Different Growth Responses to Recombinant Human Growth Hormone in Three Siblings with Isolated Growth Hormone Deficiency Type IA due to 6.7Kb Deletion of GH1 Gene

Ghosh S et al. Growth Pattern with rhGH in 3 Siblings with IGHD Type IA

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WHAT IS ALREADY KNOWN ON THIS TOPIC?
• Lack of growth response to rhGH in IGHD type IA probably suggests underlying neutralising anti-GH antibody and alternative treatment strategies should be sought.

WHAT THIS STUDY ADDS?
• IGHD type IA is a rare cause of severe proportionate short stature and this is the first reported family from India.
• 3 siblings with similar genetic abnormality have demonstrated different growth response to rhGH. Usual response in the first year of therapy was noticed in the eldest sister that waned off rapidly after 1st year, second sibling demonstrated poor response from the beginning of therapy and the third one experienced excellent response even after 3rd year of treatment.

ABSTRACT
Isolated growth hormone (GH) deficiency type IA 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 to 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 not routinely recommended. Three siblings born of a consanguineous union were referred to us with severe short stature. They were evaluated and IGHD was diagnosed in all of them. Genetic analysis revealed homozygous 6.7 Kb deletions of GH1 gene in all of them while their parents displayed a pattern of heterozygous 6.7 Kb deletions. rhGH was started at 10, 6 and 17/12 years of age. Their growth and hormonal parameters were monitored throughout the course of treatment. The eldest sibling demonstrated usual growth velocity (9.5 cm/year) after start of therapy that rapidly waned after 1st year (2.5 cm/year). The youngest sibling experienced excellent growth response even after 3rd year (10.3 cm/year) while the middle one displayed sub-optimal response from beginning (6.3cm/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 earlier.

Keywords: Isolated growth hormone deficiency type IA, GH1 gene, Anti-GH antibody

INTRODUCTION
Growth hormone deficiency (GHD) in children can present either as an isolated defect i.e. isolated GHD (IGHD) or in combination with one or more of the other pituitary hormone deficiencies i.e. combined pituitary hormone deficiency (CPHD). Defects in the growth hormone (GH) 1 or GH releasing hormone receptor (GHRHR) genes, involved in the control of GH secretion, typically cause IGHD. IGHD is classified into three categories having different mode of inheritance: type I (autosomal recessive), II (autosomal dominant) and III (X-linked). Type I IGHD is further divided into two subtypes depending on severity: IA (severe) and IB (less severe). Type IA IGHD is characterized by early onset severe short stature due to profound congenital GH, 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. Because 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 background of a typical family history is suggestive of GH1 gene deletion.

CASE REPORT:
Three siblings Case 1, Case 2, Case 3 were referred to us for evaluation of severe short stature at their 10 years, 6 years and 1.5 years of age 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 ailment.

All of them had proportionate short stature, frontal bossing, depressed nasal bridge, mid facial crowding and high pitched voice without any midline defect. Rest of the systemic examination was non-contributory. 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 have been summarized in Table 1. Sexual maturation rate in all of them were 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 has been summarized in Table 1.

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral venous blood by QIAGEN DNA extraction kit by manufacturer method. Polymerase chain reaction (PCR) amplification of the whole GH1 gene was done by using Velocity DNA Polymerase (Bioline, USA, Cat. No.-BIO-21098) and oligonucleotide primers GH1F (5'-ccagcaatgctcagggaaag-3') and GH1R (5'-tgctccagcgggtcgacctgagttc-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 GH1 gene deletion was performed according to the method by Vnencak-Jones and cols (2) modified by Mone and cols. (3). Briefly, two homologous sequences flanking GH1 gene, and the fusion fragments resulting from different GH1 gene deletions, were simultaneously amplified by PCR with the following primers: 5'-ggtctcagctaatgtctcctccgatgcagt-3' and 5'-gcctttccctctatgctcagcag-3' (GH1F and GH1R, 2R). The resulting PCR fragments were digested overnight at 37°C with Smal 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.

