

Different Growth Responses to Recombinant Human Growth Hormone in Three Siblings with Isolated Growth Hormone Deficiency Type 1A due to a 6.7Kb Deletion in the *GH1* Gene

© Sayan Ghosh¹, © Partha Pratim Chakraborty¹, © Biswabandhu Bankura², © Animesh Maiti¹, © Rajkrishna Biswas², © Madhusudan Das²

¹Medical College Kolkata, Department of Endocrinology and Metabolism, Kolkata, India

²Calcutta University Faculty of Medicine, Department of Zoology, Kolkata, India

What is already known on this topic?

Lack of growth response to recombinant human growth hormone (rhGH) in IGHD type 1A probably suggests underlying neutralising anti-GH antibody and alternative treatment strategies should be sought.

What this study adds?

IGHD type 1A is a rare cause of severe proportionate short stature and this is the first reported family from India. Three siblings with similar genetic abnormality demonstrated different growth responses to rhGH. An expected response in the first year of therapy was noticed in the eldest sister that waned rapidly after the first year. The second sibling demonstrated poor response from the beginning of therapy while the third sibling experienced an excellent response even after the third year of treatment.

Abstract

Isolated growth hormone deficiency (IGHD) type 1A is a rare, autosomal recessive disorder caused by deletion of the *GH1* gene and characterized by early onset severe short stature and typical phenotype. Lack of exposure to GH during fetal life often leads to formation of anti-GH antibody following exposure even the least immunogenic recombinant human GH (rhGH). Some patients with circulating anti-GH antibodies demonstrate lack of growth response to GH while others do not. However, the clinical significance of this antibody is unclear; hence testing is not routinely recommended. Three siblings, born of a consanguineous union, were referred with severe short stature. They were evaluated and IGHD was diagnosed in all of them. Genetic analysis revealed that all three had homozygous 6.7 Kb deletion in *GH1* gene, while their parents displayed a pattern of heterozygous 6.7 Kb deletions. rhGH was started at 10, 6 and 1.58 years of age, respectively. Growth and hormonal parameters were monitored throughout the course of treatment. The eldest sibling demonstrated expected growth velocity (9.5 cm/year) for the first year of rhGH that rapidly waned thereafter (2.5 cm/year). The youngest sibling experienced excellent growth response even after the third year (10.3 cm/year) while the middle sibling displayed sub-optimal response from rhGH initiation (6.3 cm/year). Change of rhGH brand did not work in the two elder sisters. Such a different growth response with rhGH in three siblings harbouring similar genetic abnormality has not been described previously.

Keywords: Isolated growth hormone deficiency type 1A, *GH1* gene, anti-growth hormone antibody

Introduction

Growth hormone deficiency (GHD) in children can present either as an isolated defect as in isolated GHD (IGHD) or in combination with one or more of the other pituitary hormone deficiencies, for example combined pituitary

hormone deficiency. Defects in the growth hormone 1 (*GH1*) or growth hormone releasing hormone receptor (*GHRHR*) genes, involved in the control of growth hormone (GH) secretion, typically cause IGHD. IGHD is classified into three categories having different modes of inheritance:



Address for Correspondence: Partha Pratim Chakraborty MD, Medical College Kolkata, Department of Endocrinology and Metabolism, Kolkata, India
Phone: +91 98300 92947 **E-mail:** docparthapc@yahoo.co.in **ORCID:** orcid.org/0000-0002-3316-4525

Conflict of interest: None declared
Received: 20.01.2020
Accepted: 24.08.2020

type 1 (autosomal recessive), type 2 (autosomal dominant) and type 3 (X-linked). Type 1 IGHD is further divided into two subtypes depending on severity: 1A (severe) and 1B (less severe). Type 1A IGHD is characterized by early onset severe short stature due to profound congenital GHD, a typical phenotype and an initial strong growth response following GH that is not infrequently followed by dramatic slowing of growth due to appearance of neutralizing anti-GH antibodies. As GH is not produced, even in fetal life, patients are immunologically intolerant to GH and frequently develop anti-GH antibodies when treated with any form of GH. Estimation of anti-GH antibody and mutational analysis are not yet component of routine care for patients with GHD in many countries due to lack of available laboratories, cost and utility of these tests in clinical practice. Early onset severe short stature, typical phenotype, undetected basal/stimulated GH, preserved pituitary functions without structural abnormality of the hypothalamo-pituitary area in the context of a typical family history is suggestive of *GHI* gene deletion.

