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Prepubertal Girls With Turner Syndrome and Children With Isolated SHOX Deficiency Have Similar Bone Geometry at the Radius

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Context: The low bone mineral density (BMD) and alterations in bone geometry observed in patients with Turner syndrome (TS) are likely caused by hypergonadotropic hypogonadism and/or by haploinsufficiency of the *SHOX* gene.

Objective: Our objective was to compare BMD, bone geometry, and strength at the radius between prepubertal girls with TS and children with isolated SHOX deficiency (SHOX-D) to test the hypothesis that the TS radial bone phenotype may be caused by SHOX-D.

Design and Setting: This comparative cross-sectional study was performed between March 2008 and May 2011 in 5 large centers for pediatric endocrinology.

Patients: Twenty-two girls with TS (mean age 10.3 years) and 10 children with SHOX-D (mean age 10.3 years) were assessed using peripheral quantitative computed tomography of the forearm.

Main outcomes: BMD, bone geometry, and strength at 4% and 65% sites of the radius were evaluated.

Results: Trabecular BMD was normal in TS (mean Z-score = -0.2 ± 1.1, $P = .5$) as well as SHOX-D patients (mean Z-score = 0.5 ± 1.5, $P = .3$). At the proximal radius, we observed increased total bone area (Z-scores = 0.9 ± 1.5, $P = .013$, and 1.5 ± 1.4, $P = .001$, for TS and SHOX-D patients, respectively) and thin cortex (Z-scores = -0.7 ± 1.2, $P = 0.013$, and -2.0 ± 1.2, $P < .001$, respectively) in both groups. Bone strength index was normal in TS as well as SHOX-D patients (Z-scores = 0.3 ± 1.0, $P = .2$, and 0.1 ± 1.3, $P = .8$, respectively).

Conclusions: The similar bone geometry changes of the radius in TS and SHOX-D patients support the hypothesis that loss of 1 copy of *SHOX* is responsible for the radial bone phenotype associated with TS. (***J Clin Endocrinol Metab* 98: E1241–E1247, 2013**)

Turner syndrome (TS) is a congenital disease caused by the complete or partial loss of one X chromosome and occurs in 1 in 2000 live female births (1). Short stature and ovarian failure are the main phenotypic characteristics of TS. Among the other symptoms associated with TS, low bone mineral density (BMD) has been demonstrated

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Abbreviations: BMC, bone mineral content; BMD, bone mineral density; MA, muscle area; MLPA, multiplex ligation-dependent probe amplification; pQCT, peripheral quantitative computed tomography; SHOX, short stature homeobox-containing gene; SHOX-D, SHOX deficiency; SSI, strength-strain index; TS, Turner syndrome; vBMD, volumetric BMD.

by many densitometry studies using both dual-energy x-ray absorptiometry (2, 3) and peripheral quantitative computed tomography (pQCT) (4, 5). In addition, girls and women with TS are more prone to fractures compared with the general population (6, 7). Recent pQCT studies suggest that not only low BMD but also changes in bone geometry (ie, decreased cortical thickness and reduced relative cortical bone cross-sectional area at the radius) may contribute to bone fragility in patients with TS (5, 8). The etiology of the complex changes in BMD and bone geometry has not been fully elucidated, but previous authors have speculated that the changes are due to either the influence of hypergonadotropic hypogonadism or the deletion of genes controlling bone development as a result of gonosomal haploinsufficiency (9, 10).

The short stature homeobox-containing gene (*SHOX*) discovered by Rao et al (10) has been proposed as the most probable gene locus responsible for the skeletal alterations in TS. This gene is situated at the end of the short arm of both gonosomes X and Y, specifically in pseudoautosomal region 1, which escapes X chromosome inactivation and thus acts as an autosomally inherited trait. Despite the observed link between the SHOX transcription factor and several genes regulating chondrogenesis (eg, *FGFR3*, *SOX5*/*SOX6*, *SOX9*, and *Agc1*), limited information is available on the intracellular pathways activated by SHOX (11, 12). Nevertheless, embryological and histopathological studies highlight the role of SHOX in long bone development and growth by showing that the expression of *SHOX* is specific to the midparts of the limbs and the first and second pharyngeal arches of the human embryo (13) and to the growth plates of fetuses and also children up to the cessation of pubertal growth (14). Thus, *SHOX* haploinsufficiency might cause the alterations in bone composition and geometry observed in TS.