A GH1 gene amplification yielded no product using three different genomic DNA samples of three probands as template, while their parents showed one amplicon of the expected size (Figure 2). This result was suggestive of GH1 gene deletion in the patients. Smal restriction enzyme digestion of PCR amplified two homologous sequences flanking GH1 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®, Ranbaxy Laboratories Limited) was started at 10, 6 and 17/12 years of age in Case 1, Case 2 and 3 respectively. In addition, Case 1 and Case 2 were also put on levothyroxine and thyroid stimulating hormone (TSH) values were kept below 2.5 mIU/L. The annual growth velocity (GV) has been summarized in Table 1. The dose of rhGH was gradually increased to 0.5 mg/kg/day. Due to poor response, the brand of rhGH was changed (Norditropin Nordilet®, Novo Nordisk Pharma India Ltd.) after 2nd year of therapy in Case 1 & 2 and treatment was ultimately stopped after 3rd year. The parents inadvertently stopped rhGH for 7 months in Case 3 after 2 years of therapy. Therapy was reinitiated and a height increment of 4.3 cm was observed in next 5 months (GV: 10.3 cm/year) (Figure 3). Currently the youngest sib is taller than the second sib (Figure 4).

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

DISCUSSION:

The frequency of GH1 gene deletions in children with GHD is variable and different sizes of these deletions have been described. The most frequent being 6.7 Kb, which is seen in 70-80% of such cases; the others being 7.0, 7.6, 45 Kb, as well as double deletions within the GH1 gene cluster located in the long arm of chromosome 17 (17q24.2) (4). Genetics and Neuroendocrinology of Short Stature International Study (GeNeSIS), a prospective, open-label, observational research program conducted in 30 countries at more than 800 study sites between 1999 and 2005 looked for mutations in GH1 and GHRHR in 475 patients with IGHD of which 440 patients had idiopathic GHD. GH1 mutation was found in 23 of these 475 patients (4.8%) and only 1 patient (and 1 kindred) had homozygous 6.7 Kb deletions and another one had 7.0 Kb deletion of GH1 gene (5).

Type IA IGHD due to homozygous GH1 gene deletion was first described in 1970 in three Swiss siblings with severe short stature and a particular phenotype who subsequently developed high titters of anti-GH antibodies that interfered with growth response to pituitary-extracted GH (6). The induction of recombinant human GH has significantly reduced the frequency of development of these antibodies; but have not eliminated it. In GH drug trials, measurement of anti-GH antibodies is a standard procedure and prevalence in children varies from 2 to 22% depending on aetiology and duration of follow-up. Most of the patients with type IA 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 IA IGHD, neuroendocrine dysfunction, bioinactive GH syndrome (without genetic confirmation) or constitutional delay of growth and 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 titer 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 first of therapy to 7-9 cm/year in second and third year. Progressive waning of GH efficacy in all forms of GHD is poorly understood. Binder et. al observed an insufficient response to rhGH in one sibling pair with IGHD type IA while growth of a second sibling pair was unaffected despite the fact that all were tested positive for anti-GH antibodies (7). It has also been observed that despite having the identical genetic defect and similar anti-GH antibodies 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 has been unaffected 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 levothyroxine to negate any possible detrimental effect of high TSH 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 (rhIGF)-1 instead of rhGH are the alternatives that have been proposed to optimise growth in such situation (11, 12). These options are backed by poor quality evidence and change of rhGH brand did not work in our cases.

References

Figure 1: Family tree suggestive of autosomal recessive inheritance
Figure 2: (A) GH1 gene amplification (1.5% agarose gel electrophoresis, ethidium bromide staining). GH1 gene PCR 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 6.7 Kb deletion, and the patient 1 (P1), patient 2 (P2) and patient 3 (P3) all are homozygous for 6.7 Kb deletions. L: 1 Kb Ladder; F: Father; M: Mother; P1: Patient 1; P2: Patient 2; P3: Patient 3.

Figure 3: Growth charts (Combined WHO 2006 MGRS and revised Indian Academy of Pediatrics 2015) of 3 patients from start of 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 7 months 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).