The growth response to GH treatment is greater in patients with \textit{SHOX} enhancer deletions compared to \textit{SHOX} defects

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\textbf{Abstract}

\textbf{Objective:} Short stature caused by point mutations or deletions of the short stature homeobox (\textit{SHOX}) gene (\textit{SHOX} haploinsufficiency (SHI)) is a registered indication for GH treatment. Patients with a \textit{SHOX} enhancer deletion (SED) have a similar phenotype, but their response to GH is unknown. It is uncertain if duplications of \textit{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 \textit{SHOX} and its enhancers.

\textbf{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).

\textbf{Results:} Patients with SEDs showed similar HtSDS to patients with SHI (K\textsubscript{2}2.3 and \textsubscript{K}2.6, respectively, \textit{P} = 0.2), but they were less disproportionate (SH/height ratio SDS 2.0 vs 3.1 (\textit{P} < 0.01) and extremities/trunk ratio 2.57 vs 2.43 (\textit{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 (\textit{n} = 3) was similar to the other groups.

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

\textbf{Introduction}

The short stature homeobox (\textit{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 \textit{SHOX} results in an extreme phenotype of osteodysplasia called Langer syndrome. The classical presentation of \textit{SHOX} haploinsufficiency (SHI), due to heterozygous \textit{SHOX} defects, is Lééri-Weill dyschondrostosis (LWD), characterized by the
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 SHOXα 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).
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.

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 (ΔHtSDS), height velocity (HV, cm/year) and height velocity as SDS (HVSDS) 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...
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 (ΔBA/ΔCA 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, ΔSH/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 χ² 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.
Comparison to parents’ SHOX proband’s HtSDS was closer to HtSDS of the unaffected parent (Table 1). As expected, in both groups the HtSDS was compared to the conditional target HtSDS. In case of a familial mutation, affected parents in group 1 (affected parents in group 2 were significantly less short than

| Clinical Study | S H Donze and others | GH in patients with defects of SHOX or its enhancer | 173:S | 615 |

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

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>n</th>
<th>All patients</th>
<th>Range</th>
<th>n</th>
<th>SHOX mutations or deletions</th>
<th>n</th>
<th>SHOX upstream and downstream enhancer deletions</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first visit (years)</td>
<td>80</td>
<td>8.35 (3.6)</td>
<td>1.2; 16.2</td>
<td>54</td>
<td>8.3 (3.5)</td>
<td>26</td>
<td>8.5 (3.7)</td>
<td>0.839</td>
</tr>
<tr>
<td>Male/female</td>
<td>34/46</td>
<td></td>
<td></td>
<td>23/31</td>
<td></td>
<td>11/15</td>
<td></td>
<td>0.981</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>66</td>
<td>−0.4 (1.3)</td>
<td>−3.3; 3.2</td>
<td>43</td>
<td>−0.4 (1.3)</td>
<td>23</td>
<td>−0.3 (1.3)</td>
<td>0.728</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>39</td>
<td>−1.1 (1.2)</td>
<td>−4.3; 1.5</td>
<td>23</td>
<td>−1.0 (1.2)</td>
<td>14</td>
<td>−1.1 (1.4)</td>
<td>0.823</td>
</tr>
<tr>
<td>Height SDS</td>
<td>80</td>
<td>−2.5 (0.8)</td>
<td>−4.4; −0.3</td>
<td>54</td>
<td>−2.6 (0.8)</td>
<td>26</td>
<td>−2.3 (0.8)</td>
<td>0.111</td>
</tr>
<tr>
<td>Conditional target height SDS</td>
<td>74</td>
<td>−1.0 (0.6)</td>
<td>−2.9; 0.7</td>
<td>50</td>
<td>−1.0 (0.6)</td>
<td>24</td>
<td>−0.9 (0.5)</td>
<td>0.647</td>
</tr>
<tr>
<td>Sitting height/height ratio SDS</td>
<td>72</td>
<td>2.8 (1.3)</td>
<td>−0.1; 5.5</td>
<td>50</td>
<td>3.2 (1.1)</td>
<td>22</td>
<td>1.9 (1.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>66</td>
<td>0.95 (0.03)</td>
<td>0.87; 1.01</td>
<td>21</td>
<td>0.95 (0.03)</td>
<td>12</td>
<td>0.96 (0.03)</td>
<td>0.365</td>
</tr>
<tr>
<td>Extremities/trunk ratioa</td>
<td>76</td>
<td>0.4 (1.0)</td>
<td>−2.0; 2.7</td>
<td>51</td>
<td>0.5 (0.9)</td>
<td>25</td>
<td>0.1 (1.1)</td>
<td>0.061</td>
</tr>
<tr>
<td>Comparison to parents’ phenotype:</td>
<td>32</td>
<td>2.48 (0.2)</td>
<td>2.06; 2.80</td>
<td>21</td>
<td>2.43 (0.2)</td>
<td>11</td>
<td>2.57 (0.2)</td>
<td>0.028</td>
</tr>
<tr>
<td>Height SDS affected parent</td>
<td>49</td>
<td>−2.2 (0.9)</td>
<td>−4.8; −0.3</td>
<td>28</td>
<td>−2.4 (0.9)</td>
<td>21</td>
<td>−1.9 (0.9)</td>
<td>0.032</td>
</tr>
<tr>
<td>Sitting height/height ratio SDS affected parent</td>
<td>23</td>
<td>2.7 (1.7)</td>
<td>0.5; 6.1</td>
<td>9</td>
<td>3.3 (1.4)</td>
<td>14</td>
<td>2.3 (1.8)</td>
<td>0.168</td>
</tr>
<tr>
<td>Height SDS − TH SDS (de novo mutations)</td>
<td>7</td>
<td>1.9 (0.7)</td>
<td>−3.2; −1.1</td>
<td>6</td>
<td>−1.9 (0.7)</td>
<td>1</td>
<td>−2.1</td>
<td></td>
</tr>
<tr>
<td>Height SDS − height SDS affected parent</td>
<td>46</td>
<td>−0.3 (1.1)</td>
<td>−2.2; 2.6</td>
<td>25</td>
<td>−0.2 (0.9)</td>
<td>21</td>
<td>−0.5 (1.3)</td>
<td>0.419</td>
</tr>
<tr>
<td>Height SDS − height SDS unaffected parent</td>
<td>43</td>
<td>−1.7 (0.9)</td>
<td>−3.5; 0.2</td>
<td>24</td>
<td>−1.7 (0.9)</td>
<td>19</td>
<td>−1.6 (1.0)</td>
<td>0.579</td>
</tr>
</tbody>
</table>

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

aBinder’s 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 unaffected 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, ΔHtSDS, 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.
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.

**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.

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

<sup>a</sup>Adopted from Taiwan, therefore clinical information concerning birth and height and proportions of the parents is missing.

<sup>b</sup>Patients treated with GH.

<sup>c</sup>Patients 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 ± 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 (ΔSH/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).
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...
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 −3.3 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 stunted 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 2](image-url)

**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).

![Figure 3](image-url)

**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 to disclose. J M Wit consults for OPKO, Versartis, Biopartners, and Merck-Serono. W Oostdijk received unrestricted grant support from Novo Nordisk and Ferring.

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