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Decreased bone mineral density in Costello syndrome

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ABSTRACT

Introduction: Costello syndrome (CS) is a multisystemic disorder characterized by postnatal reduced growth, facial dysmorphism, cardiac defects, cognitive impairment, skin and musculo-skeletal anomalies, and predisposition to certain cancers. CS is caused by activating germline mutations in the *HRAS* proto-oncogene. Similar to what is observed in other RASopathies, CS causative *HRAS* mutations promote enhanced signal flow through the RAF–MEK–ERK and PI3K–AKT signaling cascades. While decreased bone mineralization has been documented in other RASopathies, such as neurofibromatosis type 1 and Noonan syndrome, systematic studies investigating bone mineral density (BMD) are lacking in CS.

Materials and methods: Dual-energy X-ray absorptiometry (DXA) was utilized to assess BMD and body composition (fat and fat-free mass) in a cohort of subjects with molecularly confirmed diagnosis of CS ($n = 9$) and age-matched control individuals ($n = 29$). Using general linear regression, subtotal body (total body less head), lumbar, femoral neck and femur BMD parameters were compared considering age, sex, body mass index (BMI) and Tanner stage. Blood and urine biomarkers of bone metabolism were also assessed.

Results: All individuals with CS showed significantly lower mean values of subtotal, lumbar and femoral neck BMD compared to the control group ($p \leq 0.01$). Similarly, mean total body mass and fat-free mass parameters were lower among the CS patients than in controls ($p < 0.01$). Low 25-OH vitamin D concentration was documented in all individuals with CS, with values below the reference range in two patients. No significant correlation between vitamin D levels and BMD parameters was observed.

Discussion: CS belongs to a family of developmental disorders, the RASopathies, that share skeletal defects as a common feature. The present data provide evidence that, similar to what is recently seen in NF1 and NS, bone homeostasis is impaired in CS. The significant decrease in BMD and low levels of vitamin D documented in the present cohort, along with the risk for pathologic fractures reported in adult individuals with CS, testifies the requirement for a preventive treatment to alleviate evolutive complications resulting from dysregulated bone metabolism.

1. Introduction

Costello syndrome (CS) (OMIM #218040) is a developmental disorder characterized by distinctive facial features, postnatal reduced growth, cardiac defects and hypertrophic cardiomyopathy, intellectual disability, skin–muscle–skeletal anomalies and predisposition for certain rare cancers during childhood [1]. CS is a rare condition, with approximately 300 individuals reported worldwide [2]. It is caused by heterozygous activating mutations in *HRAS*, which encodes one member of a small family of monomeric GTPases functioning as a major node in intracellular signaling [3]. RAS signaling is implicated in several cellular functions, including cell fate determination, proliferation, survival, and differentiation, and within this network, signal flow through the RAF–MEK–ERK pathway mediates early and late developmental processes controlling morphology determination, organogenesis, synaptic plasticity and growth. Besides the role played by RAF–MEK–ERK pathway in oncogenesis [4], this intracellular pathway has recently been recognized as the cause of an emerging family of clinically related disorders known as RASopathies. This group of disorders includes an increasing number of conditions

Abbreviations: CS, Costello syndrome; NS, Noonan syndrome; CFCS, cardiofaciocutaneous syndrome; NF1, neurofibromatosis type 1; DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density; S-BMD, subtotal bone mineral density; WBLH, whole body less head; L-BMD, lumbar bone mineral density; FN-BMD, femoral neck bone mineral density; F-BMD, femur bone mineral density.

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among which Noonan syndrome (NS) and neurofibromatosis type 1 (NF1) are the most common [5]. These are caused by heterozygous mutations in genes encoding RAS proteins, regulators of RAS function and modulators of RAS interaction with downstream signal transducers effectors [6].

Several lines of evidence support the relevant role of RAS signaling in bone homeostasis and modeling [7–9]. Orthopedic problems represent an important diagnostic element of CS and related RASopathies. Bone mineral density (BMD) and skeletal abnormalities have been well studied in patients with NF1 [10–19], and recently investigated in Noonan syndrome [20]. Skeletal involvement in CS has been clinically reported in a small cohort of patients [7,21–23]. White et al. described the adult phenotype of CS, and reported six patients with osteoporosis and two with osteopenia. Stevenson et al. reported clinical BMD parameters from different body regions, using unadjusted z-score measurements in 7 affected cases. Dual energy X-ray absorptiometry (DXA) measurements in CS, however, have not yet been systematically examined with appropriate controls.

In the present study, we investigated BMD in a cohort of individuals with CS compared to a control group controlling for potential confounders such as sex, age, body mass index (BMI) and Tanner stage. Biochemical markers of bone metabolism in individuals with CS are also provided.

2. Materials and methods

Ten individuals with a clinical and molecular diagnosis of CS (7 F; 3 M) admitted to the Center for Rare Diseases of Catholic University (Rome, Italy) for regular clinical follow-up were recruited for the study. Informed written consent was obtained. DXA imaging was not performed on individuals <5 years of age.

A cohort of 29 healthy controls (18 F; 11 M) was selected from the DXA studies database of healthy subjects of the Department of Internal Medicine (Catholic University, Rome) using age and sex as selection criteria. Age range in the control group was 5.3 to 29.6 years (mean ± SD = 10.3 ± 5.3 years).

