



HAL
open science

Low bone mass in Noonan syndrome children correlates with decreased muscle mass and low IGF-1 levels

Marine Delagrangé, Vanessa Rousseau, Catie Cessans, Catherine Pienkowski, Isabelle Oliver, Béatrice Jouret, Audrey Cartault, Gwenaëlle Diene, Maithé Tauber, Jean-Pierre Salles, et al.

► To cite this version:

Marine Delagrangé, Vanessa Rousseau, Catie Cessans, Catherine Pienkowski, Isabelle Oliver, et al.. Low bone mass in Noonan syndrome children correlates with decreased muscle mass and low IGF-1 levels. *BONE*, 2021, 153, pp.116170. 10.1016/j.bone.2021.116170 . hal-03781177

HAL Id: hal-03781177

<https://ut3-toulouseinp.hal.science/hal-03781177>

Submitted on 16 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Low bone mass in Noonan syndrome children correlates with decreased muscle mass and low IGF-1 levels

Short title: Bone Health in Children with Noonan Syndrome

Marine Delagrangé ^a, Vanessa Rousseau ^b, Catie Cessans ^a, Catherine Pienkowski ^a, Isabelle Oliver ^a, Béatrice Jouret ^a, Audrey Cartault ^a, Gwenaëlle Diene ^a, Maithé Tauber ^a, Jean-Pierre Salles ^a, Armelle Yart ^c, Thomas Edouard ^{a,c*}

^a Endocrine, Bone Diseases and Genetics Unit, Reference Center for Rare Diseases of Calcium and Phosphate Metabolism, ERN BOND, OSCAR Network, Pediatric Research Unit, Children's Hospital, Toulouse University Hospital, Toulouse, France

^b MeDatAS-CIC unit, CIC1436, Toulouse University Hospital, Toulouse, France

^c RESTORE, INSERM UMR1301, CNRS UMR5070, Université Paul Sabatier, Université de Toulouse, Toulouse, France.

*** Corresponding author**

Dr. Thomas Edouard

Endocrine, Bone Diseases and Genetics Unit, Children's Hospital, Toulouse University Hospital

330 avenue de Grande-Bretagne TSA 70034

31059 Toulouse cedex 9, France

Phone: +33 (0)5 34 55 85 55; Fax: +33 (0)5 34 55 85 58; Email: edouard.t@chu-toulouse.fr

Declarations of interest: none

Abstract

Although musculoskeletal abnormalities have long been described in patients with Noonan syndrome (NS), only a few studies have investigated the bone status of these patients. The aim of this retrospective observational study was to describe the bone health of children with NS. Thirty-five patients with a genetically confirmed diagnosis of NS were enrolled. We analyzed the axial skeleton (lumbar spine) using dual energy X-ray absorptiometry and the appendicular skeleton (hand) with the BoneXpert system. Bone metabolism markers, including mineral homeostasis parameters, serum 25-hydroxy vitamin D (25-OHD) levels and markers of bone formation and resorption were also reported. Compared to the general population, axial and appendicular bone mass was significantly decreased in children with NS ($p < 0.0001$). Serum 25-OHD levels were low in about half of the patients and were negatively correlated with age ($r = -0.52$; $p < 0.0001$). Patients with NS exhibited reduced bone formation marker levels and increased bone resorption marker levels ($p < 0.0001$). No gender difference or genotype-phenotype correlations were found for the different bone parameters. Muscle mass and, to a lesser extent, serum insulin-like growth factor 1 (IGF-1) levels were independent predictors of whole-body bone mineral content ($p < 0.0001$ for both parameters; adjusted $R^2 = 0.97$). In conclusion, bone mass is reduced in children with NS and correlates with decreased muscle mass and low serum IGF-1 levels. These data justify addressing all potential threats to bone health including sufficient calcium and vitamin D intake, regular physical exercise, and hormone replacement therapy.

Keywords

Bone mass; Insulin-like growth factor 1; Muscle mass; Noonan syndrome; RASopathies; RAS/ERK signaling pathway

1. Introduction

Noonan syndrome (NS; MIM # 163950) is a rare autosomal dominant genetic disorder characterized by the association of distinctive facial features, cardiac defects, short stature, skeletal abnormalities, mild developmental delay, and cryptorchidism in males [1]. NS is caused by germline mutations in genes encoding components or regulators of the RAS/extracellular signal-regulated kinases (ERK) signaling pathway, which is essential for cell cycle differentiation, growth, and senescence [1]. Although approximately 20 genes have been implicated in NS to date, the most prevalent, detected in approximately 50% of NS patients, are heterozygous missense mutations in the *protein tyrosine phosphatase non-receptor type 11 (PTPN11)* gene, followed by the less common mutations in the *SOS1*, *RAF1*, *KRAS*, and *LZTR1* genes, which are found in 10%, 10%, <5%, and <1% of patients, respectively [1]. Mutations in gene components of the RAS/ERK pathway are also found in phenotypically close syndromes, notably NS with multiple lentigines (NSML; MIM # 151100), NS with loose anagen hair (MIM # 607721), cardiofaciocutaneous syndrome (CFCS; MIM # 115150), Costello syndrome (CS; MIM # 218040), and neurofibromatosis type 1 (NF1; MIM # 162200). Collectively, these disorders are now termed RASopathies [1]. The best-defined consequence of the gene mutations implicated in NS and other RASopathies is enhancement of RAS/ERK activation, which underlies the different defects observed in NS [1, 2].

Skeletal abnormalities (i.e. anterior chest wall anomalies, scoliosis, kyphosis, pes planus, and hand anomalies) are common and have long been described in patients with NS and other RASopathies [3]. Although several studies have reported decreased bone mass in some RASopathies, notably CS [4-6] and NF1 [7-18], only a few studies have investigated bone status in NS patients [19-22]. These studies have been performed on a small number of patients, sometimes without molecular diagnosis of NS, and using non-reference methods (such as phalangeal quantitative ultrasound and radiographic absorptiometry). Given the important role of SHP2, the protein encoded by the *PTPN11* gene [23-25] and the RAS/ERK signaling pathway [26, 27] in normal osteoblast and osteoclast differentiation and function, the investigation of bone status in NS is important.

The purpose of this retrospective study was to investigate bone mass in children and adolescents with a molecular diagnosis of NS. We analyzed the axial skeleton (lumbar spine) using dual energy X-ray absorptiometry (DXA) and the appendicular skeleton (hand) with the BoneXpert system. The bone parameters were adjusted for the short stature of these patients. Bone metabolism markers, including mineral homeostasis parameters, serum 25-hydroxy vitamin D levels, and markers of bone formation and resorption, were also reported. Finally, the influence of bone mass determinants, including gender, genotype, hormonal status, and muscle mass, were analyzed using multiple regression analyses.