Loss of one copy of *SHOX* has also been observed in non-TS patients. Mutations of *SHOX* (SHOX deficiency [SHOX-D]) have been found in as many as 15% of children of both genders with idiopathic short stature (15) and in 50% to 90% of patients with Léri-Weill dyschondrosteosis (16, 17). Biallelic *SHOX* mutations cause a severe form of skeletal dysgenesis called Langer syndrome (18, 19). The phenotype of patients with SHOX-D is not uniform. In addition to the 2 main features, short stature and a distinct grade of mesomelic limb shortening, a number of other skeletal characteristics are frequently observed in subjects with SHOX-D (bowing of the tibia, genu valgum, shortening of the fourth and fifth metacarpals, high arched palate, increased carrying angle of the elbow, scoliosis, and micrognathia) (20). Interestingly, these phenotypes are shared between TS and SHOX-D.

To test the hypothesis that the changes in BMD and bone geometry observed in TS are caused by SHOX-D, we compared volumetric BMD (vBMD) and bone geometry at the radius between girls with TS and children with isolated SHOX-D. To minimize the effect of estrogens on bone parameters, only prepubertal patients were included in this study.

Patients and Methods

Patients with TS

We examined 22 prepubertal girls with TS (median age 10.9 years, range 6.0–13.8 years) who were regularly followed at the University Hospital Motol in Prague, Czech Republic, and who had no other disease affecting bone metabolism except controlled autoimmune thyroiditis in 2 girls. The clinical characteristics of the patients are summarized in Table 1. All girls with TS presented with Tanner stage 1 breast development. Their karyotypes were either 45,X (7 of 22, 32%), various forms of mosaicism (13 of 22, 59%), or a structurally abnormal X chromosome (2 of 22, 9%).

All girls with TS were treated with recombinant human GH at a starting dose of 50 μg/kg/d, which was adjusted during therapy according to the clinical response (21). Median age at the start of GH therapy was 5.5 years (range 2.8–11.8 years), and the median duration of GH administration was 3.5 years (range 0.3–10.0 years). No other medications known to influence bone metabolism were administered except for T_4 substitution, which was used to control autoimmune thyroiditis in 2 girls who had been euthyroid for a long period before the densitometry was performed.

Patients with isolated SHOX-D

Ten prepubertal patients (4 girls and 6 boys, median age 11.0 years, range 6.7–12.7 years) from 7 unrelated families with genetically confirmed isolated SHOX-D were recruited from 4 university centers for pediatric endocrinology across the Czech Republic. The selection of patients for genetic testing was based on

Table 1. Summary of the Clinical Characteristics of the 2 Patient Groups[a]

	TS (n = 22)	SHOX-D (n = 10)	Difference (*P* value)
Age y	10.3 (2.2)	10.3 (2.1)	.950
Height age, y	8.5 (2.2)	9.4 (2.0)	.265
Height, cm	132.8 (12.9)	138.4 (11.6)	.239
Height Z-score	−1.6 (1.0)[d]	−0.81 (0.43)[d]	.003
Weight, kg	33.3 (10.4)	38.7 (8.0)	.122
Weight Z-score	−0.52 (1.0)[b]	0.56 (0.76)[b]	.003
BMI, kg/m^2	18.4 (3.0)	20.0 (1.9)	.075
BMI Z-score	0.44 (0.87)[b]	1.2 (0.83)[c]	.032

Abbreviation: BMI, body mass index.