All patients were examined by experienced pediatricians and a physical therapist. Growth measurements were evaluated by standard clinical balance (weight) and wall-stadiometer (height). Tanner stage [24,25] was assessed in both individuals with CS and controls. BMI was calculated using the standard formula: weight/height^2 (kg/m^2). No subject participating in the study had history of bone pain or evidence of bone fractures in the year prior to enrollment for BMD and biomarker evaluation. Family history of osteoporosis and non-traumatic fractures was collected in all individuals. Physical activity was evaluated qualitatively, counting the amount of hours spent playing sports or doing physical therapy per week, over a time frame of three months prior to the study.

Body composition consisting of fat free mass (FFM) and fat mass (FM) as well as bone mineral density parameters were obtained by dual-energy X-ray absorptiometry scans (MOC-DXA, Lunar Prodigy Advance, GE Healthcare). Whole body scans were obtained with the patient supine, arms placed at one's side with palms down. Subtotal body measurements were evaluated by subtracting the head region from the rest of the body. The bone parameters evaluated by DXA in the studied were: subtotal bone mineral density (whole body less head) (S-BMD g/cm^2), lumbar bone mineral density (L-BMD g/cm^2), femoral neck bone mineral density (FN-BMD g/cm^2), and femur bone mineral density (F-BMD g/cm^2).

Markers of bone metabolism were obtained from blood (*i.e.*, calcium, phosphorus, magnesium, 25-hydroxy (25-OH) vitamin D, parathyroid hormone, serum c-terminal telopeptide of the β-1 chain of type 1 collagen and IGF1), and 24 hour urine collection (*i.e.*, calcium, phosphorus, creatinine, and phosphate). 25-OH vitamin D concentrations were analyzed by automated chemiluminescence immunoassay, using a Liaison analyzer (DiaSorin-RIA). The lower detection value for this method was 7.0 ng/ml, with a total CV (coefficient of variation) lower than 10% [26,27]. Serum 25-OH vitamin D concentrations were categorized into 3 classes: optimal ≥30 ng/ml, insufficient 20–29 ng/ml, and deficient ≤20 ng/ml.

Dietary calcium intake was assessed by 7-day diet record evaluation [28]. A general linear model was used to compare the bone parameters between cases and controls, while controlling for potential confounders (*i.e.*, age, sex, BMI, and Tanner stage). Significant values were set at $p = 0.05$. Pearson correlation was used to assess the relationship between 25-OH vitamin D concentrations, and S-BMD and L-BMD.

3. Results

Thirty-nine individuals were enrolled in the study. Age range in the CS cohort ($n = 10$) was 2.2 to 28.9 years, whereas in the control group ($n = 29$) it was 5.3 to 29.6 years (Table 1). Within the CS cohort, all subjects fulfilled the criteria for a clinical diagnosis of CS and harbored disease causative mutations in *HRAS*. Specifically, the common c.34G>A missense change (p.Gly12Ser) was documented in 9 cases, while one individual was heterozygous for the less common c.37G>T substitution (p.Gly13Cys) (Table 2). One individual was younger than 5 years of age; for this reason she did not undergo DXA imaging, but did have biochemical markers of bone metabolism obtained. As expected, height and weight were significantly lower in the CS cohort compared to the control group (Table 1). All individuals were ambulant, even though multiple skeletal abnormalities, such as scoliosis, pes planus, and tight heel cords were present. Level of physical activity was annotated, together with any other significant sign or feature during physical examination (Table 2). In particular skin findings such as skin hyperpigmentation, acanthosis nigricans and hyperkeratosis were described in Table 2 and clinically compared with vit D concentrations. The review of medical records documented that CS individual #8 was on treatment with valproate; similarly, CS individual #10 was on statin and thyroid therapy due to hypercholesterolemia and hypothyroidism, respectively. Both individuals exhibited delayed puberty (ID#8 at 14 y of age and ID#10 showed delayed menarche at 19 y old but she started to have signs of puberty at 10 y of age). There was no family history of osteoporosis or non-traumatic fractures among participants. DXA analysis documented significantly lower S-BMD, L-BMD, and FN-BMD values in the CS cohort compared to the control group (Table 3), while no statistically significant differences between cohorts in femur BMD and fat mass measures were observed. There was no statistically significant correlation between 25-OH vitamin D concentrations and S-BMD measures ($p = 0.11$) or L-BMD parameters ($p = 0.12$).

Evaluation of the biochemical markers of bone metabolism in both blood and urine samples documented that serum phosphorus levels were below the assay reference interval in 4/10 individuals (range 4–7 mg/dl), while serum 25-OH vitamin D was insufficient in 3/10, deficient in 5/10, and lower than the cutoff of 7 ng/ml in 2/10 (Table 4). No information on sunlight exposure by standardized questionnaire was collected, ruling out the possibility of exploring correlations between vitamin D concentrations and hours of sunlight

Table 1
Clinical characteristics.

Subjects	Costello syndrome ($n = 10$)	Controls ($n = 29$)
Gender	7 F/3 M	11 M/18 F
Age (y)	13.13 ± 8.31	10.38 ± 5.35
Weight (kg)	25.53 ± 13.09	35.26 ± 14.67
Height (cm)	116.13 ± 25.18	134.28 ± 18.56
BMI (kg/m^2)	17.47 ± 2.50	18.76 ± 3.02
Tanner stage	4 F/stage I	8 F/stage I
	2 M/stage I	10 M/stage I
	3 F/stage IV	10 F/stages II–V
	1 M/stage IV	1 M/stage V