.29	11	-2.24	<7	218.9	3.16	84	-3.53	97.8	-1.79	13.8	gliosis	hypoplasia
F5-2	F	1.76	1	72.3	-3.59	7	-3.71	-	-	-	-	-	-	-	-	-	-
F6-1	M	3.75	1.5	81.2	-4.99	10.5	-3.72	<7	<250	4.73	85.6	-5.18	99.5	-3.19	13.9	N	hypoplasia
F6-2	M	2.00	1	70	-4.88	9.5	-2.61	<7	<250	2.50	73.2	-5.17	85.7	-4.51	12.5	N	hypoplasia
F6-3	F	6.95	3	85.9	-7.04	11.5	-4.96	<7	<250	7.95	87.1	-7.41	100.1	5.78	15	N	hypoplasia
F6-4	F	5.38	2	75.3	-7.71	9	-5.82	<7	<250	6.38	79.2	-7.93	92.5	6.01	13.3	N	hypoplasia

Abbreviations: F: female, IGF-1: insulin-like growth factor 1, IGFBP-3: insulin-like growth factor binding protein-3, M: male, MRI: magnetic resonance imaging, N: normal, rGH: recombinant growth hormone, SD: standard deviation

Table II: Height SD levels before and after rGH treatment and annual growth rates

Before growth hormone replacement	Patient (n:11)
Gender n, (%)	
Male	7 (63.6%)
Female	4 (36.4%)
Average age (year ± SD)	5.12 ± 2.55
Height SD	-5.97 ± 1.31
Mean Height Velocity (year/cm)	3.6±0.85
Growth hormone replacement first year	
Mean Height Velocity (year/cm)	13.36 ± 1.75
Height SD	-4.43 ± 1.41

Abbreviations: SD: standard deviation, rGH: recombinant growth hormone

Hypoplasia of the pituitary gland was detected in 8 (8/11, 73%) of 11(11/13, 84.6%) patients who underwent MRI examination. Only one patient had white matter gliosis. No structural anomalies were observed in the MRI examination of the remaining 10 (10/11, 91%) patients.

To investigate the genetic causes of short stature, patients underwent targeted NGS analysis. In 4 patients (F1-1, F1-2, F2-1, F3-1), a biallelic variant was detected in the *GH1* gene, while in the remaining 9 patients (F4-1, F4-2, F4-3, F5-1, F5-2, F6-1, F6-2, F6-3, F6-4), a biallelic variant was detected in the *GHRHR* gene. A nonsense variant was present in exon 2 of the *GH1* gene in patients F1-1 and F1-2, and it was identified as "pathogenic" in the ClinVar database and according to the 2015 ACMG criteria for PVS1+, PM2+, PS4+, PP5+ (NM_000515.5; c.59G>A; p.W20*). In patients F2-1 and F3-1, biallelic deletions of respectively 1.6 and 2.1 kb in size, involving exons 1-2-3-4-5 of the *GH1* gene, were detected. In F4, there was a nonsense variant (NM_000823.4; c.214G>T, p.E72*) in exon 3 of the *GHRHR* gene, which was identified as pathogenic in the databases and classified as pathogenic according to the criteria for PVS1+, PM2+, PS4+, PP5+ in the 2015 ACMG guideline. In the affected individuals of F5 and F6, a biallelic missense variant (NM_000823.4; c.190T>G, p.C64G) was identified in exon 3 of the *GHRHR* gene, which had not been reported in the databases and had no clinical significance based on the PP3+, PM2+ criteria in the 2015 ACMG guideline. The pathogenicity of the identified variants was assessed using various in silico prediction tools. According to REVEL, MutationTaster, and PolyPhen-2 software, it was predicted to have a disease-causing effect,

while it was classified as a variant of unknown clinical significance in the SIFT software (Figure 2). The variants detected in affected individuals and pathogenicity scores are shown in detail in Table III.