2. Subjects and methods

2.1. Patient population

Our retrospective study specifically focused on children whose NS had been confirmed by molecular diagnostics and who were followed up with at the Children's Hospital in Toulouse, France, between 2012 and 2019. A germline mutation in the *PTPN11*, *SOS1*, *RAF1*, *KRAS*, or *LZTR1* genes was considered for the molecular diagnosis of NS. A distinction was made between the *PTPN11* gain-of-function and loss-of-function mutations, respectively designated as NS-associated *PTPN11* mutations (*NS-PTPN11*) and NSML-associated *PTPN11* mutations (*NSML-PTPN11*). Patients receiving medication affecting bone metabolism, including growth hormones, were excluded.

This study received approval from the Institutional Research Ethics Committee (registration number: RnIPH 2020-56) and the French National Commission for Information Technology and Civil Liberties (CNIL number: 2206723 v 0). Patient information was pseudonymized and encrypted before the analysis. Following standard practice, all parents gave their written and informed consent and children assented to performing a molecular diagnosis of NS.

2.2. Clinical data

Personal history (including non-traumatic fractures and skeletal abnormalities), clinical data (including height, weight, body mass index [BMI], and Tanner stages), and biological and radiological data were obtained by retrospective chart review. Where available, multiple measurements were collected for

each parameter. French reference data were used to convert height, weight, and BMI measurements to age and sex-specific Z-scores.

2.3. Biochemical measurements

Fasting state blood samples were obtained as part of patient clinical follow-up. Serum concentrations of 25-hydroxy vitamin D (25-OHD), insulin-like growth factor 1 (IGF-1), leptin, and serum carboxy-terminal collagen crosslinks (CTX) were measured using automated chemiluminescence immunoassay systems (Cobas 8000 modular analyzer series, Roche Diagnostics; iSYS, Immunodiagnostic Systems). The Quidel enzyme immunoassay (San Diego, CA, USA) was used to determine bone alkaline phosphatase (BAP) levels. IGF-1, BAP, and CTX serum concentrations were converted to age- and sex-specific Z-scores based on published reference data [28]. The following cut-off points were used for the serum 25-OHD levels: sufficiency above 75 nmol/L (> 30 ng/mL), insufficiency between 50 to 75 nmol/L (20-30 ng/mL), and deficiency below 50 nmol/L (< 20 ng/mL).

2.4. Molecular analyses

The Robert-Debré Hospital Genetics Department in Paris, France (H. Cavé, A. Verloes) performed the molecular analyses presented in this study. Peripheral leukocytes from each patient were used to obtain DNA samples that were tested for all *PTPN11*, *SOS1*, *RAF1*, *KRAS*, and *LZTR1* coding regions as previously described [29].

2.5. Dual-energy X-ray absorptiometry (DXA)

Lean body mass (LBM), bone mineral content (BMC), and areal bone mineral density (aBMD) of the total body (TB-aBMD), as well as aBMD of the lumbar spine (LS-aBMD), were measured by DXA using a Lunar Prodigy device (GE Healthcare, Piscataway, NJ, USA). In order to take into account the short stature of NS patients, an estimate of three-dimensional bone density, commonly called volumetric BMD (vBMD, unit: mg/cm³), was calculated as described by Kroger et al. [30] using the formula: $vBMD = aBMD \times [4 / (\pi \times width)]$, width being the mean width of the vertebral bodies. LS-

vBMD provides an estimate of the trabecular bone at the lumbar level, duly corrected for vertebral body width.

Age- and sex-specific Z-scores of LBM, BMC, TB-aBMD, LS-aBMD, and LS-vBMD were obtained following van Der Sluis et al. [31] for patients above 4.5 years old or Manousaki et al. [32] for patients under 4.5 years old.

2.6. Bone age and age and bone health index

The radiographs of the left hand and wrist were collected as DICOM files. Images were analyzed using the BoneXpert system (BoneXpert, Version 2.1.0.12, Visiana, Holte, Denmark), which automatically generates the following outcome variables: bone age based on Greulich & Pyle's method, Z-scores of bone age (compared with a healthy reference population), the Bone Health Index (BHI), and Z-scores of BHI [33]. The Z-score is computed relative to healthy children of the same bone age and gender.

BHI is calculated using the cortical thickness (T) of the three middle metacarpals and the metacarpal width (W) and length (L) to compensate for the variation in stature of growing children, using the following formula: $BHI = \pi \times T \times (1 - T / W) / (L \times W)^{0.33}$. As a result, BHI can be considered an estimate of cortical volume at the metacarpal level, corrected for the length and width of the bones. It has been reported that BHI and LS-aBMD evaluated by DXA were positively and significantly correlated ($p < 0.001$) [34].

2.7. Statistical analysis

Data are expressed as either mean (standard deviation [SD]) or median (range). We initially compared the mean Z-scores for the entire NS sample and the different subgroups (genotypes) to those of the general population using the one-sample Student test. A linear mixed model was used on repeated measurements, with a robust variance estimator for mean values and confidence intervals (CIs), and to compare bone parameters (BMC, TB-aBMD, LS-aBMD, LS-vBMD, and BHI Z-score) according to gender and genotype. Factors related to total body bone mineral content (BMC) were highlighted by performing a linear regression analysis. Each potential factor was initially tested in a univariate model:

age, gender, genotype, height, serum 25-OHD, IGF-1, leptin, and LBM. Factors with a univariate significance of 20% were then inserted into a multivariate model. A stepwise selection method was used to obtain the final model, which only contained factors with a 5% level of significance. Pearson correlation or, when appropriate, Spearman rank correlation was used for determining associations. In all cases, a $P < 0.05$ was considered significant. Two-tailed tests were used throughout the study. The SAS® software, version 9.4 (SAS Institute Inc., Cary, NC, USA), was used to perform all statistical analyses.

3. Results

Thirty-five NS patients (14 females, 21 males) with a median age of 8.1 years (range: 1.4 to 17.3 years) were finally included in the study. No significant differences were found between the biochemical and radiological bone parameters between genders; thus, the results recorded for the girls and boys were analyzed as a single group.

3.1. NS patient clinical, biochemical, and radiological characteristics

Table 1 summarizes the main characteristics of the NS population analyzed in this study. Unsurprisingly, NS patients were shorter than corresponding reference ranges (median Z-score: -1.1; range -3.8 to 1.5; $p < 0.0001$) and their BMI was lower (median Z-score: -1.0; range: -2.9 to 1.0; $p < 0.0001$). Pubertal development was appropriate for all patients, with 25 prepubertal patients (71%), 10 patients (29%) having started puberty (defined as Tanner stages ≥ 2), and none with delayed puberty (defined as Tanner stage = 1 after 13 years of age in girls and 14 years of age in boys). None of our patients reported non-traumatic fractures in their clinical history.

