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Growth response to growth hormone treatment in patients with SHOX deficiency can be predicted by the Cologne prediction model

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Abstract

Background: Growth hormone (GH) treatment in children with short stature homeobox-containing gene (*SHOX*) deficiency is recognized to increase height velocity (HV) and adult height. Prediction of growth response continues to be a challenge. A comparatively accurate method is the Cologne prediction model developed in children with GH deficiency. The aim was to investigate whether this model also applies to patients with SHOX deficiency.

Methods: Included were 48 patients with SHOX deficiency confirmed by DNA analysis and treated with 0.05 mg/kg/day of somatropin. Prediction by the Cologne model uses the following variables: relative bone age (BA) retardation, baseline insulin-like growth factor-I (IGF-I), urinary deoxypyridinoline (DPD) cross-links at 4 weeks and HV at 3 months.

Results: HV and height standard deviation scores (SDS) increased significantly during the first year of treatment. Predicted and observed HV (cm/year) showed a Pearson correlation coefficient of 0.50 ($p < 0.001$; root-mean-square error = 1.63) and for first-year change in height SDS a Pearson correlation coefficient of 0.751 ($p < 0.001$; root-mean-square error = 0.32). Poor response could be adequately predicted using SDS change, with sensitivity and specificity both above 70% for certain thresholds.

Conclusions: The results demonstrate that the Cologne model can be used to predict growth response in patients with SHOX deficiency with reasonable precision in the first treatment year, comparable to prediction in patients with GH deficiency.

Keywords: growth hormone; growth prediction; height velocity; short stature homeobox-containing gene (*SHOX*); short stature.

Introduction

The short stature homeobox-containing gene (*SHOX*) codes for a homeodomain transcription factor which is expressed in developing skeletal tissue of the tibia, distal femur, radius and ulna and the first and second pharyngeal arches [1]. The coded protein SHOX exists in the growth plates and seems to play an important role in differentiation and proliferation of chondrocytes and consequently in longitudinal growth [2, 3]. *SHOX* is located in the pseudoautosomal region 1 (PAR1) on the distal end of the short arms of the Y and X chromosomes [4]. *SHOX* was discovered and analyzed in the context of the search for genes underlying growth deficit in Turner syndrome (TS). Due to the fact that individuals with TS lack all or part of their X chromosome, they have only a single copy of *SHOX* (SHOX haploinsufficiency) [4] and this is considered the main cause of the growth deficit in these patients [5]. Deletions or mutations in the *SHOX* gene or deletions in upstream or downstream enhancer regions lead to a wide range of phenotypes [6, 7] from short stature without dysmorphic signs to the typical features of Leri-Weill syndrome with mesomelic skeletal dysplasia including Madelung deformity (dinner fork-like wrist) [8, 9]. From numerous studies it appears that SHOX deficiency is the most prevalent monogenic cause of short stature [10]. SHOX anomalies are identified in 2%–15% of patients with short stature [9, 11–14]. In 2007 a human SHOX allelic variant database was created which can help to distinguish between functional variants and polymorphisms [15]. Short children with alterations in SHOX retain a reduced height in adulthood [11, 14, 16]. Therefore, two studies analyzed the effect of growth hormone (GH) treatment in children with SHOX deficiency [17, 18]. Based on these studies, GH treatment

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in children with SHOX deficiency was approved by regulatory authorities for improvement of height velocity (HV) and adult height.

However, the growth response to GH treatment varies greatly between patients. Prediction of the growth response continues to be a challenge [19]. Various prediction models have been described in the past [20, 21]. A comparatively accurate method is the Cologne prediction model developed in children with GH deficiency [21], which attempts to identify children with a poor GH treatment response early, to allow timely dose adjustments or even discontinuation of treatment. This model predicts the first-year HV after a 3-month treatment course with GH. It includes the following variables: relative bone age (BA) retardation and serum insulin-like growth factor-I (IGF-I) concentration at the start of GH treatment, deoxypyridinoline (DPD) measured 1 month after the start of treatment and annualized HV after the first 3 months [21].

Growth prediction models require validation in the various diagnostic groups for which they may be applied [22, 23]. In the present analysis we therefore evaluated the Cologne prediction model in GH treated SHOX-deficient patients with confirmed mutations in *SHOX*. In addition, the predictive power of the model when used as a screening tool to differentiate between good response and poor response to GH treatment was assessed.

