CASE REPORT

Primary GH insensitivity ‘(Laron syndrome) caused by a novel 4 kb deletion encompassing exon 5 of the GH receptor gene: effect of intermittent long-term treatment with recombinant human IGF-I

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Abstract

Objective: GH insensitivity syndrome (GHIS; Laron syndrome) is clinically characterized by severe postnatal growth failure and very low serum levels of IGF-I despite increased secretion of GH. This mainly autosomal recessive syndrome is clinically indistinguishable from isolated GH deficiency (IGHD). Fifty-one different mutations in the GH receptor (GHR) gene have been discovered, whereas only three deletions causing the disorder have been reported so far. In this report, we describe a consanguineous family from Sri Lanka with a novel deletion of 4097 bp in length encompassing exon 5.

Subjects and methods: Parents of normal phenotype presented their second child (boy) to our clinic at the age of 7 months with severe growth retardation and the clinical features of IGHD (58 cm, −6.1 standard deviation score (SDS); 5.7 kg, −3.4 SDS). Assessment, however, revealed GHIS with absent GH-binding protein. Thereafter, the patient received intermittent recombinant human IGF-I (rhIGF-I; 80 μg/kg twice daily) treatment prepubertally for 5.5 years. Genomic DNA was extracted for genetic analysis and each exon was PCR amplified individually. Further, in order to amplify the GHR gene from exon 4 to 6, Expand Long Template PCR (Roche) was carried out. In addition, RNA isolation and RT-PCR were performed.

Results: Separate PCRs of each of the exons of the GHR gene revealed that exon 5 in the patient was missing. Thereafter, 'Long PCR' from exons 4 to 6 revealed a 4097 bp deletion encompassing exon 5, in a homozygous state in the patient and in a heterozygous state in both parents. RT-PCR analysis revealed an exact absence of exon 5 resulting in a frameshift, leading to a stop codon in exon 6, which predicts a truncated, non-functional GHR protein.

Conclusion: Fifty-one different mutations within the GHR gene causing GHIS have been reported so far. In contrast, only three deletions within the GHR gene are known. We describe a patient suffering from GHIS caused by a novel 4 kb deletion of the GHR gene encompassing exon 5 and, additionally, we focus on the effect of intermittent rhIGF-I treatment during prepuberty.

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Introduction

Growth is an inherent property of life and is mainly the result of the action of growth hormone (GH). The binding of GH to GH receptors (GHR) is the first step in the action of GH. Two GHR molecules dimerize in response to GH, turning on a cascade of events leading to signal transduction by activating gene transcription in the cell nucleus (1). Defects in the GHR gene result in GH insensitivity syndrome (GHIS; Laron syndrome; OMIM: 262500). This syndrome is clinically characterized by severe postnatal growth failure and very low serum levels of insulin-like growth factor-I (IGF-I) despite increased secretion of GH. Fifty-one mutations of the GHR gene have already been discovered, whereas only three deletions causing the disorder have been reported so far (2–4) (Fig. 1).

In this report, we describe a consanguineous Tamil family from Sri Lanka with a novel deletion of over 4 kb in length encompassing exon 5 (Fig. 1). Further, the effect of intermittent recombinant human IGF-I therapy (rIGF-I; Igf; Pharmacia and Upjohn, Dübendorf, Switzerland) over 5.5 years during prepuberty in the affected boy is reported.
Subjects and methods

Subjects

The parents and the first child (girl) were of normal phenotype, whereas the second child (boy) was presented at the age of 7 months with severe growth retardation (58 cm, $-6.1$ standard deviation score (SDS); 5.7 kg, $-3.4$ SDS) and the clinical features of isolated GH deficiency to the outpatient clinic of the University Children’s Hospital in Bern, Switzerland. Detailed assessment revealed GHIS with absent GH-binding protein (GHBP). The laboratory findings at various time-points on and off rhIGF-I treatment are given in Table 1. In addition, the growth charts are presented in Fig. 2. Of note, birth weight (37th week of pregnancy: 2.9 kg, $-0.2$ SDS) as well as length (48 cm, $-0.1$ SDS) were in the normal range (First Zurich Longitudinal Study of Growth and Development (5), but growth failure became obvious during the first 6 months of life (Fig. 2). After the diagnosis, rhIGF-I (Igef; 80 $\mu$g/kg) was subcutaneously injected twice daily after meals. Because of supply problems with the rhIGF-I, the treatment was interrupted several times giving us the opportunity to analyse the impact of this intermittent long-term replacement therapy (5.5 years of therapy within a follow-up period of 8 years) before puberty. Serum GH was measured by the DSL-10-1900 active human GH ELISA kit (DSL, Webster, TX, USA), IGF-I- and IGF-binding protein 3 (IGFBP-3) measurements were performed using the IGF-I IRMA (radioisotopic assay kit; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) and the IGFBP-3 RIA kit (Nichols Institute Diagnostics) respectively. Serum GHBP was determined by a ligand-mediated immunofunctional assay (6).

