Phenotypic spectrum and responses to recombinant human IGF1 (rhIGF1) therapy in patients with homozygous intronic pseudoexon growth hormone receptor mutation

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Abstract

Background: Patients with homozygous intronic pseudoexon GH receptor (*GHR*) mutations (6Ψ) have growth hormone insensitivity (GHI) (growth failure, IGF1 deficiency and normal/elevated serum GH). We report 9 patients in addition to previously described 11 *GHR* 6Ψ patients and their responses to rhIGF1 therapy.

Methods: 20 patients (12 males, 11 families, mean age 4.0 ± 2.2 years) were diagnosed genetically in our centre. Phenotypic data and responses to rhIGF1 treatment were provided by referring clinicians. Continuous parametric variables were compared using Student *t*-test or ANOVA.

Results: 10/20 (50%) had typical facial features of GHI, 19/20 (95%) from consanguineous families and 18/20 (90%) of Pakistani origin. At diagnosis, mean height SDS: -4.1 ± 0.95 , IGF1 SDS: -2.8 ± 1.4 ; IGFBP3 SDS: -3.0 ± 2.1 and mean basal and peak GH levels: $11.9 \mu\text{g/L}$ and $32.9 \mu\text{g/L}$, respectively. 1/12 who had IGF1 generation test, responded (IGF1: $132\text{--}255 \text{ ng/mL}$). 15/20 (75%; 11M) received rhIGF1 (mean dose: $114 \mu\text{g/kg}$ twice daily, mean duration: 5.3 ± 2.5 years). Mean baseline height velocity of $4.7 \pm 1.1 \text{ cm/year}$ increased to $7.4 \pm 1.8 \text{ cm/year}$ ($P=0.001$) during year 1 of therapy. Year 3 mean height SDS (-3.2 ± 1.0) was higher than pre-treatment height SDS (-4.3 ± 0.8) ($P=0.03$). Mean cumulative increase in height SDS after year 5 was 1.4 ± 0.9 . Difference between target height (TH) SDS and adult or latest height SDS was less than that of TH SDS and pre-treatment height SDS (2.1 ± 1.2 vs 3.0 ± 0.8 ; $P=0.02$).

Conclusion: In addition to phenotypic heterogeneity in the cohort, there was mismatch between clinical and biochemical features in individual patients with 6Ψ *GHR* mutations. rhIGF1 treatment improved height outcomes.

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Introduction

Growth hormone insensitivity (GHI) is characterised by growth failure, IGF1 deficiency and normal or elevated serum GH. A continuum of genetic, phenotypic and biochemical abnormalities has been established, associated

with defects in linear growth (1). Monogenic defects in the GH-IGF1 axis leading to GHI have been discovered in *GHR* (2, 3), *STAT5B* (4, 5), *IGFALS* (6), *PAPPA2* (7) and *IGF1* (8) genes.

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Within the growth hormone receptor (*GHR*) gene, more than 70 missense, nonsense and splice mutations in over 250 patients have been described (1). The majority of *GHR* defects are homozygous or compound heterozygous mutations in the region encoding the *GHR* extracellular domain, responsible for GH binding (9, 10). *GHR* mutations cause a continuum of phenotypes ranging from severe, with classical GHI facies and undetectable IGF1 levels (11, 12), to mild with no dysmorphic features. The latter is commonly associated with heterozygous dominant negative (13, 14) or compound heterozygous *GHR* mutations (15).

The intronic *GHR* pseudoexon mutation (6Ψ) was first described in 2001 in four siblings with mild GHI from a highly consanguineous Pakistani family. This point mutation (base change A⁻¹ to G⁻¹) in intron 6 leads to aberrant splicing and activation of a pseudoexon sequence causing a spectrum of clinical and biochemical abnormalities (16). The inclusion of an additional 108 bases between exons 6 and 7 of the *GHR* gene translates to the insertion of 36 new amino acids within the extracellular domain and impaired function of the mutant *GHR* protein (17). In 2007, a further seven 6Ψ patients were reported (18) with more severe GHI phenotypes and heights as low as -6.0 SDS.

