CASE REPORT

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Severe Growth Hormone Deficiency in an Indian Boy Caused by a Novel 6 kb Homozygous Deletion Spanning the *GH1* Gene

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What is already known on this topic?

Mutations in *GH1* genes are associated with a rare condition called isolated growth hormone deficiency. The most common *GH1* deletions reported are 6.7 and 7.6 kb in size. The largest reported deletion is 45 kb in size.

What this study adds?

We report a 3-year old boy with extreme short stature with a deletion in the *GH1* gene. The deletion was 6 kb in size which has not been reported before. The proband is homozygous for the deletion and the parents, who are also short, each have a heterozygous deletion.

Abstract

Growth disorders resulting in extreme short stature (ESS) are often a result of deficiency in growth hormone (GH) released from the pituitary gland or a defective GH releasing receptor. Genetic defects in the *GH1* and *GHRHR* genes account for around 11.1-20% of ESS cases, resulting in a rare condition called isolated GH deficiency (IGHD). We describe the characterization of a *GH1* genetic defect discovered in a 3-year-old male patient with ESS, developmental failure and undetectable serum levels of GH. There was a family history of short stature, with both parents being short. Whole genome sequencing of the patient DNA revealed a large, novel 6 kb homozygous deletion spanning the entire *GH1* gene in the patient. While the deletion was homozygous in the proband, it was present in the heterozygous state in the parents. Thus, we report a novel homozygous deletion including the *GH1* gene leading to IGHD-type 1A associated with ESS.

Keywords: *GH* gene deletion, short stature, familial short stature

Introduction

Growth disorders resulting in extreme short stature (ESS) are often a result of deficiency in growth hormone (GH) released from the pituitary gland, coded for by the *GH1* gene, located on chromosome 17q23, or defective GH releasing receptor, which is coded for by the *GHRHR* gene, located on chromosome 7p14.3. Genetic defects in the *GH1* and *GHRHR* genes account for around 11.1-20% of ESS cases, resulting in a rare condition called isolated GH deficiency (IGHD). This frequency is reported to be 18.6% higher in familial cases of IGHD (1).

IGHD is a disorder with varying prevalence in different populations, ranging from 1:1800 in Sri-Lanka to 1:30,000 in the United Kingdom (2). Familial IGHD has been grouped into four main subtypes: type 1A, type 1B, type 2 and type 3 (3). These subtypes have a wide range of phenotype, including ESS, doll-like facies, central obesity, high pitched voice and puberty that is often delayed (4). Type 1A and 1B often manifest as ESS (3,5) and follow an autosomal recessive or compound heterozygous inheritance pattern (6).

GH is a peptide hormone that contains two active sites for GH receptor (GHR) binding, a class 1 cytokine

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receptor. GHRs are expressed in a broad range of tissue cellular membranes, including kidney cells, hepatocytes, adipocytes, myocytes, and many others. One GH molecule binds with two GHRs causing dimerization and this tertiary complex activates Janus kinase 2 (JAK-2) bound to GHR (7). Then JAK phosphorylates STAT5, a signal transducer and transcriptional activator, which enters the nucleus to induce GH-mediated genes expression. The mode of action of GH relies on the secretion of insulin-like growth factor-1 (IGF-1) from cells and stimulation of chondrocytes (8) leading to their differentiation. IGF-1 has an important role in stimulating growth at the end/growth plates of bones as well as in muscle cells. In addition to the JAK-STAT pathway, the dimerization of GHR further causes the initiation of other cascades, including the mitogen activating protein-kinase (MAP-K) pathway and the phosphoinositide 3 kinase (PI3K) pathways. Thus, the deficiency of endogenous GH is directly linked to perturbation of these pathways leading to short stature.

We report a 3-year-old male patient with ESS and failure to thrive with a large, novel, 6 kb homozygous deletion spanning the entire *GH1* gene in the patient. We have also review all cases of *GH1* deletion previously reported and compared with the presented case.

Case Report

A 3-year-old male child at presentation and of Indian origin was born at full term via C-section with a birth weight of 2.8 kg. The neonatal period was uneventful. At six months of age his parents noticed that he was not gaining weight. He was referred to Sidra Medicine at the age of 16 months for investigations of short stature. His weight was 6.80 kg [0th percentile, -5.03 standard deviation score (SDS), Figure 1] and his height was at 71 cm (0th percentile, -5.4 SDS, Figure 1). On examination he had severe frontal bossing, a pointed chin and a central incisor. There was no skeletal dysplasia, intellectual or developmental delay.