As previously described, NS patients had lower serum IGF-1 concentrations (median Z-score: -1.1; range: -2.7 to 0.8; $p < 0.0001$); IGF-1 and BMI Z-scores were found to be slightly positively associated ($r = 0.25$; $p = 0.05$). The levels of serum leptin assessed in 28 patients were low (median: 1.2 ng/mL; normal values: 2.0 to 5.6 in males and 3.7 to 11.1 in females) with 23 values (82%) below the normal range. All patients displayed normal mineral homeostasis, as defined by the normal ranges of measured serum calcium, phosphate, and alkaline phosphatase. Sixteen patients (46%) had a low

urinary calcium/creatinine ratio (defined as a ratio < 0.20 mmol/mmol). Despite a median serum 25-OHD concentration of 73 nmol/L being within the normal range (23 to 273 nmol/L), 7 patients (20%) presented 25-OHD deficiency (< 50 nmol/L) and 11 patients (31%) presented 25-OHD insufficiency (between 50 to 75 nmol/L). A negative correlation of serum 25-OHD levels with age was found ($r = -0.52$; $p < 0.0001$) with an increased frequency of deficiency from childhood to puberty (prepubertal group, 15%; pubertal group, 25%); however, the difference lacks statistical significance. The levels of the bone formation marker (BAP median Z-score: -1.6; $p < 0.0001$) were lower, while those of the bone resorption marker (CTX median Z-score: 1.2; $p < 0.0001$) were higher in NS patients, relative to the age- and sex-specific reference ranges.

The median bone age delay was 0.7 years (range: -0.7 to 3), correlating negatively with the patient's age ($r = -0.76$; $p < 0.0001$). We observed a significant decrease ($p < 0.0001$) in all bone (BMC, TB-aBMD, LS-aBMD, LS-vBMD, and BHI Z-scores) and muscle (LBM Z-score) parameters relative to reference data. Among these, we found a negative correlation of LS-aBMD and LBM Z-scores with age ($p = 0.02$ for both parameters) (**Figure 1**), emphasizing the fact that a bone and muscle mass decline was aggravated from childhood to adulthood.

3.2. Genotype-phenotype relationships

Among the cohort of 35 patients, 21 patients (60.0%) harbored gain-of-function mutations in *PTPN11* gene (*NS-PTPN11*), 3 (8.6%) loss-of-function mutations in *PTPN11* (*NSML-PTPN11*), 5 (14.3%) in *SOS1*, 2 (5.7%) in *RAF1*, 1 (2.8%) in *KRAS*, and 3 (8.6%) in *LZTR1*. We compared the three main genotypes (*NS-PTPN11*, *NSML-PTPN11*, and *SOS1*) to each other and to the corresponding normal reference range data using one-sample t-tests. As previously reported [35], the BMI of patients with *NS-PTPN11* was significantly lower than that of patients with *NSML-PTPN11* ($p = 0.03$). Patients with a *NS-PTPN11* also had higher bone resorption marker (CTX) levels compared to patients with a *NSML-PTPN11* ($p = 0.004$). Except for the LS-vBMD Z-score in *SOS1* patients and TB-aBMD in *NSML-PTPN11* patients, all other bone parameters (BMC, TB-aBMD, LS-aBMD, LS-vBMD, and BHI Z-score) decreased significantly in *NS-PTPN11*, *NSML-PTPN11*, and *SOS1* patients relative to

age- and sex-specific reference ranges. However, there was no difference in these bone parameters according to the three main genotypes (**Table 2**).

3.3. Determinants of bone mass

BMC Z-scores were found to be associated positively with LBM Z-scores ($r = 0.62$; $p < 0.0001$) and, to a lesser extent, with IGF-1 Z-scores ($r = 0.34$; $p = 0.0447$) (**Figure 2**).

To determine whether LBM and IGF-1 values could be independent predictors of BMC values, multiple regression analyses were performed, taking into account factors such as age, gender, genotype, height, leptin, and 25-OHD levels (**Table 3**). This showed an independent association of LBM and IGF-1 with the whole body BMC ($p < 0.0001$ for both LBM and IGF-1; adjusted $R^2 = 0.97$).

The regression equation was: $\text{BMC (gr)} = -309.1 + 1.6 \times \text{IGF-1 (ng/ml)} + 53.9 \times \text{LBM (gr)}$.

4. Discussion

In the present study, we found that children with NS had a lower axial and appendicular bone mass compared to the general population, as previously suggested by other studies [19-22]. This finding was unchanged for bone size-independent parameters (i.e. LS-vBMD and BHI), which take into account the short stature of NS children.

Regarding the genetic factors that can influence bone mass acquisition, no difference in bone parameters was observed between males and females, and gender was not related to bone mass in the multiple regression analysis. As a number of NS phenotypes, such as heart defect, short stature, and gonadal function, have been reported to correlate with specific genotypes [29, 35], we also investigated the genotype-phenotype correlation for bone parameters in our cohort. Although the three main genotypes were related to decreased bone mass compared to the general population, no differences were found between genotypes. This finding could be explained by the small patient cohort analyzed in our study, particularly for non-NS-*PTPN11* patients, or pinpoint a common mechanism, such as RAS/ERK hyperactivation, for low bone mass. Supporting this view, low bone mass was also found in other RASopathies, notably CS [4, 5] and NF1 [7-18], highlighting the role of

the RAS/ERK pathway in bone homeostasis. This is consistent with several *in vivo* studies that have reported the essential role played by ERK in osteoblast differentiation and in promoting osteoclastogenesis [24, 26]. As previously described in NS and other RASopathies [36], we found that bone resorption markers increased in NS patients, thus highlighting the key role of osteoclasts.

However, apart from the direct effect of RAS/ERK activation on bone homeostasis, additional factors probably contribute to decreased bone mass. Indeed, even if up to 80% of bone mass acquisition depends on genetic factors (including gender and genetic background), environmental factors such as calcium and vitamin D intake, weight, physical activity, and hormone levels (particularly the growth hormone [GH] / IGF-1 axis and gonadal steroids) also play important roles [37]. In this study, half of the patients had a low urinary calcium/creatinine ratio, suggesting low calcium intake, and half of the patients had inadequate vitamin D levels (i.e. vitamin D deficiency or insufficiency). This highlights the importance of providing sufficient calcium and vitamin D intake in patients with NS in order to ensure optimal peak bone mass. Multiple regression analyses were used to identify some determinants of decreased bone mass. Thus, muscle mass and, to a lesser extent, serum IGF-1 levels were found to be independent predictive factors of bone mass. This suggests that decreased muscle mass and IGF-1 deficiency could explain bone loss in NS, at least in part.