[a] Mean (SD) values are shown. The Z-scores were calculated using national reference data, and a 1-sample *t* test was used to compare the Z-scores with the healthy population.

[b] $P < .05$.

[c] $P < .01$.

[d] $P < .001$.

their phenotypic characteristics (short stature and dysmorphic signs) and/or family history (20, 22). The clinical description of the study group is summarized in Table 1. All but 2 children had been treated with GH for a median duration of 12 months (range 6–108 months).

All patients were analyzed using the commercial multiplex ligation-dependent probe amplification (MLPA) kit (Salsa P018-E1 SHOX; MRC Holland, Amsterdam, The Netherlands), which covers the *SHOX* gene, its regulatory sequences, and the adjacent X-specific region. MLPA reaction was run with 50 to 150 ng DNA, according to the manufacturer's instructions. Subsequent fragmentation analysis was conducted on an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, California). A negative control was included in every run. Visual examination of the peak patterns was performed for each sample. Peak areas were normalized according to manufacturer's recommendations. Subsequently, the ratios of the probands' peak areas vs controls' samples were determined. A personally constructed Microsoft Excel table (Microsoft Corp, Richmond, California) was used for the entry of all of these calculations. Normal peak ratios were classified within the range of 0.65 to 1.35, whereas deletions and duplications were classified as having a ratio less than 0.65 or greater than 1.35, respectively. Each positive sample was confirmed in an independent MLPA replicate.

In 6 of 7 families, whole *SHOX* gene deletions were detected, with variable telomeric or centromeric breakpoints, and X chromosome-specific sequences were not affected. More specifically, in 3 families (4 patients), the deletion included the *SHOX* gene and both the upstream promoter sequences as well as known downstream regulatory elements (enhancers *CNE4*, *CNE5*, and *CNE9*). In the other 3 families (5 patients), the deletion covered only the *SHOX* gene and its upstream promoter sequences. In the last patient, only *SHOX* promoter region was deleted. Promoter deletion is considered to have an indistinguishable phenotypic effect relative to whole *SHOX* gene deletion (23).

Measurements

On the day of the bone density assessment, each patient's height was measured with a wall-mounted stadiometer to the nearest 1 mm and their weight was measured with an electronic scale to the nearest 100 g. Body mass index was calculated as the ratio of weight (kilograms) to height squared (square meters). For these 3 anthropometric variables, Z-scores were calculated using national reference data (24). To account for the short stature of the subjects in further analyses, height age was obtained for each patient using the same national reference data. Auxological parameters (ie, height and weight) were assessed by an experienced anthropologist (D.Z.) in standardized conditions and with approved anthropometric gauges.

A pQCT (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany) was used for bone assessment at the nondominant radius. A single tomographic 2-mm–thick slice was taken at the distances corresponding to 4% and 65% of forearm length measured from the processus styloideus ulnae to the olecranon. A standard voxel size of 0.4 mm × 0.4 mm × 2.0 mm was used. Image processing and calculation of numerical values were performed using version 6.00 B of the integrated XCT software. At the distal radius (4% site), bone mineral content (BMC), total bone cross-sectional area (total bone area) and trabecular vBMD were measured. At the proximal radius (65% site), BMC, total bone area, cortical bone cross-sectional area (cortical bone area), polar strength-strain index (SSI) and muscle area (MA) were assessed. SSI was determined using a segmentation threshold of 480 mg/cm^3. Total and cortical bone areas were determined by detecting the outer and inner cortical bone contours at a threshold of 710 mg/cm^3. Relative cortical bone area was mathematically derived by dividing cortical bone area by total bone area. Cortical thickness was calculated as described by Neu et al (25). BMC divided by MA (BMC/MA ratio) was used to evaluate the muscle-bone unit. Z-scores were calculated using published reference data from the related neighboring German population (26, 27) and implementing Cole's formula (28): Z-score = [Ln-(patient's test result/M)]/S, where Ln is the natural logarithm, M corresponds to the age- and sex-specific mean value, and S is the age- and sex-specific coefficient of variation. Z-scores for relative cortical bone area and cortical thickness were computed using the reference data compiled by Neu et al (25) and applying the formula: (patient's test result − age- and sex-specific mean value)/age- and sex-specific SD. To account for the shorter height of the patients, height-specific Z-scores of bone geometry parameters were calculated using height age instead of chronological age in the described mathematical formulas.

