jcrpe-2018-0305

Case Report

Isolated Growth Hormone Deficiency Type II due to a novel *GH1* mutation: A Case Report

Ahmad Kautsar[1], Jan M. Wit[2], Aman Pulungan[1]

[1]Department of Child Health, Cipto Mangunkusumo Hospital Jakarta, Universitas Indonesia
[2]Department of Paediatrics, Leiden University Medical Center, Leiden, The Netherlands

Short title: Novel *GH1* mutation

Corresponding author: Dr. A.Pulungan
Email: amanpulungan@mac.com
Post address: Department of Child Health, Faculty of Medicine, university of Indonesia/Dr. Cipto Mangunkusumo Hospital
Jl. Diponegoro No 71, Jakarta 10430, Indonesia
Telephone: +62213160622
https://orcid.org/0000-0002-2965-5938

What is already known on this topic?
Dominantly inherited isolated growth hormone deficiency (IGHD) can be caused by multiple defects of the GH1 gene. Affected individuals show a good growth response to recombinant human GH (rhGH) and can develop multiple pituitary deficiency.

What this study adds:
In an Indonesian infant with the classical presentation of IGHD type II a novel GH1 gene mutation was found.

Abstract
Isolated growth hormone deficiency (IGHD) type II is a rare autosomal dominant disorder characterized by severe short stature with low growth hormone level. Timely diagnosis is important for optimal results of recombinant human GH (rhGH) treatment and detection of additional pituitary deficiencies in affected relatives. A male child presented at the age of one year with severe proportionate short stature (-4.9 SDS) with normal Body Mass Index (-1.1 SDS). Physical examination revealed frontal bossing, midfacial hypoplasia, normal external genitalia with no dysmorphic features. His father's and mother's height were -6.1 and -1.9 SDS. Serum IGF-1 and IGFBP-3 were undetectable and the peak GH level in a clonidine stimulation test was extremely low (0.18 ng/mL). Brain magnetic resonance (MR) showed anterior pituitary hypoplasia. Genetic analysis identified a novel heterozygous mutation (c.291+2T>G) expected to lead to splicing out exon 3 of GH1. rhGH from 2.4 years of age led to proper catch-up. In conclusions, we identified a novel GH1 gene mutation in an infant with classical IGHD type II presentation.
Keywords: Growth hormone, GH1, short stature, isolated growth hormone deficiency

Introduction

Growth hormone deficiency (GHD) is characterized by decreased growth hormone (GH) secretion as assessed by one or two GH provocation tests in addition to low serum IGF-I and IGFBP-3 levels and clinical features including linear growth failure, typical features at physical examination and bone age retardation (1). GHD can be either isolated (IGHD) or part of multiple pituitary hormone deficiency (MPHD), and can be congenital or acquired. The reported incidence of congenital GHD is 1 in 4,000 to 1 in 10,000 live births with male predominance (2,3).
When IGHD is suspected, further evaluation is urgently needed (4). Establishing the diagnosis is a multistep process involving a proper medical history, detailed physical examination including accurate measures of growth and analysis of the growth curve, biochemical testing, pituitary imaging, and genetic screening in severe and/or familial cases (4-9).
Genetic causes of IGHD can be found in 3-30% and are classically classified into four types according to the inheritance pattern: autosomal recessive inheritance (IGHD types IA and IB), autosomal dominant (IGHD type II), and X-linked inheritance (IGHD type III) (2,3,5). Mutations of the genes

encoding GH (*GH1*), GHRH receptor (*GHRHR*), the GH secretagogue receptor (*GHSR*) and several transcription factors involved in pituitary development have been described to cause IGHD (5,10). Here, we report a case of genetically proven autosomal dominant IGHD type II caused by a novel mutation of *GH1* at a position where previously two other mutations have been found (10).

Case report

A 0.99 year old son of non-consanguineous parents was referred to our pediatric endocrinology clinic because of severe short stature. His father's height was 132 cm (-6.1 SDS) and maternal height was 151 cm (-1.86 SDS). Pregnancy and the perinatal period were uneventful. Birth weight and length were 3.3 kg and 48 cm after 38 weeks of pregnancy (-0.1 and -1.0 SDS, respectively). There were no indications of any chronic disease, and psychomotor development was normal.

