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# Heterozygous GHR gene mutation in a child with idiopathic short stature

**Abstract:** Several monogenic defects have been reported to be associated with idiopathic short stature. Focusing on growth hormone receptor (GHR)-gene alterations, the heterozygosity of the same gene defect may be associated with a range of growth deficits. We found a heterozygous mutation (V144I) within exon 6 of the GHR gene in a patient with a low level of insulin-like growth factor I (IGF-I), normal level of GH, and severe short stature. Despite the lack of statistical difference, an overall tendency for reduced *wt*-GH-induction of GHR activation and Jak/Stat signalling in cells transiently expressing GHR-V144I alone or co-expressing *wt*-GHR compared to cells expressing only *wt*-GHR was found when GH doses were increased. Our results suggest that, although GHR sequence variants are responsible for some functional alterations commonly observed in children with idiopathic short stature, these changes may not explain all the height deficits observed in these subjects.

**Keywords:** GH; GHR gene mutation; idiopathic short stature; IGF-I.

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age, sex and population group, without evidence of any systemic, endocrine, nutritional or chromosomal abnormalities. In other words, ISS designates an unexplained condition even after a thorough growth evaluation (1). Specifically, children with ISS have normal birth weight and normal growth hormone (GH) secretion (1). However, an increasing amount of disorders should be excluded in the process of ISS diagnosis (1, 2). While focusing on ISS and searching for possible causes of ISS, several monogenic causes of short stature have been reported to be “associated” with ISS. Importantly, these various disorders need to be excluded before the diagnosis of ISS can be made. These mutations include defects in GH-1, growth hormone-releasing hormone receptor (GHRHR), GH receptor gene (GHR), as well as various other genes, such as signal transducer (STAT5B), IGF-I and IGF-acid labile subunit (ALS), and so on (3). With an increasing number of reports on these gene defects, it has become evident that there is no strict geno/phenotype correlation; furthermore, phenotypes are highly variable. Focusing on GHR gene alterations, the clinical features of homozygous gene defects (mutations or deletions) can vary substantially depending on the location of the genetic alteration. Furthermore, another cause of clinical variability may be the heterozygosity of the same gene defect, which may be associated with only a mild negative effect on growth or in some cases pronounced growth failure (4). In addition, it is possible that primary IGF-I deficiency may be associated with cumulative digenic or oligogenic defects, as supported by the results from genome-wide association studies, which found that height is determined by more than 180 genes (5). Therefore, in order to diagnose short stature correctly, a genetic analysis, in addition to the functional proof of any gene defect found, is essential in the diagnostic workup of a patient with short stature (4).

## Introduction

Idiopathic short stature (ISS) is defined as a condition, in which height is more than two standard deviations (SD) below the corresponding mean height for a given

## Clinical report

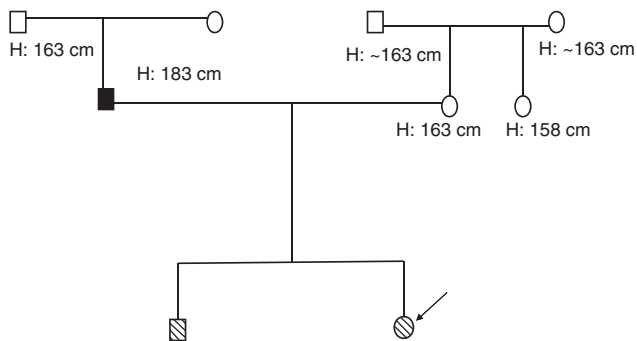
The proband was a 2-year-old female, the second child of healthy, non-consanguineous Senegalese parents. The

family history was unremarkable. She was born at term (38 weeks and 5 days) by cesarean section, length was 48 cm (25th percentile), weight of 2.40 kg (3rd–10th percentile), and a head circumference of 33 cm (25th percentile) (6). Maternal and paternal heights were 163 cm (0.13 SDS) and 183 cm (1.25 SDS), respectively, and her parental target height was 166.5 cm (0.72 SDS) (7), as shown in Figure 1. The target height was calculated using the following formula:  $[(\text{maternal height cm} + \text{paternal height cm})/2] + 6.5$  if male or  $[(\text{maternal height cm} + \text{paternal height cm})/2] - 6.5$  if female.