According to the recommendation of International Society for Clinical Densitometry (29), the precision error (expressed as the root mean square SD) was obtained from 3 consecutive measurements with repositioning in 15 healthy young adult volunteers (mean age 25.1 years). Precision errors of the primary measures were as follows: at the distal radius, BMC 0.943 mg/mm, total bone area 9.617 mm^2, and trabecular vBMD 2.905 mg/cm^3; at the proximal radius, BMC 0.745 mg/mm, total bone area 1.193 mm^2, cortical bone area 1.021 mm^2, SSI 7.685 mm^3, and MA 46.313 mm^2.

Statistical analyses

The statistical computing environment R (30) was used to carry out all statistical analyses. We report data as means (SD). The Z-scores were compared with the healthy population using a 1-sample *t* test. For 2-group comparisons, we performed a 2-sample *t* test with Welch approximation to the degrees of freedom. For all tests, the reported *P* values correspond to 2-sided alternatives.

The study was approved by the Ethics Committee of the University Hospital Motol, Prague. Informed consent was obtained from all participants and/or their guardians.

Results

The pQCT-derived bone parameters expressed as mean values and mean Z-scores are summarized separately for patients with TS and for patients with isolated SHOX-D in Table 2.

At the metaphysis of the radius, trabecular vBMD and total bone area were normal in both TS and SHOX-D patients. BMC was increased in patients with isolated SHOX-D, whereas it was normal in TS patients. No significant differences were found between the 2 groups.

At the diaphysis, both patient groups presented an increased total bone area ($P = .013$ and $P = .01$ for TS and SHOX-D, respectively) and decreased relative cortical

Table 2. Bone Geometry and BMD Parameters at the Radius in the 2 Patient Groups[a]

	TS (n = 22)	SHOX-D (n = 10)	Difference (*t* test), *P* value
Distal radius			
BMC, mg/mm	59.1 (15.7)	77.4 (19.9)	.022
BMC Z-score	0.5 (1.1)	1.2 (1.1)[b]	.13
Total bone area, mm^2	211.5 (64.4)	246.1 (93.0)	.31
Total bone area Z-score	0.4 (1.2)	0.5 (1.7)	.94
Trabecular vBMD, mg/cm^3	185.4 (33.7)	215.1 (45.8)	.088
Trabecular vBMD Z-score	−0.2 (1.1)	0.5 (1.5)	.21
Proximal radius			
BMC, mg/mm	54.1 (10.8)	58.4 (12.8)	.37
BMC Z-score	−0.2 (0.9)	−0.5 (1.3)	0.58
Total bone area, mm^2	96.6 (32.1)	116.2 (32.2)	.13
Total bone area Z-score	0.9 (1.5)[b]	1.5 (1.4)[b]	.36
Cortical bone area, mm^2	38.2 (10.8)	37.9 (9.4)	.94
Cortical bone area Z-score	−0.4 (1.0)	−1.1 (1.3)[b]	.13
Relative cortical bone area, %	42.7 (15.1)	34.1 (9.8)	.067
Relative cortical bone area Z-score	−0.9 (1.4)[c]	−2.3 (1.4)[d]	.02
Cortical thickness, mm	1.31 (0.44)	1.12 (0.29)	.15
Cortical thickness Z-score	−0.7 (1.2)[b]	−2.0 (1.2)[d]	.014
SSI, mm^3	136.5 (47.3)	158.4 (51.9)	.27
SSI Z-score	0.3 (1.0)	0.1 (1.3)	.71
MA, mm^2	1886.2 (438.9)	2171.9 (473.6)	.12
MA Z-score	0.3 (1.2)	0.4 (0.9)	.92
BMC/MA	2.91 (0.38)	2.73 (0.53)	.35
BMC/MA Z-score	−0.5 (0.9)[b]	−0.8 (1.5)	.6

[a] Mean (SD) values are shown. All Z-scores are height-specific except trabecular vBMD Z-score. A 1-sample *t* test was used to compare the Z-scores with the healthy population.

