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

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Short title: Novel GH1 mutation

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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/m² (-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 disproportionate short stature with a sitting height/height ratio of 0.65 (0.1 SDS) (12). The growth velocity following 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/m². 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>A and c.291+2>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.291+2>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 site 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
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.

In summary, we report a novel mutation in the GHRHR gene in a family with type II IGHD. The specific genetic diagnosis (splicing defect of GHRHR) increases the likelihood that with time other pituitary defects may develop. It has been reported that 3-30% of individuals 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. Mutations of relevant candidate genes have been identified in 11% of patients with severe IGHD and even in 38% of familial cases, so that it was advised to perform genetic testing in children with severe and/or familial IGHD. 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 GHI. 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. While it was previously thought that GHD is almost always associated with a normal birth weight and length, it has recently become clear that average birth size of GHD infants is decreased. 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 GHI is indicated, as was done in our patient who had a novel mutation in GHI.

In IGHD, GH secretion is very low but usually still detectable and associated with heterozygous splice site, missense, splice enhancer mutations, or intronic deletions in GHI. Most patients with type II IGHD, as our case, have mutations within the first six nucleotides of intron 3 of GHI, 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. 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. 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.

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In summary, we report a novel mutation in GHI 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
Figure 1. Characteristic clinical features of the patient. Frontal bossing, midfacial hypoplasia, lobulated subcutaneous fat and normal genitalia are noted.

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).
Figure 3. Brain MR of the index case, demonstrating anterior pituitary hypoplasia
Figure 4. Height data of the patient plotted on the WHO growth chart. The arrow indicates the beginning of rhGH injections.

![WHO Growth Chart](image)

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

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<th>Age</th>
<th>Height cm</th>
<th>Height SDS</th>
<th>Weight kg</th>
<th>Weight SDS</th>
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<th>HC SDS</th>
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<th>HV SDS</th>
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Abbreviations: HC, head circumference; HV, height velocity