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Real-life long-term efficacy and safety of recombinant human growth hormone therapy in children with short stature homeobox-containing deficiency

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Abstract

Objective: This Italian survey aims to evaluate real-life long-term efficacy and safety of recombinant human growth hormone (rhGH) therapy in children with short stature homeobox-containing gene deficiency disorders (SHOX-D) and to identify potential predictive factors influencing response to rhGH therapy.

Design and methods: This is a national retrospective observational study collecting anamnestic, anthropometric, clinical, instrumental and therapeutic data in children and adolescents with a genetic confirmation of SHOX-D treated on rhGH. Data were collected at the beginning of rhGH therapy (T0), yearly during the first 4 years of rhGH therapy (T1, T2, T3 and T4) and at near-final height (nFH) (T5), when available.

Results: One hundred and seventeen SHOX-D children started rhGH therapy (initial dose 0.23 ± 0.04 mg/kg/week) at a mean age of 8.67 ± 3.33 years (74% prepubertal), 99 completed the first year of treatment and 46 reached nFH. During rhGH therapy, growth velocity (GV), standard deviation score (SDS) and height (H) SDS improved significantly. Mean H SDS gain from T0 was $+1.14 \pm 0.58$ at T4 and $+0.80 \pm 0.98$ at T5. Both patients carrying mutations involving intragenic SHOX region (group A) and ones with regulatory region defects (group B) experienced a similar beneficial therapeutic effect. The multiple regression analysis identified the age at the start of rhGH treatment ($\beta = -0.31$, $P = 0.030$) and the GV during the first year of rhGH treatment ($\beta = 0.45$, $P = 0.008$) as main

Key Words

- ▶ SHOX deficiency
- ▶ child
- ▶ rhGH
- ▶ efficacy
- ▶ safety
- ▶ long-term effects

independent predictor factors of height gain. During rhGH therapy, no adverse event of concern was reported.

Conclusions: Our data confirm the efficacy and safety of rhGH therapy in SHOX-D children, regardless the wide variety of genotype.

Significance Statement: Among children with idiopathic short stature, the prevalence of SHOX-D is near to 1/1000–2000 (1.1–15%) with a wide phenotypic spectrum. Current guidelines support rhGH therapy in SHOX-D children, but long-term data are still few. Our real-life data confirm the efficacy and safety of rhGH therapy in SHOX-D children, regardless of the wide variety of genotypes. Moreover, rhGH therapy seems to blunt the SHOX-D phenotype. The response to rhGH in the first year of treatment and the age when rhGH was started significantly impact the height gain.

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Introduction

The short stature homeobox-containing (SHOX) gene, located in pseudo-autosomal region 1 of the short arms of the X and Y chromosomes, encodes a homeodomain transcription factor involved in the regulation of longitudinal growth and acting in chondrocyte proliferation and differentiation in the growth plate. SHOX gene escapes X inactivation, requiring the expression of both alleles. Its homozygous deficiency causes Langer-type mesomelic dysplasia, while its haploinsufficiency (SHOX-D) ranges into a large phenotypic spectrum and heterogeneity: from normal or short stature with or without dysmorphic features and/or normal body proportions to mesomelic skeletal dysplasia, as in Léri–Weill dyschondrosteosis (1, 2). The most frequent (around 80%) SHOX mutations encompass deletions in the SHOX gene itself or in the regulatory enhancer regions downstream or upstream of the SHOX gene, leaving the gene itself intact (3). Intragenic microdeletions and point mutations occur in the rest of the cases, but, over the past years, advancements in genetics suggested that also duplications in enhancer regions inhibit the proper expression of the gene and could cause SHOX-D phenotype (1, 4). Up to now, more than 1200 unique allelic variants have been reported in the SHOX database (6), and among children and adolescents with idiopathic short stature, the prevalence of SHOX-D is documented to range between 1.1 and 16.9% (6), as confirmed by recent Italian data (1/1000–2000 (1.1–15%)) (7). Nevertheless, no clear and consonant data clarify the genotype–phenotype association in SHOX-D yet, and many authors still claim the indistinguishability of phenotypes generated from enhancer mutations and the ones from single-nucleotide variants and deletions affecting the SHOX-coding region (8).

Recombinant human growth hormone (rhGH) therapy is already approved for use in patients with SHOX-D in the USA, Europe and other countries (9), and it seems to have a growth-promoting activity similar to patients with Turner syndrome (TS) (10). Nonetheless, to date, data on the long-term effectiveness of rhGH treatment in this category of paediatric patients are still too scarce (10, 11, 12, 13), and no conclusive data indicate whether different causative genotype could influence the response to rhGH therapy (14, 15).

The aim of our study is to report the real-life Italian long-term experience about the efficacy and safety of rhGH therapy in SHOX-D children and adolescents. Therefore, we collected data from several tertiary paediatric endocrine centres, affiliated to the Italian Society of Pediatric Endocrinology and Diabetology Study Group on Growth Factors and Puberty. Moreover, basing on the large national cohort, we would like to identify the potential predictive factors influencing the response to rhGH therapy, including different causative genotype.

Materials and methods

This is a national retrospective longitudinal cohort study enrolling patients with a genetically confirmed diagnosis of SHOX-D, treated on rhGH therapy at 14 Italian Pediatric Endocrine Clinics (Modena, Turin, Bologna, Genoa, Firenze, Naples, Milan, Reggio Emilia, Alessandria, Varese, Messina, Gallipoli, Trieste and Bari) from January 2010 to June 2022. Patients affected by other endocrine diseases (e.g. central precocious puberty, congenital adrenal hyperplasia, hypothyroidism and type 1 diabetes mellitus), organic brain lesion and systemic diseases as well

as patients taking drugs interfering with height growth, body weight and glucose metabolism (e.g. gonadotrophin-releasing hormone (GnRH) analogues, corticosteroids and chemotherapies) were excluded.

All anthropometric, laboratory, instrumental and therapeutic data were collected by experienced paediatric endocrinologists at baseline, at the beginning of rhGH treatment (time 0 (T0)), yearly on rhGH therapy (T1, T2, T3 and T4) and at near-final height (nFH) (T5), when available, and were anonymously recorded in a database using an alphanumeric and progressive identification code.