He underwent a glucagon GH stimulation test, which revealed undetectable serum GH levels. This suggests severe GH deficiency most likely due to a defect in the *GH1* gene. His free thyroxine level was 11.8 pmol/L (normal: 9.5-17.8 pmol/L) indicated normal thyroid functioning. Pituitary magnetic resonance imaging scan was structurally normal. The rest of the pituitary function tests were also normal. Table 1 shows the results of biochemical tests done.

Family History

His parents also have short stature, the father in particular (Figure 2) with a height of 152 cm (-3 SDS), while the mother's height was 151 cm. The mid-parental height of the child is 158 cm.

Follow-up and Management

The patient is being treated with recombinant human GH at 0.029 mg/kg/day with a growth velocity of 10 cm/year. His current height at age 3 years is 81.5 cm (<-2 SDS) and weight is 8.4 kg (<1 SDS).

Genetic Testing Methodology

Informed consent was obtained from parents. DNA samples were extracted from peripheral blood specimens of subject

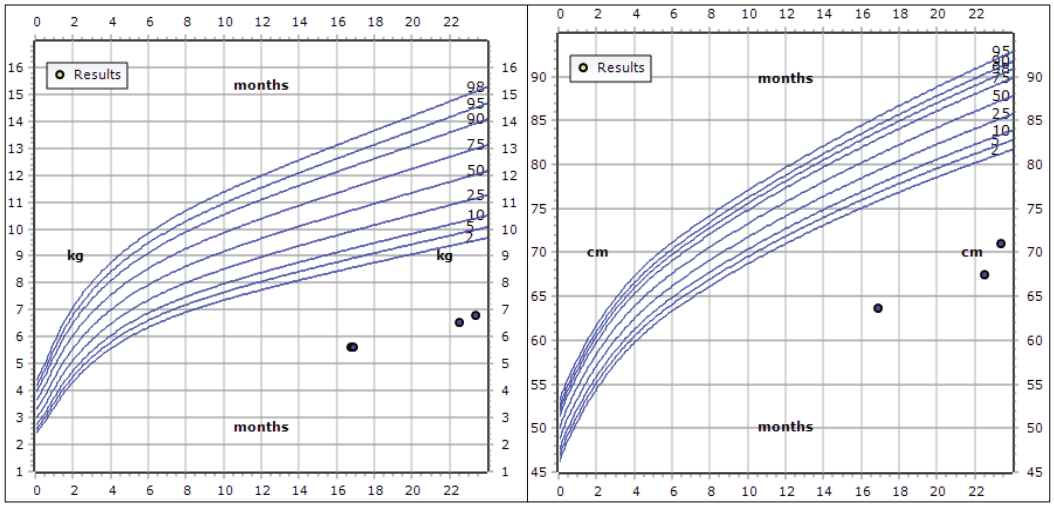

Figure 1. Weight-for-age and height-for-age growth chart for males aged 0-2 years. At the time of recruitment, the patient's weight was 6.80 kg (0th percentile, -5.03 SDS) indicating that he was severely underweight. The patient has severe short stature with a height of 71 cm (0th percentile, -5.4 SDS). The mid parental height is 158 cm

SDS: standard deviation score

and parents. Whole genome sequencing (WGS) was performed on an Illumina HiSeq platform using a 150-base paired-end single-index-read format. Reads in FASTQ files were then mapped to the NCBI human reference genome GRGh37/hg19 using Burrows-Wheeler Aligner (BWA-MEM) version 0.7.8. All subjects underwent variant calling using GATK (v3.6) and annotation was performed using SNPEff. The variants file was normalized and decomposed using vt. Additionally, vcfanno was used to annotate VCF file with extensive available data resources, including gnomad, exomes.r2.0.2, gnomad.genomes.r2.0.2.sites, 1K genome, and Exac. Genomic variants belonging to genes already known to be implicated in familial short stature were extracted.