At birth, she was treated with erythropoietin for 2 days (dosage not recorded) for severe anemia (Hb: 10.5 g). At the age of 7.5 months, she was admitted to the hospital for reduced food intake with stunted growth and weight loss, exhibiting height of 65 cm (–2.06 SDS) and a BMI of 13.54 (–2.09 SDS) (7). She presented with proportionate short stature and normal psychomotor development, an umbilical hernia, and some craniofacial features such as frontal bossing and saddle nose. Chronic diseases, including hypothyroidism, celiac and metabolic diseases, were excluded.

At the age of 1.1 year, her height was 69 cm (–3.09 SDS) and growth velocity was 8 cm/year (–3.32 SDS) (7). At the age of 2 years, her height was 77 cm (–2.3 SDS) and her BMI was 14.34 (–1.29) (7) (Figure 2). An endocrine evaluation showed a very high GH basal value (47 ng/mL), which was unchanged after pharmacological stimulus (chloridrate arginine infusion) (8), and a very low level of basal IGF1 (<25 ng/mL).

The discrepancy between the very high GH basal values and the very low levels of IGF-I suggested GH insensitivity as the cause of short stature. To verify insensitivity to GH, an IGF-I stimulation test was performed according to the classical scheme of 0.1 IU/kg/die GH for 4 days. No IGF-I increase (from 50 to 56 ng/mL) was observed, thus confirming the diagnosis of GH insensitivity (9).



**Figure 1** Patient's pedigree. The known heights of the patient's relatives are indicated.

Treatment with rIGF-I injections was proposed to improve growth, but it was rejected by the family because of side effects. At her last visit at the age of 2.4 years, her height was 80.7 cm (–2.3 SDS) and BMI was 13.82 (–1.57 SDS) (6) (Figure 3).

## Materials and methods

### Molecular studies

Genomic DNA was isolated from the peripheral blood mononuclear cells of the patient and her immediate family (father, mother, and brother). Mutation analysis was carried out by direct sequencing of all exons and the flanking region of the GHR gene, using the sequence kit Dye Terminator (Applied Biosystems, Monza, Italy) with on ABI PRISM 310 automatic sequencer (Applied Biosystems, Monza, Italy).

### Luciferase reporter gene assay of Stat5 activation

Hek293 TLA cells were transiently transfected with pcDNA 1 *wt*-GHR and/or pcDNA GHR-V144I plasmids using the calcium-phosphate transfection agent (Invitrogen, Life Technologies Europe B.V., Zug, Switzerland) according to the manufacturer's instructions. After 48 h of incubation, cells were seeded in 12-well plates at a density of 160,000 cells/well and the Stat5 activation assay was performed as previously described (10, 11). Briefly, cells were transfected with a Stat5-responsive luciferase reporter gene construct (12, 13) and treated with increasing amounts of *wt*-GH for 6 h. Luciferase expression was then measured by the dual luciferase reporter assay (Promega, Dubendorf, Switzerland) on a luminometer (Mediators PhL, Aureon Biosystems, Vienna, Austria).

## Results

### Genetic analysis

Genetic analysis revealed a heterozygous mutation at nucleotide 484 (first base position of the codon 144) within exon 6 of the GHR gene of the proband, resulting in a predicted change from valine to isoleucine (V144I). The patient's brother and father were heterozygous and homozygous, respectively, for the mutation.

### In vitro study

In order to test and compare *wt*-GH-induced activation of the Jak/Stat pathway of *wt*-GHR and/or the GHR-V144I

