The growth response to GH treatment is greater in patients with *SHOX* enhancer deletions compared to *SHOX* defects

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Abstract

Objective: Short stature caused by point mutations or deletions of the short stature homeobox (*SHOX*) gene (*SHOX* haploinsufficiency (SHI)) is a registered indication for GH treatment. Patients with a *SHOX* enhancer deletion (SED) have a similar phenotype, but their response to GH is unknown. It is uncertain if duplications of *SHOX* or its enhancer (SDUP) cause short stature. This study aimed to describe the clinical characteristics and growth response to GH treatment in patients with aberrations of *SHOX* and its enhancers.

Design: In this retrospective multi-center study (2002–March 2014) clinical information was available from 130 patients (72 SHI, 44 SED, and 14 SDUP) of whom 52 patients were treated with GH. We evaluated height, sitting height (SH), arm span, dysmorphic features and indicators of the growth response to GH (delta height SDS, height velocity, and index of responsiveness).

Results: Patients with SEDs showed similar HtSDS to patients with SHI (−2.3 and −2.6, respectively, $P=0.2$), but they were less disproportionate (SH/height ratio SDS 2.0 vs 3.1 ($P<0.01$) and extremities/trunk ratio 2.57 vs 2.43 ($P=0.03$)). The 1st year growth response to GH treatment was significantly greater in prepubertal patients with SEDs than SHI. None of the patients with an SDUP was disproportionate and SDUP cosegregated poorly with short stature; their growth response to GH treatment ($n=3$) was similar to the other groups.

Conclusions: Patients with SEDs are equally short, but less disproportionate than patients with SHI, and show a greater response to GH.

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Introduction

The short stature homeobox (*SHOX*) gene encodes a homeodomain protein that acts as a transcriptional activator (1). It is strongly expressed in the developing limb and is thought to contribute to longitudinal growth by regulating proliferation and differentiation of the chondrocytes in the growth plate (1). The gene is located on the short arm of the X- and Y-chromosomes in the pseudoautosomal

region 1 and escapes X-inactivation, which leads to a pseudoautosomal inheritance pattern (2, 3, 4). A homozygous deletion or point mutation of *SHOX* results in an extreme phenotype of osteodysplasia called Langer syndrome. The classical presentation of *SHOX* haploinsufficiency (SHI), due to heterozygous *SHOX* defects, is Léri–Weill dyschondrosteosis (LWD), characterized by the

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typical clinical triad of short stature, mesomelia and Madelung deformity. However, *SHOX* defects can also be found in short children without dysmorphic features and normal body proportions, who had initially been considered as having idiopathic short stature.

Recent studies have shown that deletions in the downstream enhancer region of *SHOX*, and (less frequently) in the upstream enhancer region (*SHOX* enhancer deletions (SEDs)), can result in a clinical picture similar to that of SHI (5, 6, 7, 8, 9, 10). In about two-thirds of cases LWD is caused by intragenic point mutations or deletions of the complete coding sequence of *SHOX*, and in one-third of cases by deletions in the enhancer sequences in the 3'- or 5'-flanking region of *SHOX*, leaving the gene itself intact (11).

A duplication of *SHOX* would be expected to be associated with tall stature. The relatively tall height of individuals with Klinefelter syndrome and XXX syndrome is indeed thought to be caused by increased *SHOX* expression. Surprisingly, duplications of *SHOX* and/or its enhancers (SDUPs) have also been found in short children (12, 13, 14), although only in a few of them additional clinical features characteristic for *SHOX* dysfunction were noted, and parents carrying the duplication were usually of normal stature. Therefore, the pathogenicity of SDUPs is still uncertain (12, 13, 14, 15).

Growth hormone (GH) treatment is registered for children with short stature caused by SHI, currently defined as a deletion or point mutation in *SHOX* itself, based on studies showing a significant increase in height SDS (HtSDS) during the first 2 years of treatment (16, 17). The effect of GH treatment, however, had not yet been evaluated for patients with an SED or SDUP (17).

The principal aim of this study is to investigate whether the effect of GH treatment in children with an SED is comparable to that in children with SHI. Because of the phenotypic similarity between the two groups (2, 10), we hypothesized that the effect of GH treatment in children with a deletion of the downstream or upstream enhancer region of *SHOX* would be similar to the effect in children with a *SHOX* point mutation or deletion. In addition, the clinical characteristics of patients with point mutations or deletions of the *SHOX* gene, deletions of the *SHOX* enhancer regions, or duplications of *SHOX* or its enhancer were investigated.

Subjects and methods

Study design

In this retrospective study from 2002 onwards, patients were included who were diagnosed with SHI, SEDs, and

SDUPs in the Laboratories for Diagnostic Genome Analysis of the Departments of Clinical Genetics of the Leiden University Medical Center (LUMC, Leiden), VU University Medical Center (VUMC, Amsterdam), Erasmus Medical Center (EMC, Rotterdam), and University Medical Center Groningen (UMCG, Groningen) in The Netherlands.

Deletions and duplications of *SHOX* and its enhancer regions were diagnosed with multiplex ligation probe amplification (MLPA) with the P018 kit from MRC-Holland (Amsterdam, The Netherlands) using standard procedures. Most deletions and duplications were characterized with the P018-D1 MLPA kit but other versions of the kit had been used as well. A graphical picture of the position of the various probes is shown in [Supplementary Figure 1](#), see section on [supplementary data](#) given at the end of this article. If a deletion of the *SHOX* gene was detected, Sanger sequencing was not performed. If MLPA analysis revealed no deletions in the *SHOX* gene or if it revealed a deletion of the *SHOX* enhancer region, Sanger sequence analysis of the complete coding region of *SHOX*, including intron–exon boundaries, was performed (18). The *SHOXa* isoform was sequenced using the Ensembl transcript 'ENST00000381575' (NM_000451.3).