In our study, muscle mass was significantly lower in children with NS compared to healthy age-matched controls, even after adjusting for height (i.e. LBM/height ratio). This decrease in muscle mass was already evident in younger children and worsened from childhood to adulthood. This finding is consistent with several clinical studies that have reported muscle abnormalities, such as diminished strength and endurance, coupled with muscle weakness in NS patients [38-40]. Muscle mass and physical activity have long since been positively related to whole body bone mass in healthy children [41, 42]. Thus, bone adapts to the mechanical forces to which it is subjected in order to maintain the strain on the bone at a constant set point [43]. A decrease in muscle mass therefore correlates with a decrease in bone strength. The origin of the decrease in muscle mass and strength is unknown and could be due to low activity levels either from central or peripheral neuropathies, or a primary myopathy. Interestingly, mitochondrial dysfunction has been reported in muscle biopsies performed in

5 patients carrying mutations in the RAS/ERK pathway [44]. Whatever the mechanism underlying the muscle abnormalities, it can be assumed that NS patients may benefit from physical training. This type of exercise program should be investigated for children and adolescents with NS.

Another important finding of our study is the inverse relationship between serum IGF-1 levels and bone mass. While there have been reports on GH deficiency or neurosecretory dysfunction, patients with NS (notably those with *PTPN11* mutations) usually display normal or slightly increased GH levels associated with low serum IGF1 levels, suggesting GH insensitivity [45]. Consistent with this finding, a NS-causing *Ptpn11* mutant impairs the systemic production of IGF-1 *in vivo* through hyperactivation of the RAS/ERK signaling pathway [2]. It has long since been recognized that endocrine signals modulate bone turnover and bone mass acquisition. Before puberty, bone growth is largely dependent on the growth hormone / IGF-1 axis, which acts directly on bone and muscle cells [46]. Thus, children presenting growth hormone deficiency and low IGF-1 levels display decreased muscle and bone mass, which is corrected during GH replacement therapy [47]. A similar effect of growth hormone therapy on muscle and bone mass was also reported in a small pediatric NS cohort [22].

Decreased bone mass in NS could also be due to low androgen and/or estrogen levels: these hormones are crucial for the sharp increase in bone mass during puberty. Indeed, it has been reported that hypogonadism leads to reduced bone mass and increased fracture risk and that hormone replacement therapy is associated with bone mass improvement [48]. This hypothesis was not confirmed in our study because all of our patients had appropriate pubertal development without delayed puberty; however, hormonal status was not checked in our patients' sample. Osteoporosis associated with hypogonadism has been reported in a few adult male patients with NS [49, 50]. Finally, other hormones, such as leptin, presenting low levels in NS patients, may also play a role in bone mass acquisition. Although leptin levels positively correlated with BMC Z-scores, they were not bone mass-independent predictive factors. Further studies will be required to clarify the role of this hormone in NS.

Despite the fact that none of our patients had suffered any pathological fractures, they may nevertheless be at increased risk of developing osteoporosis and fragility fractures later on in life. Bone mass acquisition during childhood and adolescence leads to peak bone mass acquisition by early adulthood at the end of the growth period [37]. This peak bone mass, which corresponds to the maximum amount of bone mass in the life of an individual, is a major determinant of bone strength and fragility throughout life. Interestingly, as regards other RASopathies, an increased fracture rate has been highlighted in adult patients with NF1 [17], and pathological fractures have been reported in patients with CS [5]. Further longitudinal studies are needed to describe bone mass progression and to evaluate the long-term risk of bone fragility, especially in adults and the elderly. To date, except in clinical research studies, we cannot recommend systematically performing DXA in patients with NS; however, this exam can be discussed in patients with non-traumatic fractures.

Although our study is one of the largest series involving children with molecular diagnosis of NS investigated for their bone health, it has several limitations. Only a few patients were followed up with longitudinally and, with the exception of *NS-PTPN11* patients, this study was restricted by the small dataset available for analysis of the other sub-groups. Unfortunately, due to the retrospective design of this study, it was not possible to assess important predictors of bone mass, notably calcium intake as well as muscle strength and physical activity. The urinary calcium / creatinine ratio, which correlates with calcium intake, was used as a surrogate of dietary intake. Regarding the interaction of muscle and bone, further prospective studies are required to investigate the role of diminished strength and endurance reported in NS on the decreased bone mass, as well as the potential positive effect of physical training in these patients. Further accurate hormonal investigations are also required to assess the role of the different hormonal insufficiencies (decreased IGF1, leptin, androgen and/or estrogen levels) displayed by patients with NS on muscle and bone abnormalities.

5. Conclusion

Compared to the general population, axial and appendicular bone mass is decreased in children with NS. No genotype-phenotype correlation for bone parameters was found in our cohort. Muscle mass

and, to lesser extent, serum IGF-1 levels were independent predictive factors of bone mass, suggesting that muscle hypoplasia and IGF-1 deficiency could account for bone loss in NS, at least in part. These data justify addressing all potential threats to bone health, including sufficient calcium and vitamin D intake, regular physical exercise, and hormone replacement therapy (GH / IGF-1 and gonadal steroids). Further longitudinal studies are needed to investigate the long-term risk of bone fragility, especially in adults and the elderly. By identifying the main factors causing bone loss in NS patients, this small retrospective study lays the foundation for larger prospective interventional studies on the potential positive effect of physical training and/or hormone replacement therapy in these patients.

6. Funding

This work was supported by grants from the European Joint Programme on Rare Diseases (EJP RD COFUND-EJP No. 825575).

7. Acknowledgments

We wish to thank the NS patients and their families who participated in this study. We are also extremely grateful to F. Auriol and I. Kieffer at the Pediatric Clinical Investigation unit for their valuable contribution.

8. Data Availability Statement

All of the data supporting the results presented in this paper are available on request.

9. Tables

Table 1. Clinical, biochemical, and radiological characteristics of patients with Noonan syndrome.

Variable	Patients, n	Measurements, n	Median (min; max)	Mean (SD)	P value *
Gender (female/male)	35 (14/21)	-	-	-	-
Age (years)	35	60	8.1 (1.4; 17.3)	8.5 (4.7)	-
Height (Z-score)	35	60	-1.2 (-3.8; 1.5)	-1.3 (1.1)	< 0.0001
BMI (Z-score)	35	60	-1.0 (-2.9; 1.0)	-0.9 (0.9)	< 0.0001
IGF-1 (Z-score)	35	59	-1.1 (-2.7; 0.8)	-1.1 (0.8)	< 0.0001
BAP (Z-score)	34	50	-1.6 (-3.7; 1.6)	-1.6 (1.0)	< 0.0001
CTX (Z-score)	34	50	1.2 (-0.4; 5.3)	-1.0 (1.3)	< 0.0001
Delayed bone age (years)	23	39	0.7 (-0.7; 3.0)	1.0 (1.0)	< 0.0001
BMC (Z-score)	23	36	-2.3 (-4.3; -0.8)	-2.3 (0.8)	< 0.0001
TB-aBMD (Z-score)	35	60	-1.1 (-1.4; -0.4)	-0.9 (0.8)	< 0.0001
LS-aBMD (Z-score)	30	45	-1.2 (-3.5; 0.8)	-1.3 (0.9)	< 0.0001
LS-vBMD (Z-score)	30	45	-0.5 (-2.7; 1.6)	-0.6 (0.9)	< 0.0001
BHI (Z-score)	23	39	-2.0 (-3.6; -0.2)	-1.9 (0.8)	< 0.0001
LBM (Z-score)	23	36	-2.5 (-4.5; -0.9)	-2.5 (0.8)	< 0.0001
LBM / height (percentile)	23	44	34.5 (0; 100)	38.1 (29.4)	0.0104