Length and weight at first presentation were 64 cm (-4.9 SDS) and 6.3 kg (-4.8 SDS), respectively (calculated based on the WHO growth charts) (11), BMI was 15.4 kg/m^2 (-1.1 SDS) and head circumference 44 cm (-1.6 SDS). Physical examination revealed frontal bossing, midfacial hypoplasia, normal external genitalia and no dysmorphic features (**Figure 1**). Further anthropometric data showed proportionate short stature with a sitting height/height ratio of 0.65 (0.1 SDS) (12). The growth velocity foregoing the first observation was 3 cm over 6 months (-3.5 SDS) (11). Bone age was 6 months at a chronological age of 1.0 year.

Laboratory examination revealed a normal free thyroxine level (FT4, 1.23 ng/dL) and TSH (2.74 µU/mL) and undetectable levels of IGF-I (< 25 ng/mL) and IGFBP-3 (<0.5 mg/L). His father also demonstrated a low serum IGF-I (<25 ng/ml).

The pedigree of the family is shown in **Figure 2**. The height of the paternal grandfather and grandmother was reportedly approximately 165 cm (≈ -1.6 SDS) and 150 cm (-2.0 SDS), respectively.

The patient then underwent a GH stimulation test using clonidine 0.15 mg/m^2. Peak GH level was extremely low (0.18 ng/ml). An MRI of the brain showed anterior pituitary hypoplasia (**Figure 3**).

Because of financial restraints it took more than a year before recombinant human growth hormone (rhGH) (Saizen, Merk-Serono) replacement therapy could be started at the age of 2 years and 5 months in a daily dose of 20-24 µg/kg body weight. This resulted in a proper catch-up growth (**Figure 4** and **Table 1**). Growth velocity after 1.5 year of treatment was 9.5 cm/year in a 13 months' interval.

Screening for other deficiencies of other pituitary hormones (FSH, LH, TSH, and morning cortisol) showed normal results. Examination of his father's other pituitary and related hormones (FSH, LH, testosterone, FT4, TSH, Prolactin, ACTH and cortisol) also proved to be normal.

Sanger sequencing of *GH1* was performed in the laboratory of Centogene AG (Rostock, Germany) and showed a novel heterozygous mutation (c.291+2T>G) expected to lead to splicing out exon 3.

Mutation analysis of his father's DNA has not been performed, but the extremely short stature and low IGF-I make it highly likely that he carries the same mutation, which appears to be *de novo* according to the normal heights of the paternal grandparents and the father's brothers.

All clinical investigations were conducted in accordance with the guidelines by the Declaration of Helsinki. The parents gave informed consent to clinical and genetic studies, as well as for publication of the clinical information and pictures.

Discussion

In this report we describe a novel splice site mutation of *GH1* leading to severe short stature in the index patient and his father characteristic for type II IGHD. No other relatives with severe short stature are known in this family, so we assume that the mutation occurred *de novo* in the patient's father. The mutation is located at a base known to be vital for correct splicing, since previously mutations c.291+2T>A and >C have been discovered with an autosomally inherited and similarly severe phenotype (13-15), with lower GH peaks upon provocation compared with those with missense mutations (13). The hypoplastic anterior pituitary in the patient is consistent with previous observations in 60% of patients with splice spite mutations (13). The severe IGHD with early onset in this condition is thought to be caused by a disturbance of GH storage and secretion due to misfolded mutant GH (16).

The combination of early-onset severe proportionate growth failure, bone age delay and classical physical signs (midface hypoplasia and frontal bossing) makes the *a priori* likelihood of congenital GHD very high. This should always lead to laboratory testing (serum IGF-I and IGFBP-3, and one or more GH stimulation tests), followed by MRI of the hypothalamic-pituitary region (8). If one parent is very short and GH deficient, type II IGHD is almost certain, but it is still important to confirm this by genetic testing. In such cases, rhGH treatment in a substitution dose is highly effective in leading to rapid catch-up growth followed by a normal growth pattern and a normal adult height (6,9,14).

Infants with severe congenital GHD can present with neonatal hypoglycaemia, prolonged postpartum hyperbilirubinemia and elevated liver function tests and microphallus (1,4). Although the data of blood glucose during neonatal period of our patient could not be obtained, the absence of reported neonatal

seizures argue against a past history of hypoglycaemia. Neonatal hypoglycaemia is less frequent in isolated GHD than in multiple pituitary hormone deficiency (17,18).