None of enrolled patients was previously involved in other protocols and/or publications.

The study was conducted in agreement with the Declaration of Helsinki and was approved by the Ethical Committee of the coordinator centre (Modena – protocol number 0016074/2019).

Parental written informed consent and patient assent were obtained at recruitment and before starting the data collection.

Anthropometric measures

All recruited patients underwent a complete past medical history (recorded data: chronological age (CA), gender, ethnicity, gestational age and birth anthropometric data, concomitant medical conditions and results of SHOX genetic testing). The anthropometric measurements were performed at any study time according to the Anthropometric Standardization Reference Manual in every centre by well-trained paediatric endocrine clinicians (16), and they included standing height (H), measured to the nearest 0.1 cm with a calibrated well-mounted Harpenden's stadiometer (Crymych, UK), compared with age-matched reference values and expressed as standard deviation score (H SDS) (17); sitting height, measured from the highest point of the head to the sitting surface; arm span, measured from the fingertips of one hand to the other with the arms raised parallel to the ground (18); body weight, measured to the nearest 0.1 kg with a calibrated scale, and pubertal staging, assessed using the Marshall and Tanner maturity scale (19, 20). Body mass index (BMI) was calculated by dividing weight in kilogram by square of the height (m²) and was standardized to SDS (BMI SDS) according to age using the appropriate Italian chart (17). nFH was defined when growth velocity (GV) was less than 1 cm/year during last 6 months and/or when the hand and wrist x-ray showed a process of epiphyseal fusion (bone age (BA) > 16 years in males, > 14 years in females) (21). Height

gain was defined as the difference between H at T4 or at T5 and H at the beginning of treatment (T0) and expressed in SDS. At each study time, the Rappold's scoring system, evaluating the presence of any combination of body disproportions (reduced arm span/H ratio (<96.5%), increased sitting H/H ratio (>55.5%)), the above-average BMI and the presence of Madelung deformity, of cubitus valgus, of short or bowed forearms and of dislocation of the ulna at the elbow or of muscular hypertrophy, was calculated and considered as an indicator of SHOX-D when ≥ 4 (22). Sitting H/H ratio was compared with age- and gender-matched reference values (23) and expressed both as absolute number and as its SDS (sitting H/H ratio SDS). The presence of other SHOX-D-related clinical features as tibial varus, muscular pseudo-hypertrophy and ogival palate was also verified and collected at each study time.

All patients were submitted to rhGH treatment according to the Italian Agency for Drugs guidelines (25).

Biochemical analysis

Fasting glucose, insulin and insulin-like growth factor 1 (IGF1) values were measured by local laboratories using specific commercially available kits at each study time. IGF1 levels were expressed as SDS: in our database, each IGF1 measurement has been documented with its specific age- and gender-adjusted reference ranges, constituting two s.d. above and below the age and gender adjusted mean IGF1, respectively. IGF1 SDS was calculated as the difference between measured IGF1 and mean IGF1, divided by the s.d. (25). Insulin resistance was defined by homeostatic model assessment index, compared to specific paediatric percentiles according to gender and pubertal stage (26), and by a fasting glucose/insulin ratio of <7 (27).

Genetic analysis of SHOX

The genetic analysis of SHOX gene was performed at different Italian centres according to local clinical practice.

Diagnostic imaging

The skeletal maturation was assessed on roentgenograms of the non-dominant hand and wrist, and the BA was determined according to the method of Greulich and Pyle (28). The presence of radiological dysmorphic features and bone abnormalities, including carpal wedging (pyramidization of the carpal row), distal radial lucency, shortness of fourth and/or fifth metacarpals,

the shape of distal radial epiphysis (flat, round, convex, triangular or trapezoidal) and/or Madelung deformity of the wrist, was evaluated by well-trained paediatric endocrine clinicians.

Statistical analysis

Data were checked for normal distribution using the Kolmogorov–Smirnov test. Continuous data are reported as mean \pm S.D., while categorical ones are reported as per cent values. The statistical analysis was performed in the total study population as well as in groups defined according to the SHOX-D genotype: patients carrying SHOX intragenic mutations or deletions (group A) and patients with SHOX enhancer mutations (group B). Between-group comparisons were performed using Mann–Whitney *U*-test (for two numerical variables), Kruskal–Wallis ANOVA (for more than two numerical variables) and Pearson χ^2 test (for per cent values). The Friedman ANOVA test was used for longitudinal comparison of variables in total population as well as in each group. Spearman correlation was used to identify the association between variables. A significative model (model SE = 0.40, $R^2 = 0.24$, $P = 0.005$) comprising height gain SDS at T4 as dependent variable and age when rhGH was started, rhGH starting dose and GV during the first and the second year of rhGH treatment as independent variables was used to perform the multiple regression analysis.

For each test, statistical significance was for $P < 0.05$.

The statistical analysis was performed using the STATISTICA™ software (StatSoft Inc., Tulsa, OK, USA).

Results

Baseline data

The initial total population comprises 117 children and adolescents (93% Caucasian, 74% prepubertal and 52% male) with a confirmed genetic diagnosis of SHOX deficiency, 75 with intragenic point mutation or deletion of SHOX gene and 42 with SHOX enhancer mutations (Tables 1 and 2). Anthropometric data at birth were normal (gestational age 38.71 ± 1.90 weeks, birth weight 3008.51 ± 534.09 g and length 48.22 ± 6.04 cm). rhGH was started at a mean age of 8.67 ± 3.33 years at a dose of 0.23 ± 0.04 mg/kg/week (range 0.12–0.35 mg/kg/week). Before starting rhGH treatment, patients showed a growth impairment ($H = -2.37 \pm 0.67$ SDS,

H adjusted for $TH = -1.11 \pm 1.11$ and $GV = -1.37 \pm 1.65$ SDS), mild BA delay ($BA - CA: -1.07 \pm 1.09$ years) as well as slight SHOX-D stigmata: arm span/height ratio equal to 0.97 ± 0.07 , sitting height/height ratio 0.56 ± 0.02 and its SDS 2.19 ± 1.27 and mean Rappold's score 7.51 ± 4.86 (range 0–24). Other phenotypic SHOX-D features such as tibial varus, muscular pseudohypertrophy, high-arched palate and radiological bone abnormalities were encountered in 34, 45, 17 and 46% of the total population, respectively. In the total population, IGF1 SDS was -0.99 ± 0.93 . The baseline clinical presentation was uniform among groups, regardless of different genotypes (Table 2).