Case Report

Three siblings case 1, case 2, and case 3 were referred for evaluation of severe short stature at the ages of 10 years, 6 years and 1.5 years, respectively. Born of a consanguineous union (Figure 1), all of them had cephalic presentation and were delivered vaginally at term. The birth weights were 3 kgs, 2.7 kgs and 2.8 kgs respectively. Other than prolonged neonatal jaundice in case 1, they had had uncomplicated perinatal periods. Motor milestones in case 1 and case 2 were slightly delayed. One of their siblings died immediately after birth due to unknown cause.

All of them had proportionate short stature, frontal bossing, depressed nasal bridge, mid facial crowding and high pitched voice without any midline defect. The rest of the systemic examination was unremarkable. The mid parental height was 145.35 cm with a standard deviation score (SDS) of -2.6. The auxologic parameters, expressed in cm and SDS according to Indian references, are summarized in Table 1. Sexual maturation rate in all of them was Tanner B1P1. Baseline investigations, including complete blood count, renal function tests, liver function tests, electrolytes, and urine and stool microscopy were normal. Hormonal and radiological evaluation is summarized in Table 1.

Genomic DNA was isolated from peripheral venous blood using the QIAGEN DNA extraction kit and following the manufacturer recommended method. Polymerase chain reaction (PCR) amplification of the whole *GHI* gene was performed using Velocity DNA polymerase

(Bioline, USA, Cat. No.-BIO-21098) and oligonucleotide primers GH1F (5'-ccagcaatgctcagggaaag-3') and GH1R (5'-tgtcccaccgggtgggcatggcaggtagcc-3') (1). PCR mixtures were denatured for 2 minutes at 98 °C and submitted to 32 cycles at 98 °C for 30 seconds, 68 °C for 30 seconds, and 72 °C for 1 minute, followed by final extension at 72 °C for 10 minutes. The resulting PCR product (2700 bp) was visualized by agarose gel electrophoresis and ethidium bromide staining. Characterization of *GHI* gene deletion was performed according to the method of Vnencak-Jones et al (2), modified by Mone et al (3). Briefly, two homologous sequences flanking the *GHI* gene, and the fusion fragments resulting from different *GHI* gene deletions, were simultaneously amplified by PCR with the following primers: 5'-tccagcctcaaagagcttacagtc-3' (GH1_2F) and 5'-cgttttctctagtctagatcttcccagag-3' (GH1_2R). The resulting PCR fragments were digested overnight at 37 °C with *SmaI* restriction endonuclease (Cat. No.-RO141S, New England Biolabs, MA, USA) according to the manufacturer's protocol, and the digested products were visualized by ethidium bromide staining after electrophoresis on a 1 % agarose gel.

GHI gene PCR amplification yielded no product using three different genomic DNA samples of three probands as template, while her parents showed one amplicon of the expected size (Figure 2). This result was suggestive of *GHI* gene deletion in the patients. *SmaI* restriction enzyme digestion of PCR amplified two homologous sequences flanking the *GHI* gene, suggested that all three patients were carrying homozygous 6.7 Kb deletions, while their parents displayed a pattern of heterozygous 6.7 Kb deletion (Figure 2).