Subjects and methods

Study design/patients

This multinational, prospective, open-label, randomized study was conducted at 33 study sites in 14 countries and comprised three periods [24]: (i) screening for eligible patients including DNA analysis for *SHOX* gene mutations/deletions, (ii) a 2-year randomized control period [18] and (iii) an extension to final height (FH) or study closure [24]. Patients with confirmed SHOX deficiency were randomized to the GH treatment group (n = 27) or the non-treatment control group (n = 25). All patients who completed the control period were offered GH treatment in the extension until attainment of FH or study closure. Data collection for growth prediction under GH treatment of the patients in the control group was therefore delayed by 2 years. The study was performed in accordance with the Declaration of Helsinki and good clinical practice and was approved by the Ethics Committees of the participating institutions. Written informed consent was obtained from the patients' parent(s)/legal guardian(s) before conducting any study-related procedure.

Patients were included in the trial if they fulfilled the following entry criteria: confirmed SHOX deficiency, chronological age (CA) of at least 3 years and prepubertal stage, height <3rd percentile of the local reference range or height <10th percentile with HV <25th percentile, BA <10 years for boys and <8 years for girls or <9 years for TS, no GH deficiency or resistance, no chronic disease and no known growth-influencing medications. The patients were treated with a daily subcutaneous injection of recombinant hGH (Humatrope® [somatropin, rDNA origin], Eli Lilly and Company, Indianapolis, IN, USA) at a dose of 0.05 mg/kg/day. Because of the 2-year delay of GH treatment in the control group, BA of six girls and three boys had progressed to more than 8 and 10 years, respectively, by entry into the treatment extension period.

The total sample of confirmed SHOX-deficient patients with GH treatment for at least 1 year consisted of 52 children. Complete datasets were available in 48 of these 52 patients [24].

Molecular/genetic analyses for *SHOX* gene defects

SHOX gene deletions or mutations were confirmed in two central laboratories (Department of Molecular Human Genetics, Heidelberg, Germany; Esoterix, Calabasas, CA, USA) by microsatellite analysis, fluorescence in situ hybridization, denaturing high-performance liquid chromatography (HPLC), and, if indicated, by DNA sequencing [6]. The diagnosis was confirmed in all subjects within the previous treatment trial [18]. Of 52 subjects, 34 had a complete deletion of the *SHOX* gene, four had partial gene deletions and 14 had point mutations. Of the 14 subjects with point mutations, three had a nonsense mutation, 10 had a missense mutation and one subject had an insertion. Missense mutations were judged to be clinically relevant if they presented in vitro testing with a loss of function. In cases where a severe phenotype was present, or the short stature phenotype segregated with the genotype in pedigree analysis, or the mutation had previously been described in the literature as clinically relevant, detected mutations were judged as relevant and causing the clinical phenotype [18].

Clinical and biochemical assessments

Enrolled subjects were evaluated at baseline (at the start of GH treatment [24]) for standing height and pre-existing conditions. Subsequent evaluation was performed after 3 months and 1 year of treatment. Standing height was measured using standard wall mounted stadiometers. German height references were used because most of the patients originated from Central Europe [24]. X-rays of the left hand for BA were obtained at baseline (start of GH treatment) and after 1 year and were assessed centrally according to the standards of Greulich and Pyle [25]. Relative BA retardation was calculated as BA minus CA divided by CA. Blood was drawn for routine safety assessment and IGF-I concentrations at baseline and at 3, 6 and 12 months.

Twenty-four-hour urine was collected after 4 weeks of GH treatment for analysis of DPD and creatinine at the osteologic laboratory of the University Children's Hospital Cologne by enzyme immunoassay (Pyrilinks-D; Metra Biosystems Inc., Mountain View, CA, USA).

Growth prediction model

The growth prediction model has been described in detail by Schoenau and colleagues [21]. The model was first established in children with GH deficiency during a prospective multicenter trial where patients were treated with GH at a fixed dosage. The present model combines four relevant parameters to predict the first-year growth response to GH treatment: relative BA retardation calculated in

association with the CA by ([BA – CA]/CA), pre-treatment serum IGF-I concentration, DPD measured 1 month after the start of treatment and annualized HV after the first 3 months (±3 weeks) of treatment. The predicted HV in the first year of treatment can be calculated after 3 months of treatment, using the following equation:

$$\text{First-year HV} = 3.543 - (2.337 \times \text{baseline relative BA retardation}) - (0.010 \times \text{baseline IGF-I}) + (0.100 \times \text{DPD at 4 weeks}) + (0.299 \times \text{HV at 3 months}).$$

Statistical analysis

The predictive accuracy of the original Cologne model was assessed by comparing the predicted and measured 1-year HVs using graphical and linear regression methods, and by calculating the Pearson correlation coefficient and the root-mean-square error of prediction.