Efficacy

In our cohort, rhGH therapy lasted about 5.94 ± 2.00 years (range 1.25–11.30 years), and it led to an overall height gain of $+0.80 \pm 0.98$ SDS from baseline until the achievement of nFH. Throughout the therapy, patients experienced a significant improvement of GV SDS, especially during the first 2 years of treatment, and a concomitant gradual increase of H SDS (Table 1 and Figs. 1 and 2). The therapy did not affect BMI SDS, and the bone delay reduced gradually over follow-up (Table 1). Globally, rhGH seemed to longitudinally prevent a worsening of both body proportions and Rappold's score and to limit the increase of prevalence of clinical SHOX-D features such as tibial varus, muscular pseudohypertrophy and high-arched palate (Table 1). The mean dose of rhGH did not change significantly along follow-up (Table 1). At the time of the analysis, among 117 SHOX-D children and adolescents started on rhGH at T0, 18 did not complete the first year of treatment, other 9 the second year, other 18 the third year and other 14 the fourth year of rhGH. No therapeutic dropout was registered along study time. By now, 46 patients reached nFH (nFH group). When the longitudinal analysis was performed exclusively in the nFH group, results were mostly overlapping the ones referred to the total population: a notable increase of H SDS and GV SDS was confirmed across rhGH, together with a statistical amelioration of body proportion, specifically a reduction of sitting height/H ratio SDS and a stability of Rappold's score (Table 3). Unlike the total cohort, in the nFH group BMI SDS increased at T5. Moreover, a little but significant increase of rhGH dose was detected from the third year of treatment (Table 3). Baseline anthropometric data did not differ between total and nFH groups.

A beneficial effect on growth of rhGH was demonstrated both in patients carrying mutations

Table 1 Baseline and longitudinal features of the total population.

	T0	T1	T2	T3	T4	T5	χ^2	<i>p</i>
Number of patients	117	99	90	72	58	46	n.a.	n.a.
Prepubertal (%)	87/117 (74%)	59/99 (60%)	45/90 (50%)	23/72 (32%)	14/58 (24%)	0/46 (0%)	51.57	<0.05 ^a
Male (%)	61/117 (52%)	51/99 (51%)	45/90 (50%)	39/72 (54%)	30/58 (52%)	19/46 (41%)	1.12	0.95
CA (years)	8.67 ± 3.33	9.68 ± 3.38	10.49 ± 3.33	11.25 ± 3.34	11.92 ± 3.06	15.64 ± 1.49	164.04	<0.05 ^a
BA (years)	7.94 ± 3.15	9.06 ± 3.25	9.85 ± 3.58	10.81 ± 3.98	12.44 ± 3.21	14.97 ± 1.36	23.09	0.00012^a
BA delay (years)	-1.07 ± 1.09	-0.89 ± 1.10	-0.77 ± 1.01	-0.67 ± 1.33	-0.32 ± 1.19	n.a.	5.82	0.21
H SDS	-2.37 ± 0.67	-1.91 ± 0.64	-1.64 ± 0.73	-1.45 ± 0.62	-1.39 ± 0.64	-1.65 ± 0.94	107.14	<0.05 ^a
BMI SDS	-0.07 ± 0.94	-0.09 ± 0.97	-0.05 ± 1.01	-0.13 ± 0.92	-0.02 ± 0.84	0.28 ± 1.04	13.19	0.02
GV SDS	-1.37 ± 1.65	1.85 ± 2.08	1.32 ± 2.23	0.99 ± 2.02	0.36 ± 2.19	-0.99 ± 2.21	18.27	0.002^a
Arm span/H ratio	0.97 ± 0.07	0.98 ± 0.13	0.97 ± 0.03	0.97 ± 0.03	0.97 ± 0.04	0.97 ± 0.02	2.13	0.83
Sitting H/H ratio	0.56 ± 0.02	0.56 ± 0.05	0.55 ± 0.02	0.55 ± 0.01	0.55 ± 0.02	0.56 ± 0.02	19.36	0.0016^a
Sitting H/H ratio SDS	2.19 ± 1.27	2.12 ± 1.35	2.10 ± 1.31	2.10 ± 1.28	2.11 ± 1.37	2.24 ± 1.12	9.74	0.08
Rappold's score	7.51 ± 4.86	7.72 ± 5.10	7.14 ± 5.67	7.08 ± 5.41	6.35 ± 5.36	7.57 ± 5.66	1.24	0.94
Tibial varus (%)	35/101 (34%)	38/69 (55%)	32/79 (40%)	28/65 (43%)	24/50 (48%)	22/42 (52%)	9.12	0.34
Muscular pseudohypertrophy (%)	45/100 (45%)	42/71 (59%)	36/80 (45%)	28/65 (43%)	21/50 (42%)	2/4 (50%)	7.3	0.56
High-arched palate (%)	15/89 (17%)	12/73 (16%)	12/67 (18%)	10/53 (19%)	8/38 (21%)	1/4 (25%)	4.5	0.67
rhGH dose (mg/kg/week)	0.23 ± 0.04	0.24 ± 0.05	0.24 ± 0.06	0.24 ± 0.06	0.24 ± 0.06	n.a.	4.97	0.28
IGF1 SDS	-0.99 ± 0.93	0.69 ± 1.30	0.87 ± 1.20	0.95 ± 1.42	1.16 ± 1.17	n.a.	48.56	<0.05 ^a

^a*P* < 0.05 from longitudinal analysis (T0–T5) using Friedman ANOVA test. Bold values represent statistical significance.

