SHOX GENE DELETION SCREENING by FISH in CHILDREN with SHORT STATURE and MADELUNG DEFORMITY, and THEIR CHARACTERISTICS

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Introduction

Idiopathic short stature (ISS) is defined as a condition in which a person's height is more than two standard deviations (SDs) below the average height for a specific age, sex, and population in the absence of other systemic, endocrine, nutritional, or chromosomal abnormalities or history of intrauterine growth retardation and low weight for gestational age. Furthermore, hundreds of variants clustered in specific genomic loci play roles in human height (2). One of these is the short stature homeobox-containing (*SHOX*) gene, which strongly affects height. Previous estimates have suggested that 1.1–16.9% of ISS patients (4–15) and 60–100% of patients with Leri–Weill dyschondrosteosis syndrome (LWS) have haploinsufficiency in *SHOX* .

SHOX is located in the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes. Therefore, both females and males have two functional copies of *SHOX* . Langer mesomelic dysplasia results from genetic changes involving both copies of *SHOX* in each cell, and LWS results from genetic changes involving just one copy of *SHOX*. Mutations or deletions of entire *SHOX* have been identified in some people of short stature. However, some people with short stature and changes in *SHOX* have been found to have subtle skeletal abnormalities.

We investigated the rate of *SHOX* haploinsufficiency via fluorescence *in situ* hybridisation (FISH) in a population of patients with short stature and described anthropometric measurements of the 8 patients with MD, ie LWS.

Patients and Methods

Between 2010 and 2017, we evaluated 86 patients (70 females, 16 males; age 4.3–18 years old) with a clinical diagnosis of short stature. The patients were selected based on the following inclusion criteria: a diagnosis of short stature based on height < -2.5 SD of the mean height for a given age, sex, and population group according to our national database; body disproportion, evaluated based on arm span–height difference and sitting height/standing height ratio; normal karyotype (for girls); no evidence of chronic disease (e.g., chronic renal failure, chronic anaemia, celiac disease, malabsorption, malnutrition, chronic hepatic disease, chronic infectious disease, or congestive heart failure); no growth hormone (GH) deficiency or GH resistance; MD (clinical or radiological) and/or family history suggestive of short stature and/or MD in a first-degree relative.

Mutation Analyses

Only girls with a normal karyotype and boys were included in subsequent FISH analyses. FISH analyses were performed on metaphase and interphase chromosome spreads using a probe specific to *SHOX*. Detection was performed using the Aquarius SHOX probe (Catalogue number LPU 025; Cytocell, Cambridge, UK) according to the manufacturer's instructions.

Results

According to our inclusion criteria, 78 of 86 patients (70 females, 16 males) had short stature and a family history suggestive of short stature. Eight patients had short stature, a family history suggestive of short stature, and MD. Afterwards these eight patients were further evaluated and presented in Table 1. The patient 1 receiving long-term GH therapy was presented in table 2.

Table 1. Clinical findings of patients with Madelung deformity with and without SHOX haploinsufficiency

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Sex	Female	Female	Female	Female	Female	Female	Boy	Female
Birth weight Gestational age (weeks)	2450 38	n/a 40	2250 40	3000 39	2100 36	2750 36	2600 37	3000 40
Maternal height (cm)	161.3 (-0.28 SDS)	142.9 (-3.1)	137.4 (-3.94 SDS)	147.6 (-2.4)	144.7 (-2.8)	160 (-0.5)	133.4 (-4.6)	160 (-0.5)
Paternal height (cm)	164.4 (-1.66 SDS)	170.2 (-0.87 SDS)	153 (-3.23 SDS)	150 (-3.6)	150.3 (-3.6)	173 (-0.5)	147 (-4.1)	172 (-0.6)
Target height SDS	165.4 cm (-1.04 SDS)	150.05 (-2 SDS)	138.7 (-3.74SDS)	142.3 (-3.2)	141 (-3.4)	160 (-0.5)	146.7 (-4.1)	159.5 (-0.6)
Parent with Madelung deformity	No	No	No	No	No	No	Yes	No
Age at admission for short stature and/or dyschondrosteosis (years)	6.9	13.6	6.6	13.4	9.1	9.7	17.9	8.8
Bone age	5.8 years	12	5.8 years	13	8.8	8.8	18	7.8
Height at admission (cm)	105.6 (-3.03 SDS)	134.4 (-4.2 SDS)	93.6 (-5.14)	127.5 (-5.3)	117 (-2.65)	112.3(-3.1)	160(-2.6)	108.3(-4)
Weight at admission	19.4 (-1.02 SDS)	33.4 (-3.2 SDS)	13.25 (-3.58)	36 (-2.5)	34.4 (0.88)	28.9(0.3)	58.9 (-1.5)	17.9 (-2.8)
BMI at referral (kg/m^2)	17.4 (0.87 SDS)	18.49 (-0.8 SDS)	15.12 (-0.26)	22.2 (0.7)	24.1 (2.2)	22.9(2.1)	23(0)	15.3(-0.5)
Arm span: Height (cm)	-13.1 (<-2 SDS)	-7.3 (<-2SDS)	-11.1 (<-2 SDS)	3 (1 to 2)	-3 (mean to -1)	-20 (<-2)	4.5 (mean to1)	-0.3 (mean to 1)
Sitting height (SH)/height (H) ratio (SDS)	0.55 (mean)	0.51 (-2 to -1)	0.54 (-1)	0.52 (mean)	0.56 (+1)	0.57 (+2)	0.54 (mean to 1)	0.53 (-1)
Increased SH/H ratio	No	No	No	No	No	Yes	No	No
Madelung deformity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Scoliosis	No	No	No	Yes	No	No	No	No
Muscular hyperthrophy	Yes	No	No	No	No	No	No	No
Short neck	Yes	Yes	No	No	No	No	No	Yes
Cubitus valgus	Yes	Yes	No	No	No	No	No	Yes
Leri–Weill dyschondrosteosis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Bone age – Chronological age	-1.04	-1.5	-1	-0.4	-0.3	-0.9	0.1	-1
Pubertal condition at admission	Prepubertal	Pubertal	Prepubertal	Pubertal	Prepubertal	Prepubertal	Pubertal	Prepubertal
GH treatment	Yes	No	No	No	No	No	No	No
Age at GH treatment start	7.4	-	No	No	No	No	No	9, Used for a short time
Additional anomaly	No	No	Ebstein's anomaly, VSD, ASD	No	No	No	No	No
SHOX deletion	Yes	Yes	Yes	No	No	No	No	No