We have identified nine further 6Ψ subjects and report the clinical and biochemical features in the cohort of twenty patients. Additionally, we describe growth responses to rhIGF1 therapy, which has not previously been reported.

Subjects and methods

Patients

Between 2001 and 2014, 20 patients (11 families, 10 with parental consanguinity) were diagnosed with the intronic *GHR* 6Ψ mutation in our centre. There were 12 males and 8 females, mean age at presentation was 4.0±2.2 years (range 0.7–13.0 years). The patients were investigated in 5 UK and 1 US paediatric endocrinology centres.

Clinical, auxological and biochemical data

The patients were investigated at their home institutions and the referring physicians completed a proforma detailing the clinical and biochemical details at the time of sending the DNA sample for genetic analysis. Height measurements were obtained using a wall-mounted

stadiometer. Pubertal staging was done using Tanner stages (19, 20). Pre-pubertal patients were Tanner stage 1 genital development or breast development for boys and girls, respectively. Pubertal patients were Tanner stage 2 or above genital or breast development for boys or girls, respectively.

Birth weight, parental height, height and BMI values were expressed as SDS according to the appropriate UK-WHO growth national standards (21, 22, 23). Biochemical investigations included: basal and/or peak GH, basal IGF1 and peak IGF1 during an IGF1 generation test (IGFGT) and basal IGFBP-3 levels. Basal GH levels and GH provocation tests (glucagon, clonidine or arginine stimulation tests or insulin tolerance tests) were performed in the local centres. IGF1 and IGFBP3 values were expressed as SDS based on the age and sex appropriate ranges provided by the host institution. Where serum IGF1 was undetectable (less than the lower limit of the assay) ($n=7$), the lowest detectable SDS was calculated for statistical analysis. IGFGTs were performed locally as previously published (dose of GH 0.033 mg/kg body weight daily for 4 days) (24, 25). An increase in IGF1 level of >15 ng/mL between basal and peak values in the IGFGT was considered a positive response (24).

Therapy with rhIGF1

Patients were treated with recombinant human IGF1 (rhIGF1) at their local centres by the referring paediatric endocrinologists. Auxology data (height and weight) at different time points of treatment and the relevant clinical data (e.g. pubertal stage, concomitant treatment, etc.) were provided by the referring clinicians. Auxology data were excluded from statistical analysis if the patient had greater than 6 months interruption of rhIGF1 treatment.

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes (Qiagen DNeasy Kit). Each exon of the *GHR*, plus the pseudoexon (6Ψ), including their intronic boundaries, were amplified by PCR using specific primers (primer sequences available on request). PCR products were visualized on 1% agarose gel and sent subsequently for Sanger sequencing. Sanger sequencing was performed by the Barts and the London Genome Centre (<http://www.smd.qmul.ac.uk/gc/>) or GATC Biotech (<https://www.gatc-biotech.com>).

Ethical approval

Informed written consent for genetic research and publication of their clinical details and clinical images were obtained from patients and/or their parents. The study was approved by the Health Research Authority, East of England – Cambridge East Research Ethics Committee (REC reference: 17/EE/0178).

Statistical analysis

For responses to rhIGF1 therapy, the primary end point was height velocity (HV) at the end of the first year of treatment. Pearson correlation coefficient assessed the following correlations: height SDS and IGF1 SDS, height SDS and IGFBP-3 SDS, first year HV and age at initiation of treatment, sex of patient, baseline height SDS and baseline IGF1 SDS.

Pre-treatment HV/height SDS and HV/height SDS during years 1, 2 and 3 of rhIGF1 treatment were compared with ANOVA with Bonferroni correction for multiple comparisons. The difference between TH SDS and pre-treatment height SDS was compared to the difference between TH SDS and adult height/height at latest assessment by unpaired two-tailed Student's *t*-test. A *P* value of ≤ 0.05 was considered significant.