It is customary to report standard deviation scores (SDS) for measurements such as height, which are thus adjusted to the mean and SD of the measurement in the normal population of corresponding age and sex. We converted the measured and predicted heights to SDS and then calculated the change in SDS over the 1-year period. Prediction accuracy was then assessed for SDS change. For conversion to age- and sex-related SDS, central height references were used [26].

In order to assess the relevance of each explanatory variable in the Cologne model to SHOX-deficient patients, multiple linear regressions were used to refit the parameters of that model to the data of the patients with SHOX deficiency.

In order to characterize the ability of the Cologne model to diagnose poor growth in advance, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were computed for a range of threshold values of HV and SDS change.

Sensitivity is defined as the proportion of cases with poor growth whose predicted growth is poor; specificity is the proportion of cases without poor growth whose predicted growth was not poor. PPV is the proportion of cases predicted to have poor growth whose growth was actually poor; NPV is the proportion of cases without poor growth whose growth was actually not poor. Accuracy is the proportion of all cases for which the prediction (poor growth or not) agrees with the actual growth.

SDS change below 0.5 within the first treatment year was defined as poor response based on statements by Bang et al. [27].

Results

Baseline characteristics

Baseline characteristics of the study population at the start of GH treatment are shown in Table 1. The mean age was 7.5 years, the mean BA SDS was –1.1 and the mean height SDS was –3.20.

Out of 48 subjects, 24 presented with Leri-Weill syndrome and 22 with non-syndromic short stature (apparent idiopathic short stature). For two subjects the phenotype was not specified.

Table 1: Characteristics of the study cohort (n = 48) at the start of GH treatment.

Variable	Mean ± SD (except gender)
Females/males, n	25/23
Chronological age at start, years	7.5 ± 2.4
Bone age at start, years	6.6 ± 2.4
Bone age SDS at start	–1.1 ± 1
IGF-I SDS at start	–0.8 ± 1.1
Height at start, cm	111 ± 13
Height SDS at start	–3.2 ± 0.9
Pre-treatment HV, cm/year	5.1 ± 1.5
Pre-treatment HV SDS	–0.64 ± 1.3

HV, height velocity (cm/year); SDS, standard deviation score.

Table 2: Predicted and observed annualized first-year height velocity and change in height SDS.

Variable	Mean ± SD	Correlation coefficient	RMS error
Predicted HV, cm/year	7.8 ± 1.3	0.51	1.63
Observed HV, cm/year	8.3 ± 1.7		
Predicted change in height SDS	+0.49 ± 0.38	0.75	0.32
Observed change in height SDS	+0.61 ± 0.45		

HV, height velocity (cm/year); RMS, root mean square; SDS, standard deviation score.

Growth prediction

Table 2 depicts the means of predicted and observed annualized first-year absolute HV and predicted change in height SDS during the GH treatment period. In the 38 patients for whom the baseline HV was available, HV increased significantly ($p < 0.001$) from 5.1 ± 1.5 cm/year to 7.6 ± 2 cm/year during the first-year of treatment with GH. The association of the observed first-year HV and predicted HV is presented in Figure 1A (Pearson's correlation coefficient $r = 0.51$, $p < 0.001$). The root-mean-square error of prediction was 1.63 cm/year. Residuals (observed minus predicted HV) vs. predicted HV are presented in Figure 2A.

The standing height SDS increased from –3.2 ± 0.9 to –2.6 ± 0.9. Hence, the change in height SDS in the first year of treatment was +0.6. The association between the observed and predicted change in height SDS is presented in Figure 1B (Pearson's correlation coefficient $r = 0.75$, $p < 0.001$). The root-mean-square error of prediction was

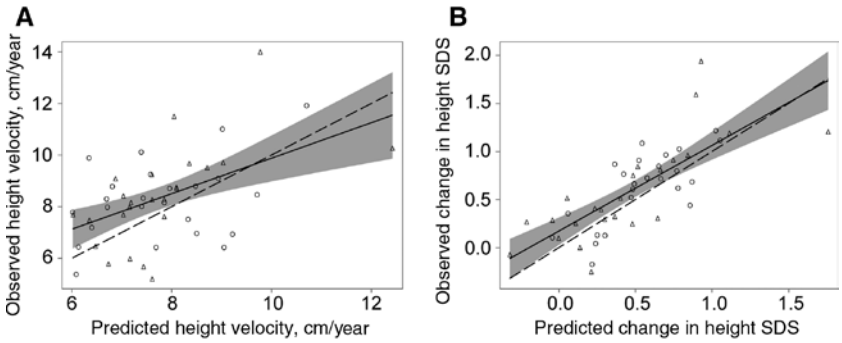

Figure 1: Prediction of height velocity vs. observed height velocity after 1 year in response to growth hormone treatment.
Scatterplot of the predicted vs. observed values of height velocity (A) and change in height SDS (B) after 1 year in response to GH treatment. Triangles represent patients with non-syndromic short stature and circles represent patients with Leri-Weill syndrome phenotype. Regression line (solid line) and line of identity (hatched line) are shown. The shaded area depicts the 95% confidence interval for the regression line.

