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Decreased bone mineralization in children with Noonan syndrome: Another consequence of dysregulated RAS MAPKinase pathway?

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ABSTRACT

Introduction: Noonan syndrome (NS) is a disorder of RAS- mitogen activated protein kinase (MAPK) pathway with clinical features of skeletal dysplasia. This pathway is essential for regulation of cell differentiation and growth including bone homeostasis. Currently, limited information exists regarding bone mineralization in NS.

Materials and methods: Using dual-energy X-ray absorptiometry (DXA), bone mineralization was evaluated in 12 subjects (mean age 8.7 years) with clinical features of NS. All subjects underwent genetic testing which showed mutations in *PTPN11* gene ($N=8$) and *SOS1* gene ($N=1$). In a subgroup of subjects with low bone mass, indices of calcium-phosphate metabolism and bone turnover were obtained.

Results: 50% of subjects had low bone mass as measured by DXA. *Z*-scores for bone mineral content (BMC) were calculated based on age, gender, height, and ethnicity. Mean BMC *z*-score was marginally decreased at −0.89 {95% CI −2.01 to 0.23; $p=0.1$}. Mean total body bone mineral density (BMD) *z*-score was significantly reduced at −1.87 {95% CI −2.73 to −1.0; $p=0.001$}. Mean height percentile was close to - 2 SD for this cohort, thus total body BMD *z*-scores were recalculated, adjusting for height age. Adjusted mean total body BMD *z*-score was less reduced but still significant at −0.82 {95% CI −1.39 to −0.25; $p=0.009$}. Biochemical evaluation for bone turnover was unremarkable except serum IGF-I and IGF-BP3 levels which were low-normal for age.

Discussion: Children with NS have a significantly lower total body BMD compared to age, gender, ethnicity and height matched controls. In addition, total BMC appears to trend lower in children with NS compared to controls. We conclude that the metabolic bone disease present resulted from a subtle variation in the interplay of osteoclast and osteoblast activity, without clear abnormalities being defined in the metabolism of either. Clinical significance of this finding needs to be validated by larger longitudinal studies. Also, histomorphometric analysis of bone tissue from NS patients and mouse model of NS may further elucidate the relationship between the RAS-MAPK pathway and skeletal homeostasis.

1. Introduction

Noonan syndrome (NS) (OMIM ID: 163950) is an autosomal dominant disorder characterized by dysmorphic facies, short stature, delayed puberty, cardiac defects, and chest and spine deformities. About 50% of the NS patients have a missense *PTPN11* mutation along with less common mutations in *KRAS*, *SOS1*, *NRAS*, and *RAF1* genes. [1–4]. *PTPN11* encodes Src homology 2 domain containing tyrosine phosphatase 2 (SHP-2), a protein tyrosine phosphatase that acts in the RAS-mitogen activated protein kinase (MAPK) signal transduction pathway. *PTPN11* mutations in NS are mostly gain of function, disrupting SHP-2's activation-inactivation mechanism. Abnormally increased phosphatase activity of mutant SHP-2 has also been implicated in leukemogenesis in patients with NS [5].

Others and we have reported that children as well as adults with neurofibromatosis type 1 (NF1) have low bone mass [6–14]. The *NF1* gene encodes neurofibromin, a multidomain molecule involved in regulation of several intracellular processes, including the ERK, RAS-MAPK cascade, adenylyl cyclase, and cytoskeletal assembly. Recent studies investigating neurofibromin have suggested several

Abbreviations: NS, Noonan syndrome; NF1, Neurofibromatosis type 1; BMD, Bone mineral density; BMC, Bone mineral content; DXA, Dual-energy X-ray absorptiometry; IRB, Institutional Review Board.

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important roles in skeletal development and growth. *Nf1* inactivation in murine undifferentiated mesenchymal cells leads to bowing of tibia and diminished growth associated with decreased stability of the cortical bone, higher degree of porosity, decreased stiffness and reduction in the mineral content. At the cellular level, osteoblasts showed an increase in proliferation and a decreased ability to differentiate and mineralize *in vitro* [15]. In addition to an osteoblastic defect, $Nf1^{+/-}$ mice were found to contain elevated numbers of osteoclasts with increased resorptive activity [16]. Also, osteoclasts from patients with NF1 were reported to be insensitive to bisphosphonates *in vitro*. Ras inhibitor FTS counteracted the insensitivity of osteoclasts to bisphosphonates [17]. These reports suggest that RAS-MAPK pathway is important in postnatal skeletal homeostasis.

NS is also a disorder of RAS-MAPK pathway. While clinical features such as short stature, chest and spine deformities suggest an underlying skeletal dysplasia; little information is known about mineralization of bone and associated function of osteoblasts and osteoclasts on a tissue level. Stevenson et al. studied urine markers of bone resorption in patients with RAS-MAPK pathway disorders ($N=49$), which included 14 patients with NS. Their data suggested increased bone resorption in these patients compared to control subjects ($N=99$) [18]. Takagi et al. reported 2 adult male patients with NS with osteopenia and postulated estrogen deficiency as a possible etiology [19]. Noordam et al. studied 16 children with Noonan syndrome before and after growth hormone therapy and reported low trabecular vBMD at baseline with improvement following growth hormone therapy [20]. Also, Reinker et al. reported 2 adult patients with NS with osteopenia but no information was provided regarding how the diagnosis was made [21].