In general, patients with short stature were referred for DNA analysis by pediatric endocrinologists or clinical geneticists. After approval from the Medical Ethical Committee of the Leiden University Medical Center, patients diagnosed with an aberration of *SHOX* or its enhancer region were asked to participate in this study. Written informed consent was obtained from the participating subjects or from the subjects' parents.

Participants

Until March 2014, 390 patients were diagnosed with defects of *SHOX* or its enhancer regions ([Fig. 1](#)). Patients living in other countries than The Netherlands ($n=23$) and patients in whom the *SHOX* variants were considered non-pathogenic ($n=27$) were not asked to participate in the study. Eight patients were not included because the treating physicians were reluctant to ask the patients and their parents for permission, and another eight patients were diagnosed with other disorders also contributing to short stature: multiple epiphyseal dysplasia ($n=2$), Smith Magenis syndrome ($n=1$), insulin-like growth factor 1 (*IGF1*) mutation ($n=1$), Albright's hereditary osteodystrophy ($n=1$), an additional deletion of chromosome 6 ($n=1$), bio-inactive GH ($n=1$), and an isodicentric Y-chromosome ($n=1$). The affected parents of these patients were excluded as well ($n=4$).

Figure 1

Flow chart of study design and patient distribution.

The remaining 320 patients were asked to participate in this study. At closure of the database, we had received 157 informed consent forms and collected clinical information from 130 patients (88 probands, from 74 families). Fifty-six patients from 47 families were treated with a variety of commercially available recombinant GH products.

Clinical assessment

Clinical data from the referring hospital and the Dutch National Registry for GH Treatment in children located at the Dutch Growth Research Foundation ('Stichting Kind en Groei', Rotterdam, The Netherlands) were collected from all patients who provided informed consent. These included: birth length, birth weight, head circumference at birth, height, weight, sitting height (SH), arm span, forearm length, dysmorphic signs (Madelung deformity, cubitus valgus, bowing of the radius and ulna or muscular hypertrophy), serum IGF1 and IGFBP3, and skeletal age. Information about linear growth, serum IGF1, IGFBP3, and skeletal age, was collected every 12 ± 3 months up to 4 years for patients treated with GH. From

the parents, height, body proportions, and *SHOX* test results were obtained.

Patient analysis

Patients were divided into three groups: group 1 consisted of patients with SHI; group 2 of patients with a SED; and group 3 of patients with an SDUP. Subgroups of each group were treated with GH (Fig. 1). For all patients, we analyzed the available clinical data and compared the clinical data of probands to those of their parents.

To assess the effect of GH therapy in patients with a deletion in the *SHOX* enhancer region and patients with a *SHOX* defect, we used four outcome measures for the first 4 years of treatment. The first three of these, the difference in height SDS (Δ HtSDS), height velocity (HV, cm/year) and height velocity as SDS (HVSIDS) for age (based on the Swiss longitudinal growth study (19)), are parameters of the growth response. The fourth measure is the 'index of responsiveness (IoR)', based on prediction models for growth during the first 4 years of GH treatment in girls with Turner syndrome (20). We chose this approach because the growth response to GH treatment is reported

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as being similar for patients with *SHOX* defects and patients with Turner syndrome (21). Because previous studies have shown that prepubertal growth is largely independent of sex, we applied the prediction models for Turner syndrome in males and females (22). For each patient, the predicted growth velocity (based on clinical predictors of the growth response) as described by Ranke *et al.* (20, 23, 24) was subtracted from the observed height velocity and transformed into a studentized residual. A studentized residual above 0 is an indicator of a relatively high responsiveness to GH treatment, adjusted for potential confounders. Strictly speaking, the prediction formulas for Turner syndrome are not applicable for children who undergo spontaneous puberty. However, since prepubertal children only represented a relatively small part of the total number of patients treated with GH, we also applied the formulas to all patients, irrespective of their pubertal status, and compared the results between groups 1 and 2.

Secondary outcome measures included the increase in bone age/calendar age ratio during GH therapy ($\Delta\text{BA}/\Delta\text{CA}$ ratio), the increase in serum IGF1 SDS during GH treatment (ΔIGF1) and the effect of GH on body proportions (the change in SH/height SDS, $\Delta\text{SH}/\text{height SDS}$). Skeletal age, serum IGF1, and SH/height SDS closest to the start of GH were compared to the last measured BA, serum IGF1 and SH/height SDS. All analyses were carried out separately for patients who remained prepubertal during the observation period and for all patients. Results for prepubertal children were compared to published data on the growth response to GH treatment in patients with SHI. In this study by Blum *et al.* (21), the percentage of *SHOX* deletions and mutations was similar to our study, as was the GH dose.

Statistical analysis

HtSDS and SH/height SDS were calculated based on Dutch nation-wide references (25, 26). Height velocity SDS was calculated based on the Swiss longitudinal study (19). Midparental height adjusted for gender, assortative mating, and parent-offspring correlations, adapted to the most recent Dutch population reference (26), was expressed as SDS (conditional target HtSDS) (27). BMI SDS was calculated based on the 1980 Dutch nation-wide growth study (28). Birth weight and birth length for gestational age were transformed to SDS values using the standards of Niklasson *et al.* (29). IGF1 and IGFBP3 data were collected as measured by local laboratories. Since the IGF1 assays in The Netherlands have been harmonized and titrated on the original assay used for preparing reference data (30), levels were expressed as SDS. BA of

patients was analyzed as reported by the treating physicians. The extremities/trunk ratio, which compares extremities length to trunk length, was calculated as follows: (calculated subischial length + arm span)/SH (31).