Table 1. Results of biochemical tests done on the patient at the time of recruitment

Test	Patient results	Reference level
Growth hormone at 0, 60, 120, 180 mins (mcg/L)	Undetectable	>10 mcg/L at any time point
IGF-1 (ug/dL)	Undetectable	0.06-0.57
IGF binding protein-3 (mcg/mL)	<0.5	0.7-3.6
Morning ACTH (pg/mL)	146	7.2-63.3
Random blood glucose (mg/dL)	82.8	70-110
Total calcium (mg/dL)	10.2	8.8-10.8
TSH (mIU/L)	3.61	0.76-4.64
Free T4 (ng/dL)	0.92	0.74-1.38
Prolactin (mIU/L)	302	70-390
Cortisol (ug/dL)	7.94	2.17-12.79

IGF-1: insulin-like growth factor-1, ACTH: adrenocorticotropic hormone, TSH: thyroid-stimulating hormone

Copy number variation of the WGS analysis detected a novel, homozygous, 6 kb deletion on the long arm of chromosome 17, 17q23.3 with coordinates (GRCh37/hg19 17:61993713-62000168) in the proband. However, the parents were heterozygotes for the deletion. This deletion was manually identified using integrated genome viewer and spans the entirety of *GH1*, which is the main candidate gene and consistent with the phenotype of the patient (Figure 3a). We further confirmed the deletion using Samplot by visualizing and comparing the coverage of the structural variant with the surrounding regions (Figure 3b).

Discussion

IGHD Type IA is the second most commonly reported type of IGHD and these cases often have a variety of mutations

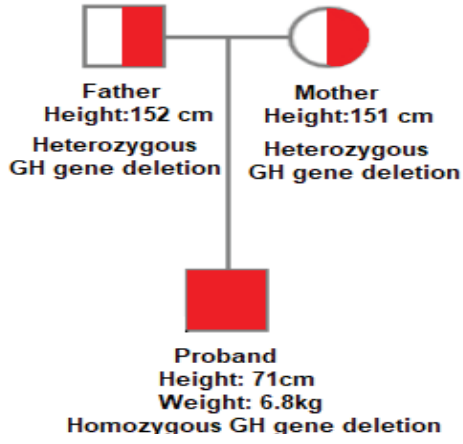

Figure 2. Family pedigree for this patient with IGHD type 1A

IGHD: isolated growth hormone deficiency

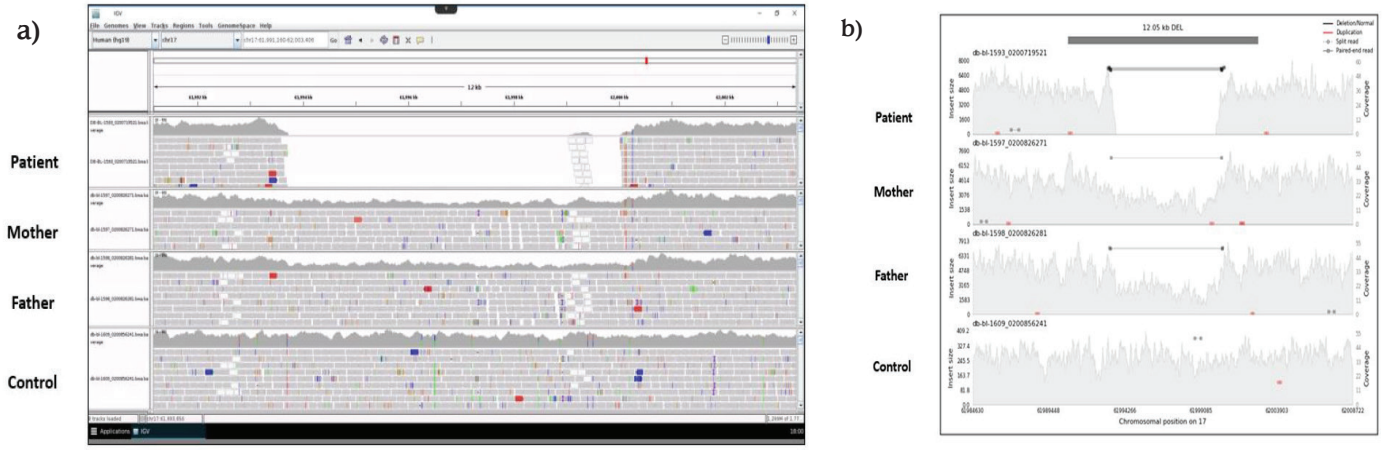

Figure 3. Results of genetic analysis for the patient and parents. a) Integrated genome viewer track showing homozygous 6 kb deletion in the proband (top), heterozygous deletion in both parents (middle) and wild type control (bottom) from the bam-files of the samples. b) Samplot analysis of whole genome sequencing showing structural variant. A 6 kb deletion spanning the entire *GH1* gene on chromosome 17 (GRCh37/hg19 17:61993713-62000168)