Results

Phenotypic details

Clinical and biochemical details are shown in Table 1. The mean height SDS of the subjects was -4.1 ± 0.95 (range: -1.7 to -5.9); mean IGF1 SDS was -2.8 ± 1.4 (range: -1.0 to -6.8); mean IGFBP-3 SDS was -3.0 ± 2.1 (range: -0.6 to -8.9); mean basal GH level was $11.9 \mu\text{g/L}$ (range: 0.1 – 19.3) and mean peak GH level was $32.9 \mu\text{g/L}$ (range: 10.0 to >40). Ten out of 20 (50%) patients had classical facial features of GHI (defined as mid-facial hypoplasia, depressed nasal bridge and prominent forehead (26)); 19/20 (95%) were from consanguineous families and 18/20 (90%) are of Pakistani origin. Consistent with the previous results, wide ranges of short stature and biochemical abnormalities are noted.

Variable phenotypic and biochemical features between and within kindreds

Patient A6 is related to the previously described highly consanguineous Pakistani family (A1–A5) (16, 18). Unlike the other family members, she had facial features of GHI with mid-facial hypoplasia, depressed nasal bridge and prominent forehead. Patients A2 and A5, from the same family, had similar or more severe degrees of short

Table 1 Clinical and auxological details of the patients with homozygous *GHR* pseudoexon (6Ψ) mutations.

Family	Patient	Age (years)	Sex	Height SDS	BMI SDS	Birth weight SDS	Target height SDS	Ethnicity/consanguinity	GHI classical facial features
A	1*	1.3	M	−1.7	−4.9	−0.2	−2.2	Pak/+	No
	2*	3.7	M	−5.9	−2.0	0.3	−2.2	Pak/+	No
	3*	8.3	M	−3.3	−0.4	NK	−1.6	Pak/+	No
	4*	3.8	M	−3.6	−0.5	−0.1	−1.6	Pak/+	No
	5*	1.2	F	−4.4	+1.8	0.7	−2.4	Pak/+	No
	6	2.5	F	−4.4	−0.1	−1.8	NK	Pak/+	Yes
B	1*	1.6	F	−5.6	−2.4	−1.4	−1.4	Pak/+	Yes
C	1*	NK	M	−5.0	NK	NK	NK	Palestine-Arab/+	Yes
D	1*	3.3	M	−4.9	0.1	NK	NK	Pak/+	No
	2*	8.1	M	−3.3	−2.4	−1.5	NK	Pak/+	No
E	1*	5.4	F	−3.5	0.02	NK	NK	Pak/+	No
	2*	NK	F	−4.0	NK	NK	NK	Pak/+	No
F	1	7.0	M	−4.2	−0.5	−0.5	−0.9	Pak/+	No
G	1	2.6	M	−3.8	−2.9	−2.9	−1.3	Pak/+	Yes
	2	3.7	F	−4.2	−0.9	0.1	0.7	Pak/+	Yes
H	1	5.7	M	−3.0	−0.7	0.7	−0.7	Pak/+	Yes
	2	1.5	F	−4.7	−1.2	NK	−0.7	Pak/+	Yes
I	1	2.3	F	−4.3	−1.7	−1.7	−1.6	Ind/−	Yes
J	1	5.3	F	−4.0	0.4	0.1	−1.6	Pak/+	Yes
K	1	4.3	F	−4.1	−0.2	−0.3	−0.9	Pak/+	Yes

Age and height SDS are at presentation; GHI facial features: frontal bossing, mid-facial hypoplasia.

*Patients previously reported (16, 18).

+, parents consanguineous; −, parents not consanguineous; Ind, Indian; NK, not known; Pak, Pakistani.