[b] $P < .05$.

[c] $P < .01$.

[d] $P < .001$.

bone area ($P = .007$ and $P < .001$ for TS and SHOX-D, respectively), with more obvious changes in patients with isolated SHOX-D (Figure 1). As a consequence, cortical thickness was decreased in TS as well as in isolated SHOX-D patients ($P = .013$ and $P < .001$, respectively). All Z-scores of bone geometry parameters at the radial diaphysis were height-specific by using height age.

Patients with TS did not differ from isolated SHOX-D patients in SSI, MA, or BMC/MA ratio (Table 2).

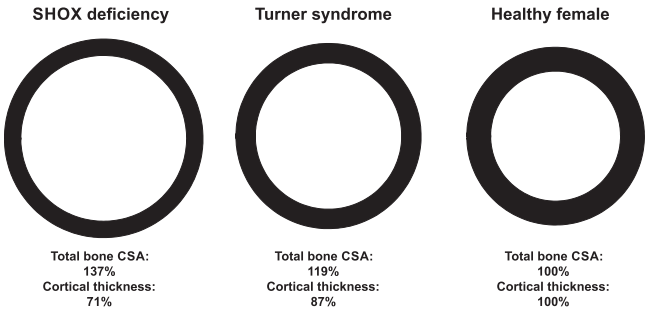

Figure 1. Schematic illustration of the bone geometry at the diaphysis of the radius in SHOX-D, TS, and healthy girls, respectively. The cross-sections are proportional. Height-specific Z-scores presented in this study were compared with reference data for prepubertal healthy children (25).

Discussion

This study compares vBMD and bone geometry of the radius between patients with TS and patients with isolated SHOX-D. We show that subjects with TS and SHOX-D share similar changes in bone geometry at the proximal radius (increased total bone area, decreased relative cortical bone area, and a thin cortex) and that some of these changes are more pronounced in cases of isolated SHOX-D. Our findings support the hypothesis that SHOX haploinsufficiency is responsible for the changes in shape and geometry of the radius observed in TS.

In our previous studies, we examined patients with TS and SHOX-D separately. We observed that girls with TS have an alteration in cortical bone (enlarged total bone area and decreased cortical thickness). Low trabecular vBMD was observed in pubertal and postpubertal patients but not in prepubertal girls (8). Interestingly, patients with SHOX-D presented with similar changes in cortical bone, but their trabecular vBMD was normal (31). Investigating the etiology of changes in bone geometry and bone structure in TS is challenging because the influence of estrogen deficiency on the bone microstructure must be considered.

Therefore, to make the two patient groups as comparable as possible, we included only prepubertal subjects while keeping in mind that also these children are exposed to the minimal amounts of estrogens, which can potentially affect the bone. A thinner bone cortex and an enlarged total bone area at the diaphysis of the radius were present in TS as well as in SHOX-D patients. The interpretation of these findings may be that this phenotype arises as a result of an adjustment of long bones with a disrupted cortex to the mechanical loading aimed at increasing the bone strength. Physiologically, an enlargement of the cross-section of a bone, leading to higher bone strength, has been described as an adaptation of the bone to mechanical loading mediated by skeletal muscle contraction (32). This complies with an important finding in both patient groups of our study, which is the normal SSI. SSI is a calculated surrogate of the resistance of the bone to bending and torsion and has been validated in ex vivo studies (33). Therefore, we may conclude that prepubertal TS as well as SHOX-D patients retain adequate radial bone strength.