BMI: body mass index; IGF-1: insulin-like growth factor 1; BAP: bone alkaline phosphatase; CTX: carboxy-terminal collagen crosslinks; BMC: bone mineral content; BMD: bone mineral density; TB-aBMD: total body areal BMD; LS-aBMD: lumbar spine areal BMD; LS-vBMD: lumbar spine volumetric BMD; BHI: bone health index; LBM: lean body mass.

Where available, multiple measurements were collected for each parameter; the number of measurements (n) is specified in the Table.

French reference data for the general pediatric population were used to convert height, weight, and BMI measurements to age and sex-specific Z-scores.

Values are expressed as mean (SD) and median (min; max).

* P values for comparison to normal reference data using the one-sample t-test.

		Patients, n	Measurements, n	Median (IQR)	Mean (SD)	Estimated mean (95% CI)	P value *
BMC (Z-score)	All patients	23	36	-2.3 (-2.8; -1.7)	-2.3 (0.8)	-2.3 (-2.7; -1.9)	< 0.0001†
	Genotype						
	<i>NSML-PTPN11</i>	2	4	-1.4 (-1.9; -1.2)	-1.6 (0.6)	-1.8 (-3.1; -0.5)	0.01†
	<i>NS-PTPN11</i>	15	23	-2.4 (-3.1; -1.9)	-2.5 (0.9)	-2.3 (-2.9; -1.8)	< 0.0001†
	<i>SOS1</i>	4	7	-2.4 (-2.6; -1.5)	-2.2 (0.7)	-2.1 (-3.1; -1.2)	0.0002†
	Comparisons						
	<i>NSML-PTPN11</i> vs. <i>NS-PTPN11</i>	3 vs. 15	4 vs. 23	-	0.9	0.6 (-0.9; 2.0)	0.4
<i>NS-PTPN11</i> vs. <i>SOS1</i>	15 vs. 4	23 vs. 7	-	-0.3	-0.2 (-1.3; 0.9)	0.7	
TB-aBMD (Z-score)	All patients	35	60	-1.1 (-1.4; -0.4)	-0.9 (0.8)	-1.0 (-1.4; -0.5)	< 0.0001†
	Genotype						
	<i>NSML-PTPN11</i>	3	8	0.2 (-0.6; 0.6)	0.0 (0.9)	-0.4 (-1.7; 0.8)	1.0†
	<i>NS-PTPN11</i>	21	34	-1.0 (-1.5; -0.5)	-1.0 (0.8)	-1.0 (-1.5; -0.4)	< 0.0001†
	<i>SOS1</i>	5	12	-1.3 (-1.4; -1.2)	-1.1 (0.8)	-1.1 (-2.2; -0.1)	0.03†
	Comparisons						
	<i>NSML-PTPN11</i> vs. <i>NS-PTPN11</i>	3 vs. 21	8 vs. 34	-	1.0	0.5 (-0.8; 1.9)	0.41
<i>NS-PTPN11</i> vs. <i>SOS1</i>	21 vs. 5	34 vs. 12	-	0.1	0.14 (-1.0; 1.3)	0.8	
LS-aBMD (Z-score)	All patients	30	45	-1.2 (-1.9; -0.7)	-1.3 (0.9)	-1.2 (-1.6; -0.8)	< 0.0001†
	Genotype						
	<i>NSML-PTPN11</i>	3	5	-1.1 (-1.1; -0.8)	-1.1 (0.5)	-1.2 (-2.3; 0.01)	0.008†
	<i>NS-PTPN11</i>	17	25	-1.2 (-1.8; -0.7)	-1.4 (0.8)	-1.3 (-1.8; -0.7)	< 0.0001†
	<i>SOS1</i>	5	10	-1.3 (-1.9; 0.2)	-1.0 (1.2)	-1.0 (-1.8; -0.1)	0.03†
	Comparisons						
	<i>NSML-PTPN11</i> vs. <i>NS-PTPN11</i>	3 vs. 17	5 vs. 25	-	0.3	0.1 (-1.1; 1.4)	0.9
<i>NS-PTPN11</i> vs. <i>SOS1</i>	17 vs. 5	25 vs. 10	-	-0.5	0.1 (-1.0; 1.3)	0.8	
LS-vBMD (Z-score)	All patients	30	45	0.5 (-1.4; 0.1)	-0.6 (0.9)	-0.5 (-0.9; -0.1)	< 0.0001†
	Genotype						
	<i>NSML-PTPN11</i>	3	5	-0.5 (-0.6; -0.4)	-0.7 (0.5)	-0.7 (-1.8; 0.4)	0.03†
	<i>NS-PTPN11</i>	17	25	-0.5 (-1.6; 0.1)	-0.8 (1.0)	-0.6 (-1.1; -0.1)	0.0004†
	<i>SOS1</i>	5	10	-0.5 (-0.9; 0.4)	-0.2 (0.9)	-0.2 (-1.0; 0.7)	0.6†
	Comparisons						
	<i>NSML-PTPN11</i> vs. <i>NS-PTPN11</i>	3 vs. 17	5 vs. 25	-	0.2	-0.1 (-1.2; 1.1)	0.9
<i>NS-PTPN11</i> vs. <i>SOS1</i>	17 vs. 5	25 vs. 10	-	-0.7	-0.5 (-1.4; 0.5)	0.3	
BHI (Z-score)	All patients	23	39	-2.0 (-2.6; -1.3)	-1.9 (0.8)	-1.9 (-2.4; -1.5)	< 0.0001†
	Genotype						
	<i>NSML-PTPN11</i>	2	5	-2.6 (-2.7; -1.5)	-2.2 (0.7)	-2.1 (-3.3; -0.9)	0.003†
	<i>NS-PTPN11</i>	12	19	-1.7 (-2.6; -1.3)	-1.2 (0.9)	-2.0 (-2.6; -1.4)	< 0.0001†

	<i>SOSI</i>	4	10	-2.1 (-2.6; -2.0)	-2.1 (0.5)	-2.1 (-3.0; -1.3)	< 0.0001†
	Comparisons						
	<i>NSML-PTPN11</i> vs. <i>NS-PTPN11</i>	2 vs. 12	5 vs. 19	-	-0.3	-0.1 (-1.4; 1.2)	0.9
	<i>NS-PTPN11</i> vs. <i>SOSI</i>	12 vs. 4	19 vs. 10	-	0.2	0.1 (-1.2; 1.8)	0.7

Table 2. Comparisons of bone parameters in Z-score in children with Noonan syndrome with the normal reference values and according to genotype.