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stature (height SDS: -5.4 and -4.4 , respectively) but, lacked abnormal facial features. Patient B had a moderate clinical phenotype, height: -5.6 SDS but IGF1 SDS was only slightly subnormal (-2.3 SDS). Families G and H (2 pairs of siblings) showed more phenotypic variability with moderate short stature (height SDS: -3.4 to -4.7), relatively mild biochemical features (IGF1 SDS: -2.3 to -3.1) and variable peak GH (18 to >33 $\mu\text{g/L}$) but all had classical facial GHI features. Similarly, patients I and K had mild-to-moderate phenotypes and abnormal facial features. In contrast, families D and E (2 pairs of siblings) and patient F had moderate clinical and biochemical features, similar to patients A6, I and K but lacked facial abnormalities. Finally, patient J (distant cousin of A5) had typical GHI facial features and a severe biochemical phenotype but height was moderately low (height: -4.0 SDS).

IGF1 generation test (IGFGT)

Twelve out of 20 subjects underwent IGFGT (Table 2). Only 1 patient (D2) showed a response, with increase of IGF1 from 132 to 255 ng/mL. His height was -4.9 SDS, and he had normal facial features (Fig. 1).

Relationships between height and IGF1 and IGFBP-3

There was no positive correlation between height SDS and basal IGF1 SDS or between height SDS and IGFBP-3 SDS.

Responses to rhIGF1 therapy

15 out of 20 patients (75%; 11M) received rhIGF1 treatment. The mean age at initiation of rhIGF1 in all subjects was 9.0 ± 2.7 years (range: 5.7–15.3) and the mean duration of treatment was 5.3 ± 2.5 years (range: 1.5–7.6). The mean dose of rhIGF1 was 114 (range: 110–130) $\mu\text{g/kg}$ twice a day. 5 of 15 patients had received combination rhIGF1/IGFBP-3 therapy as part of a previous study (27). Of these 5 patients, in the first 5 years of treatment, 1 had >6 months interrupted rhIGF1 treatment between years 2 and 3, the rest had uninterrupted rhIGF1 therapy. 10 of 15 patients were treatment naïve. In this group, 5 patients had treatment gaps of >6 months between years 4 and 5 of therapy. Height outcomes were analysed at baseline ($n=15$), year 1 ($n=15$), year 2 ($n=14$) and year 3 ($n=10$) (Figs 2 and 3).

Mean cumulative height SDS change over 5 years of treatment was calculated in 9 patients (4 previously treated and 5 treatment naïve). 3 of 15 patients were pubertal at the start of rhIGF-I therapy and were concomitantly commenced on GnRH analogue therapy.

Change in HV during years 1, 2 and 3 of rhIGF1 therapy

Baseline mean HV was 4.7 ± 1.1 cm/year and increased to 7.4 ± 1.8 cm/year during the first year of treatment ($P=0.001$) (Fig. 2). The first year HV in the treatment-naïve patients ($n=10$) was 7.9 ± 1.6 cm/year, which was

Table 2 Biochemical details of patients with homozygous GHR pseudoexon (6Ψ) mutations.

Family	Patient	Basal GH ($\mu\text{g/L}$)	Peak GH ($\mu\text{g/L}$)	IGF1 SDS	IGFGT basal/peak (ng/mL)	IGFBP3 SDS
A	1*	11.0	10.0	-2.5	23.0/24.0	-6.0
	2*	6.0	14.3	-2.5	21.0/26.0	-8.9
	3*	1.8	53.3	-1.7	29.0/36.0	-2.9
	4*	17.5	90.0	-2.0	20.0/20.0	-3.4
	5*	0.1	18.8	-2.2	ND	-1.72
	6	3.4	26.7	NK	ND	ND
B	1*	13.0	>33.3	-2.3	6.9/7.6	-2.4
C	1*	0.6	NK	NK	NK	NK
D	1*	10.2	15.4	-2.3	36.0/41.0	-2.6
	2*	0.3	28.4	-0.7	132.0/255.0*	-1.6
E	1*	2.5	27.0	-1.0	ND	-2.3
	2*	8.3	37.7	-1.4	ND	-2.3
F	1	2.0	40.0	-2.5	41.2/29.7	-2.6
G	1	4.0	>33.0	-2.3	63.3/16.8	ND
	2	16.9	33.3	-2.5	ND	ND
H	1	17.5	90.0	-2.9	1.5/8.4	-2.4
	2	0.1	18.8	-3.1	ND	ND
I	1	3.4	26.7	-2.1	ND	ND
J	1	19.3	>40.0	-6.8	$<25.0/<25.0$	ND
K	1	0.6	NK	-4.0	$<22.9/<22.9$	-2.4