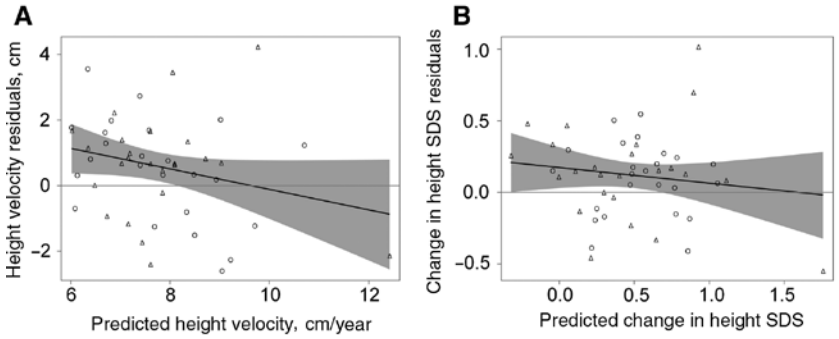

Figure 2: Residuals of prediction of height velocity after 1 year in response to growth hormone treatment.
Scatterplot of individual residuals after 1 year in cm (A) and individual residuals after 1 year presented in SDS (B). Triangles represent patients with non-syndromic short stature and circles represent patients with Leri-Weill syndrome phenotype. Regression line (solid line) and line of identity (hatched line) are shown. The shaded area depicts the 95% confidence interval for the regression line.

0.32 SDS. Residuals (observed minus predicted height SDS change) vs. predicted delta height SDS are presented in Figure 2B.

Operational characteristics for prediction of poor growth

Operational characteristics (sensitivity, specificity, PPV, NPV and accuracy) of the prediction model were tested with the following thresholds for the first-year HV and first-year height SDS changes:

- HV of 7; 8; 9; 10 cm/year
- height SDS change of 0.3; 0.4; 0.5; 0.6; 0.7; 0.8.

Results of the cut-point analyses are displayed in Table 3. For HV, no threshold could be found which provided both high sensitivity and high specificity, whereas for height SDS change a range of thresholds yielded sensitivities and specificities both over 70% (with high PPV and NPV also).

Regression analyses

The multiple regression analysis was performed for the first-year HV and first-year change in height SDS as dependent variables.

For both dependent variables the following explanatory variables (i.e. those in the Cologne model) were analyzed:

- baseline relative BA retardation ([BA – CA]/CA)
- baseline IGF-I
- DPD at 4 weeks
- HV (cm/year) at 3 months.

For first-year HV, a significant positive correlation with the HV (cm/year) at 3 months was confirmed with $p < 0.001$. Analysis of the influence of baseline relative BA retardation ([BA – CA]/CA), baseline IGF-I and DPD at 4 weeks could not confirm any significant relationship ($p = 0.30$, $p = 0.37$, $p = 0.13$, respectively).

Table 3: Absolute results of operational characteristics for first-year HV and change in height SDS.

Cut-point of height velocity, cm/year			7	8	9	10
Sensitivity, %			36.4	77.8	91.2	100
Specificity, %			75.7	43.3	35.7	33.3
PPV, %			30.8	45.2	77.5	91.3
NPV, %			80	76.5	62.5	100
Accuracy, %			66.7	56.3	75	91.7
Cut-point of change in height SDS	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**
Sensitivity, %	84.6	88.2	90	91.3	88.9	93.6
Specificity, %	85.7	87.1	71.4	64	47.6	41.2
PPV, %	68.8	79	69.2	70	68.6	74.4
NPV, %	93.8	93.1	90.9	88.9	76.9	77.8
Accuracy, %	85.4	87.5	79.2	77.1	70.8	75

NPV, negative predictive value; PPV, positive predictive value.

Similarly, for first-year change in height SDS, a significant positive correlation with the change in height SDS at 3 months was shown, with $p < 0.001$. Analysis of the influence of baseline relative BA retardation ([BA – CA]/CA), baseline IGF-I and DPD at 4 weeks could not confirm any significant correlation ($p = 0.53$; $p = 0.65$; $p = 0.91$, respectively).