To calculate the predicted height velocity, according to the prediction model for Turner syndrome, height and weight were converted to SDS using the height standards of Prader *et al.* (19) and the weight standards of Freeman *et al.* (32), and mid-parental height was calculated as (father's HtSDS + mother's HtSDS)/1.61 (33).

Data were expressed as means and s.d. and compared between groups with the Student's *t*-test for continuous variables with a Gaussian distribution and χ^2 test for categorical variables. If continuous variables were not normally distributed the Mann-Whitney *U* test was used. Significance was considered at the 5% level ($P < 0.05$).

Results

Of the 88 probands (from 74 families), 55 (from 42 families) inherited the mutation from one of the parents, 26 from their father (47%) and 29 from their mother (53%). Seven patients had a *de novo* defect, and for the remaining cases ($n=26$) no information was available.

Mutations of *SHOX* and its enhancer

Of the 88 probands, 54 were diagnosed with a point mutation ($n=11$, from ten families) or deletion ($n=43$, from 35 families) of *SHOX* (SHI, group 1). Growth data were similar, except for a slight but statistically significant difference in HtSDS (-3.0 for *SHOX* point mutations, -2.5 for *SHOX* deletions, $P=0.04$). There was no statistically significant correlation between HtSDS of the index cases and that of the affected or unaffected parents. In order to increase statistical power, we clustered both types of SHI in one group.

There were 26 patients (from 22 families) with a deletion of the *SHOX* downstream or upstream enhancer region (SED, group 2). Table 1 shows the clinical characteristics at first visit of groups 1 and 2. Children from group 2 were less disproportionate than children from group 1 (SH/height SDS 2.0 vs 3.1, $P < 0.01$ and extremities/trunk ratio 2.57 vs 2.43, $P=0.03$). Madelung deformity was present in 23 out of 74 individuals (31%), 17 in group 1 and six in group 2 ($P=0.11$). Information on other dysmorphic features, such as cubitus valgus, bowing of the forearm and muscular hypertrophy were often not reported and could therefore not be compared between groups.

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Table 1 Clinical characteristics at first visit in patients with *SHOX* mutations and deletions and *SHOX* enhancer deletions, mean (s.d.).

Clinical characteristics	n	All patients	Range	n	<i>SHOX</i> mutations or deletions	n	<i>SHOX</i> upstream and downstream enhancer deletions	P*
Age at first visit (years)	80	8.35 (3.6)	1.2; 16.2	54	8.3 (3.5)	26	8.5 (3.7)	0.839
Male/female		34/46			23/31		11/15	0.981
Birth weight SDS	66	−0.4 (1.3)	−3.3; 3.2	43	−0.4 (1.3)	23	−0.3 (1.3)	0.728
Birth length SDS	37	−1.1 (1.2)	−4.3; 1.5	23	−1.0 (1.2)	14	−1.1 (1.4)	0.823
Height SDS	80	−2.5 (0.8)	−4.4; −0.3	54	−2.6 (0.8)	26	−2.3 (0.8)	0.111
Conditional target height SDS	74	−1.0 (0.6)	−2.9; 0.7	50	−1.0 (0.6)	24	−0.9 (0.5)	0.647
Sitting height/height ratio SDS	72	2.8 (1.3)	−0.1; 5.5	50	3.2 (1.1)	22	1.9 (1.3)	<0.01
Armspan/height ratio	33	0.95 (0.03)	0.87; 1.01	21	0.95 (0.03)	12	0.96 (0.03)	0.365
BMI SDS	76	0.4 (1.0)	−2.0; 2.7	51	0.5 (0.9)	25	0.1 (1.1)	0.069
Extremities/trunk ratio ^a	32	2.48 (0.2)	2.06; 2.80	21	2.43 (0.2)	11	2.57 (0.2)	0.028
Comparison to parents' phenotype:								
Height SDS affected parent	49	−2.2 (0.9)	−4.8; −0.3	28	−2.4 (0.9)	21	−1.9 (0.9)	0.032
Sitting height/height ratio SDS affected parent	23	2.7 (1.7)	0.5; 6.1	9	3.3 (1.4)	14	2.3 (1.8)	0.168
Height SDS – TH SDS (<i>de novo</i> mutations)	7	−1.9 (0.7)	−3.2; −1.1	6	−1.9 (0.7)	1	−2.1	
Height SDS – height SDS affected parent	46	−0.3 (1.1)	−2.2; 2.6	25	−0.2 (0.9)	21	−0.5 (1.3)	0.419
Height SDS – height SDS unaffected parent	43	−1.7 (0.9)	−3.5; 0.2	24	−1.7 (0.9)	19	−1.6 (1.0)	0.579

*P value from t-test comparing patients with *SHOX* mutations and deletions to patients with *SHOX* upstream and downstream enhancer deletions.

^aBinders' extremities/trunk ratio compares the extremities length to the trunk length and is calculated as follows: (calculated subischial length + arm span) / sitting height. The normal values are dependent on height. A lower score indicates more severe disproportionate stature.

HtSDS of patients with a *de novo* mutation was compared to the conditional target HtSDS. In case of a familial mutation, HtSDS was compared to HtSDS of the affected and the unaffected parent (Table 1). As expected, in both groups the proband's HtSDS was closer to HtSDS of the parent carrying the *SHOX* aberration than to the other parent's HtSDS. Affected parents in group 2 were significantly less short than affected parents in group 1 (−1.9 vs −2.4, $P=0.03$).

Duplications of *SHOX* and its enhancer

The clinical features of the eight patients (from seven families) with duplications of *SHOX* or its enhancer (group 3) are shown in Table 2. In none of them was SH/height SDS above +2 SDS, and only one parent carrying the duplication was short. The duplicated *SHOX* probes of these patients are shown in Supplementary Figure 1.