While in this and similar cases the dominant inheritance and the classical phenotype made the diagnosis of type II IGHD straightforward, the diagnosis of less severe IGHD is much more challenging. In such cases the clinician has to make an assessment of the likelihood of IGHD based on the growth pattern, bone age delay, observations at physical examination, and the result of the usual screening test (serum IGF-I) (6,8,9,19,20). If the likelihood appears sufficiently high, the next step is a GH stimulation test, which should be repeated if a low GH peak is observed, to prevent too many false positive results (1,21). With regard to the growth pattern of children with GHD, height velocity can be very low in severe GHD, particularly in the first years of life, but in other cases height SDS can stabilize for a number of years at or below the -2 SDS line of the population (but considerably below target height SDS), so that height velocity appears normal for its height SDS position. While in most cases height SDS is lower than TH SDS, the dominant form of IGHD that is present in our case and other type II IGHD cases can present with a height SDS close to the height SDS of one of the parents, so that for this subtype if IGHD the distance to TH is not a strong predictor (6,9,21).

Due to its pulsatile nature, physiological and pharmacological GH provocation tests are the key to asses GH secretion (9). The average GH response to various stimuli is slightly different and the level of adiposity is an important determinant of the GH peak, but usually still a single cut-off is used (1,21). With time, this moved upwards from 7 to 10 ng/mL (1,16), but due to the increased potency of GH standards a more rational cut-off may be at 7 ng/mL (22). Although few comparative studies have been performed, clonidine (through its stimulation of GHRH release) is thought to be a powerful stimulant for GH secretion, to a similar extent as insulin (1,23).

Each patient with a congenital GHD needs to be evaluated with a brain MRI to search for anatomic abnormalities of pituitary anatomy (24). MRI is an important tool to forecast future endocrine dysfunction, since individuals with abnormal pituitary anatomy are more likely to have or develop multiple endocrinopathies (25). MRI imaging in our patient demonstrated anterior pituitary hypoplasia, in line with the majority of patients with type II IGHD. The specific genetic diagnosis (splicing defect of *GH1*) increases the likelihood that with time other pituitary defects may develop (26).

It has been reported that 3-30% of individual with isolated GHD have a genetic basis, but the likelihood of a genetic cause is considerably higher in children with a positive family history and/or severe short stature (5). Mutations of relevant candidate genes have been identified in 11% of patients with severe IGHD and even in 38% of familial cases (13), so that it was advised to perform genetic testing in children with severe and/or familial IGHD (13,27,28). Children with proportionate short stature and a low peak GH after stimulation without additional pituitary deficiency should be considered for mutation screening for *GHRHR* and *GH1*. Another potential genetic cause is a *GHSR* mutation, although the wide phenotypic spectrum of published patients with such mutations do not allow for strong statements about their pathogenicity (28). While it was previously thought that GHD is almost always associated with a normal birth weight and length (1,19,21), it has recently become clear that average birth size of GHD infants is decreased (18). A positive family history of severe short stature of one of the parents strongly suggests an autosomal dominant inheritance pattern, which makes type II IGHD very likely, so that full gene sequencing of *GH1* is indicated, as was done in our patient (10,13,27-29)

In IGHD type II, GH secretion is very low but usually still detectable and associated with heterozygous splice site, missense, splice enhancer mutations, or intronic deletions in *GH1* (5,10,27-29). Most patients with type II IGHD, as our case, have mutations within the first six nucleotides of intron 3 of *GH1*, resulting in skipping of exon 3. The result is the production of the 17.5-kDa isoform, which lacks amino acids 32-71 and, hence, the loop that connects helix 1 and helix 2 in the tertiary structure of GH. This isoform exerts a dominant negative effect upon secretion of the full-length GH molecule and may disturb the secretion of other pituitary hormones, such as TSH, LH, and prolactin (5,10,29-32). Pre-treatment thyroid hormone level in this patient was normal as well as other anterior pituitary hormones 1.5 year after start of rhGH treatment. The probability of having other pituitary hormone deficiency in IGHD rises around puberty, and the first hormone to be affected is ACTH at around 8 years of age (33). The normal results of pituitary testing of the patient's father suggest that the risk of additional pituitary insufficiencies in this family may be limited.

In summary, we report a novel mutation in *GH1* leading to type II IGHD in an Indonesian child with a classical phenotype. Genetic testing is indicated in severe and or familial IGHD, particularly if one parent is also affected.