SD: Standard deviation; IQR: interquartile range; CI: confidence interval

* Estimated mean values, confidence intervals (CI), and p-values were calculated using a linear mixed model for repeated measurements with a robust variance estimator.

† Mean Z-scores were compared to those of the general population using the one-sample Student test, assuming an average Z-score of 0 in the general population.

Table 3. Determinants of bone mineral content (BMC) evaluated using multiple regression analyses.

	Univariate model*			Full model**			Final model***		
	Beta	95% CI	P value	Beta	95% CI	P value	Beta	95% CI	P value
Gender	204.3	-104.1; 512.8	0.2	-	-	-	-	-	-
Gene	-13.3	-140.7; 114.1	0.8	-	-	-	-	-	-
Age (years)	98.5	84.7; 112.3	<0.0001	24.1	-6.4; 54.6	0.1	-	-	-
Height (cm)	17.9	15.2; 20.6	<0.0001	-2.9	-8.7; 2.8	0.3	-	-	-
25-OHD (nmol/L)	-6.4	-10.9; -2.0	0.006	-0.2	-1.2; 0.8	0.7	-	-	-
Leptin (ng/mL)	206.0	94.7; 317.2	0.0006	-27.1	-62.6; 8.4	0.1	-	-	-
IGF1 (ng/mL)	6.0	4.5; 7.5	<0.0001	1.9	1.0; 2.7	0.0001	1.6	0.9; 2.3	<0.0001
LBM (kg)	64.3	59.0; 69.5	<0.0001	49.4	36.5; 62.2	<0.0001	53.9	47.7; 60.1	<0.0001

25-OHD: 25-hydroxy vitamin D; IGF-1: insulin-like growth factor 1; LBM: lean body mass.

* Univariate model: only one explanatory variable. A total of 7 models.

** Full model: only explanatory variables significant at 5% in univariate models.

*** Final model: obtained using a stepwise selection method with, at first step, insertion of explanatory variables significant at 20% in univariate models.

10. Figures

Figure 1. Changes in lean body mass (LBM) (A) and lumbar spine areal bone mineral density (LS-aBMD) (B) by age and genotype. *(in black in the printed version)*

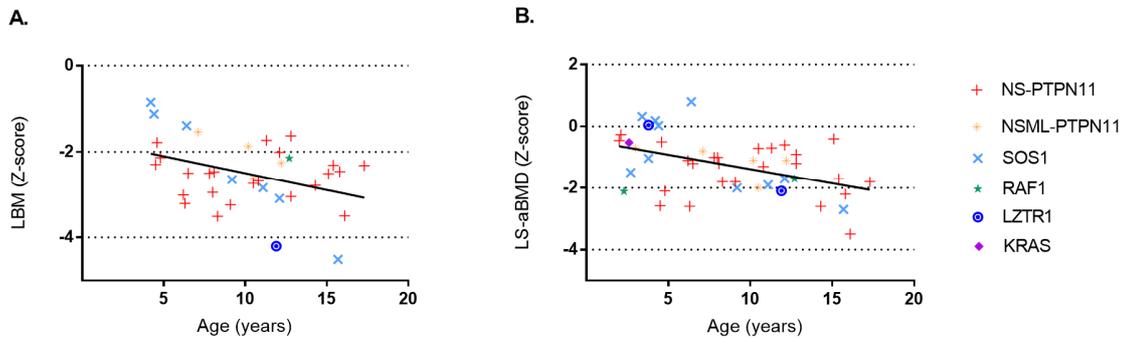
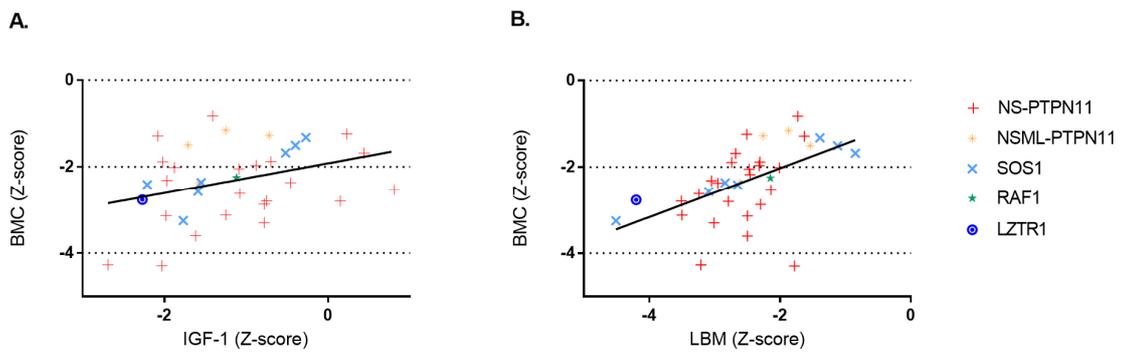


Figure 2. Relationship between bone mineral content (BMC), IGF-1 (A) and lean body mass (LBM) (B). *(in black in the printed version)*