Growth response to GH

From the 56 probands who were treated with biosynthetic GH, we obtained clinical information during GH

treatment about 37 patients from group 1 (66%), 12 patients from group 2 (22%), and three patients from group 3 (5%) (Fig. 1). Table 3 shows the baseline data and growth response for patients from groups 1 and 2 who remained prepubertal. For comparing HV and IoR between groups 1 and 2, we only included patients of whom HV before start of GH was available. Except for a small, but statistically significant, difference in HV before start of GH treatment (5.0 for group 1 and 6.1 for group 2, $P=0.02$), there were no significant differences between the two groups at start of GH. In the 1st year, all three parameters of growth response, Δ HtSDS, HV, and HV SDS were significantly greater in group 2. The difference between the two groups for the IoR was just above the level of statistical significance (Table 3). A graphical representation of these data is shown in Figs 2 and 3, showing HtSDS and HV in response to GH treatment for four cohorts of children who remained prepubertal during GH treatment for 1–4 years, in comparison to data reported by Blum *et al.* (16). In both groups the growth response was greatest in the 1st year of GH treatment and declined thereafter.

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Table 2 Clinical characteristics of patients with *SHOX* or *SHOX* enhancer duplications at first visit ($n=8$). The size of the duplications are shown in [Supplementary Figure 1](#).

Clinical characteristics	1 ^a	2	3	4	5 ^{b,c}	6 ^{b,c}	7 ^b	8
Age at first visit (years)	5.6	3.3	11.9	4.5	9.2	5.5	3.8	3.2
Male (M)/female (F)	F	F	F	F	M	M	M	F
Birth weight SDS	–	–	0.22	–1.7	–0.5	–0.5	–1.0	–3.6
Birth length SDS	–	–	0.9	–1.1	–	–	–	–4.8
Height SDS	–2.4	–3.5	–2.4	–2.4	–2.2	–2.7	–3.4	–2.9
Conditional target height SDS	–	–0.9	–0.8	–1.7	–1.1	–1.1	–1.3	0.2
Sitting height/height ratio SDS	1.5	–	1.8	–	0.7	1.3	1.9	0.8
Armspan/height ratio	–	–	0.94	–	–	–	–	–
BMI SDS	0.6	0.2	–1.2	–1.9	–1.5	–1.1	–1.3	0
IGF1 SDS	0.8	–	–2.7	–	–1.4	–2.1	–0.3	–
IGFBP3 SDS	0.6	–	–0.1	–	–2.7	–4.3	–3.5	–
Parents' phenotype:								
Carrier parent								
Height SDS	–	1.0	–0.4	–1.5	–1.4	–1.4	–2.5	1.2
Sitting height/height ratio SDS	–	–	–	3.1	–	–	1.4	–
Unaffected parent								
Height SDS	–	–3.5	–1.5	–3.0	–1.6	–1.6	–1.0	–0.5
Sitting height/height ratio SDS	–	–	3.2	1.8	–	–	1.5	–
Growth response and responsiveness								
First year of GH								
Δ Height SDS	–	–	–	–	1.0	0.7	0.8	–
HV (cm/year)	–	–	–	–	10.5	8.7	9.6	–
IoR	–	–	–	–	2.9	0.8	1.0	–

^aAdopted from Taiwan, therefore clinical information concerning birth and height and proportions of the parents is missing.

^bPatients treated with GH.

^cPatients 5 and 6 are siblings.

For all patients from groups 1 and 2, irrespective of pubertal status during treatment, numerical data are shown in [Supplementary Table 1](#), see section on [supplementary data](#) given at the end of this article, and graphical representations in [Supplementary Figures 2 and 3](#). All parameters of the growth response and responsiveness in the 1st year of GH treatment were significantly greater for group 2 compared with group 1.

Only three patients from group 3 (SDUP), all pre-pubertal, were treated with GH, and their response is shown in [Table 2](#). In the 1st year of GH treatment, these patients gained on average 0.8 SDS in height and achieved a HV of 9.6 cm in 1 year, which is similar to patients from groups 1 and 2. Because of the uncertainty about the pathogenicity of these duplications, and the small number of patients, no further statistical analysis was carried out.

Effect of GH on serum IGF1, skeletal maturation, and body proportions

Serum IGF1 SDS (mean $\pm$ s.d.) increased similarly in groups 1 and 2 (2.4 ± 1.0 , $n=29$ and 2.4 ± 0.9 , $n=9$ respectively). Baseline median IGF1 was -0.4 SDS (range -3.3 to 0.9 SDS)

and on treatment median (s.d.) serum IGF1 was 1.9 (range 0 to 2.9 SDS), after a median treatment duration of 2.7 (0.2–5) years. The ratio between BA and CA increased by 0.1 in both groups over a median duration of 2.7 years (range 1–5 years after start GH treatment), and body proportions did not significantly change during treatment (Δ SH/height ratio SDS of 0.4 and -0.1 in groups 1 and 2, respectively). There were no significant differences between the two groups.

Discussion

Children with a deletion of the *SHOX* enhancer region are equally short, but less disproportionate than patients with a *SHOX* deletion or point mutation and have a similar frequency of Madelung deformity. Their parents carrying the variant are less short and disproportionate than parents carrying a *SHOX* deletion or point mutation. For children who remained prepubertal during GH treatment, the growth response was slightly, but statistically significantly, greater in patients with SEDs, even though the serum IGF1 response was similar for both groups. The growth response of patients with SHI was similar to that reported previously (16).

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Table 3 Clinical characteristics at start of GH and growth response to GH in prepubertal patients with *SHOX* mutations and deletions and *SHOX* enhancer deletions, mean (s.d.).