Acknowledgements

The authors are grateful for the collaboration of the index case and his parents. The authors would also like to thank the local crowdfunding platform (wecare.id) for supporting genetic testing for index case.

Author contribution

AK and AP initiated the study, carried out the clinical investigations, requested genetic testing and wrote the first draft of the manuscript. JMW advised to collect additional information and assisted in writing subsequent versions of the manuscript.

References

1. Ranke MB. Growth hormone deficiency: diagnostic principles and practice. In: Ranke MB, Mullis PE, (eds). Diagnostics of endocrine function in children and adolescents. 4th edition. Basel, Karger, 2011; 102-137.
2. Lacey KA, Parkin JM. Causes of short stature. A community study of children in Newcastle upon Tyne. Lancet 1974;I:42-45.
3. Rona RJ, Tanner JM. Aetiology of idiopathic growth hormone deficiency in England and Wales. Arch Dis Childhood 1977;52:197-208.
4. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. J Clin Endocrinol Metab 2000;85:3990-3993.
5. Alatzoglou KS, Webb EA, Tissier PL, Dattani MT. Isolated growth hormone deficiency (GHD) in childhood and adolescence: recent advances. Endocrine Rev 2014;35:376-432.
6. Stanley T. Diagnosis of growth hormone deficiency in childhood. Curr Opin Endocrinol Diabetes Obes 2012;19:47-52.
7. Alatzoglou KS, Dattani MT. Genetic causes and treatment of isolated growth hormone deficiency - an update. Nat Rev Endocrinol 2010;6:562-567.
8. Oostdijk W, Grote FK, Keizer-Schrama SMPF, Wit JM. Diagnostic approach in children with short stature. Horm Res 2009;72:206-217.
9. Chinoy A, Murray PG. Diagnosis of growth hormone deficiency in the paediatric and transitional age. Best Pract Res Clin Endocrinol Metab 2016;30:737-747.
10. Wit JM, Losekoot M, Baumann G. Growth hormone-releasing hormone receptor and growth hormone gene abnormalities. In: Cohen LE, editor. Growth hormone deficiency physiology and clinical management. 1st edition. Switzerland, Springer, 2016;149-175.
11. The WHO Child Growth Standards [Internet]. World Health Organization. World Health Organization; 2016 [cited 2018May12]. Available from; https://www.who.int/childgrowth/standards/en/
12. Fredriks AM, Buuren SV, Van Heel WJM, Dijkman-Neerincx RHM, Verloove-vanhorick SP, and Wit JM. Nationwide age references for sitting height, leg length, and sitting height/height ratio, and their diagnostic value for disproportionate growth disorders. Arch Dis Child 2005;90:807-812.
13. Alatzoglou KS, Turton JP, Kelberman D, Clayton PE, Mehta A, Buchanan C, et al. Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. J Clin Endocrinol Metab 2009;94:3191-3199.
14. Binder G, Iliev DI, Mullis PE, Ranke MB. Catch-up growth in autosomal dominant isolated growth hormone deficiency (IGHD type II). Growth Horm IGF-1 Res 2007:17:242-248.
15. Fofanova OV, Evgrafov OV, Polyakov AV, Poltaurus AB, Peterkova VA, Dedov II. A novel IVS2-2A>T splicing mutation in the GH-1 gene in familial isolated growth hormone deficiency type II in the spectrum of other splicing mutations in the russian population. J Clin Endocrinol Metab 2003;88:820-826.
16. Binder G, Keller E, Mix M, Massa GG, Stokvis-Bratnsma WH, Wit JM, et al. Isolated GH deficiency with dominant inheritance: new mutations, new insights, J. Clin. Endocrinol Metab 2001;86: 3877–3881.
17. Binder G, Weidenkeller M, Blumenstock G, Langkamp M, Weber K, Franz AR. Rational Approach to the diagnosis of severe growth hormone deficiency in the newborn. J Clin Endocrinol Metab 2010;95:2219-2226.
18. Mehta A, Hindmarsh PC, Stanhope RG, Turton JP, Cole TJ, et al. The role of growth hormone in determining birth size and early postnatal growth, using congenital growth hormone deficiency (GHD) as a model. Clin Endocrinol 2005;62:223-231.
19. Rose SR, Vogiatzi MG, Copeland KC. A general pediatric approach to evaluating a short child. Pediatr in Rev 2005;26:410-420.
20. Pulungan AB, Delemarre-Van de Waal HA. Management of growth disorders. Pediatr Indones 2002:42:225-238.
21. Webb EA, Dattani MT. Diagnosis of growth hormone deficiency. In: Hindmarsh PC, editor. Current indication for growth hormone therapy. 2nd edition. Basel, Karger, 2010;55-66.
22. Guzzetti C, Ibba A, Pilia S, Beltrami N, Di Iorgi N, Rollo A, et al. Cut-off limits of the peak GH response to stimulation tests for the diagnosis of GH deficiency in children and adolescents: study