Based on the normative data of the First Zurich Longitudinal Study of Growth and Development (5), height

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before rhIGF-I</th>
<th>On rhIGF-I</th>
<th>Before rhIGF-I</th>
<th>On rhIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>12 months</td>
<td>18 months</td>
<td>7 years 6 months</td>
<td>8 years 2 months</td>
</tr>
<tr>
<td>GH basal (ng/ml)</td>
<td>$77.5-150.8$</td>
<td>Not measured</td>
<td>125.5</td>
<td>$&lt;6.8$ ($-4.5$)</td>
</tr>
<tr>
<td>IGF-I (ng/ml) (SDS)</td>
<td>$&lt;6.8$ ($-4.2$)</td>
<td>$45$ ($+0.75$)</td>
<td>(146; 96–289)</td>
<td>(146; 96–289)</td>
</tr>
<tr>
<td>IGFBP3 (mg/l) (SDS)</td>
<td>$0.53$ ($-4.22$)</td>
<td>$0.85$ ($-2.33$)</td>
<td>$0.33$ ($-4.45$)</td>
<td>$0.33$ ($-3.58$)</td>
</tr>
<tr>
<td>GHBP (pmol/l)</td>
<td>Absent</td>
<td>$1.29; 0.94–1.76$</td>
<td>$1.62; 1.12–2.33$</td>
<td>$2.11; 1.32–3.38$</td>
</tr>
<tr>
<td>Peak GH after provocation test (arginine, 0.5 g/kg i.v.)</td>
<td>$172.4$</td>
<td>$172.4$</td>
<td>$172.4$</td>
<td>$172.4$</td>
</tr>
<tr>
<td>IGF-generation test*</td>
<td>$&lt;6.8/ 6.8$</td>
<td>$0.72/0.56$</td>
<td>$0.72/0.56$</td>
<td>$0.72/0.56$</td>
</tr>
</tbody>
</table>

* Daily injection of recombinant human GH (0.1 IU/kg per day and 0.2 IU/kg per day) for 4 and 3 days respectively.

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data are expressed as SDS (5). Following the calculation of the body mass index (BMI; kg/m²), the values were transformed into SDS according to the normative values obtained from the First Zurich Longitudinal Study of Growth and Development (5). Height velocity was calculated only if height measurements were available for 6 successive months. Radiographs were taken of the left knee and later of the left hand and wrist for assessment of skeletal age according to Pyle & Hoerr (7) and Greulich & Pyle (8). The experimental protocol was approved by the Ethical Committee of the University Children’s Hospital as well as by the equivalent of SwissMedic (governmental office in charge to supervise the drugs used not registered in Switzerland). Informed consent was obtained from the parents.

Methods

PCR of each exon of the GHR gene Genomic DNA was extracted from the whole blood of the patient and his family using the QIAamp-blood extraction kit (QIAGEN AG, Basel, Switzerland). Each exon of the GHR gene was first amplified using the primers as described in Table 2. PCR amplification was performed for 30 cycles with an initial denaturation step at 94°C for 5 min, thereafter 94°C for 1 min, annealing at 54°C (for exon 4), 56°C (for exon 8), 58°C (for exons 3, 5, 7), and 60°C (for exons 2, 6, 9) for 1 min, and with an extension at 72°C for 2 min. A final extension at 72°C for 5 min was done. PCR products were purified with the QIAEX II gel extraction kit (QIAGEN). Purified PCR products were amplified again using the Big Dye Terminator Sequencing kit (Perkin Elmer, Applied Biosystems, Rotkreuz, Switzerland). Products were analyzed on an ABI 373 automated DNA sequencing system (Applied Biosystems).

Long-range PCR ‘Long PCR’ was carried out to amplify the GHR gene from exon 4 to exon 6 using

Table 2 Primer sequences.