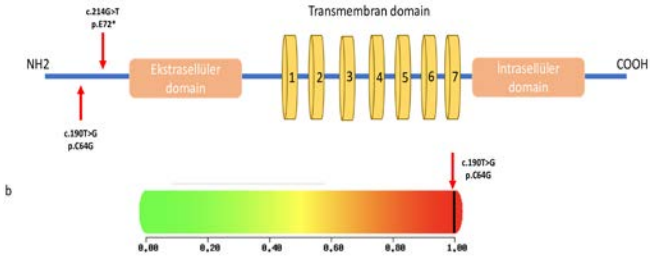

Figure 2. a) Schematic diagram of the GHRHR protein. The locations of the *GHRHR* variants identified in the study are shown according to the GHRHR protein domains (UniProt: Q02643). **b)** Diagram showing the potential pathogenic impact of the p.C64G missense variant on the structure and function of the human protein, as predicted by the PolyPhen-2 tool.

Table III: Evaluation of the pathogenicity of the variants detected in the study with public databases and in silico prediction tools

Family	Gene	Variant	Patient genotype	dbSNP	HGVS nomenclature	Pathogenicity	CADD score	SIFT	Mutation Taster	Revel
F1	*GH1*	c.59G>A	homozygous	rs137853219	NM_000515.5: c.59G>A NP_000506.2: p.W20*	ClinVar pathogenic ACMG-2015 pathogenic (PVS, PM2, PS4, PP5)	39	-	Pathogenic	-
F2 F3	*GH1*	Exon 1-2-3-4-5 deletion	homozygous	-	-	-	-	-	-	-
F4	*GHRHR*	c.214G>T	homozygous	rs121918117	NM_000823.4: c.214G>T NP_000814.2: p.E72*	ClinVar pathogenic ACMG-2015 pathogenic (PVS1, PM3, PM2, PP5)	40	-	Pathogenic	-
F5 F6	*GHRHR*	c.190T>G	homozygous	rs1319200922	NM_000823.4: c.190T>G NP_000814.2: p.Cys64Gly	Clinvar no data avaliable ACMG-2015 VUS (PP3, PM2)	28.5	VUS	Pathogenic	Pathogenic

DISCUSSION

Combined pituitary hormone deficiency (CPHD) or IGHD occurs in 16-20% of children with marked short stature (height SD ≤ -2.5). While most cases of IGHD are idiopathic, it can also result from acquired causes or genetic conditions[9]. The frequency of familial IGHD has been reported as 9% in Northern Europe, 14% in the Mediterranean Region, 16% in Türkiye, and 18.7% in Asia[10,11]. Although biallelic disease-causing variants in the *GHRHR* and *GH1* genes are the most common cause of familial IGHD, it can also rarely occur as a result of heterozygous variants in the *HESX1* gene or in the genes encoding the transcription factor *SOX3*[12,13]. IGHD is inherited in three patterns: Types IA and IB are inherited autosomal recessively (AR), type II is passed down in an autosomal dominant (AD) manner, while type

III is transmitted through an X-linked recessive inheritance pattern.