Table 2. Deletion size and phenotype of all reported subjects with GH deletion causing short stature

	Deletion size	Deletion zygosity	Phenotype
1	7.5 kb [10]	Homozygous	- Reported four patients from three families with severe IGHD. - Extreme short stature. - Absence of GH production. - Formation of anti-hGH-antibodies in high titers after hGH therapy.
2	7.5 kb [13]	Homozygous	- Reported four unrelated Jewish patients with IGHD. - All patients carry 7.5 kb deletion. - All four patients showed good response for hGH therapy.
3	6.7 kb and 7.6 kb [14]	Compound heterozygous	- Severe GH deficiency, truncal obesity, acromicria, low IGF-1 and IGFBP-3 and severe anterior pituitary hypoplasia.
4	6.7 kb and 2 bp [15]	Compound heterozygous	- Severe growth deficiency. - GH therapy resulted in catch-up growth at 9 years and 2 months with development of anti-GH antibodies.
5	7.6 kb [16]	Homozygous	- Two sibling patients with short stature. - Responded well to GH substitution. - No formation of blocking antibodies occurred. - *GH1* and *CSHL1* gene affected by the deletion.
6	6.7 kb and 7.6 kb [17]	Homozygous	- A patient with IGHD. - Very low response of GH secretion. - Undetectable levels of IGF-1 and IGFBP-3. - MRI showed a severe anterior pituitary hypoplasia.
7	6.7 kb or 7.6 kb [9]	Homozygous	- Ten patients with either 6.7 kb (8/10) or 7.6 kb (2/10) deletions. - 3 North European, 3 Mediterranean and 4 Turkish patients. - All the patients presented with severe growth retardation, decreased growth rate, and retarded bone age.
8	45 kb [18]	Homozygous	- An Italian family 3 affected IGHD patients. - These three patients showed heterogeneity in growth response and antibody formation on hGH replacement therapy.
9	45 kb [19]	Homozygous	- A Turkish family with three affected boys presenting with growth retardation. - The deletion involved GH and somatotropin gene clusters.
10	40 kb [20]	Homozygous	- A French family with two affected siblings, with severe congenital growth deficiency. - Both patients developed antibody after the hGH therapy.
11	6.7 kb [21]	Homozygous	- A girl with short stature and cystic fibrosis. - Developed anti hGH antibodies after 2 months of hGH replacement.
12	7.1 kb [22]	Homozygous	- A Chilean patient with short stature and repeated hypoglycemic episodes (neonatal period). - hGH treatment discontinued after the patient developed anti hGh antibodies and switched to IGF-1 treatment.
13	3.8 kb [23]	Homozygous	- A patient with severe congenital GH deficiency. - Non-detectable plasma GH levels in response to pharmacological stimulation tests. - A round face, small nose with depressed nasal bridge. - Treated with biosynthetic methionyl (met) GH but developed antibody after 5 years.
14	6.7 kb [24]	Homozygous	- Three Indian siblings with short stature. - Patients carried exactly same deletion but showed heterogeneity in rhGH treatment response.
15	22 kb and c.10 + 1G > T [25]	Compound heterozygous	- A 1 year and 9 months patient with growth retardation. - Auxiliary examinations showed low GH, low IGF-1 and elevated TSH.
16	6.7 kb and/or 7.6 kb [26]	Homozygous and compound heterozygous	- 12 patients with IGHD: - 10/12 (homozygous 6.7 kb del). - 1/12 (homozygous 7.6 kb del). - 1/12 (compound HT 6.7 and 7.6 kb del). - All patients had growth failure, very low GH, IGF-1 and IGFBP-3. - MRI showed hypoplastic adenohypophysis.
17	7.6 kb [27]	Homozygous	- Two Hispanic sisters with short stature and high body fat. - Developed antibodies against rhGH exposure.
18	c.1G > T 7.6 kb [28]	Compound Heterozygous	- A Japanese patient with growth retardation confirmed after provocative GH testing. - Responded well to hGH therapy. - Did not develop anti-hGH antibody.
19	6.7 kb or 7.6 kb [29]	Homozygous	- 3 Brazilian patients with GH deficiency. - 2 patients had 6.7 kb del (developed anti-GH antibodies after therapy) and 1 patient with 7.6 kb del (no anti-GH antibodies developed). - All the 3 patients had common phenotype: large forehead, low nasal bridge, increased subcutaneous fat, thin hair, and a high-pitched voice.