involving SHOX exons (group A) and in ones with SHOX enhancer mutations (group B), as shown in Table 2 (group A vs group B: H gain at T4 +1.11 ± 0.59 vs +1.17 ± 0.59 SDS, *P*=0.64; overall H gain +0.88 ± 0.96 vs +0.67±1.02 SDS, *P*=0.29; mean rhGH duration 5.82 ± 2.03 vs 6.12 ± 2.00 years, *P*=0.34). Even if short stature was similar in both groups, group B presented milder phenotype, as expressed in a persistently lower Rappold's score in group B than in group A along follow-up, especially reaching a statistical significance on therapy (at T3 and at T4) (Table 2). Moreover, only in group B, sitting height/height ratio, but not its SDS, ameliorated during rhGH therapy and a significant increase of rhGH dosage has been documented during the study period. This dose adequacy could be consequent to a significant decrease of H SDS and of GV SDS in group B than in group A during the second year of rhGH treatment (Table 2). During the course of rhGH treatment, IGF1 SDS increased in both groups.

In our cohort, H gain at T4 correlated with GV during first year (*r*=0.30), second year (*r*=0.37), third year (*r*=0.47) and fourth year (*r*=0.32) of rhGH treatment. Similarly, the overall H gain correlated with GV during the first (*r*=0.40), second (*r*=0.35) and third year (*r*=0.35) of rhGH treatment, but not with age when rhGH treatment was started (*r*=-0.15) and not with the initial rhGH dose (*r*=-0.26).

Nevertheless, the multiple regression analysis (model SE=0.40, *R*²=0.24, *P*=0.005) identified age when rhGH was started (β =-0.31, *P*=0.030) and GV during the first year of rhGH treatment (β =0.45, *P*=0.008) as the main independent predictor factors of height gain SDS at T4.

Safety

IGF1 levels increased during the course of the treatment and were significantly superior in group A than group B at T4. Even if some patients experienced high IGF1 SDS on therapy (IGF1 SDS above 2 SDS in 12.5% of patients at T1, in 14% at T2, in 16.3% at T3 and in 18% at T4, $\chi^2=8.5$, *P*=0.67), no related discontinuation, even transient, was documented, and no other adverse event of concern was reported.

A condition of initial insulin resistance was described already in 12% (8/68) of patients before the start of rhGH therapy, and this prevalence did not change significantly on treatment (T1: 11/84 (13%); T2: 12/73 (16%); T3: 11/58 (19%); T4 13/52 (25%), $\chi^2=6.56$, *P*=0.58).

Discussion and conclusion

Our data strongly confirm the efficacy of rhGH in SHOX-D patients. In fact, rhGH therapy led to an overall height gain of +0.80 ± 0.98 SDS from baseline until the achievement of nFH. In the last years, the access to rhGH treatment became gradually easier for SHOX-D children, first due to earlier clinical suspicion and more rapid genetic diagnosis than before and, secondly, due to the authorization of rhGH treatment in SHOX-D patients independent of the presence of GH deficiency. If not treated, SHOX-D final height is estimated around 2 SDS below the mean. If treated, about 40% of SHOX-D patients on rhGH therapy seemed to reach an appropriate FH in contrast to 4% of

Table 2 Baseline and longitudinal features of the study population according to genotype.

	T0	T1	T2	T3	T4	T5	χ^2	P
	Group A: SHOX intragenic point mutations or deletions (81% prepubertal at diagnosis, 39% reached nFH), n = 75							
CA (years)	8.07 ± 3.34	9.67 ± 3.48	10.68 ± 3.52	11.45 ± 3.52	12.12 ± 3.18	15.79 ± 1.54	94.33	<0.05 ^a
H SDS	-2.34 ± 0.64	-1.87 ± 0.62	-1.50 ± 0.74 ^{b(P = 0.018)}	-1.41 ± 0.66	-1.37 ± 0.69	-1.62 ± 1.00	67.65	<0.05 ^a
BMI SDS	-0.12 ± 0.91	-0.09 ± 0.82	0.03 ± 0.85	-0.04 ± 0.94	0.07 ± 0.89	0.29 ± 0.93	8.42	0.13
GV SDS	-1.28 ± 1.74	2.01 ± 1.98	1.78 ± 2.40 ^{b(P = 0.014)}	1.01 ± 2.08	0.55 ± 2.17	-0.51 ± 2.28	10.76	0.048^a
Arm span/H ratio	0.96 ± 0.09	0.98 ± 0.16	0.97 ± 0.02	0.97 ± 0.02	0.96 ± 0.04	0.97 ± 0.02	1.28	0.93
Sitting H/H ratio	0.56 ± 0.02	0.56 ± 0.06	0.54 ± 0.02	0.55 ± 0.01	0.55 ± 0.03	0.56 ± 0.02	9.83	0.07
Sitting H/H ratio SDS	2.29 ± 1.29	2.24 ± 1.43	2.18 ± 1.44	2.25 ± 1.07	2.29 ± 1.12	2.38 ± 1.15	7.29	0.19
Rappold's score	7.87 ± 4.57	8.65 ± 4.68	8.12 ± 5.60	8.33 ± 5.32 ^{b(P = 0.02)}	8.61 ± 5.43 ^{b(P = 0.01)}	8.31 ± 5.33	1.74	0.88
rhGH dose (mg/kg/week)	0.23 ± 0.04	0.24 ± 0.06	0.23 ± 0.06	0.23 ± 0.06	0.23 ± 0.06	n.a.	3.64	0.30
IGF1 SDS	-1.03 ± 0.88	0.54 ± 1.14	1.07 ± 1.20	1.01 ± 1.42	1.44 ± 1.18 ^{b(P = 0.03)}	n.a.	25.12	<0.05 ^a
	Group B: SHOX enhancer mutations (82% prepubertal at diagnosis, 40% reached nFH), n = 42							
CA (years)	8.56 ± 3.37	9.65 ± 3.30	10.14 ± 3.04	10.83 ± 3.09	11.65 ± 2.94	15.37 ± 1.40	69.72	<0.05 ^a
H SDS	-2.41 ± 0.74	-1.94 ± 0.67	-1.84 ± 0.65 ^b	-1.49 ± 0.53	-1.41 ± 0.59	-1.72 ± 0.84	40.57	<0.05 ^a
BMI SDS	0.01 ± 1.01	-0.07 ± 1.20	-0.15 ± 1.25	-0.30 ± 0.88	-0.17 ± 0.76	0.27 ± 1.24	7.41	0.19
GV SDS	-1.57 ± 1.44	1.65 ± 2.25	0.66 ± 1.74 ^b	0.87 ± 1.95	0.11 ± 2.23	-1.89 ± 1.93	11.88	0.03^a
Arm span/H ratio	0.98 ± 0.03	0.98 ± 0.02	0.98 ± 0.04	0.99 ± 0.03	0.97 ± 0.03	0.97 ± 0.03	4.30	0.50
Sitting H/H ratio	0.56 ± 0.03	0.56 ± 0.02	0.55 ± 0.02	0.55 ± 0.02	0.54 ± 0.01	0.55 ± 0.01	11.79	0.03^a
Sitting H/H ratio SDS	2.44 ± 1.26	2.31 ± 1.24	2.30 ± 1.13	2.38 ± 1.19	2.11 ± 1.26	2.46 ± 1.10	2.69	0.74
Rappold's score	6.61 ± 5.02	6.31 ± 5.45	5.96 ± 5.63	4.80 ± 4.46 ^b	4.42 ± 4.58 ^b	6.56 ± 6.12	1.19	0.94
rhGH dose (mg/kg/week)	0.24 ± 0.05	0.23 ± 0.05	0.25 ± 0.06	0.26 ± 0.05	0.25 ± 0.05	n.a.	11.36	0.009^a
IGF1 SDS	-0.93 ± 1.01	0.92 ± 1.55	0.51 ± 1.15	0.87 ± 1.47	0.76 ± 1.06 ^b	n.a.	26.18	<0.05 ^a

