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 post-natal 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.

European Journal of Endocrinology 150 635–642

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 (rhIGF-I; Igefi; Pharmacia and Upjohn, Dübendorf, Switzerland) over 5.5 years during prepuberty in the affected boy is reported.

	DNA	mRNA
<i>GHR</i> -gene (wild type) ⁽¹⁾
deletion exon 3 ^(2,3)
discontinuous deletion of exons 3, 5 and 6 ⁽⁴⁾
partial deletion exon 5 ⁽³⁾
partial deletion exon 9 ⁽³⁾
deletion exon 5 4097 bp encompassing exon 5 (patient reported)

Figure 1 Variable forms of *GHR* gene deletions at the DNA and mRNA level. Superscript numbers in parentheses relate to the references.

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 µg/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 immuno-functional assay (6).

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

Table 1 Clinical and laboratory findings before and on rhIGF-I (80 µg/kg twice daily) treatment in infancy and childhood.

Parameter	Before rhIGF-I	On rhIGF-I	Before rhIGF-I	On rhIGF-I
Age	12 months	18 months	7 years 6 months	8 years 2 months
GH basal (ng/ml)	77.5–150.8	Not measured	125.5	Not measured
IGF-I (ng/ml) (SDS)	<6.8 (< -4.2)	45 (+0.75)	<6.8 (< -4.5)	218 (+1.0)
(normal mean; range)	(33; 10–69)		(146; 96–289)	(146; 96–289)
IGFBP3 (mg/l) (SDS)	0.53 (-4.22)	0.85 (-2.33)	0.33 (-4.45)	0.59 (-3.56)
(normal mean; range)	(1.29; 0.94–1.76)	(1.62; 1.12–2.33)	(2.11; 1.32–3.38)	(2.11; 1.32–3.38)
Serum GHBP (pmol/l)	Absent			
Peak GH after provocation test (arginine, 0.5 g/kg i.v.)	172.4			
Before/after IGF-generation test*				
IGF-I (ng/ml)	<6.8/<6.8			
IGFBP3 (mg/l)	0.72/0.56			

* 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.

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.

data are expressed as SDS (5). Following the calculation of the body mass index (BMI; kg/m^2), 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

Table 2 Primer sequences.

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

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

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.

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

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.

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

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

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 6 Effect of treatment with rhIGF-I on facial maturation and soft tissue growth.

Table 3 Auxological characteristics of the patient.

Age (years)	Treatment	Duration (months)	Height SDS (start/end)	Δ height SDS	Height velocity (cm/year)	BMI (start/end)	Δ BMI	BMI SDS (start/end)	Δ BMI SDS
6/12–16/12	Off	12	–6.0/–7.1	–1.1	3	16.6/16.1	–0.5	0.04/–0.83	–0.87
16/12–2	On	6	–7.1/–4.7	+2.4	16	16.1/16.6	+0.5	–0.83/0.07	+0.9
2–27/12	Off	7	–4.7/–7.6	–2.9	1.8	16.6/16.1	–0.4	0.07/–0.05	–1.2
27/12–41/12	On	18	–7.6/–5.6	+2.0	8.9	16.1/19.2	+3.1	–0.05/3.37	+3.42
41/12–47/12	Off	6	–5.6/–7.1	–1.5	1.3	19.2/17.4	–1.8	3.37/1.8	–1.57
47/12–63/12	On	20	–7.1/–5.7	+1.4	6.3	17.4/20.4	+3.0	1.8/4.2	+2.4
63/12–76/12	Off	15	–5.7/–6.4	–0.7	1.6	20.4/19.6	–0.8	4.2/3.2	–1.0
76/12–94/12	On	22	–6.4/–5.7	+0.7	6.1	19.6/20.1	+0.5	3.2/2.6	–0.6
Overall									
16/12–94/12	On/off	94	–7.1/–5.7	+1.4	5.6	16.1/20.1	+4.0	0.04/2.6	+2.2

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).

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

wt GHR mRNA

exon 4 exon 5 exon 6
 ... t t c t a t a c c a g a a g | g a a c a c t c a a g a a t g g g t t g a t g a a a t a g | t g c a a c c a g a t c c a
 ... F Y T R R | N T Q E W V D E I V | Q P D P

del GHR mRNA

exon 4 exon 6
 c t g t t c t a t a c c a g a a g | t g c a a c c a g a t c c a c c c a t t g c c t c a a c t g g a c t t t a c t g a a c g t c a g t t
 L F Y T R S | A T R S T H C P Q L D F T E R Q F

 t a a c t g g g a t t c a t g c a g a t a t c c a a g t g a g a t g g g a a g c a c c a c g c a a t g c a g a t a t t c a g a a a g g a t
 N W D S C R Y P S E M G S T T Q C R Y S E R M

 g g a t g g t t c t g g a g t a t g a
 D G S G V *

Figure 7 Prediction of the sequence of the GHR mRNA isolated from the patient (del) compared with the normal mRNA sequence of the same part of the *GHR* gene (wt). *stop codon.

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 analyse 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 Δ height SDS of +1.4 (height SDS at the end minus height SDS at the beginning of therapy), which was similar to the Δ height SDS of +1.6 reported by the GHIS Collaborative Group (9) in patients treated constantly for an identical period of time. Secondly, whenever patients with similar height SDS (-6.5 to -7.5) at the beginning of the rhIGF therapy (-7.1 in our patient) were compared with each other in terms of effect on height gain, a mean of +1.5 (Δ height SDS) following a median treatment duration of approximately 5 years was found (9, 23). In our patient, Δ height SDS was +1.4, and therefore identical. Thirdly, data were analysed 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 (Δ 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 (Δ height SDS) was +2.7 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 Δ BMI SDS of +2.2, 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.

Acknowledgements

We should like to thank Dr Jane McDougall for reviewing this article and her highly appreciated contribution.

This study was supported by a grant to P E M by the Swiss National Science Foundation (SNF 3200-064623.01).

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Received 4 November 2003

Accepted 6 February 2004