Variants in the *GH1* and *GHRHR* genes are the most common reason of congenital IGHD, which is associated with AR inheritance [14-16]. The *GH1* gene is situated on chromosome 17q22-24 and encodes GH, a protein essential for growth and development. This gene consists of five exons, which are the coding regions of the gene that are transcribed into mRNA and eventually translated into the mature GH protein, and four introns between the exons that are removed during mRNA processing to produce the final mature mRNA transcript. The mature molecule produced from the *GH1* gene usually weighs about 22 kDa (kilodaltons). To date, over 60 different mutations causing IGHD, including missense, splice-site, large deletions, and nonsense, have been reported in the *GH1* gene[6]. The occurrence of biallelic *GH1* gene deletions, which can range in size from 6.5 kb to 45 kb, differs across various ethnic groups. In individuals diagnosed with IGHD, *GH1* gene deletions have been found to occur in less than 5% of cases, with a higher prevalence in closely related or consanguineous populations[6,10,11]. The size of the deletion of *GH1* gene is heterogeneous and a 6.7 kb deletion is associated with the absence of truncated or non-functional GH, and the frequency has been reported as 70–80% in the literature[10,11]. The rates of *GH1* gene deletions have been demonstrated as 8.7%, 11.8% and 18.7% in Northern Europeans, Mediterranean populations and Asians, respectively. In our cohort, only two patients (2/13, 1.5%) (F2-1, F3-1) had homozygous deletions of respectively 1.6 and 2.1 kb in size, involving all coding exons of the *GH1* gene (exons 1-5). In other two patients (F1-1, F1-2), the biallelic nonsense variant detected in the *GH1* gene (NM_000515.5; c.59G>A; p.W20*) was a pathogenic variant previously reported in the literature in two Turkish patients with severe short stature and diagnosed with IGHD[17]. In this family, both normal (204 bp) and abnormal (121 and 83 bp) fragments were detected in heterozygous individuals as a result of cutting the fragments formed after polymerase chain reaction (PCR) amplification of the *GH1* gene in carrier and affected family members by restriction endonucleases. In two patients, abnormal (121 and 83 bp) fragments were detected, and the *GH1* c.59G>A; p.W20* variant was shown to inhibit mature GH mRNA expression.

The *GHRHR* gene, resided on chromosome 7p15, comprises 13 exons and encodes a protein with seven transmembrane domains, consisting of 423 amino acids. This protein is part of the G-protein-coupled cell-surface receptor (GPCR) family. These receptors are composed of three domains: the extracellular N-terminal domain, seven transmembrane α-helical domains, and the intracellular C-terminal domain. The integrity and functionality of these three domains are crucial for maintaining the stability of the GHRH receptor protein and facilitating its role in promoting somatotroph proliferation and GH secretion. Over 80 biallelic and/or compound heterozygous mutations—including missense, nonsense, splice-site mutations, deletions, and regulatory variants—have been reported in the *GHRHR* gene (https://www.lovd.nl/). Most reported mutations in the *GHRHR* gene result in the introduction of a premature stop codon or cause mRNA frameshifting, leading to truncated proteins. This results in receptors that lack the C-terminal domain and/or a significant portion of the transmembrane domains. Such variants prevent the defective receptor from localizing on the cell membrane or from interacting with G proteins. Others do not alter the cell surface expression of the mutant receptor but cause the ligand binding capacity of the receptor to change. It is known that most of the variants that have been detected in the *GHRHR* gene so

far are clustered in exon 31, 6. In our study, the p.E72* biallelic variant reported in the literature was detected in F4, while the missense p.C64G variant was detected in patients in F5 and F6. Both variants were found to be located in exon 3 and affected the N-terminal domain of the GHRHR protein (Figure 2). Functional studies of these variants have demonstrated that the mutant receptor cannot bind to its ligand (GHRH), thereby preventing intracellular signaling required to stimulate GH secretion[18].

Patients with the *GH1* variant show some differences in terms of phenotypic findings that distinguish them from affected individuals who have the *GHRHR* variant, which may be guiding in the diagnosis. Affected individuals with mutations in the *GH1* gene often have a prominent forehead, flat nasal bridge, and a small face, whereas these features are not observed in affected individuals with mutations in the *GHRHR* gene. In males, microphallus is a common genital anomaly in IGHD type IA. In our study, the twins in F1 and patients F2-1 and F3-1 had dysmorphic findings previously reported in the literature, such as prominent forehead, deep-set eyes, triangular face, and mild midface hypoplasia (Figure 1). No dysmorphic findings were observed in our patients with variants detected in the *GHRHR* gene.