Clinical characteristics	n	All patients	Range	n	<i>SHOX</i> mutations and deletions	n	<i>SHOX</i> upstream and downstream enhancer deletions	P*
At start of GH								
Age (years)	33	8.07 (2.3)	4.0; 12.1	25	8.21 (2.2)	8	7.65 (2.7)	0.585
Male/female		13/20			8/17		5/3	0.130
Height SDS	33	-2.8 (0.7)	-4.3; -1.8	25	-2.9 (0.7)	8	-2.6 (0.6)	0.227
HV year before GH (cm/year)	28	5.4 (1.1)	3.1; 7.2	20	5.1 (1.0)	8	6.1 (0.8)	0.042
HV SDS before GH	28	-0.5 (1.0)	-2.8; 1.2	20	-0.7 (1.0)	8	0.0 (0.8)	0.075
BMI SDS	33	0.6 (0.9)	-1.4; 2.7	25	0.7 (1.0)	8	0.4 (0.7)	0.581
IGF1 SDS	29	-1.0 (1.1)	-3.3; 0.7	22	-0.9 (1.2)	7	-1.5 (0.5)	0.053
IGFBP3 SDS	9	0.1 (0.8)	-1.6; 1.1	5	-0.2 (0.9)	4	0.4 (0.7)	0.327
BA/CA ratio	21	0.9 (0.1)	0.5; 1.1	15	0.9 (0.1)	6	0.8 (0.2)	0.099
GH dose (mg/m ² per day)	33	1.24 (0.2)	0.6; 2.0	25	1.27 (0.2)	8	1.14 (0.2)	0.179
GH dose (μg/kg per day)	33	48.0 (9.4)	28.6; 77.2	25	49.1 (10.0)	8	44.7 (6.5)	0.208
After 1 year of GH therapy								
HV (cm/year)	28	9.2 (1.3)	7.0; 12.8	20	8.9 (1.0)	8	10.2 (1.6)	0.028
HV SDS	28	3.5 (1.9)	0.2; 8.6	20	3.1 (1.6)	8	4.6 (2.3)	0.048
Δ Height SDS	33	0.6 (0.2)	0.2; 1.2	25	0.6 (0.2)	8	0.8 (0.3)	0.036
IoR	28	0.9 (0.9)	-0.8; 3.2	20	0.6 (0.7)	8	1.5 (1.0)	0.053
After 2 years of GH therapy								
HV (cm/year)	19	7.9 (1.9)	4.4; 14.1	13	7.7 (0.9)	6	8.3 (3.2)	0.537
HV SDS	19	2.5 (2.1)	-1.2; 9.4	13	2.2 (1.0)	6	3.1 (3.6)	0.396
Δ Height SDS	23	0.3 (0.2)	-0.3; 0.6	17	0.3 (0.1)	6	0.3 (0.3)	0.731
IoR	19	0.9 (1.6)	-1.8; 6.2	13	0.7 (0.8)	6	1.2 (2.7)	0.930
After 3 years of GH therapy								
HV (cm/year)	14	6.9 (0.9)	5.3; 8.2	9	6.7 (0.9)	5	7.2 (0.7)	0.292
HV SDS	14	1.6 (1.1)	0.1; 3.6	9	1.5 (1.1)	5	1.7 (1.3)	0.739
Δ Height SDS	15	0.3 (0.2)	0; 0.5	10	0.2 (0.2)	5	0.3 (0.2)	0.635
IoR	14	0.6 (0.9)	-1.3; 1.6	9	0.5 (0.8)	5	0.8 (1.2)	0.463
After 4 years of GH therapy								
HV (cm/year)	11	6.2 (0.9)	4.7; 7.7	8	6.0 (0.9)	3	7.0 (0.7)	0.066
HV SDS	11	0.8 (0.8)	-0.2; 2.8	8	0.5 (0.4)	3	1.7 (0.9)	0.014
Δ Height SDS	11	0.2 (0.2)	-0.1; 0.6	8	0.1 (0.1)	3	0.3 (0.2)	0.074
IoR	11	0.5 (0.9)	-0.9; 1.8	8	0.3 (0.9)	3	0.9 (0.7)	0.414

*P value from t-test comparing patients with *SHOX* mutations and deletions to patients with *SHOX* upstream and downstream enhancer deletions.

The results of genetic testing in our patients, showing deletions of *SHOX* and its enhancer in 35 and 22 out of 74 families (47 and 30% respectively), far more than *SHOX* point mutations (10/74 families, 14%), demonstrates the efficiency of our stepwise diagnostic approach, consisting of an MLPA for the detection of copy number variants followed by Sanger sequencing. Deletions of *SHOX* or its enhancer region were found in around 80% of patients in other reports as well (34, 35).

Our findings confirm those of previous reports (5, 6, 10) that patients with SEDs show a similar degree of short stature compared to patients with SHI. We also confirm the remarkable heterogeneity of statural growth in carriers of *SHOX* defects both in probands and affected parents, as well as in carriers of SEDs. Similarly to body stature, as well as for body proportions, the variability was wide for

both groups. Patients with SEDs were on average less disproportionate than patients with SHI if the SH/height ratio and the extremities/trunk ratio were taken as criteria, but the arm span/height ratio was not different. Consistent with an earlier report (35), patients with SEDs also had a slightly lower BMI than patients with SHI, although mean BMI SDS in both groups was close to average for the population. These observations suggest that the predictive value of a BMI above the mean as part of the clinical score developed by Rappold *et al.* (36) may have been overestimated and that normal body proportions should not be considered a contraindication for *SHOX* testing.