^aP < 0.05 from longitudinal analysis (T0–T5) using Friedman ANOVA test, in each group, ^bP < 0.05: group A vs B using Mann–Whitney U-test (for numerical variables) and Pearson χ^2 test (for per cent values) at the same study timepoint. Bold values represent statistical significance.

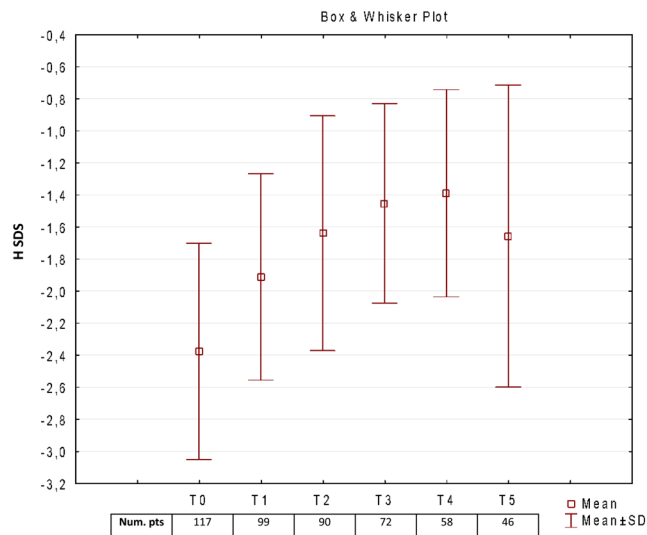


Figure 1 Longitudinal changes in height SDS in total SHOX-D population (χ^2 107.14, $P < 0.05$).

non-treated ones (4). First supporting data came from the use of rhGH in patients with TS, a condition that is associated with the loss of one SHOX gene because of the numerical or structural aberration of the X chromosome (29, 30). Subsequently, several studies supported short-term rhGH efficacy in SHOX-D (30, 31, 32). Nevertheless, up to now, long-term data are still lacking, and in the literature, to our knowledge, no more than 200 SHOX-D patients treated on rhGH and who achieved FH or nFH have been described (10, 11, 12, 13). In 2017, data from

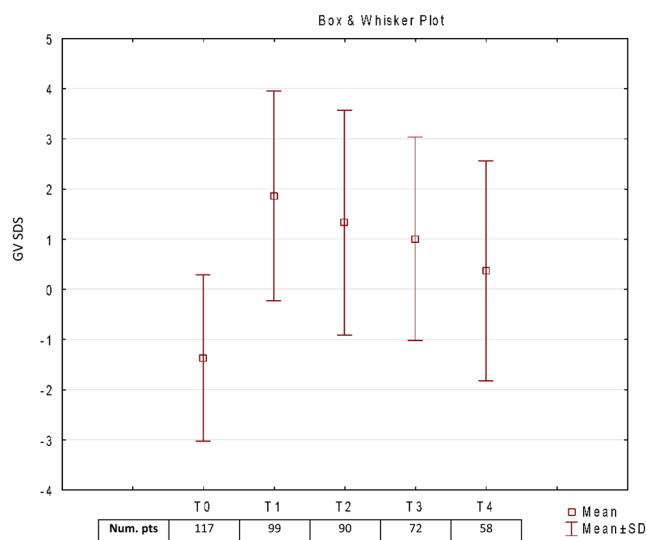


Figure 2 Longitudinal changes in growth velocity (GV) SDS in total SHOX-D population (χ^2 18.27, $P = 0.002$).