<table>
<thead>
<tr>
<th>Oligo name</th>
<th>Sequence (5’–3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHR2F</td>
<td>GTC TGC TTT TAA TTT CTG GGC</td>
</tr>
<tr>
<td>GHR2R</td>
<td>ACA CTG AGG GTG GAA ATG GA</td>
</tr>
<tr>
<td>GHR3F</td>
<td>CTT TCT GTT TCA GCA AC</td>
</tr>
<tr>
<td>GHR3R</td>
<td>GGA TAG TAG TGT AAA TAC ACT A</td>
</tr>
<tr>
<td>GHR4F</td>
<td>AGG ATC ACA TAT GAC TCA CCT</td>
</tr>
<tr>
<td>GHR4R</td>
<td>AGT GTA TTT TAG GTA CAT C</td>
</tr>
<tr>
<td>GHR5F</td>
<td>TAA GCT ACA ACA TGA TTT TTT G</td>
</tr>
<tr>
<td>GHR5R</td>
<td>TTA GTG TAA AAC TAT GTC AAA TG</td>
</tr>
<tr>
<td>GHR6F</td>
<td>GTG TCT GTC TGT GTA CTA ATG</td>
</tr>
<tr>
<td>GHR6R</td>
<td>AGA AAA GTC AAA GTG TAA GGT G</td>
</tr>
<tr>
<td>GHR7F</td>
<td>TAG TGT TCA TGT GCA TTG AG</td>
</tr>
<tr>
<td>GHR7R</td>
<td>ACA AAA GCC AGG TTA GCT AC</td>
</tr>
<tr>
<td>GHR8F</td>
<td>AAA CTG TGC TTC AAC TAG TCG</td>
</tr>
<tr>
<td>GHR8R</td>
<td>GGT CTA ACA CAA GTG GTA CA</td>
</tr>
<tr>
<td>GHR9F</td>
<td>GAA TAT GTA GCT TTT AAG ATG TC</td>
</tr>
<tr>
<td>GHR9R</td>
<td>CAT ATG ACA GGA GTC TTC AGG TG</td>
</tr>
<tr>
<td>GHR11kb.s</td>
<td>CCA CTG GAC AGA TGA GTG TCA TCA</td>
</tr>
<tr>
<td>GHR11kb.as</td>
<td>TGG TAC AAA G</td>
</tr>
<tr>
<td>GHRi5.as</td>
<td>CTC ACT TGG ATA TCT GCA TGA ATC</td>
</tr>
<tr>
<td></td>
<td>CCA GTC AAA C</td>
</tr>
<tr>
<td></td>
<td>GGA ATC CCT CTG TTT G</td>
</tr>
</tbody>
</table>

Figure 2 Growth chart. In the left-hand chart the finer lines indicate the intrauterine period and the heavier lines the extrauterine period. Percentiles are shown on the extreme left and right. SSW, weeks of pregnancy; solid circles, height and weight measurements; open circles, bone ages (7, 8); hatched bars, duration of rhIGF-I treatment.
the Expand Long Template PCR System (Roche, Basel, Switzerland). For this amplification (approximately 11 kb), primers GHR11 kb.s and GHR11 kb.as (Table 2 and Fig. 3) were used. PCR conditions were as follows: initial denaturation at 94 °C for 3 min; 10 cycles of 94 °C for 15 s, 65 °C for 30 s, 68 °C for 8 min; followed by 20 cycles of 94 °C for 15 s, 65 °C for 30 s, 68 °C for 8 min 20 s; and final elongation at 68 °C for 7 min. In order to obtain a shorter fragment (approximately 7 kb) for the sequencing, a second 'Long PCR' was performed with a second anti-sense primer (GHRi5.as) located in intron 5, in combination with GHR11 kb.s (Fig. 3). Importantly, the whole 7 kb fragment was planned to be sequenced from both sides using the so-called 'genome walking method' until the exact ends of the deletion were about to be defined (Fig. 3).

**RNA isolation/RT-PCR** RNA from cytomegalovirus-immortalized leucocytes was isolated and converted to cDNA using reverse transcriptase (RT-M-MLV; Gibco-BRL, Life Technologies, Basel, Switzerland). The PCR amplified products were purified and sequenced as described above.

**Results**

**Clinical diagnosis and follow-up on rhIGF-I treatment**

One boy originally from Sri Lanka was diagnosed with GHIS (pedigree in Fig. 4A and Table 1). According to the variable rhIGF-I supply the boy was treated intermittently with rhIGF-I in two daily doses of 80 μg/kg, following meals (Fig. 2). Besides the impressive lipohypertrophy (Fig. 5), no side-effect which could be attributed to this therapy was noted. In particular, no hypoglycaemic episodes were observed as the injection was given following a meal and the blood sugar was closely followed in the first 2 weeks on treatment. As shown in Fig. 6, there is also a clear overgrowth of the facial soft tissues in this prepubertal boy, leading to a more prominent glabella and thickening of the eyebrows, nasal tip, philtrum and especially lips.