*Positive response during IGFGT.

IGFGT, IGF1 generation test; ND, not done; NK, not known.

Figure 1

Patient with homozygous *GHR* pseudoexon mutation and normal facial features. A patient with the homozygous *GHR* pseudoexon mutation but no dysmorphic facial features i.e. no frontal bossing or mid-facial hypoplasia.

comparable to HV in the previously treated group ($n=5$) (6.3 ± 1.9 cm/year; $P=0.12$). There was no significant correlation between year 1 mean HV or year 1 mean HV SDS with sex, age at rhIGF1 initiation, baseline height SDS, baseline BMI SDS or baseline IGF1 SDS.

Mean HV during the years 2 and 3 of rhIGF1 treatment were 5.6 ± 1.8 cm/year and 5.3 ± 1.9 cm/year, respectively. Although these values were above baseline, the difference was not significant ($P=0.11$ and 0.36 , respectively) (Fig. 2). In treatment-naïve group, there were also no significant differences in mean HV at year 2 and 3 compared to baseline.

Change in height SDS during years 1, 2 and 3 of rhIGF1 therapy

Mean height SDS at year 1 and year 2 of rhIGF1 therapy were -3.8 ± 0.9 and -3.4 ± 1.0 , respectively. These values were not significantly different from pre-treatment height SDS (-4.3 ± 0.8 , Fig. 3). In the treatment-naïve group, there were also no significant differences in height SDS at year 1

Figure 2

Height velocity at four different time points during treatment with rhIGF1. Box and whisker plots show the median, upper and lower quartiles and range; IQR, interquartile range; n , number of patients data available/included for each time point; P values calculated by ANOVA with Dunn–Bonferroni *post hoc* pairwise comparison; $*P=0.001$.

and 2 compared to baseline. Mean height SDS at year 3 of treatment (-3.2 ± 1.0) was, however, significantly higher than pre-treatment height SDS ($P=0.03$) (Fig. 3). In the naïve group, mean height SDS also increased significantly from -4.1 ± 0.8 at baseline to -2.9 ± 1.0 at year 3 ($P=0.01$). The mean cumulative change in height SDS at year 5 of continuous treatment in 9 treated patients was 1.4 ± 0.9 (range: 0.2 – 3.2).

Adult height (AH) at discontinuation of rhIGF1 therapy and height at latest assessment (LH) for patients with ongoing rhIGF1 therapy

12 (8M) of 15 treated patients have completed linear growth (AH). 7 of 12 were naïve to rhIGF1 therapy and 5 had received rhIGF1/IGFBP-3 therapy previously (27). The mean AH SDS was -3.3 ± 1.3 SDS (-5.7 to -1.8), compared to pre-treatment height SDS (-4.3 ± 0.9 SDS; -5.9 to -3.2) ($P=0.05$). Mean AH in the treatment-naïve group ($n=7$) was -3.1 ± 1.3 SDS (-5.7 to -1.8), and this was also higher than the pre-treatment mean height SDS -4.1 ± 0.9 SDS (-5.9 to -3.2) ($P=0.08$). The individual growth curves for 8 male and 4 female patients are shown in Fig. 4A and B, respectively.