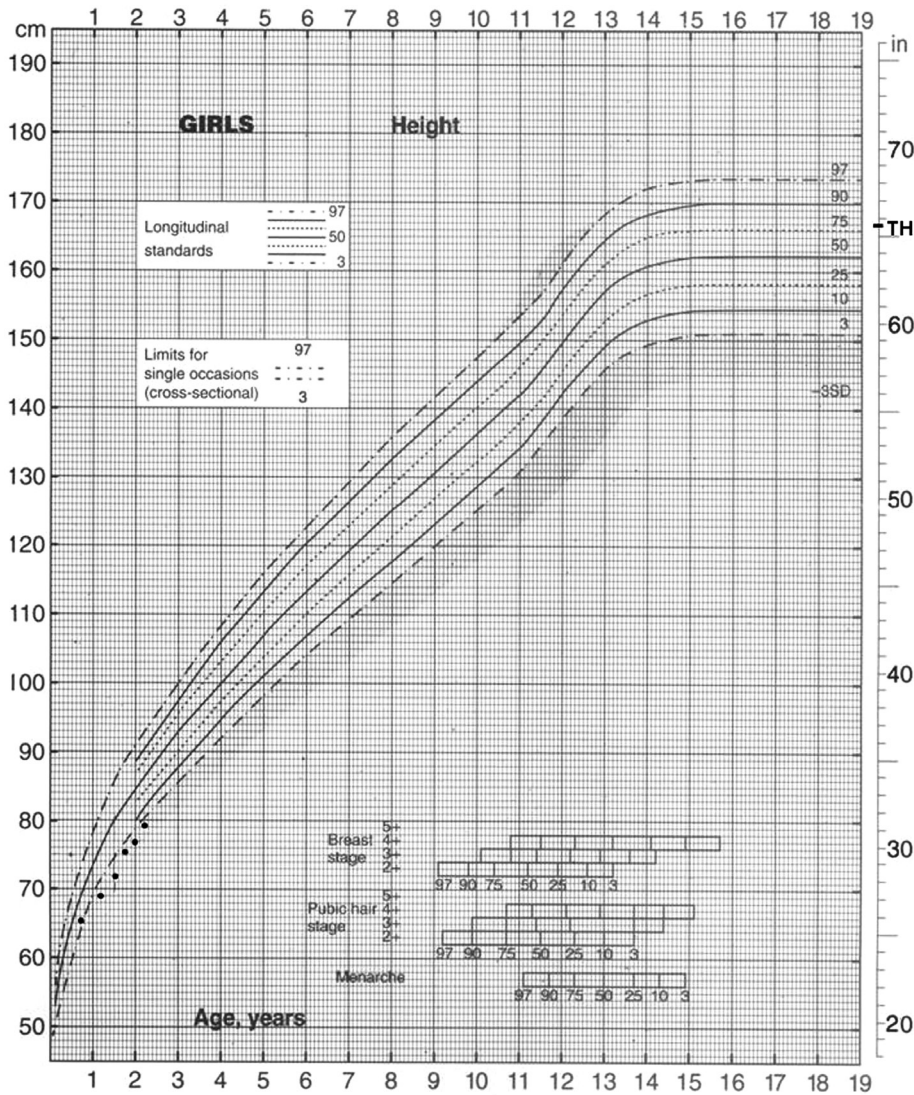


Figure 2 Patient’s growth chart. All height measurements taken from 7.5 months of age to 2.4 years of age.

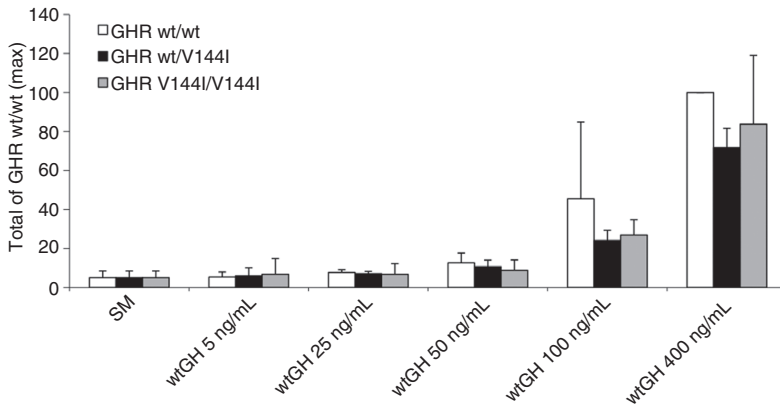


Figure 3 Patient at 2.4 years of age exhibiting a frontal bossing, prominent forehead, hypoplastic nasal bridge, and scleras blue.

mutant, the Stat5 activation assay was performed using Hek293 TLA cells transiently expressing *wt*-GHR, GHR-V144I mutant, or equal amounts of both (GHR *wt/wt*, GHR V144I/V144I, GHR *wt/V144I*). With increasing rhGH exposure, we observed an overall tendency of reduced Jak/Stat signalling in cells expressing GHR *wt/V144I* and GHR V144I/V144I compared with GHR *wt/wt* when stimulated with 100 and 400 ng/mL of *wt*-GH. However, the results were not statistically significant (Figure 4).

## Discussion

It is a considerable challenge for clinicians to distinguish ISS from partial GH deficiency. There is no gold standard



**Figure 4** Jak2/Stat5 signaling capacity of GHR wt and/or GHR V144I evoked by wt-GH. The normalized firefly/renilla luciferase activity of wt-GH-stimulated cells was divided by the normalized activity of unstimulated samples (SM), yielding the fold induction. Furthermore, activation of Jak/Stat signalling of GHR wt evoked by 400 ng/mL wt-GH [GHR wt/wt (max)] was set at 100% and other measurements were compared against this. The results are given as the means $\pm$ SD of three separate experiments performed in duplicate.

pharmacological test that can establish normal GH secretion due to arbitrary cut-off levels and low accuracy (14). Thus, children with test results may be false-positive diagnosed as partial GHD, and vice versa false-negative may be labeled as ISS (15).