SDS: standard deviation score; BMI: body mass index; GH: growth hormone; n/a: not available; VSD: ventricular septal defect; ASD: atrial septal defect

Table 2. Clinical and laboratory findings of patient 1.

Treatment status	Chronological age (Yr.)	Bone age	Height (cm)/SDS	Weight (cm)/SDS	BMI/SDS	Pubertal status	Growth velocity (cm/year)	GH dose (mg/kg/week)	IGF–1 (ng/mL) (SDS)	IGFBP3 (ng/mL) (SDS)	IGF-1/IGFBP3 ratio (SDS)
At start of treatment	7.3	5,75	107/-3.2	21.6/-0.7	18.9/1.3	Prepubertal	4.3	0.35	155.5 (Mean–1)	4270 (mean–1)	0.04
3 mo								0.30*	466 (>2)	4610 (1–2)	0.1
6 mo								0.30	309 (1–2)	4870 (1–2)	0.06
12 mo	8.3	7.8	117.2/-2.1	28.5/0.3	20.7/1.6	Prepubertal	10.2	Stop	811 (>2)	6610 (1–2)	0.13 (>2)
13 mo								0.3**	295 (1–2)	5510 (1–2)	0.05
14 mo								0.25*	831 (>2)	5580 (1–2)	0.14 (>2)
21 mo								0.25	491 (>2)	5810 (1–2)	0.08
24 mo	9.3	10 yr.	123.4/-1.8	32.4/0.3	21.3/1.5	Prepubertal	6.2	Stopped	503 (>2)	5100 (mean–1)	0.09 (>2)
25 mo								0.2**	162 (-1 to m)	4240 (mean–1)	0.04
28 mo								Stopped	641 (>2)	6400 (1–2)	0.1 (>2)
38 mo	10.6		126.8/-2.2	40.6/0.7	25.2/2.1	Tanner stage 2	3.4	0.17**	242 (m-1)	5130 (mean–1)	0.05
39 mo								Stopped	611 (>2)	6130 (1–2)	0.09 (2)
48 mo	11.3	12 yr.	129.3/-2.9	42.2/0.2	25.2/1.9	Tanner stage 2	2.5	0.17**	218 (-1 to m)	5410 (m–1)	0.04
55 mo	12		133.3/-3.1	44.6/-0.04	25.3/1.7	Tanner stage 3	4 cm	Stopped	365 (m–1)	4940 (-1 to mean)	0.07

SDS: standard deviation score, mo: months; *Therapy ended, **Therapy resumed, m: mean

Conclusion

Our clinical findings suggest that patients with shortened arm span relative to height, MD, cubitus valgus, and muscular hypertrophy are the most likely to have *SHOX* haploinsufficiency. Furthermore, the incidence of MD may have been higher in the cohort if X-rays were performed in all individuals.

In our study, we could only give GH treatment to the first patient, and it had to be interrupted due to the detection of high levels of IGF-1/IGFBP3 during follow-up. This likely explains why the patient's height SD did not increase. Still, it is noteworthy that there have been no long-term evaluations of patients with *SHOX* haploinsufficiency treated with GH. In conclusion, we identified *SHOX* deletions in three children, corresponding to 37.5% of individuals with MD, whilst no deletions were detected in the 78 patients with only short stature. Since we likely missed cases due to our methodology, the routine analysis for SHOX screening should be firstly multiplex ligation-dependent probe amplification. Due to the importance of early diagnosis and treatment, screening for *SHOX* haploinsufficiency among children of disproportionately short stature should be considered.

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