In 3 of 15 patients who remained on rhIGF1 therapy (all naïve to rhIGF1, ages at latest assessment 9.2, 11.0 and

Figure 3

Height SDS at four different time points during treatment with rhIGF1. Box and whisker plots show the median, upper and lower quartiles and range; IQR, interquartile range; *n*, number of patients data available/included for each time point; *P* values calculated by ANOVA with Dunn–Bonferroni *post hoc* pairwise comparison; **P*=0.03.

12.3 years), LH was -3.1 ± 0.1 SDS (-3.2 to -3.0), and this was higher than pre-treatment height SDS -4.2 ± 0.6 SDS (-4.8 to -3.6) (*P*=0.03).

The difference between target height (TH) SDS and AH/LH SDS was less than that of TH SDS and pre-treatment height SDS (2.1 ± 1.2 vs 3.0 ± 0.8 ; *P*=0.02) (Fig. 5).

Heights in the untreated patients

In the 3 untreated patients, AH SDS was -3.5 and -5.0 and LH SDS (at age of 5.0 years) was -4.4 SDS.

Discussion

It is well established that GHR gene mutations cause a continuum of phenotypes, even within families with the same mutation (11, 29, 30). Our cohort of 20 patients with the rare intronic GHR pseudoexon mutation (6Ψ) provides further insights into the phenotypic variation of GHI caused by a single mutation. Consistent with the previous report (18), the spectrum of phenotypic variability is marked. The 6Ψ GHR mutation leads to aberrant splicing, resulting in an aberrant splice product of the GHR gene.

Figure 4

Individual growth curves for homozygous GHR pseudoexon mutation patients who have completed rhIGF-I therapy. (A) Individual growth and adult height data of 8 male patients, compared with the UK-WHO growth standards (21, 22, 23) (upper shaded area; mean represents the 50th centile; +2 s.d. represents the 91st centile; -2 s.d. represents the 2nd centile on the UK-WHO charts) and the mean $\pm$ 2 s.d. for height for untreated Laron syndrome patients (lower shaded area; represents reference range for patients with presumed GH receptor abnormalities (28)). (B) Individual growth and adult height data of 4 female patients, compared with the UK-WHO growth standards (21, 22, 23) (upper shaded area; mean represents the 50th centile; +2 s.d. represents the 91st centile; -2 s.d. represents the 2nd centile on the UK-WHO charts) and the mean $\pm$ 2 s.d. for height for untreated Laron syndrome patients (lower shaded area; represents reference range for patients with presumed GH receptor abnormalities (28)).

Figure 5

Difference between target height (TH) and heights pre- and post-treatment with rhIGF1. Box and whisker plot showing A: Difference between target height (TH) SDS and pre-treatment baseline height SDS and B: Difference between target height SDS and height SDS at final adult height (AH) or at latest assessment (LH) during treatment with rhIGF1 therapy. Box plots show the median, upper and lower quartiles and range; IQR, interquartile range; *P* values calculated by Student's unpaired *t*-test; **P*=0.02.

This splicing process is highly variable; hence, variable quantities of normal and abnormal transcripts will be generated. Gene transcript heterogeneity i.e. the ratio of abnormal (mutated GHR) to normal (wild-type GHR) proteins and the role of genetic and environmental factors in defining this ratio have been postulated to play a role in the clinical variability (16, 18). However, this needs to be further explored in 6Ψ patients with a range of phenotypes to establish whether patients with more severe phenotypes have relatively more mutant protein transcript.

The characteristic facial features seen in severe GHI, namely, mid-facial hypoplasia and prominent forehead, reflect the underdevelopment of the facial bones secondary to IGF1 deficiency (12, 31). As such, it has been proposed that the degree of craniofacial changes are likely to be more prominent in patients with more severe short stature and/or a greater degree of IGF1 deficiency (31, 32). However, in our cohort, the presence or absence of abnormal facial features did not correlate with either the degree of short stature or the biochemical abnormalities.