In the present study, we investigated the bone mineral status in a group of children with clinical and molecular diagnosis of NS compared to age, gender, and ethnicity matched controls using DXA. Our aim was to determine whether gain of function of RAS-MAPK signal transduction seen in NS can result in abnormal bone mass due to altered bone formation or resorption. We also assessed bone metabolic markers in patients with low BMD as defined by *z*-score of <-2.

2. Materials and methods

The IRB approved the study for Human Subject Research at Baylor College of Medicine. Informed, written consent was obtained from parents. Based on variability between subjects and DXA scan, sample size of 10 for NS cohort was calculated to detect a difference of 10%. This assumes a type I error of 0.05 and power of 0.8.

Twelve patients were recruited from Pediatric Endocrinology Clinic and/or Medical Genetics Clinic at Texas Children's Hospital, Houston, Texas. All the subjects were diagnosed with NS based on clinical features and underwent genetic testing. These subjects were unselected for skeletal problems. Patients with metabolic bone disorders or on medications known to affect bone metabolism such corticosteroids and growth hormone were excluded. Height (± 0.5 cm) was measured with a stadiometer, and weight (± 0.1 kg) was measured on a standard clinical balance. The body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Dietary calcium intake was assessed by detailed food frequency questionnaire about dairy products [22]. Drug intake and fracture history were also included in the medical history.

Bone mineral content (BMC) and areal bone mineral density (BMD) measurements were obtained with a Hologic Delphi-A instrument (Software version 11.2). The following bone parameters were obtained: BMC, bone area, and BMD. DXA results are presented as *z*-scores {$z=$(subject BMD - matched control BMD)/control SD} where controls were matched for age, gender and ethnic background from the database maintained at the Body Composition Laboratory of the Children's Nutrition Research Center for healthy children [23]. We used only total body measurements since on adjusting for height age, most of the children were <8 years old and *z*-score for lumbar spine measurements were not available for ages <8 years.

Full bone metabolism work up including serum calcium, phosphorus, magnesium, intact parathyroid hormone levels by immunochemiluminometric assay, 25 hydroxy vitamin D (by liquid chromatography, tandem mass spectrometry), osteocalcin by immunoradiometric assay, and urinary collagen I N-telopeptide by enhanced chemiluminescence were done at clinic visit after DXA showed BMD *z*-score of <-2. IGF-I and IGFBP3 levels were measured by immunoassay.

We used SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA) for statistical analysis. The distribution of *z*-scores was normal (using one-sample Kolmogorov–Smirnov test) and hence comparison of *z*-score mean with the zero mean reference value was made with the parametric two-sided one-sample *t*-test. 95th percentile confidence intervals were calculated.

3. Results

Twelve children with NS (9 males, 3 females) participated in the study. This cohort included different ethnicities representative of the region and diversity of the Clinics at Texas Children's Hospital: Caucasian ($n=6$), Hispanic ($n=4$) and Asian ($n=2$). Mean age of patients was 8.7 ± 4.2 years (range: 3.8–15.8 years). Eleven subjects were prepubertal at Tanner stage 1 and one subject was at Tanner stage 3. Eleven of the twelve patients had facial dysmorphism characteristic of NS. These patients also had variable degree of developmental delay. Pulmonary stenosis was the most common congenital heart disease found in 9 out of 12 patients. One patient who had mutation in the *SOS-1* gene did not have any cardiac abnormalities. One patient also had Tetralogy of Fallot and another one had pulmonary stenosis as a part of hypoplastic right heart syndrome. Two patients had coagulopathy and four male subjects had history of cryptorchidism.

Eight patients had mutation in *PTPN11* gene and one patient in *SOS1*. Mutation analysis of *PTPN11, KRAS, RAF-1*, and *SOS-1* was negative in three patients (Table 1). The mutation in the *PTPN11* gene in patient 3 resulted in amino acid change of Valine to Leucine at codon 354 (p.V354L). This is a novel variant but is not considered to be causative, even though he had several clinical features of NS. Array comparative genomic hybridization analysis showed that this patient and his mother had a gain of one clone on the subtelomeric region of the short arm of chromosome 20 [arr cgh>20p13(RP11-371L19) x3.nuc ish 20p13(RP11-371L19x3)] (chr20: 659,205–785,463). The *SIRPA* gene is located centromeric to this region (chr20: 1822813–1868540), which encodes a product that acts as docking protein and induces translocation of PTPN6, PTPN11 and other binding partners from the cytosol to the plasma membrane.

As expected, the mean height *z*-score was reduced at -2.14. The mean BMI was 50th %tile $\pm$ 30.01. The mean calcium intake was 942.8 ± 254 mg/day. None of the subjects enrolled were taking corticosteroids, growth hormone or any other medication affecting bone metabolism. None of our patients had history of fractures.

The mean BMC *z*-score was reduced at -0.88 {95% CI -2.01; 0.23}, $p=0.109$. Mean total body BMD *z*-scores was -1.87 {95% CI -2.73; -1.0}, $p=0.001$ (Fig. 1). The use of *z*-score does not consider differences in body size between healthy children and children with NS of the same age. As most of our patients' heights were below -2 SD for general population, we recalculated total body BMD *z*-score based on their height age. The recent prediction models for adjusting for stature published by Zemel et al. could not be utilized as most of our cohort was of less than 7 years of age. The mean adjusted total body BMD *z*-score was -0.82 {95% CI -1.39; -0.25}, $p=0.009$ (Fig. 1).

In patients with low BMD as defined by *z*-score <-2 ($N=6$), we evaluated several markers of bone metabolism (Table 2). All biochemical parameters measured were within the normal range. Serum IGF-I and IGFBP3 were in the low normal range for the respective age groups.