Further, most interestingly, the growth pattern in relation to the rhIGF-I therapy could be followed starting at 18 months in this boy. The details of the auxological...

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**Figure 3** Schematic representation of the GHR exon (E) 5 deletion and the sequences at the breakpoints. The 5' breakpoint lies approximately 3.7 kb upstream of exon 5. The 3' breakpoint lies approximately 200 bp downstream of exon 5. The primers are shown. wt, wild-type; del, deleted allele.

**Figure 4** (A) Pedigree of the patient’s family. The distribution of the 4 kb deletion is shown. The open symbol represents wild-type, whereas the solid symbol represents homozygosity for the deletion. The open symbols containing smaller solid symbols represent heterozygosity. (B) ‘Long PCR’ products across the deleted region with primers GHR11kb.s and GHR11kb.as. Marker II is shown on the left of the gel. wt, wild-type; del, deleted allele.
evaluation are presented in Table 3. Without the rhIGF-I treatment the height velocity decreased drastically and height did not increase at all. Moreover, the increase in weight, and therefore BMI, was parallel to the acceleration of the growth rate. On treatment, IGF-I in the circulation was well within the normal range, whereas the effect on IGFBP-3 was insufficient; this has been reported previously (Table 1) (9–11).

**Figure 5** Lipohypertrophy at the injection site following rhIGF treatment (80 μg/kg) twice daily.

**Figure 6** Effect of treatment with rhIGF-I on facial maturation and soft tissue growth.
Analysis of the GHR gene

Mutation detection involving PCR amplification of all GHR gene exons failed to generate a product for exon 5 (data not shown), suggesting the presence of a deletion of the whole exon 5. This deletion was verified by ‘Long PCR’ amplification from exon 4 to exon 6, which confirmed the hypothesis by revealing a deletion of approximately 4 kb, encompassing exon 5 totally (Fig. 4B). This deletion was found to be in a homozygous state in the affected child, and in a heterozygous state in both parents. The sister does not carry the deleted allele.

In order to detect the 5' and 3' breakpoints, a second ‘Long PCR’ was performed. Thereafter, the resulting wild-type fragment (approximately 7 kb) and the fragment bearing the deletion (approximately 3 kb) were sequenced. The 5' breakpoint lies in intron 4. 3719 bp upstream of exon 5. The 3' breakpoint was within intron 5. 206 bp downstream of exon 5. Therefore, the deletion spanned exactly 4097 bp (Figs 3 and 7). This deletion was verified by ‘Long PCR’ amplification from exon 4 to exon 6, which confirmed the hypothesis by revealing a deletion of approximately 4 kb, encompassing exon 5 totally (Fig. 4B). This deletion was found to be in a homozygous state in the affected child, and in a heterozygous state in both parents. The sister does not carry the deleted allele.

With exon 5 being completely absent (Figs 1 and 3), a perfect junction of exon 4 with exon 6 at the mRNA level can be predicted. The resulting frameshift leads to a stop codon in exon 6 and, further, to a non-functional truncated GHR protein (Fig. 7). This hypothesis was proven by RT-PCR and sequencing of cDNA.

Discussion

In 1966, Laron et al. (12) reported several Oriental Jewish families suffering from GHIS. Studies of the GHR gene in these Israeli patients revealed that some contained gene deletions (4). These deletions, interestingly, included exons 3, 5 and 6, suggesting that an unusual rearrangement may have occurred (4). However, after additional studies were performed in placenta and an unaffected individual was found to be heterozygous for the exon 5 and 6 deletion but homozygous for the exon 3 deletion, it was suggested that the exon 3 deletion might be a polymorphism (2, 3, 13).

To date, nearly 300 patients have been identified worldwide, mostly from the Mediterranean, mid-eastern region and from Ecuador (14–17). Further, GHIS in these subjects is caused by a great variety of homozygous point mutations and about 30 different mutations (missense, nonsense or abnormal splicing) have been subsequently described (14–20). In contrast to this high number of patients suffering from GHIS caused by a wide variety of GHR gene mutations, there are only a few reports on patients with GHR gene deletions (3, 4, 21). In addition to the deletions of exons 5 and 6 mentioned above, a partial deletion of exon 5 has been reported in one patient from Cambodia (3). Moreover, in a Caucasian girl, a 13 bp deletion within exon 9 was found (3). This partial deletion within exon 9 led to a frameshift and insertion of 15 missense amino acids before termination within exon 10 (3).