the Genetics and Neuroendocrinology of Short Stature International Study (GeNeSIS) observational study and clinical trial reported nFH of 90 and 28 SHOX-D patients, respectively: height gain was +0.83 SDS after 4.4 years of rhGH for the full GeNeSIS cohort (+1.19 SDS if patients were prepubertal at the beginning of the treatment) compared with +1.25 SDS for patients in the clinical trial after 6 years of therapy (13). These data are entirely in line with our real-life experience despite differences between the cohorts: a major prevalence of mutations in SHOX-regulatory regions (36% vs 4%) and a lower dose of initial rhGH (0.23 ± 0.04 vs 0.30 ± 0.09 mg/kg/week) characterized our cohort with respect to GeNeSIS's. Recently, also Dantas and colleagues published data on adult height in 18 SHOX-D patients that received rhGH therapy (10 on combined therapy with GnRH analogues) and in 13 followed without treatment: a negative median change in height from baseline to adult height of -0.8 SDS was documented in the latter, while the treated ones showed a median positive change in height of about $+0.6$ SDS (11). Our results are more promising than Dantas's linked to a larger studied population and despite the use of a lower median dose of rhGH (0.24 vs 0.34 mg/kg/week). In fact, in our report, the real-life dose of rhGH used in SHOX-D is lower with respect to the one that should be offered and usually overlapping Turner's indication: at least 0.35 mg/kg/week. In SHOX-D, the effect of rhGH therapy seems to be associated with the induction of the same intracellular signalling response triggered by endogenous GH and, in most cases, with an increased serum concentrations of the GH-dependent peptides such as IGF1. Thus, a strong dose-dependent growth-promoting effect of rhGH is expected (8). Analysing our data, we could justify the use of a general low-medium rhGH dose due to the retrospective design of the study itself: many patients with long follow-up may start rhGH as a replacement rather than a pharmacological therapy, maybe because they met the criteria to be classified as GH deficient. In the same way, we can only speculate that it is also the cause of the lack of a dose adjustment across follow-up, together with the real practice of each clinician, which maybe supported by a good anthropometric and biochemical response to the rhGH therapy itself among her/his patients, as shown in our report. In fact, despite the use of a low-medium rhGH dose, we demonstrated a significant increase of IGF1 SDS in response to rhGH therapy. Moreover, in our cohort, young age at the beginning of rhGH rather than initial dosage seems to influence long-term growth improvement, as revealed by the results of the multi-regression analyses. An earlier initiation of rhGH therapy

Table 3 Baseline and longitudinal features of nFH group (*n* = 46).

	T0	T1	T2	T3	T4	T5	χ^2	<i>p</i>
CA (years)	9.67 ± 2.50	10.71 ± 2.55	11.69 ± 2.58	12.26 ± 2.45	13.20 ± 2.38	15.64 ± 1.49	154.04	<0.05 ^a
BA (years)	8.84 ± 2.83	9.94 ± 2.89	11.21 ± 2.81	12.06 ± 2.91	13.30 ± 2.74	14.97 ± 1.36	15.13	0.004^a
BA delay (years)	-0.89 ± 1.00	-1.00 ± 1.25	-0.75 ± 0.96	-0.24 ± 1.10	-0.21 ± 1.35	n.a.	3.46	0.48
H SDS	-2.39 ± 0.68	-1.87 ± 0.51	-1.61 ± 0.68	-1.53 ± 0.47	-1.48 ± 0.51	-1.65 ± 0.94	99.77	<0.05 ^a
BMI SDS	-0.13 ± 1.03	-0.19 ± 0.99	-0.13 ± 1.03	-0.16 ± 0.89	-0.11 ± 0.73	0.28 ± 1.04	12.44	0.02^a
GV SDS	-1.80 ± 1.47	1.51 ± 1.99	0.82 ± 2.15	0.80 ± 2.09	0.46 ± 2.12	-0.99 ± 2.21	18.27	<0.05 ^a
Arm span/H ratio	0.97 ± 0.03	0.97 ± 0.03	0.96 ± 0.03	0.97 ± 0.02	0.96 ± 0.03	0.97 ± 0.02	2.13	0.83
Sitting H/H ratio	0.56 ± 0.01	0.57 ± 0.07	0.55 ± 0.01	0.55 ± 0.01	0.55 ± 0.02	0.56 ± 0.02	20.18	0.001^a
Sitting H/H ratio SDS	2.53 ± 1.06	2.51 ± 0.89	2.31 ± 0.93	2.48 ± 1.01	2.58 ± 1.16	2.24 ± 1.12	11.84	0.036^a
Rappold's score	7.19 ± 4.64	7.42 ± 4.85	7.09 ± 5.46	6.70 ± 5.07	6.66 ± 4.95	7.57 ± 5.66	1.25	0.939
rhGH dose (mg/kg/week)	0.25 ± 0.05	0.25 ± 0.05	0.25 ± 0.06	0.26 ± 0.05	0.27 ± 0.05	n.a.	17.74	0.001^a
IGF1 SDS	-0.92 ± 1.09	0.66 ± 1.56	1.05 ± 1.16	0.94 ± 1.49	1.19 ± 1.24	n.a.	22.75	<0.05 ^a

^a*P* < 0.05 from longitudinally analysis (T0-T4 or T5) using Friedman ANOVA test. Bold values represent statistical significance.

seems to strengthen growth in the prepubertal period and to preserve it from a gradual worsening in puberty, as occurred in untreated SHOX-D patients (13). As in other rhGH therapeutic indications, the main part of the catch-up growth happened in the first 2 years of treatment (13, 30, 32), but our current data demonstrated its long-term persistence: in fact, in our cohort, height gain after 4 years of rhGH treatment correlated with the GV not only on the first 2 years but also on the third and the fourth year of treatment.

Treatment response could be also affected by intrinsic individual sensibility and SHOX-D genotype as well as by external factors, including therapeutic adherence, concomitant replacement of other hormones, nutritional status and duration of treatment. In 2015, Donze SH and colleagues published data collected in a retrospective study from 130 children and adolescents (72 with SHOX point mutations or deletions, 44 with SHOX enhancer deletion and 14 with duplications of SHOX or its enhancer), of whom 52 were treated with rhGH (37/72 in the first group, 12/44 in the second group and 3/14 in the last group): patients with SHOX enhancer deletion seemed to be equally short but less disproportionate in comparison to patients with SHOX point mutations or deletions, and they showed a greater response to rhGH (14). Our data only partially confirm Donze's finding, while they are in line with other publications (33): in fact, we document that children carrying mutations involving SHOX enhancer seemed to present a milder phenotype, but not a less severe growth impairment at baseline, than ones with intragenic mutations. The amelioration of sitting height/height ratio in this group on rhGH therapy is only apparent because, considering the strong influence of age on this ratio, SDS is more reliable than the absolute values and it did not change during therapy in patients with SHOX enhancer