hGH: human growth hormone, GH: growth hormone, IGHD: isolated growth hormone deficiency, IGF-1: insulin-like growth factor-1, IGFBP-3: IGF binding protein-3, MRI: magnetic resonance imaging, TSH: thyroid-stimulating hormone, rhGH: recombinant human growth hormone

involving the *GH1* gene (3). Specifically, 66.7% of familial cases of IGHD type 1A involve *GH1* aberrations (6). Eighty percent of reported *GH1* deletions are of the 6.7 kbs size, while many others are of 7.6 kbs in length (9,10). These deletions are caused by imbalanced recombination between 98% homologous 454-592bp flanking regions of the *GH1* gene (9,11). Due to these mutations, IGHD type 1A patients will often have undetectable serum levels of GH due to a lack of endogenous GH and rhGH treatment may frequently lead to an antibody response against GH (3,10,12). Affected proportionate short stature patients will have heights ranging from -3 to -9.6 SDS (6). In keeping with this, undetectable serum GH levels were reported in the proband on stimulation testing.

Genetic testing also revealed that both parents, who are both affected with short stature, even though they were heterozygous with only one identical copy of the deletion. This suggested that a heterozygous deletion with this variant in *GH1* may also contribute to the defects in short stature. However, we could not find any previous reports of heterozygous deletions of *GH1* causing short stature.

We performed an electronic literature review of the PubMed database to identify relevant articles about *GH1* that lead to short stature, written in English and published up to March 2022. The following terms were used to search the database: "growth hormone 1 gene deletion", "*GH1* deletion", "pituitary growth hormone gene deletion", "IGHD1A and deletion", and "short stature". Twenty articles were identified with *GH1* deletion. Table 2 shows full list of deletions with clinical phenotype and size of deletions. These studies included different ancestries (Europeans, Asians, South/North Americans, and Arabs), however, most of the studies were conducted in European populations. The majority of the patients were diagnosed with severe short stature in early infancy. Our analysis showed that the most common size of *GH1* deletion reported is 6.7 or 7.6 kb and the largest deletion reported was 45 kb in size. The majority of subjects displayed a similar phenotype with development of GH antibodies after hGH therapy after varying durations of therapy. Most of the patients with 6.7 kb deletion developed GH antibodies in response to GH therapy while patients who carry the 7.6 kb deletion tend to exhibit more immunological tolerance when treated with exogenous GH. However, there is heterogeneity in the development of GH antibodies, even between members of the same family. The proband in this case report is still young and has been on GH therapy for two years, with a promising increase in height but not in weight gain. A limitation of our study was that GH antibodies were not measured.

Conclusion

We report a 3-year-old male patient of Indian origin with ESS, failure to thrive and a family history of short stature. GH levels were undetectable on stimulation testing. WGS revealed a large, novel, 6 kb homozygous deletion spanning the entire *GH1* gene in the patient leading to IGHD-type 1A associated with ESS. Both parents were heterozygous for the same variant and were also of short stature.

Ethics

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: Basma Haris, Khalid Hussain, Concept: Basma Haris, Diksha Shirodkar, Khalid Hussain, Design: Basma Haris, Diksha Shirodkar, Khalid Hussain, Data Collection or Processing: Basma Haris, Diksha Shirodkar, Idris Mohammed, Analysis or Interpretation: Basma Haris, Diksha Shirodkar, Idris Mohammed, Umm-Kulthum Ismail Umlai, Khalid Hussain, Literature Search: Basma Haris, Diksha Shirodkar, Idris Mohammed, Khalid Hussain, Writing: Basma Haris, Diksha Shirodkar, Idris Mohammed, Umm-Kulthum Ismail Umlai, Khalid Hussain.

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