Whether bone quality (ie, cortical vBMD) plays any part in the described changes of radial bone geometry in patients with TS and SHOX-D is not clear. In accordance with a previous study (5), we observed decreased cortical vBMD relative to age-specific reference data in both groups (mean Z-scores = −2.0, $P < .001$, and −2.2, $P = .001$, for TS and SHOX-D, respectively). However, these findings could be substantially underestimated due to the partial volume effect (34). To cope with this issue, we recalculated the Z-scores with cortical vBMD values corrected by the Rittweger's formula (34). Interestingly, after correcting for the thin cortex, cortical vBMD was rather increased in TS (mean Z-score = 1.1 ± 1.1, $P < .001$) as well as SHOX-D patients (Z-score = 1.3 ± 0.8, $P < .001$). The role of technical setting (beam hardening) or biological factors (age, height, and pubertal status) has been discussed previously (8) but still remains speculative. Because we cannot definitely determine the cortical vBMD in these specific groups of patients with thin cortices and large total bone areas using pQCT, we have excluded this measure from further analyses.

Interestingly, changes in some of the bone geometry parameters at the proximal radius (ie, relative cortical bone area and cortical thickness) were more pronounced in patients with isolated SHOX-D than in TS. This is in agreement with the higher prevalence of characteristic skeletal changes in SHOX-D compared with TS patients (the short forearm and Madelung deformity are 6 times more prevalent, and the shorter lower leg is 4 times more frequent) (20) and also with the more severe phenotypes (higher triangularization index) observed in SHOX-D patients (35). The moderate bone phenotypes of patients with a complete loss of an X chromosome (TS) compared with the more pronounced alterations in those with only 1 gene deletion (SHOX) are not well understood. One possible explanation is that the bone phenotype in TS is blunted by the karyotype heterogeneity, specifically by preserved SHOX function in patients with mosaicism. However, we failed to prove that there is any influence of karyotype on BMD or bone geometry in TS patients in our previous study (8), which is in agreement with many other studies (2, 6, 7, 36, 37). A single study showed a normal lumbar spine BMD T-score in patients with 45,X/46,XX mosaicism compared with a decreased BMD T-score in patients with the classical 45,X karyotype (38). However, bone geometry parameters were not assessed in that study. Whether other locus deletions that occur as a result of widespread loss of the X chromosome in TS mitigate the effects of isolated *SHOX* mutations remains to be elucidated.

A second possible explanation was proposed by Binder et al (35) who suggested that a low serum estrogen level could suppress the development of the Madelung deformity. This hypothesis was based on the observations that skeletal changes are less frequent in males than females in Léri-Weill dyschondrosteosis (18, 39) and that a lower prevalence of the Madelung deformity was reported among hypogonadal patients with TS relative to patients with isolated SHOX-D who have normal sex steroid production (20, 35). Our results contradict this idea. If estrogens drive the changes in cortical bone geometry in isolated SHOX-D, we would expect that bone phenotypes would not differ between TS and isolated SHOX-D during the prepubertal period, which was not the case in the present study.

Because GH is thought to impact bone metabolism, one could speculate about the role of GH therapy on the described skeletal changes in our patients. However, previous studies performed on TS patients showed no difference in metacarpal cortical thickness between GH-treated and untreated girls with TS (40) and no significant influence of the duration of GH therapy on bone geometry as assessed by pQCT (8). It is therefore very unlikely that GH therapy significantly contributes to the skeletal phenotype observed at the proximal radius in patients with a loss of 1 copy of *SHOX*.

In conclusion, our finding of shared bone geometry of the radius in prepubertal patients with the loss of 1 copy of *SHOX* (TS and isolated SHOX-D) clarifies our understanding of their skeletal phenotypes and suggests that increased total bone area and a thin cortex may represent the typical bone features in subjects with SHOX-D. These results agree with the hypothesis that the loss of

SHOX is responsible for diaphyseal radial changes associated with TS.

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