It has been suggested that GHR-gene alterations may account for up to 5% of all ISS patients, and that these mutations should be taken into consideration when other causes of short stature have been ruled out (16). Thus, the correlation between heterozygous mutations in the GHR gene and ISS has been explored in various studies with conflicting results.

Several abnormal forms of the GHR have been identified, which result in abnormal GH binding, defective receptor dimerization, and defective signal transduction (17). However, it is uncertain whether heterozygous mutations in GHR play a major role in determining growth (18, 19).

In our case, we found a transition from G to A at position 1 of codon 144 of the GHR gene, resulting in the non synonymous amino acid (aa) change from valine to isoleucine. The same change has been reported by Sanchez et al. (19). Another nucleotide change in the same amino acid (second base pair of codon 144 as opposed to the first in our patient) has also been found in a compound heterozygous patient with Laron syndrome in a report by Amselem et al. (20).

Our patient presented with low levels of IGF-I, normal values of GH and clinically severe short stature, which did not correlate with her mid-parental height. These parameters are often associated with the V144I heterozygous mutation in the GHR gene. However, her father who was homozygous for the same mutation, and her brother, who carried the mutation in a heterozygous state, were both of normal height, thus underlining the absence of a strict

geno-/phenotype correlation. In addition, our finding that family members of normal stature carrying the same sequence change as the patient challenges the concept that GHR-V144I plays a major role in determining height velocity, growth and eventually final height, although some impacts on GHR signaling cannot be ruled out.

In previous studies, Sanchez et al. (19) and Amselem et al. (20) have found a correlation between this mutation and its function. In fact, Sanchez et al. reported that one patient presented with partial growth hormone insensitivity (GHI) and that family members with the same heterozygous mutations are of short stature (19). Furthermore, a patient described by Amselem et al. (20) has been found to be compound heterozygous with an aa change at the same locus, but resulting in a valine to aspartic acid substitution that, in turn, causes GHIS. In contrast with our results, these studies suggest that the valine at position 144 is likely to be important for proper GH-receptor function. However, in both cases, the significance of the V144I mutation has yet to be determined using functional studies.

A dominant negative effect of a heterozygous mutation can be expected in some cases, where the dimerization of the mutant with the wild-type variant interferes in terms of formation and, thereafter, the activity of the hormone-receptor complex. Importantly, this fact must be analyzed by functional studies, but bearing in mind that in vitro results are not always predictive of a clinical effect (3).

Nevertheless, we tested the Jak/Stat signaling capacity of wt-GHR and/or GHR-V144I mutant and found an overall tendency for a reduced Jak/Stat signalling in cells expressing GHR wt/V144I and GHR V144I/V144I compared to GHR wt/wt after stimulation with wt-GH, but the data did not reach statistical significance (Figure 4).

Finally, although sequence variants within the GHR gene are very common in children with ISS and a high frequency of sequence changes in the GHR gene in short children with characteristics of GHI has been reported (18), our results suggest that these changes may not play a major role in determining growth and eventually final height (19–21). Along this line, the presence of the identical sequence change in family members of normal stature suggests that these sequence variants do not influence the function of GHR at all. Furthermore, it is possible that other factors, such as environmental, epigenetics as well as other more important genes and their interaction, may explain the insensitivity to GH in children with GHI (3–5).

For instance, Lango et al. (22) have reported that hundreds of genetic variants, in at least 180 loci, influence adult height, indicating that height and growth are highly heritable and classic polygenic traits. These loci are non-randomly clustered within biologically relevant pathways

and are enriched for genes involved in growth-related processes, which underlie syndromes of abnormal skeletal growth, and are directly relevant to growth-modulating therapies.

In conclusion, we present a patient suffering from a heterozygous GHR gene mutation, previously described as causing short stature and GHI. Functional analysis data, however, do not fully support the concept that this V144I GHR variant is the unique cause for short stature in this girl. Importantly, as growth is a classic polygenic trait involving nearly 200 genes along with environmental and epigenetic factors, there is a need to broaden the analyses when studying possible causes for short stature.

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