Recombinant human GH (rhGH) (Headon®, Manufacturer: M/S Shanghai United Cell Biotechnology Co.,Ltd. 1150 Guiqiao Road, China (Shanghai) Pilot Free Trade Zone, 201206, P.P.China; Imported and Marketed by: SUN Pharmaceutical Industries Ltd.) was started at 10, 6 and 1.58 years of age in case 1, 2 and 3 respectively. In addition, case 1 and case 2 were also put on 12.5 mcg of levothyroxine and thyroid stimulating hormone (TSH) values were kept below 2.5 mIU/L. The annual growth velocity (GV) data is summarized in Table 1. The dose of rhGH was gradually increased to 0.05 mg/kg/day. Due to poor response, the brand of rhGH was changed (Norditropin Nordilet®, Manufacturer: Novo Nordisk India Pvt Ltd, Plot No. 32, 47-50, EPIP Area, Whitefield, Bangalore - 560 066, India) after the second year of therapy in case 1 and 2 and treatment was ultimately stopped after the third year. The parents inadvertently stopped rhGH for seven months in case 3 after two years of therapy. Therapy was restarted and a height increase of 4.3 cm was observed in the subsequent five months (GV: 10.3 cm/year) (Figure

Table 1. Clinical characteristic and summary of investigations at baseline

	Case 1	Case 2	Case 3
Date of birth	11.10.2006	08.12.2010	15.05.2015
Chronological age	10 years	6 years	1.5 years
Height	77.5 cm	71.5 cm	55.5 cm
Height SDS	-9.2	-7.8	-8.7
Height age	18 months	9 months	3 months
Weight	8 kgs	8 kgs	4.1 kgs
Weight age	9 months	9 months	3 months
US: LS	1	1.1	1.18
Investigations			
Bone age (estimated by Greulich and Pyle method)	4.5 years	2 years	2 years
IGF-1 (ng/mL)	< 25 (Ref: 75-546)	34.4 (Ref: 53-250)	< 25 (Ref: <25-258)
IGFBP-3 (µg/mL)			0.2 (Ref: 0.7-3.6)
Basal GH (ng/mL)	< 0.05	< 0.05	< 0.05
Clonidine stimulated GH (ng/mL)	All values < 0.05	All values < 0.05	All values < 0.05
fT4 (ng/dL) (at baseline)	1.31 (Ref: 0.8-1.8)	1.1 (Ref: 0.8-1.8)	1.42 (Ref: 0.9-2.4)
TSH (mIU/L)	6.37 (Ref: 0.7-6.4)	6.35 (Ref: 0.7-6.4)	3.35 (Ref: 0.7-6.4)
8:00 am cortisol (µg/dL)	9.4 (Ref: 5-25)	10.2 (Ref: 5-25)	7 (Ref: 5-25)
Post-synacthen peak cortisol (µg/dL)	22.1	19.8	18.2
MRI of hypothalamo-pituitary area	Small anterior pituitary, normal stalk and eutopic bright spot	Small anterior pituitary, normal stalk and eutopic bright spot	Small anterior pituitary, normal stalk and eutopic bright spot
Growth velocity after rhGH			
First year of therapy	9.5 cm	6.3 cm	13.5 cm
Second year of therapy	2.5 cm	2.4 cm	15 cm
Third year of therapy	1.5 cm	1.9 cm	4.9 cm (0.6 cm over first 7 months and 4.3 cm in next 5 months) (rhGH inadvertently stopped for first 7 months)

SDS: standard deviation score, TSH: thyroid stimulating hormone, US: upper segment, LS: lower segment, IGF: insulin like growth factor, IGFBP: IGF binding protein, GH: growth hormone, rhGH: recombinant human GH, fT4: free thyroxine, MRI: magnetic resonance imaging