in patients with organic GHD. Eur J Endocrinol 2016;175:41-47.
23. Zadik Z, Chale SA, Kowarski A. Assessment of growth hormone secretion in normal stature children using 24-hour integrated concentration of GH and pharmacological stimulation. J Clin Endocrinol Metab 1990;71:932-936.
24. Tsai SL, Laffan E. Congenital growth hormone deficiency: a review focus on neuroimaging. Eur Endocrinol 2013;9:136-140.
25. Tsai S, Laffan E, Lawrence S. A retrospective review of pituitary MRI findings in children on growth hormone therapy. Pediatr Radiol 2012;42:799-804.
26. Mullis PE, Robinson ICAF, Salemi S, Eble A, Besson A, Vuissoz JM. Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. J Clin Endocrinol Metab 2005;90:2089-2096.
27. Mullis PE. Genetic of isolated growth hormone deficiency. J Clin Res Pediatr Endocrinol 2010;2:52-62.
28. Wit JM, Kiess W, Mullis P. Genetic evaluation of short stature. Best Pract Res Clin Endocrinol Metab 2011;25:1-17.
29. Dauber A, Rosenfeld RG, Hirchhorn JN. Genetic evaluation of short stature. J Clin Endocrinol Metab 2014;9:3080-3092.
30. Hayashi Y, Yamamoto M, Ohmori S, Kajimoto T, Ogawa M, Seo H. Inhibition of growth hormone secretion by a mutant GH-1 gene product in neuroendocrine cells containing secretory granules: an implication for isolated GH deficiency inherited in an autosomal dominant manner. J Clin Endocrinol Metab 1999;84:2134-2139.
31. Lee MS, Wajnrajch MP, Kim SS, Plotnick LP, Wang J, Gertner JM, et al. Autosomal dominant growth hormone deficiency type II: the Del32-71- GH deletion mutant supresses secretion of wild-type GH. Endocrinology 2000;141:883-890.
32. Mcguinnes L, Mogoulas C, Sesay AK, Mathers K, Carmignac D, Manneville JB, et al. Autosomal dominant growth hormone deficiency disrupts secretory vesicles in vitro and in vivo in transgenic mice. Endocrinology 2003;144:720-731.
33. Blum WF, Deal C, Zimmermann AG, Sharikova EP, Child CJ, Quigley CA, dkk. Development of additional pituitary hormone deficiencis in pediatric originally diagnosed with idiopathic isolated GH deficiency. Eur J Endocrinology 2014;17:13-21.

Figure 1. Characteristic clinical features of the patient. Frontal bossing, midfacial hypoplasia, lobulated subcutaneous fat and normal genitalia are noted.

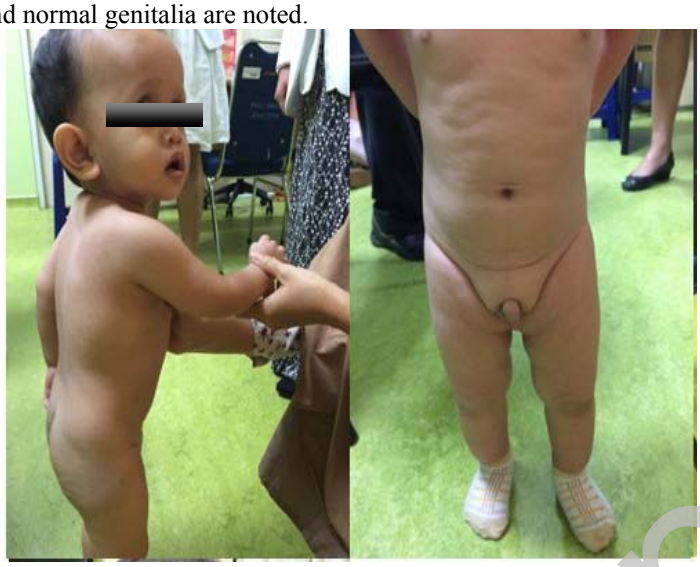

Fig 1

Figure 2. The pedigree of the family of the index patient with autosomal dominant type II GHD. Filled squares indicate affected members (the index patient (arrow) and the father).