In the literature, it has been found that most patients diagnosed with GHD are male. These findings suggest that the male gender may be a risk factor for GHD. In our study, consistent with previous studies, the majority of patients were also male (8/13, 61.5%). The mean chronological age of children diagnosed with GHD at diagnosis was found as 11.3-11.5 years in studies respectively conducted in Turkey[19,20]. The mean age at initiation of rGH treatment was found to be 9.2 ± 4.1 years in the USA and 8.5 years in a study conducted in Canada[21,22]. In our study, the mean age at diagnosis of GHD was 3.8± 2.47 years, while the mean age at initiation of rGH treatment was found as 5.12 ± 2.55 years, which was lower compared to the literature and other studies conducted in Türkiye. We believe that the reason for this is the increased awareness of IGHD as a result of the conducted studies and publications in recent years; therefore, when patients present to pediatric outpatient clinics with severe short stature and growth retardation and are evaluated by physicians, the treatment is initiated earlier. The frequency of IGHD in patients with severe growth retardation (height SD -4.5) is approximately 20%. It has been reported that the height SD at diagnosis in patients with familial IGHD is below -4.5 in Caucasians and below -7.0 in Asians. In this study, the mean height SD at initial diagnosis was found as -5.6 ± 1.4, which was very close to the height SD value found in the Asian population. After rGH treatment, the mean height SD value and the mean annual height growth rate were found to be -4.43 ± 1.41 and 13.36 ± 1.75 cm, respectively. In the comparison between the pre- and post-treatment values, it was observed that all affected individuals responded well to rGH treatment, and there was an improvement in post-treatment height SD values compared to pre-treatment height SD values.

Radiological imaging of the hypothalamic-pituitary region is crucial for patients diagnosed with GHD to detect any potential pathologies[23,24]. The main purpose of neuroradiological imaging is to detect central nervous system (CNS) tumors as well as to help the clinician in diagnosis and prognosis by determining abnormalities in the anatomy of the pituitary gland. It is known that the most common neuroradiological imaging finding in IGHD types IA and IV is anterior pituitary hypoplasia. In a previously conducted study, it was found that in approximately 75% of patients, IGHD was accompanied by anterior pituitary hypoplasia, while this rate was found at 47-61% in other studies. However, other

pituitary gland abnormalities, such as empty sella, ectopic posterior pituitary gland, and other CNS anomalies, such as Arnold-Chiari malformation, have also been reported in patients. In our study, consistent with the data in the literature, anterior pituitary hypoplasia was detected in 72% of the affected individuals. Only in F5-1, white matter gliosis was observed in addition to anterior pituitary hypoplasia. In another study, it has been reported that although 71.4% of patients with variants in the *GHRHR* gene exhibit anterior pituitary hypoplasia on MRI, the size of the anterior pituitary can vary even among affected individuals with the same variant[6]. In our study, pituitary hypoplasia was observed in the twins F1-1 and F1-2, who carry the same homozygous variant in the *GH1* gene, and in two of the 3 siblings in F4, who have the same variant in the *GHRHR* gene. The pituitary size in the other sibling was found to be within normal limits according to age and gender. This finding shows that in addition to clinical differences between patients, there may be inter- and intra-familial variability in terms of neuroradiological imaging findings despite the fact that they carry the same variant.

CONCLUSION

Isolated growth hormone deficiency (IGHD) is a rare but important cause of short stature in childhood. Patients with severe short stature and growth and developmental delay should be evaluated in terms of familial IGHD. Early diagnosis is very important as it is known that initiating rGH treatment at an early age is associated with a better growth response. Especially in IGHD type IA, dysmorphic findings may provide important clues in distinguishing the patients from individuals with other types of IGHD. Since IGHD is accompanied by anterior pituitary hypoplasia in approximately 75% of patients, radiological imaging of the hypothalamic-pituitary region should be performed in every patient. Determining the underlying molecular genetic cause facilitates providing accurate, up-to-date and comprehensive information on the clinical course of the disease, prognosis, complications, available treatment methods and prenatal or preimplantation genetic diagnosis options. In regions where the rate of consanguineous marriage is high, it should be kept in mind that there may be more than one affected individual in the same family and family screening should be recommended.

Ethics Committee Approval: This study was conducted in accordance with the principles of the 2008 Declaration of Helsinki, and the ethical approval for the study was obtained from the Local Ethics Committee (approval date/number: May 24, 2024/74).

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