In this report we describe a patient suffering from GHIS caused by a novel 4 kb deletion in the GHR gene. This 4 kb deletion encompassing exon 5 is predicted to result in a perfect junction of exon 4 with exon 6 at the mRNA level, in a similar way to that described by Gastier et al. (3) in the family from Cambodia carrying the partial deletion in exon 5. A frameshift in the mutant mRNA leads to a severely truncated GHR protein lacking part of the extracellular domain and all of the transmembrane and intracellular domain. Further, the fact that GHBP could not be measured in the patient’s blood and mRNA of the GHR peptide was not detectable in the RT-PCR underlines the instability of the mutant mRNA, which is probably rapidly degraded within the cell (22).

We observed that treatment with rhIGF-I improves growth rate best at a very young age (Table 3 and Fig. 2), confirming the view that treatment should therefore be started as early as possible (23). Another most interesting finding is that the reported decline in height velocity (23-25) during the second and following years of treatment may be overcome by using a treatment protocol including rhIGF-I-free intervals. When our overall auxological data were compared with data obtained from studies where similar patients (age at the beginning of therapy) were identically treated (doses) and followed (duration of treatment), one notices that both height velocity and effect on height gain were identical (9, 15, 23, 24). Therefore, although
the final outcome in terms of height gain achieved might not be different between the patients on constant IGF-I therapy and those on a treatment regimen which includes periods of off-treatment, the years off-treatment are at least cost effective.

Compared with the GH substitution in patients with severe early onset GH deficiency, it is of importance to analyze the auxological data and, therefore, the effect of IGF-I treatment in more detail with reference to duration of the therapy, and height as well as age at diagnosis. First, focusing on the duration of treatment, the overall treatment effect over 7 years (94 months), with years without rhIGF therapy being included, resulted in a mean height velocity of 5.6 cm/year and a $D$ height SDS of $þ1.4$ (height SDS at the end minus height SDS at the beginning of therapy), which was similar to the $D$ height SDS of $þ1.6$ reported by the GHIS Collaborative Group (9) in patients treated constantly for an identical period of time. Secondly, whenever patients with similar height SDS ($26.5$ to $27.5$) at the beginning of the rhIGF therapy ($27.1$ in our patient) were compared with each other in terms of effect on height gain, a mean of $þ1.5$ ($D$ height SDS) following a median treatment duration of approximately 5 years was found (9, 23). In our patient, $D$ height SDS was $þ1.4$, and therefore identical. Thirdly, data were analyzed with respect to age at diagnosis bearing in mind that there is a reverse relationship between age at start of rhIGF-I treatment and total height gain ($D$ height SDS) in patients treated for more than 4 years (23). Whenever treatment was started in the affected children before the age of 5, the increase in height SDS ($D$ height SDS) was $þ2.7$ over treatment duration of approximately 6 years (9, 23). Therefore, our regimen would result in a definitive loss of final height. However, as the number of young children treated with rhIGF-I and reported in the literature is rather small, no final conclusion can be drawn and further studies are needed to clarify this issue.

It has been previously reported that statural growth in patients suffering from GHIS is accompanied by a significant weight gain, which parallels the increment in height (9, 23, 24). This pattern was also observed during the follow-up of this boy, but as BMI increased from 16.1 to 20.1, which represented a $D$ BMI SDS of $þ2.2$, the off-treatment intervals did not have an overall positive effect on the development of obesity (Table 3). This may explain the remarkable effect on overgrowth of soft tissue as well as the dramatic lipohypertrophy seen in this patient as shown in Figs 5 and 6. The pathogenetic basis of this finding, however, remains speculative. Possible explanations are (a) the absence of sufficient IGFBP-3 levels (23, 25, 26) and (b) an overall IGF-I excess which may act via insulin receptors as adipocytes lack IGF-I receptors (27, 28). Therefore, the addition of IGFBP-3 to IGF-I may lead to more stable availability of IGF-I at the tissue level, resulting in a decrease of the stimulation of the soft tissue, the different organs as well as the adipose tissue and eventually a better effect in terms of growth (16).

In conclusion, we have described a boy suffering from GHIS with a novel deletion within the $GHR$ gene, 4097 in length, which encompasses exon 5. In addition, the effect of interval therapy of IGF-I is reported.

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References


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