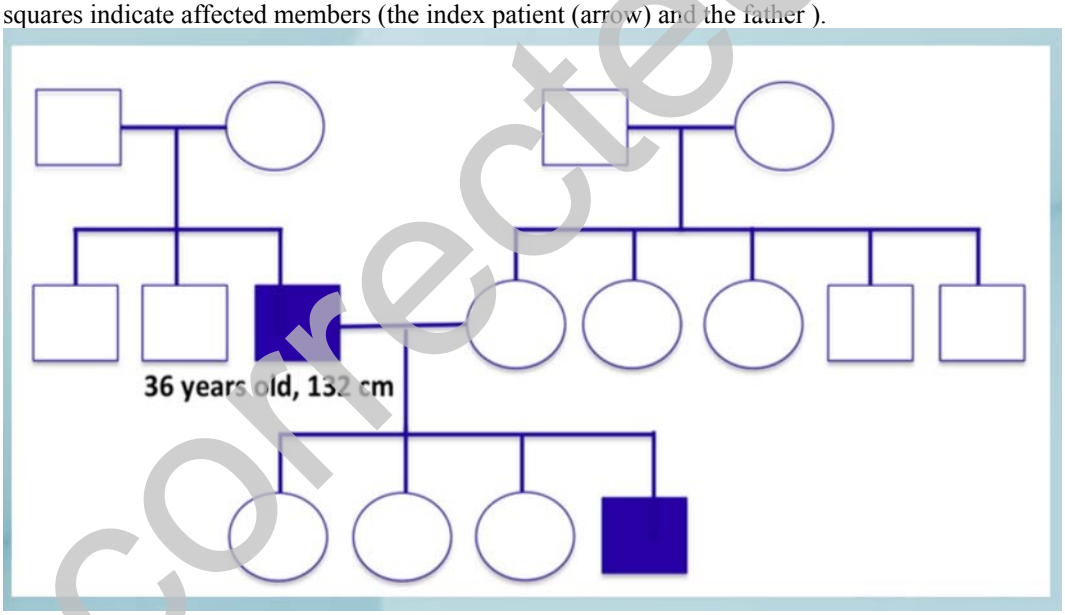

Fig 2

Figure 3. Brain MR of the index case, demonstrating anterior pituitary hypoplasia