While duplications of *SHOX* have been reported to be associated with normal to tall stature (37, 38, 39), there are also reports on a possible association with short stature (12, 13, 14). In one of these reports, it was hypothesized

Figure 2

Mean height SDS in children of groups 1 and 2 who remained prepubertal during GH treatment in four cohorts (followed for 1–4 years). The dotted lines with circles represent group 1, the lines with squares group 2, and the dashed lines with triangles represent data as reported by Blum *et al.* (16). One year of GH treatment (A): group 1 ($n=25$) and group 2 ($n=8$). Two years of GH treatment (B): group 1 ($n=17$) and group 2 ($n=6$). Three years of GH treatment (C): group 1 ($n=10$) and group 2 ($n=5$). Four years of GH treatment (D): group 1 ($n=8$) and group 2 ($n=3$).

that patients with smaller duplications may be more severely affected than patients with larger duplications (13). In another report (12), it was speculated that patients with duplications which included the *SHOX* enhancer could impair gene regulation by interfering with the three dimensional chromatin spatial organization, resulting in impaired contact between the enhancer and promoter (40). The phenotype of these patients is quite variable (13, 39), and the clinical characteristics of patients with duplications of only the *SHOX* downstream enhancer region have not been described so far. Based on these reports, we tried to collect as much as possible clinical information about the children and their parents with *SHOX* duplications ($n=5$) and duplications of the enhancer region ($n=9$) (Table 2). HtSDS in our patients ranged from -3.5 to -2.2 , and SH/height SDS from 0.7 to 1.9 . HtSDS of parents carrying the duplication varied from -2.5 to $+1.2$ SDS and in unaffected parents from -3.5 to -0.5 . In some patients a causal association between the *SHOX* duplication and the child's short stature appeared unlikely, for example, in the child born severely small for

gestational age (SGA) with normal body proportions and a normally statured parent carrying a small duplication (Table 2, case 8). Even though the absence of body disproportion might be partially explained by the young age of the majority of these children, the observation that body disproportion is absent in all children, and the absence of clinical features in their parents, cast doubt on the pathogenicity of these genetic variants.

This is the first study assessing the effect of GH treatment in patients with SEDs in comparison with SHI. The growth response in patients with SHI was similar to previously reported data (16), but the conventional outcome measures of the 1st year growth response (height velocity, height velocity SDS, and change in HtSDS) showed a greater effect in prepubertal patients with SEDs. The GH dose between the two groups was similar, and the recommended dose for SHI as reported by Blum *et al.* (16), seems efficacious for patients with *SHOX* defects as well as enhancer deletions. Since the growth response in patients

Figure 3

Mean height velocities in children of groups 1 and 2 who remained prepubertal during GH treatment in four cohorts (followed for 1–4 years). The dotted lines with circles represent group 1, the lines with squares group 2, and the dashed lines with triangles represent data as reported by Blum *et al.* (16). One year of GH treatment (A): group 1 ($n=20$) and group 2 ($n=8$). Two years of GH treatment (B): group 1 ($n=13$) and group 2 ($n=6$). Three years of GH treatment (C): group 1 ($n=9$) and group 2 ($n=5$). Four years of GH treatment (D): group 1 ($n=8$) and group 2 ($n=3$). Statistically significant differences are indicated with an asterisk.

with SHI has been reported as similar to that of girls with Turner syndrome, we used the prediction models for Turner syndrome (20) to calculate the IoR. The IoR in group 2 was slightly higher than the IoR in group 1, but this difference was not significantly different in prepubertal children. The IoR was, however, significantly different if all patients were included, regardless of their pubertal status. The reason why children with SEDs seem to respond slightly greater to GH treatment remains unclear. We speculate that if GH, via downstream GH-dependent transcription factors, promotes expression of *SHOX*, the presence of two intact (functional) copies of *SHOX* in SED may cause a higher responsiveness to GH. Another possible explanation for the higher responsiveness to GH could be that *SHOX* deficiency is less severe in the presence of enhancer deletions. This hypothesis would explain the lower degree of skeletal disproportion and Madelung deformity observed, but not the same degree of short stature.

This study has several limitations. First, there may be an ascertainment bias towards patients with typical clinical features of LWS, because screening for *SHOX* deficiency is not performed routinely in all cases of short stature. Second, due to the retrospective character of the study, not all variables were available and we had to depend on data as reported by multiple physicians. Third, we were only able to investigate 40% of potentially eligible cases. However, it seems unlikely that this has generated sampling bias. Fourth, similarly to previous studies, the effect of GH treatment could not be compared with untreated controls. However, our data are well in line with previous reports (16, 17), and there is little doubt that GH is effective for this indication. Finally, the number of patients with *SHOX* or *SHOX* enhancer duplications was too small to draw any firm conclusion on the pathogenicity of these duplications, and on their growth response to GH treatment.

In conclusion, we show that children with SEDs are less disproportionate than children with SHI, and that in both groups HtSDS and body proportions vary widely. Children with a SED show a slightly greater growth response to GH treatment than children with SHI. The clinical significance of SDUPs remains uncertain.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-15-0451>.

Declaration of interest

S H Donze, C R Meijer, S G Kant, G R J Zandwijken, A H van der Hout, R M L van Spaendonk, A M W van den Ouweland, and M Losekoot have nothing

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References

- 1 Sabherwal N, Bangs F, Roth R, Weiss B, Jantz K, Tiecke E, Hinkel GK, Spaich C, Hauffa BP, van der Kamp H *et al.* Long-range conserved non-coding *SHOX* sequences regulate expression in developing chicken limb and are associated with short stature phenotypes in human patients. *Human Molecular Genetics* 2007 **16** 210–222. (doi:10.1093/hmg/ddl470)
- 2 Benito-Sanz S, Thomas NS, Huber C, Gorbenko del Blanco D, Aza-Carmona M, Crolla JA, Maloney V, Rappold G, Argente J, Campos-Barros A *et al.* A novel class of pseudoautosomal region 1 deletions downstream of *SHOX* is associated with Leri-Weill dyschondrosteosis. *American Journal of Human Genetics* 2005 **77** 533–544. (doi:10.1086/449313)
- 3 Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M *et al.* Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nature Genetics* 1997 **16** 54–63. (doi:10.1038/ng0597-54)
- 4 Kant SG, van der Kamp HJ, Kriek M, Bakker E, Bakker B, Hoffer MJ, van Bunderen P, Losekoot M, Maas SM, Wit JM *et al.* The jumping *SHOX* gene – crossover in the pseudoautosomal region resulting in unusual inheritance of Leri-Weill dyschondrosteosis. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E356–E359. (doi:10.1210/jc.2010-1505)
- 5 Fukami M, Kato F, Tajima T, Yokoya S & Ogata T. Transactivation function of an approximately 800-bp evolutionarily conserved sequence at the *SHOX* 3' region: implication for the downstream