11. References

- [1] M. Tajan, R. Paccoud, S. Branka, T. Edouard, A. Yart, The Rasopathy family: Consequences of germline activation of the RAS/MAPK pathway, *Endocr Rev* (2018).
- [2] A. De Rocca Serra-Nedelec, T. Edouard, K. Treguer, M. Tajan, T. Araki, M. Dance, M. Mus, A. Montagner, M. Tauber, J.P. Salles, P. Valet, B.G. Neel, P. Raynal, A. Yart, Noonan syndrome-causing SHP2 mutants inhibit insulin-like growth factor 1 release via growth hormone-induced ERK hyperactivation, which contributes to short stature, *Proc Natl Acad Sci U S A* 109(11) (2012) 4257-62.
- [3] D.A. Stevenson, F.C. Yang, The musculoskeletal phenotype of the RASopathies, *Am J Med Genet C Semin Med Genet* 157C(2) (2011) 90-103.
- [4] C. Leoni, D.A. Stevenson, L. Martini, R. De Sanctis, G. Mascolo, F. Pantaleoni, S. De Santis, I. La Torraca, S. Persichilli, P. Caradonna, M. Tartaglia, G. Zampino, Decreased bone mineral density in Costello syndrome, *Mol Genet Metab* 111(1) (2014) 41-5.
- [5] S.M. White, J.M. Graham, Jr., B. Kerr, K. Gripp, R. Weksberg, C. Cytrynbaum, J.L. Reeder, F.J. Stewart, M. Edwards, M. Wilson, A. Bankier, The adult phenotype in Costello syndrome, *Am J Med Genet A* 136(2) (2005) 128-35.
- [6] C. Leoni, D.M. Romeo, M. Pelliccioni, M. Di Gia, R. Onesimo, V. Giorgio, E. Flex, M. Tedesco, M. Tartaglia, D. Rigante, A. Valassina, G. Zampino, Musculo-skeletal phenotype of Costello syndrome and cardio-facio-cutaneous syndrome: insights on the functional assessment status, *Orphanet J Rare Dis* 16(1) (2021) 43.
- [7] L. Armstrong, K. Jett, P. Birch, D.L. Kendler, H. McKay, E. Tsang, D.A. Stevenson, D.A. Hanley, D. Egeli, M. Burrows, J.M. Friedman, The generalized bone phenotype in children with neurofibromatosis 1: A sibling matched case-control study, *American Journal of Medical Genetics Part A* 161(7) (2013) 1654-1661.
- [8] N. Brunetti-Pierri, S.B. Doty, J. Hicks, K. Phan, R. Mendoza-Londono, M. Blazo, A. Tran, S. Carter, R.A. Lewis, S.E. Plon, W.A. Phillips, E. O'Brian Smith, K.J. Ellis, B. Lee, Generalized metabolic bone disease in Neurofibromatosis type I, *Molecular Genetics and Metabolism* 94(1) (2008) 105-111.
- [9] C. Caffarelli, S. Gonnelli, L. Tanzilli, R. Vivarelli, S. Tamburello, P. Balestri, R. Nuti, Quantitative Ultrasound and Dual-Energy X-ray Absorptiometry in Children and Adolescents With Neurofibromatosis of Type 1, *Journal of Clinical Densitometry* 13(1) (2010) 77-83.
- [10] S. Dulai, J. Briody, A. Schindeler, K.N. North, C.T. Cowell, D.G. Little, Decreased Bone Mineral Density in Neurofibromatosis Type 1: Results From a Pediatric Cohort, *Journal of Pediatric Orthopaedics* 27(4) (2007) 472-475.
- [11] O. Duman, S. Ozdem, D. Turkkahraman, N.D. Olgac, F. Gungor, S. Haspolat, Bone metabolism markers and bone mineral density in children with neurofibromatosis type-1, *Brain and Development* 30(9) (2008) 584-588.
- [12] T. Illés, V. Halmai, T. de Jonge, J. Dubousset, Decreased Bone Mineral Density in Neurofibromatosis-1 Patients with Spinal Deformities, *Osteoporosis International* 12(10) (2001) 823-827.
- [13] T. Kuorilehto, M. Pöyhönen, R. Bloigu, J. Heikkinen, K. Väänänen, J. Peltonen, Decreased bone mineral density and content in neurofibromatosis type 1: Lowest local values are located in the load-carrying parts of the body, *Osteoporosis International* 16(8) (2005) 928-936.
- [14] M. Lammert, M. Kappler, V.-F. Mautner, K. Lammert, S. Störkel, J.M. Friedman, D. Atkins, Decreased bone mineral density in patients with neurofibromatosis 1, *Osteoporosis International* 16(9) (2005) 1161-1166.
- [15] S. Seitz, C. Schnabel, B. Busse, H.U. Schmidt, F.T. Beil, R.E. Friedrich, T. Schinke, V.F. Mautner, M. Amling, High bone turnover and accumulation of osteoid in patients with neurofibromatosis 1, *Osteoporosis International* 21(1) (2010) 119-127.
- [16] D.A. Stevenson, L.J. Moyer-Mileur, M. Murray, H. Slater, X. Sheng, J.C. Carey, B. Dube, D.H. Viskochil, Bone Mineral Density in Children and Adolescents with Neurofibromatosis Type 1, *The Journal of Pediatrics* 150(1) (2007) 83-88.
- [17] T. Tucker, C. Schnabel, M. Hartmann, R.E. Friedrich, I. Frieling, H.-P. Kruse, V.-F. Mautner, J.M. Friedman, Bone health and fracture rate in individuals with neurofibromatosis 1 (NF1), *Journal of Medical Genetics* 46(4) (2009) 259-265.