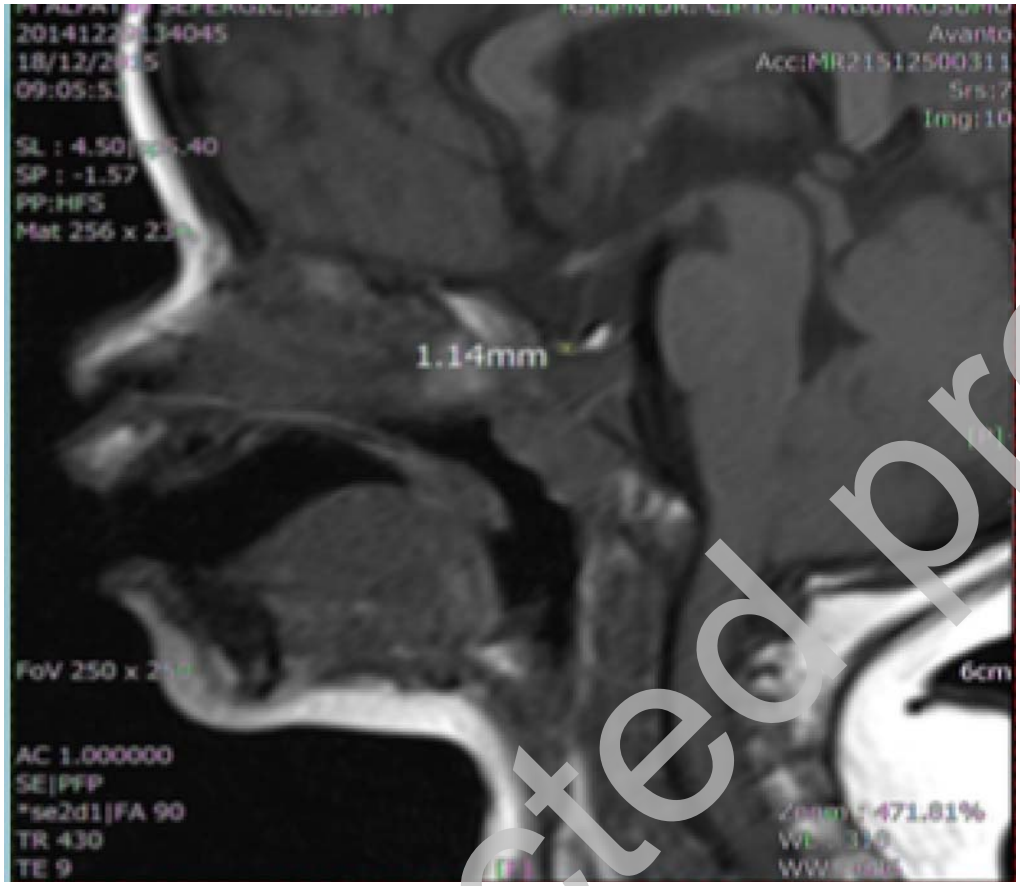

Fig. 3.

Figure 4. Height data of the patient plotted on the WHO growth chart. The arrow indicates the beginning of rhGH injections.

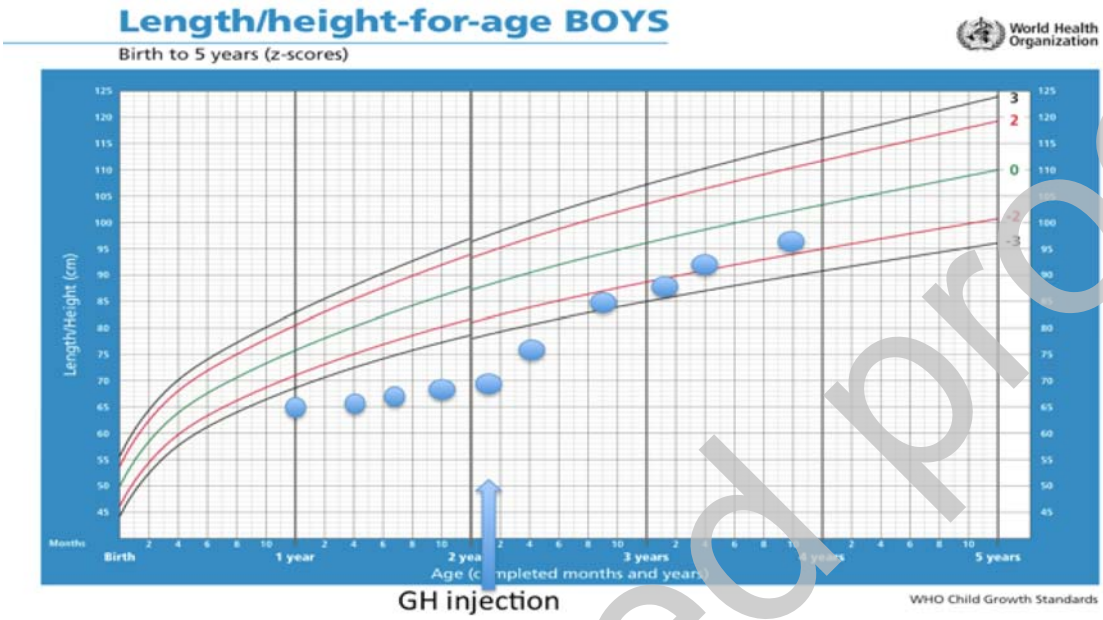

Fig. 4.

Table 1. Summary of antropometric data of the patient during rhGH therapy

Age	Height cm	Height SDS	Weight kg	Weight SDS	HC cm	HC SDS	HV	HV SDS
2.41	78	-3.68	8.64	-5.14	47.5	-0.97	18 cm/year	14
2.75	85	-2.43	10.1	-3.24	48	-0.87	21 cm/year	20.5
3.08	87	-2.36	10.21	-3.72	48.5	-0.73	12 cm/year	-3
3.33	91	-1.69	13	-1.28	49	-0.52	14 cm/year	21
3.75	96	-1.12	14.8	0.05	49.5	-0.38	9.5 cm/year	10

Abbreviations: HC, head circumference; HV, height velocity