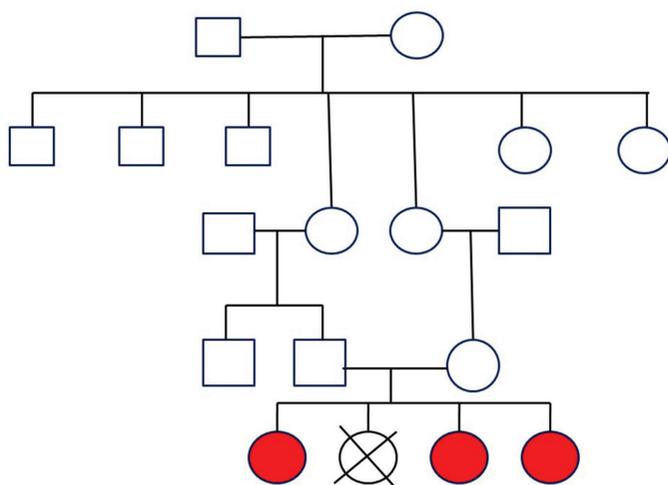


Figure 1. Family tree suggestive of autosomal recessive inheritance

3). Currently the youngest sibling is taller than the middle sibling (Figure 4).

Informed consent from the parents of the patients was taken for publication of these three cases.

Discussion

The frequency of *GH1* gene deletions in children with GHD is variable and deletions of different sizes have been described. The most frequently reported deletion size is 6.7 Kb, which is seen in 70-80 % of such cases. Other sizes reported include 7.0, 7.6, and 45 Kb. In addition, there have been reports of double deletions within the *GH* gene cluster located in the long arm of chromosome 17 (17q24.2) (4). Genetics and Neuroendocrinology of Short Stature International Study, a prospective, open-label, observational research program conducted in 30 countries at more than 800 study sites between 1999 and 2015 looked for mutations in *GH1* and *GHRHR* in 475 patients with IGHD of which 440 patients

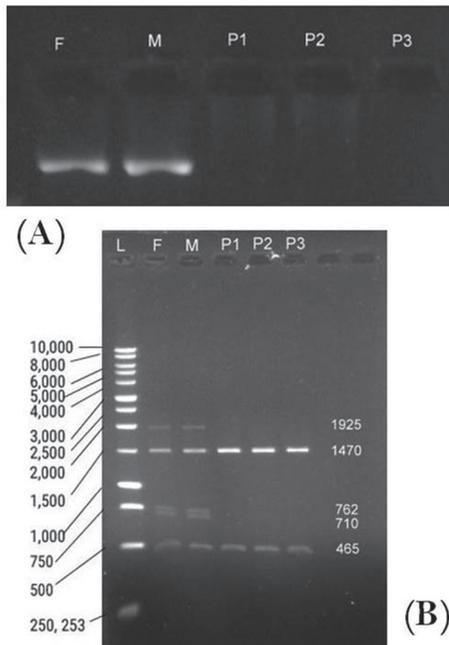


Figure 2. (A) *GH1* gene amplification (1.5% agarose gel electrophoresis, ethidium bromide staining). *GH1* gene polymerase chain reaction amplification yielded no product using the genomic DNA of probands as template (P1, P2, P3), while their parents (M, F) showed one band of the expected size (2,700 bp). (B) *SmaI* digestion (1% agarose gel electrophoresis, ethidium bromide staining). Fragment pattern was consistent with the father (F) and mother (M) being heterozygous carrier for the 6.7 Kb deletion, and patient 1 (P1), patient 2 (P2) and patient 3 (P3) are all homozygous for 6.7 Kb deletions. L: 1 Kb Ladder; F: Father; M: Mother; P1: Patient 1; P2: Patient 2; P3: Patient 3

had idiopathic GHD. *GH1* mutation was found in 23 of these 475 patients (4.8%) but only one patient (and one kindred) had a homozygous 6.7 Kb deletion and another had a 7.0 Kb deletion of the *GH1* gene (5).

Type 1A IGHD due to homozygous *GH1* deletion was first described in 1970 in three Swiss siblings with severe short stature and a particular phenotype, who subsequently developed high titers of anti-GH antibodies that interfered with growth response to pituitary-extracted GH (6). The widespread availability and use of rhGH has significantly reduced the frequency of development of these antibodies but has not eliminated it. In GH drug trials, measurement of anti-GH antibodies is a standard procedure and its prevalence in children varies from 2% to 22% depending on aetiology and duration of follow-up. Most of the patients with type 1A IGHD have undetectable circulatory GH levels and subsequently develop anti-GH antibodies when exposed to rhGH. In a recently published retrospective study 13 GH-treated patients with either type 1A IGHD, neurosecretory dysfunction, bioinactive GH syndrome (without genetic confirmation) or constitutional delay of growth and