Discussion

The present analyses evaluated the Cologne growth prediction model in patients with SHOX deficiency. Our results show that growth response to GH treatment in patients with SHOX deficiency can be predicted to a certain extent by this model.

Growth prediction by the Cologne prediction model was applied earlier to patients with GH deficiency [21, 23]. Compared to our data in patients with SHOX deficiency, prediction of growth response in GH deficiency seems to be more accurate [21–23]. Based on the fact that the growth response in the first year is associated with the long-term outcome, it is reasonable to evaluate the growth response as early as possible to predict the long-term outcome [28–30]. Although the experience with GH therapy over 30 years suggests that GH treatment is safe [31, 32], discussions about adverse effects especially in the long-term flared up again recently because of results from the French cohort of the Safety and Appropriateness of Growth Hormone Treatments in Europe (SAGhE) study [33, 34]. Moreover, treatment costs are very high. Therefore, the likely efficacy of this treatment in an individual patient should be critically assessed [27]. Cut-off points for poor and good response of HV or change in height SDS have been suggested [27]. No guidelines authored by scientific societies are available yet to address the cut-off levels for poor and good treatment response. It has to be kept in mind that the response to GH treatment also depends on the underlying condition. Poor and good growth responders in this analysis were defined according to their changes in height SDS (good response if delta height SDS > 0.5 SD, poor response if delta height SDS < 0.5 SD) based on the mean height gain SDS over 1 year of +0.6. This arbitrary definition is similar to the cut-off determination in other trials [35]. When used as a screening tool in SHOX deficiency to identify poor responders, our model had to predict SDS changes of <0.5 SD reliably. In these analyses we evaluated the prediction by the Cologne prediction model for both the absolute HV per year and the change of height SDS within the treatment period. It was demonstrated that prediction was more precise in changes in height SDS compared to 1-year HV. This might be explained by the fact that SDS values are adjusted for age and gender. Furthermore, it supports the work of Bang and colleagues where criteria for growth response to GH were determined [27].

Sensitivity, specificity, accuracy, PPV and NPV were all >70% for height SDS changes <0.5. Compared to the earlier application in GH deficiency, the Cologne model showed sufficient reliability to identify patients with SHOX deficiency at risk of poor response. Therefore, the Cologne model may be recommended for use in counseling patients with SHOX deficiency regarding their likely response to GH treatment and their individual benefit/risk profile.

Our results are limited by the fact that based on the trial design our cohort was split for 2 years into a control and a treatment arm. Therefore data collection of one half of the cohort was performed 2 years later and four patients had entered puberty (Tanner stage 2 or greater). No subgroup analyses were performed to evaluate the influence of gender and pubertal changes on the accuracy of the prediction model because of the resulting small sample size. The evaluated model showed reasonable precision for growth prediction in SHOX-deficient patients. In the long term it should be aimed to optimize the model for children with SHOX deficiency. Due to the small sample size we were neither able to estimate the predictive power of each component variable in the Cologne prediction model, nor to re-estimate regression coefficients.

The average dose in the study for developing the Cologne prediction model in patients with GHD was 0.16 mg/kg/week. Because of the stringent design as a clinical trial, the variance of GH doses in both studies

was very small, not allowing to make dose a meaningful explanatory variable in regression models. In a less stringent observational study (GeNeSIS) with a greater variance of GH dose, dose did not show up as a variable that contributed significantly to the first-year growth prediction [22, 23] and validation of the Cologne model in various indications yielded reasonable results. The explanation may be that the GH response variables 4-week DPD and 3-month HV reflect already the impact of GH dose due to high colinearity.

The strength of our analyses is that the data were collected in a prospective clinical trial setting within defined time frames. The drop-out rate of four patients out of 52 was very low, in view of the known problems with adherence to GH treatment in routine clinical practice [36]. Out of 52 enrolled patients, follow-up data were unavailable for only two patients. For two other patients, one parameter of the Cologne prediction model was missing, so that these were not included in the final analyses. Although the sample size is relatively small, the analyzed cohort is a very well-documented group of clinical trial subjects and to the best of our knowledge there are no other SHOX-deficient cohorts available with adequate follow-up and the required variables for verifying the Cologne growth prediction model.

In conclusion, the Cologne prediction model can be applied in patients with SHOX deficiency to predict the growth response with reasonable precision in the first year of treatment. The change in height SDS over the year is better predicted than the absolute HV. In general, the growth prediction in patients with SHOX deficiency seems to be less reliable than in patients with GH deficiency. However, the detection of poorly responding children with the Cologne prediction model was reasonable. To avoid potentially futile treatment with GH, prediction of poor response may be useful in patients with SHOX deficiency as an individual decision-making aid.

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