Clinical Study	S H Donze and others	GH in patients with defects of SHOX or its enhancer	173:5	620
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- enhancer. *American Journal of Human Genetics* 2006 **78** 167–170. (doi:10.1086/499254)
- 6 Chen J, Wildhardt G, Zhong Z, Roth R, Weiss B, Steinberger D, Decker J, Blum WF & Rappold G. Enhancer deletions of the SHOX gene as a frequent cause of short stature: the essential role of a 250 kb downstream regulatory domain. *Journal of Medical Genetics* 2009 **46** 834–839. (doi:10.1136/jmg.2009.067785)
 - 7 Durand C, Bangs F, Signolet J, Decker E, Tickle C & Rappold G. Enhancer elements upstream of the SHOX gene are active in the developing limb. *European Journal of Human Genetics* 2010 **18** 527–532. (doi:10.1038/ejhg.2009.216)
 - 8 Kenyon EJ, McEwen GK, Callaway H & Elgar G. Functional analysis of conserved non-coding regions around the short stature hox gene (shox) in whole zebrafish embryos. *PLoS ONE* 2011 **6** e21498. (doi:10.1371/journal.pone.0021498)
 - 9 Evers C, Heidemann PH, Dunstheimer D, Schulze E, Haag C, Janssen JW, Fischer C, Jauch A & Moog U. Pseudoautosomal inheritance of Leri–Weill syndrome: what does it mean? *Clinical Genetics* 2011 **79** 489–494. (doi:10.1111/j.1399-0004.2010.01488.x)
 - 10 Kant SG, Broekman SJ, de Wit CC, Bos M, Scheltinga SA, Bakker E, Oostdijk W, van der Kamp HJ, van Zwet EW, van der Hout AH *et al.* Phenotypic characterization of patients with deletions in the 3'-flanking SHOX region. *PeerJ* 2013 **1** e35. (doi:10.7717/peerj.35)
 - 11 Benito-Sanz S, Royo JL, Barroso E, Paumard-Hernandez B, Barreda-Bonis AC, Liu P, Gracia R, Lupski JR, Campos-Barros A, Gomez-Skarmeta JL *et al.* Identification of the first recurrent PAR1 deletion in Leri–Weill dyschondrosteosis and idiopathic short stature reveals the presence of a novel SHOX enhancer. *Journal of Medical Genetics* 2012 **49** 442–450. (doi:10.1136/jmedgenet-2011-100678)
 - 12 Iughetti L, Capone L, Elsedfy H, Bertorelli R, Predieri B, Bruzzi P, Forabosco A & El Kholi M. Unexpected phenotype in a boy with trisomy of the SHOX gene. *Journal of Pediatric Endocrinology & Metabolism* 2010 **23** 159–169. (doi:10.1515/JPEM.2010.23.1-2.159)
 - 13 Benito-Sanz S, Barroso E, Heine-Suner D, Hisado-Oliva A, Romanelli V, Rosell J, Aragones A, Caimari M, Argente J, Ross JL *et al.* Clinical and molecular evaluation of SHOX/PAR1 duplications in Leri–Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). *Journal of Clinical Endocrinology and Metabolism* 2011 **96** E404–E412. (doi:10.1210/jc.2010-1689)
 - 14 Caliebe J, Broekman S, Boogaard M, Bosch CA, Ruivenkamp CA, Oostdijk W, Kant SG, Binder G, Ranke MB, Wit JM *et al.* IGF1, IGF1R and SHOX mutation analysis in short children born small for gestational age and short children with normal birth size (idiopathic short stature). *Hormone Research in Paediatrics* 2012 **77** 250–260. (doi:10.1159/000338341)
 - 15 Wit JM, van Duyvenvoorde HA, van Klinken JB, Caliebe J, Bosch CA, Lui JC, Gijsbers AC, Bakker E, Breuning MH, Oostdijk W *et al.* Copy number variants in short children born small for gestational age. *Hormone Research in Paediatrics* 2014 **82** 310–318. (doi:10.1159/000367712)
 - 16 Blum WF, Crowe BJ, Quigley CA, Jung H, Cao D, Ross JL, Braun L & Rappold G. Growth hormone is effective in treatment of short stature associated with short stature homeobox-containing gene deficiency: two-year results of a randomized, controlled, multicenter trial. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 219–228. (doi:10.1210/jc.2006-1409)
 - 17 Massart F, Bizzi M, Baggiani A & Miccoli M. Height outcome of the recombinant human growth hormone treatment in patients with SHOX gene haploinsufficiency: a meta-analysis. *Pharmacogenomics* 2013 **14** 607–612. (doi:10.2217/pgs.13.44)
 - 18 Bunyan DJ, Baker KR, Harvey JF & Thomas NS. Diagnostic screening identifies a wide range of mutations involving the SHOX gene, including a common 47.5 kb deletion 160 kb downstream with a variable phenotypic effect. *American Journal of Medical Genetics. Part A* 2013 **161A** 1329–1338. (doi:10.1002/ajmg.a.35919)
 - 19 Prader A, Largo RH, Molinari L & Issler C. Physical growth of Swiss children from birth to 20 years of age. First Zurich longitudinal study of growth and development. *Helvetica Paediatrica Acta. Supplementum* 1989 **52** 1–125.
 - 20 Ranke MB, Lindberg A, Brosz M, Kaspers S, Loftus J, Wollmann H, Koltowska-Haggstrom M & Roelants M. Accurate long-term prediction of height during the first four years of growth hormone treatment in prepubertal children with growth hormone deficiency or Turner syndrome. *Hormone Research in Paediatrics* 2012 **78** 8–17. (doi:10.1159/000339468)
 - 21 Blum WF, Ross JL, Zimmermann AG, Quigley CA, Child CJ, Kalifa G, Deal C, Drop SL, Rappold G & Cutler GB Jr. GH treatment to final height produces similar height gains in patients with SHOX deficiency and Turner syndrome: results of a multicenter trial. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E1383–E1392. (doi:10.1210/jc.2013-1222)
 - 22 Ranke MB & Lindberg A. Observed and predicted growth responses in prepubertal children with growth disorders: guidance of growth hormone treatment by empirical variables. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 1229–1237. (doi:10.1210/jc.2009-1471)
 - 23 Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, Albertsson-Wikland K & Price DA. Prediction of long-term response to recombinant human growth hormone in Turner syndrome: development and validation of mathematical models. KIGS International Board. Kabi International Growth Study. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4212–4218. (doi:10.1210/jcem.85.11.6976)
 - 24 Ranke MB & Lindberg A. Predicting growth in response to growth hormone treatment. *Growth Hormone & IGF Research* 2009 **19** 1–11. (doi:10.1016/j.gthr.2008.08.001)
 - 25 Fredriks AM, van Buuren S, van Heel WJ, Dijkman-Neerincx RH, Verloove-Vanhorick SP & Wit JM. Nationwide age references for sitting height, leg length, and sitting height/height ratio, and their diagnostic value for disproportionate growth disorders. *Archives of Disease in Childhood* 2005 **90** 807–812. (doi:10.1136/adc.2004.050799)
 - 26 Schonbeck Y, Talma H, van Dommelen P, Bakker B, Buitendijk SE, HiraSing RA & van Buuren S. The world's tallest nation has stopped growing taller: the height of Dutch children from 1955 to 2009. *Pediatric Research* 2013 **73** 371–377. (doi:10.1038/pr.2012.189)
 - 27 van Dommelen P, Schonbeck Y & van Buuren S. A simple calculation of the target height. *Archives of Disease in Childhood* 2012 **97** 182. (doi:10.1136/archdischild-2011-301095)
 - 28 Cole TJ & Roede MJ. Centiles of body mass index for Dutch children aged 0–20 years in 1980 – a baseline to assess recent trends in obesity. *Annals of Human Biology* 1999 **26** 303–308. (doi:10.1080/030144699282633)
 - 29 Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C & Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977–1981). *Acta Paediatrica Scandinavica* 1991 **80** 756–762. (doi:10.1111/j.1651-2227.1991.tb11945.x)
 - 30 Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G & Wit JM. Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Hormone Research* 1998 **50** 166–176. (doi:10.1159/000023268)
 - 31 Binder G, Ranke MB & Martin DD. Auxology is a valuable instrument for the clinical diagnosis of SHOX haploinsufficiency in school-age children with unexplained short stature. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4891–4896. (doi:10.1210/jc.2003-030136)
 - 32 Freeman JV, Cole TJ, Chinn S, Jones PR, White EM & Preece MA. Cross sectional stature and weight reference curves for the UK, 1990. *Archives of Disease in Childhood* 1995 **73** 17–24. (doi:10.1136/adc.73.1.17)
 - 33 Cole TJ. Some questions about how growth standards are used. *Hormone Research* 1996 **45** (Suppl 2) 18–23. (doi:10.1159/000184843)