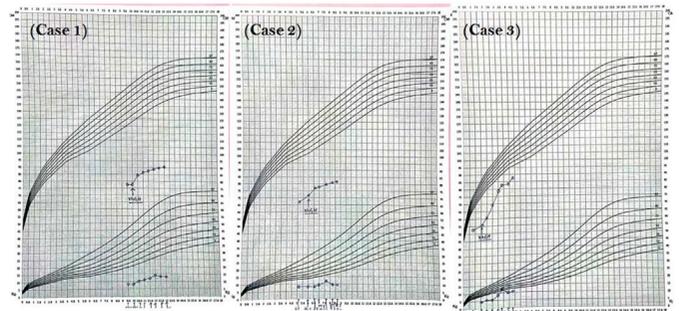


Figure 3. Growth charts (combined WHO 2006 MGRS and revised Indian Academy of Pediatrics 2015) of the three patients from the start of recombinant human growth hormone (rhGH) treatment. Note lack of growth response after 1st year of therapy in case 1 and case 2. No growth was also evident when rhGH was inadvertently stopped for seven months in the youngest sibling after 24 months of therapy



Figure 4. Current clinical profile of patients (from left to right: case 3, case 2, case 1). Note that the current height of the youngest sibling (case 3) is more than her elder sister (case 2)

puberty out of a cohort of 66 (19.7%) tested positive for these antibodies (7). The biological significance of anti-GH antibodies seems to be limited to some rare patients with very severe GHD with very high titres of neutralizing antibodies, encountered mostly in those with IGHD type 1A. Daily GH at the recommended doses typically accelerates growth in a GH-deficient child from a pre-treatment rate of 3-4 cm/year to 10-12 cm/year in the first year of therapy to 7-9 cm/year in the second and third years. Progressive waning of GH efficacy in all forms of GHD is poorly understood. Binder et al (7) observed an insufficient response to rhGH in one

sibling pair with IGHD type 1A while growth of a second sibling pair was unaffected, despite the fact that all tested positive for anti-GH antibodies. It has also been observed that despite having the identical genetic defect and similar anti-GH antibody titres, growth response to GH treatment may be quite heterogeneous, depending on the neutralizing effects of these antibodies (8,9). This is also evident in our cases, as the growth of the youngest sibling was unaffected which was in contrast to the other two. Though we could not estimate the anti-GH antibodies in these children due to non-availability of the test, the other possible causes of poor growth response to rhGH (poor compliance, incorrect injection techniques, subclinical hypothyroidism, excessive glucocorticoid therapy, prior irradiation of the spine epiphyseal fusion, coexisting systemic disease or alternate diagnosis of short stature) were confidently ruled out and the lack of response was attributed to anti-GH antibodies (10). TSH values in case 1 and case 2 were close to the upper reference limit and they were put on 12.5 mcg of levothyroxine to negate any possible detrimental effect of subclinical hypothyroidism on growth. Parents of the children were taught about the injection techniques and advised to administer injections themselves. Compliance to therapy was assured by the parents and cross checked with amount of rhGH used every month. Temporary cessation of rhGH therapy, changing the rhGH brand and recombinant human insulin like growth factor-1 instead of rhGH are the alternatives that have been proposed to optimise growth in such situation (11,12). However, these options are backed by poor quality evidence and change of rhGH brand did not work in our cases.

Conclusion

Type 1A IGHD, the most severe form of inherited isolated GH deficiency, results from homozygous *GHI* gene deletion. Many children with this disease demonstrate insufficient growth response to rhGH secondary to development of neutralizing anti-GH antibody. However, there is significant inter-individual variation in growth response to rhGH even in siblings with identical genetic defect. Loss of response is unpredictable and noticed at variable point of time after initiation of therapy.