deletion. Nevertheless, at the same time, we prove a similar response to rhGH in the two groups not only in clinical parameters (improvement of H SDS, GV SDS and H gain SDS) but also in biochemical ones (increase of IGF1 SDS). The difference in IGF1 SDS at T4 between the two groups seemed, in our opinion, to be linked to a possible difference in the rate of puberty progression rather than to inter-groups difference of rhGH sensibility. In their paper, Donze and colleagues speculate that, if rhGH promotes the expression of SHOX via downstream GH-dependent transcription factors, the presence of two intact copies of SHOX in patients with enhancer mutation may cause a higher responsiveness to rhGH (14). Our population is wider than Donze's but still genetically heterogeneous, and there is still a relatively small number of patients within each variant that do not allow us for individual variant comparisons. We believe that only the study of the residual functionality of SHOX in each genetic variants could explain the phenotype in terms of degree of skeletal disproportion, in terms of clinical presentation and/or in terms of growth impairment as well as in terms of therapeutic response. By now, we can only conclude that rhGH therapy seems to blunt the SHOX-D phenotype, limiting a worsening of the body proportions and of the clinical SHOX-D stigmata (Rappold's score, tibial varus, muscular pseudohypertrophy and high-arched palate) that usually became more pronounced with age in un-treated SHOX-D patients (8). This effect could be mediated by the rhGH-related improvement of linear growth, especially involving long bones.

Concerning the safety of rhGH, our results did not evidence any new or unexpected rhGH-related short-term and long-term adverse events in SHOX-D patients (34).

Our current study presents several limitations, especially the possible variability of registered parameters

due to the enrolment of patients in different clinical centres and the absence of a uniform population at each time of study due to its retrospective design. At the same time, these conditions could be also considered as strengths of the study itself because it allowed us to study longitudinally a wide SHOX-D population (national cohort) in a real-life clinical setting. To date, the retrospective design of the study imposed us to collect some parameters such as Rappold's score currently used in the clinical practice but discussed in the literature if reliable (35). Some limitations could be overcome in a prospective phase of the study that is already scheduled.

Our data confirm the efficacy and safety of rhGH therapy in SHOX-D children, regardless of the wide variety of genotype. Moreover, the response to rhGH in the first year of treatment and the age when rhGH was started significantly impact the overall height gain.

Declaration of interest

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

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Author contribution statement

LI and PB conceptualized the study, analysed and interpreted the data and wrote the manuscript. All the authors acquired their patient data, contributed to fulfil the final database and approved the final version, and all agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

- Marchini A, Ogata T & Rappold GA. A track record on SHOX: from basic research to complex models and therapy. *Endocrine Reviews* 2016 **37** 417–448. (<https://doi.org/10.1210/er.2016-1036>)
- Mastromauro C & Chiarelli F. Novel insights into the genetic causes of short stature in children. *TouchREVIEWS in Endocrinology* 2022 **18** 49–57. (<https://doi.org/10.17925/EE.2022.18.1.49>)
- Kang MJ. Novel genetic cause of idiopathic short stature. *Annals of Pediatric Endocrinology and Metabolism* 2017 **22** 153–157. (<https://doi.org/10.6065/apem.2017.22.3.153>)
- Fanelli A, Vannelli S, Babu D, Mellone S, Cucci A, Monzani A, Al Essa W, Secco A, Follenzi A, Bellone S, *et al.* Copy number variations residing outside the SHOX enhancer region are involved in Short Stature and Léri-Weill dyschondrosteosis. *Molecular Genetics and Genomic Medicine* 2022 **10** e1793. (<https://doi.org/10.1002/mgg3.1793>)
- Leiden Open Variation Database (LOVD). All genes - global variome shared LOVD. Leiden, The Netherlands: Leiden University Center, 2023. (available at: <https://databases.lovd.nl/shared/genes>)
- Kurnaz E, Savaş-Erdeve Ş, Çetinkaya S & Aycan Z. SHOX gene deletion screening by FISH in children with short stature and Madelung deformity and their characteristics. *Journal of Pediatric Endocrinology and Metabolism* 2018 **31** 1273–1278. (<https://doi.org/10.1515/jpem-2018-0038>)
- Orso M, Polistena B, Granato S, Novelli G, Di Virgilio R, La Torre D, D'Angela D & Spandonaro F. Pediatric growth hormone treatment in Italy: a systematic review of epidemiology, quality of life, treatment adherence, and economic impact. *PLoS One* 2022 **17** e0264403. (<https://doi.org/10.1371/journal.pone.0264403>)
- Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. *Hormone Research in Paediatrics* 2011 **75** 81–89. (<https://doi.org/10.1159/000324105>)
- Savage MO. The changing face of paediatric human growth hormone therapy. *Endocrines* **3** 419–427. (<https://doi.org/10.3390/endocrines3030033>)
- Blum WF, Ross JL, Zimmermann AG, Quigley CA, Child CJ, Kalifa G, Deal C, Drop SLS, 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. (<https://doi.org/10.1210/jc.2013-1222>)
- Dantas NCB, Funari MFA, Vasques GA, Andrade NLM, Rezende RC, Brito V, Scalco RC, Arnhold IJP, Mendonça BB & Jorge AAL. Adult height of patients with SHOX haploinsufficiency with or without GH therapy: a real-world single-center study. *Hormone Research in Paediatrics* 2022 **95** 264–274. (<https://doi.org/10.1159/000524374>)
- Blum WF, Cao D, Hesse V, Fricke-Otto S, Ross JL, Jones C, Quigley CA & Binder G. Height gains in response to growth hormone treatment to final height are similar in patients with SHOX deficiency and Turner syndrome. *Hormone Research* 2009 **71** 167–172. (<https://doi.org/10.1159/000197874>)
- Benabbad I, Rosilio M, Child CJ, Carel JC, Ross JL, Deal CL, Drop SLS, Zimmermann AG, Jia N, Quigley CA, *et al.* Safety outcomes and near-adult height gain of growth hormone treated children with SHOX deficiency: data from an observational study and a clinical trial. *Hormone Research in Paediatrics* 2017 **87** 42–50. (<https://doi.org/10.1159/000452973>)
- Donze SH, Meijer CR, Kant SG, Zandwijken GR, van der Hout AH, van Spaendonck RM, van den Ouweland AM, Wit JM, Losekoot M & Oostdijk W. The growth response to GH treatment is greater in patients with SHOX enhancer deletions compared to SHOX defects. *European Journal of Endocrinology* 2015 **173** 611–621. (<https://doi.org/10.1530/EJE-15-0451>)
- 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. (<https://doi.org/10.1136/jmg.2009.067785>)
- Lohman TG, Roche AF & Martorell R, Eds. *Anthropometric Standardization Reference Manual*. Champaign IL: Human Kinetics Books, 1988.
- Cacciari E, Milani S, Balsamo A, Spada E, Bona G, Cavallo L, Cerutti F, Gargantini L, Greggio N, Tonini G, *et al.* Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). *Journal of Endocrinological Investigation* 2006 **29** 581–593. (<https://doi.org/10.1007/BF03344156>)
- Bogin B & Varela-Silva MI. Leg length, body proportion, and health: a review with a note on beauty. *International Journal of Environmental Research and Public Health* 2010 **7** 1047–1075. (<https://doi.org/10.3390/ijerph7031047>)
- Marshall WA & Tanner JM. Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood* 1969 **44** 291–303. (<https://doi.org/10.1136/adc.44.235.291>)
- Marshall WA & Tanner JM. Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood* 1970 **45** 13–23. (<https://doi.org/10.1136/adc.45.239.13>)