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- 34 Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. *Hormone Research in Paediatrics* 2011 **75** 81–89. (doi:10.1159/000324105)
- 35 Rosilio M, Huber-Lequesne C, Sapin H, Carel JC, Blum WF & Cormier-Daire V. Genotypes and phenotypes of children with SHOX deficiency in France. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E1257–E1265. (doi:10.1210/jc.2011-3460)
- 36 Rappold G, Blum WF, Shavrikova EP, Crowe BJ, Roeth R, Quigley CA, Ross JL & Niesler B. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. *Journal of Medical Genetics* 2007 **44** 306–313. (doi:10.1136/jmg.2006.046581)
- 37 Ogata T, Matsuo N & Nishimura G. SHOX haploinsufficiency and overdosage: impact of gonadal function status. *Journal of Medical Genetics* 2001 **38** 1–6. (doi:10.1136/jmg.38.1.1)
- 38 Adamson KA, Cross I, Batch JA, Rappold GA, Glass IA & Ball SG. Trisomy of the short stature homeobox-containing gene (SHOX), resulting from a duplication–deletion of the X chromosome. *Clinical Endocrinology* 2002 **56** 671–675. (doi:10.1046/j.1365-2265.2002.01504.x)
- 39 Thomas NS, Harvey JF, Bunyan DJ, Rankin J, Grigeliuniene G, Bruno DL, Tan TY, Tomkins S & Hastings R. Clinical and molecular characterization of duplications encompassing the human SHOX gene reveal a variable effect on stature. *American Journal of Medical Genetics. Part A* 2009 **149A** 1407–1414. (doi:10.1002/ajmg.a.32914)
- 40 Li Q, Barkess G & Qian H. Chromatin looping and the probability of transcription. *Trends in Genetics* 2006 **22** 197–202. (doi:10.1016/j.tig.2006.02.004)

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