Ethics

Informed Consent: Informed consents were obtained from the parents of the children.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Sayan Ghosh, Partha Pratim Chakraborty, Animesh Maiti, Concept: Sayan Ghosh, Partha

Pratim Chakraborty, Biswabandhu Bankura, Animesh Maiti, Design: Sayan Ghosh, Partha Pratim Chakraborty, Data Collection or Processing: Sayan Ghosh, Partha Pratim Chakraborty, Analysis or Interpretation: Biswabandhu Bankura, Rajkrishna Biswas, Madhusudan Das, Literature Search: Sayan Ghosh, Partha Pratim Chakraborty, Writing: Partha Pratim Chakraborty.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Moseley CT, Mullis PE, Prince MA, Phillips JA. An exon splice enhancer mutation causes autosomal dominant GH deficiency. *J Clin Endocrinol Metab* 2002;87:847-852.
2. Vnencak-Jones CL, Phillips JA, Wang DF. Use of polymerase chain reaction in detection of growth hormone gene deletions. *J Clin Endocrinol Metab* 1990;70:1550-1553.
3. Mone CM, Nigro V, Rotondi M, Del Buono A, Mazziotti G, Riondino M, Sinisi AM, Ghizzoni L, Phillips JA, Bellastella A, Carella C. An improved polymerase chain reaction (PCR) protocol for unambiguous detection of growth hormone gene deletions. *J Pediatr Endocrinol Metab* 1998;11:563-568.
4. Mullis PE. Genetics of isolated growth hormone deficiency. *J Clin Res Pediatr Endocrinol* 2010;2:52-62. Epub 2010 May 1
5. Blum WF, Klammt J, Amselem S, Pfäffle HM, Legendre M, Sobrier ML, Luton MP, J Child C, Jones C, Zimmermann AG, Quigley CA, Cutler Jr GB, Deal CL, Lebl J, Rosenfeld RG, Parks JS, Pfäffle RW. Screening a large pediatric cohort with GH deficiency for mutations in genes regulating pituitary development and GH secretion: frequencies, phenotypes and growth outcomes. *EBioMedicine* 2018;36:390-400. Epub 2018 Sep 25
6. Illig R, Prader A, Ferrandez M, Zachmann M. Hereditary prenatal growth hormone deficiency with increased tendency to growth hormone antibodies formation ("A-type" of isolated growth hormone deficiency). *Acta Paediatr Scand* 1971(Suppl);60:607.
7. Binder G, Heidenreich L, Schnabel D, Dunstheimer D, Oeverink R, Kiess W, Körner A, Kratzsch J. Biological significance of anti-gh antibodies in children treated with rhGH. *Horm Res Paediatr* 2019;91:17-24. Epub 2019 Apr 4
8. Rivarola MA, Phillips JA, Migeon CJ, Heinrich JJ, Hjelle BJ. Phenotypic heterogeneity in familial isolated growth hormone deficiency type I-A. *J Clin Endocrinol Metab* 1984;59:34-40.
9. Laron Z, Kelijman M, Pertzalan A, Keret R, Shoffner JM, Parks JS. Human growth hormone gene deletion without antibody formation or growth arrest during treatment--a new disease entity? *Isr J Med Sci* 1985;21:999-1006.
10. Cooke DW, Divall SA, Radovick S. Normal and aberrant growth in children. In: Melmed S, Auchus RJ, Goldfine AB, Koenig RJ, Rosen CJ (eds), *Williams Textbook of Endocrinology*. 14th ed. Philadelphia, Elsevier 2020;937-1022.
11. Rougeot C, Marchand P, Dray F, Girard F, Job JC, Pierson M, Ponte C, Rochiccioli P, Rappaport R. Comparative study of biosynthetic human growth hormone immunogenicity in growth hormone deficient children. *Horm Res* 1991;35:76-81.
12. Ahangari G, Ostadali MR, Rabani A, Rashidian J, Sanati MH, Zarindast MR. Growth hormone antibodies formation in patients treated with recombinant human growth hormone. *Int J Immunopathol Pharmacol* 2004;17:33-38.