Previous studies have shown that serum IGF1 and IGFBP-3 levels correlate with height SDS values in patients with *GHR* mutations causing severe GHI i.e. the more severe the IGF1 deficiency (IGFD), the more severe the height deficiency (29). The mismatch between clinical phenotype (i.e. degree of short stature) and the biochemical deficiency (IGF1 SDS) in our cohort is striking. IGF1 levels were measured at the 6 referral centres; hence, several different IGF1 assays were used. However, taking this limitation into account, many of the most severely affected patients (height SDS –4.0 to –5.9) have IGF1 SDS values, which are in the normal range or mildly reduced (–2.9 to –1.4). The reason for this discrepancy is unclear but may be a result of additive molecular defects in other proteins downstream from the *GHR* resulting in a greater degree of short stature e.g. the IGF1 receptor or signalling molecules of RAS-MAP kinase pathway and/or the PI3-K/Akt pathway. Other genetic and/or environmental factors involved in the *GHR* processing, trafficking and receptor degradation pathways may also be implicated (18). The use of different, rather than standardized/centralized IGF-1 assays, may also contribute to the observed discrepancy.

The majority of reported patients with *GHR* 6Ψ mutations are of Pakistani origin and previous work by our group suggests the presence of a common ancestor (18). Although most of the families were reportedly unrelated, patients J1 and A5 were distant cousins.

Response to rhIGF1 therapy has not been previously assessed in patients with 6Ψ *GHR* mutations. Given that a number of patients in our cohort had a mild degree of IGF1 deficiency, it is tempting to speculate that the response to rhIGF1 therapy would be sub-optimal. However, the first year growth response, demonstrated by the significant increase in height velocity (baseline HV: 4.7 ± 1.1 cm/year and year 1 HV: 7.4 ± 1.8 cm/year) in our patients, was comparable to that reported in patients with other homozygous *GHR* defects (baseline HV: 4.7 ± 1.3 cm/year and year 1 HV: 8.2 ± 0.8 m/year) (33) and other patients with severe IGF1 deficiency (baseline values: 2.8–4.0 cm/year and year 1 HV: 7.4–8.5 cm/year) (34, 35, 36, 37). Contrary to reported data from a large European cohort of patients on rhIGF1 (38), the increase in 1st year HV in our cohort did not correlate with age of rhIGF1 initiation or lower baseline height SDS. Furthermore, similar to other studies (34, 35), the growth-promoting effects of rhIGF1 appeared to persist, as there was a significant improvement in height SDS at year 3 of treatment. The mean change in height SDS in our cohort following 5 years of treatment was 1.4 ± 0.9 and is comparable to another

published study of patients with GHI (mean change 1.4 after 6 years of therapy) (36). Similar to other studies (34, 35), our patients who had completed rhIGF1 therapy, did not achieve adult heights in the normal range. However, the AH was higher than the pre-treatment height SDS and indicates a positive effect of rhIGF1 on growth outcome (34). Overall, the effect of rhIGF1 therapy on height outcomes in our cohort was encouraging.

Only one subject, D2, responded during the IGFGT. His height was -4.9 SDS, and he had normal facial features. Although he was treated with rhIGF1 therapy, data on his clinical course and response to treatment were unavailable; hence, he was not included in the 15 treated patients described in this manuscript.

In summary, the homozygous intronic 6Ψ *GHR* mutation caused both severe and mild GHI phenotypes, even in individuals within the same kindred. The presence or absence of abnormal facial features did not correlate with either the degree of short stature or the biochemical abnormalities. There was often a mismatch between the clinical and biochemical features in individual patients. rhIGF1 treatment improved long-term height outcomes as has been demonstrated in GHI patients with other homozygous *GHR* mutations and primary IGF1 deficiency.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

S C, S J R, T M, P E C, S B T, A B, U K, R D and H L S contributed to patient recruitment, data collection and analysis. L S and L A M performed the genetic analysis. S C performed phenotypic and statistical analyses. S C wrote the manuscript with input from M O S and H L S.

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