- 21 Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausk SD, Savage MO, Wit JM, 2007 ISS Consensus Workshop participants. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4210–4217. (<https://doi.org/10.1210/jc.2008-0509>)
- 22 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. (<https://doi.org/10.1136/jmg.2006.046581>)
- 23 Fredriks AM, van Buuren S, van Heel WJM, Dijkman-Neerincx RHM, Verloove-Vanhorick SP & Wit JM. Nationwide age references for sitting height, leg length, and sitting/height ratio, and their diagnostic value for disproportionate growth disorders. *Archives of Disease in Childhood* 2005 **90** 807–812. (<https://doi.org/10.1136/adc.2004.050799>)
- 24 AIFA. Modifica della Nota AIFA 39 di cui alla determina AIFAn. 458/2020. (Determina n. DG 390/2021). Rome, Italy: Agenzia Italiana del Farmaco (AIFA), 2021. (available at: https://www.aifa.gov.it/documents/20142/1491706/Determina_AIFA_390_2021_modifica_nota_39.pdf)
- 25 Tirosh A, Toledano Y, Masri-Iraqi H, Eizenberg Y, Tzvetov G, Hirsch D, Benbassat C, Robenshtok E & Shimon I. IGF-I levels reflect hypopituitarism severity in adults with pituitary dysfunction. *Pituitary* 2016 **19** 399–406. (<https://doi.org/10.1007/s11102-016-0718-1>)
- 26 d'Annunzio G, Vanelli M, Pistorio A, Minuto N, Bergamino L, Iafusco D, Lorini R & Diabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetes. Insulin resistance and secretion indexes in healthy Italian children and adolescents: a multicentre study. *Acta Bio-Medica: Atenei Parmensis* 2009 **80** 21–28.
- 27 Silfen ME, Manibo AM, McMahon DJ, Levine LS, Murphy AR & Oberfield SE. Comparison of simple measures of insulin sensitivity in young girls with premature adrenarche: the fasting glucose to insulin ratio may be a simple and useful measure. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2863–2868. (<https://doi.org/10.1210/jcem.86.6.7537>)
- 28 Greulich WW. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, 2nd ed. Stanford, CA: Stanford University Press 1959. (available at: <http://www.abebooks.com/book-search/title/radiographic-atlas-skeletal-development-hand-wrist>)
- 29 Li P, Cheng F & Xiu L. Height outcome of the recombinant human growth hormone treatment in Turner syndrome: a meta-analysis. *Endocrine Connections* 2018 **7** 573–583. (<https://doi.org/10.1530/EC-18-0115>)
- 30 Blum WF, Crowe BJ, Quigley CA, Jung H, Cao D, Ross JL, Braun L, Rappold G & SHOX Study Group. 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. (<https://doi.org/10.1210/jc.2006-1409>)
- 31 Munns CF, Berry M, Vickers D, Rappold GA, Hyland VJ, Glass IA & Batch JA. Effect of 24 months of recombinant growth hormone on height and body proportions in SHOX haploinsufficiency. *Journal of Pediatric Endocrinology and Metabolism* 2003 **16** 997–1004. (<https://doi.org/10.1515/jpem.2003.16.7.997>)
- 32 Iughetti L, Vannelli S, Street ME, Pirazzoli P, Bertelloni S, Radetti G, Capone L, Stasiowska B, Mazzanti L, Gastaldi R, *et al.* Impaired GH secretion in patients with SHOX deficiency and efficacy of recombinant human GH therapy. *Hormone Research in Paediatrics* 2012 **78** 279–287. (<https://doi.org/10.1159/000345354>)
- 33 Shapiro S, Klein GW, Klein ML, Wallach EJ, Fen Y, Godbold JH & Rapaport R. SHOX gene variants: growth hormone/insulin-like growth factor-1 status and response to growth hormone treatment. *Hormone Research in Paediatrics* 2015 **83** 26–35. (<https://doi.org/10.1159/000365507>)
- 34 Cianfarani S. Safety of pediatric rhGH therapy: an overview and the need for long-term surveillance. *Frontiers in Endocrinology (Lausanne)* 2021 811–846. (<https://doi.org/10.3389/fendo.2021.811846>)
- 35 Jorge AAL & Arnhold IJP. Anthropometric evaluation of children with SHOX mutations can be used as indication for genetic studies in children of short stature. *Journal of Medical Genetics* 2007 **44** e90.

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