- [18] K. Yilmaz, M. Ozmen, S. Bora Goksan, N. Eskiurt, Bone mineral density in children with neurofibromatosis 1, *Acta Paediatrica* 96(8) (2007) 1220-1222.
- [19] G. Baldassarre, A. Mussa, D. Carli, C. Molinatto, G.B. Ferrero, Constitutional bone impairment in Noonan syndrome, *Am J Med Genet A* 173(3) (2017) 692-698.
- [20] K.S. Choudhry, M. Grover, A.A. Tran, E. O'Brian Smith, K.J. Ellis, B.H. Lee, Decreased bone mineralization in children with Noonan syndrome: another consequence of dysregulated RAS MAPKinase pathway?, *Mol Genet Metab* 106(2) (2012) 237-40.
- [21] D.A. Stevenson, E.L. Schwarz, J.C. Carey, D.H. Viskochil, H. Hanson, S. Bauer, H.Y. Weng, T. Greene, K. Reinker, J. Swensen, R.J. Chan, F.C. Yang, L. Senbanjo, Z. Yang, R. Mao, M. Pasquali, Bone resorption in syndromes of the Ras/MAPK pathway, *Clin Genet* 80(6) (2011) 566-73.
- [22] C. Noordam, J. Span, R.R. van Rijn, E. Gomes-Jardin, C. van Kuijk, B.J. Otten, Bone mineral density and body composition in Noonan's syndrome: effects of growth hormone treatment, *J Pediatr Endocrinol Metab* 15(1) (2002) 81-7.
- [23] T.J. Bauler, N. Kamiya, P.E. Lapinski, E. Langewisch, Y. Mishina, J.E. Wilkinson, G.-S. Feng, P.D. King, Development of severe skeletal defects in induced SHP-2-deficient adult mice: a model of skeletal malformation in humans with SHP-2 mutations, *Disease Models & Mechanisms* 4(2) (2011) 228-239.
- [24] T. Matsushita, Y.Y. Chan, A. Kawanami, G. Balmes, G.E. Landreth, S. Murakami, Extracellular Signal-Regulated Kinase 1 (ERK1) and ERK2 Play Essential Roles in Osteoblast Differentiation and in Supporting Osteoclastogenesis, *Molecular and Cellular Biology* 29(21) (2009) 5843-5857.
- [25] W. Yang, J. Wang, D.C. Moore, H. Liang, M. Dooner, Q. Wu, R. Terek, Q. Chen, M.G. Ehrlich, P.J. Quesenberry, B.G. Neel, Ptpn11 deletion in a novel progenitor causes metachondromatosis by inducing hedgehog signalling, *Nature* 499(7459) (2013) 491-495.
- [26] Y. He, K. Staser, S.D. Rhodes, Y. Liu, X. Wu, S.-J. Park, J. Yuan, X. Yang, X. Li, L. Jiang, S. Chen, F.-C. Yang, Erk1 Positively Regulates Osteoclast Differentiation and Bone Resorptive Activity, *PloS one* 6(9) (2011) e24780.
- [27] J.-M. Kim, Y.-S. Yang, K.H. Park, H. Oh, M.B. Greenblatt, J.-H. Shim, The ERK MAPK Pathway Is Essential for Skeletal Development and Homeostasis, *International Journal of Molecular Sciences* 20(8) (2019) 1803.
- [28] D.-C. Fischer, A. Mischek, S. Wolf, A. Rahn, B. Salweski, G. Kundt, D. Haffner, Paediatric reference values for the C-terminal fragment of fibroblast-growth factor-23, sclerostin, bone-specific alkaline phosphatase and isoform 5b of tartrate-resistant acid phosphatase, *Annals of Clinical Biochemistry* 49(Pt 6) (2012) 546-553.
- [29] S. Moniez, C. Pienkowski, B. Lepage, S. Hamdi, M. Daudin, I. Oliver, B. Jouret, A. Cartault, G. Diene, A. Verloes, H. Cavé, J.-P. Salles, M. Tauber, A. Yart, T. Edouard, Noonan syndrome males display Sertoli cell-specific primary testicular insufficiency, *European Journal of Endocrinology* 179(6) (2018) 409-418.
- [30] H. Kroger, P. Vainio, J. Nieminen, A. Kotaniemi, Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology, *Bone* 17(2) (1995) 157-9.
- [31] I.M. van der Sluis, M.a.J. de Ridder, A.M. Boot, E.P. Krenning, S.M.P.F. de Muinck Keizer-Schrama, Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults, *Archives of Disease in Childhood* 87(4) (2002) 341-347; discussion 341-347.
- [32] D. Manousaki, F. Rauch, G. Chabot, J. Dubois, M. Fiscaletti, N. Alos, Pediatric data for dual X-ray absorptiometric measures of normal lumbar bone mineral density in children under 5 years of age using the lunar prodigy densitometer, *Journal of Musculoskeletal & Neuronal Interactions* 16(3) (2016) 247-255.
- [33] H.H. Thodberg, R.R. van Rijn, T. Tanaka, D.D. Martin, S. Kreiborg, A paediatric bone index derived by automated radiogrammetry, *Osteoporos Int* 21(8) (2010) 1391-400.
- [34] A.D. Leijten, B. Hampsink, M. Janssen, W.M. Klein, J.M.T. Draaisma, Can digital X-ray radiogrammetry be an alternative for dual-energy X-ray absorptiometry in the diagnosis of secondary low bone quality in children?, *Eur J Pediatr* 178(9) (2019) 1433-1441.
- [35] C. Cessans, V. Ehlinger, C. Arnaud, A. Yart, Y. Capri, P. Barat, B. Cammas, D. Lacombe, R. Coutant, A. David, S. Baron, J. Weill, B. Leheup, M. Nicolino, J.P. Salles, A. Verloes, M. Tauber, H.

- Cave, T. Edouard, Growth patterns of patients with Noonan syndrome: correlation with age and genotype, *Eur J Endocrinol* 174(5) (2016) 641-50.
- [36] D.A. Stevenson, F.-C. Yang, The musculoskeletal phenotype of the RASopathies, *American Journal of Medical Genetics Part C: Seminars in Medical Genetics* 157(2) (2011) 90-103.
- [37] C.M. Gordon, B.S. Zemel, T.A.L. Wren, M.B. Leonard, L.K. Bachrach, F. Rauch, V. Gilsanz, C.J. Rosen, K.K. Winer, The Determinants of Peak Bone Mass, *The Journal of Pediatrics* 180 (2017) 261-269.
- [38] E.A. Croonen, M. Essink, I. van der Burgt, J.M. Draaisma, C. Noordam, M.W.G. Nijhuis-van der Sanden, Motor performance in children with Noonan syndrome, *Am J Med Genet A* 173(9) (2017) 2335-2345.
- [39] E.A. Croonen, M. Harmsen, I. Van der Burgt, J.M. Draaisma, K. Noordam, M. Essink, M.W. Nijhuis-van der Sanden, Perceived motor problems in daily life: Focus group interviews with people with Noonan syndrome and their relatives, *Am J Med Genet A* 170(9) (2016) 2349-56.
- [40] D.A. Stevenson, S. Allen, W.E. Tidymann, J.C. Carey, D.H. Viskochil, A. Stevens, H. Hanson, X. Sheng, B.A. Thompson, M.J. Okumura, K. Reinker, B. Johnson, K.A. Rauen, Peripheral muscle weakness in RASopathies, *Muscle Nerve* 46(3) (2012) 394-9.
- [41] M. Behringer, S. Gruetzner, M. McCourt, J. Mester, Effects of Weight-Bearing Activities on Bone Mineral Content and Density in Children and Adolescents: A Meta-Analysis: WEIGHT-BEARING ACTIVITIES ON BMC AND BMD IN CHILDREN AND ADOLESCENTS, *Journal of Bone and Mineral Research* 29(2) (2014) 467-478.
- [42] I. Sioen, E. Lust, S. De Henauw, L.A. Moreno, D. Jiménez-Pavón, Associations Between Body Composition and Bone Health in Children and Adolescents: A Systematic Review, *Calcified Tissue International* 99(6) (2016) 557-577.
- [43] H.M. Frost, Bone's mechanostat: A 2003 update, *The Anatomical Record* 275A(2) (2003) 1081-1101.
- [44] T. Kleefstra, S.B. Wortmann, R.J. Rodenburg, E.M. Bongers, K. Hadzsiev, C. Noordam, L.P. van den Heuvel, W.M. Nillesen, K. Hollody, G. Gillessen-Kaesbach, M. Lammens, J.A. Smeitink, I. van der Burgt, E. Morava, Mitochondrial dysfunction and organic aciduria in five patients carrying mutations in the Ras-MAPK pathway, *Eur J Hum Genet* 19(2) (2011) 138-44.
- [45] A.C. Malaquias, A.A.L. Jorge, Activation of the MAPK pathway (RASopathies) and partial growth hormone insensitivity, *Mol Cell Endocrinol* 519 (2021) 111040.
- [46] D.D. Bikle, C. Tahimic, W. Chang, Y. Wang, A. Philippou, E.R. Barton, Role of IGF-I signaling in muscle bone interactions, *Bone* 80 (2015) 79-88.
- [47] A.M. Boot, M.A.M.J. Engels, G.J.M. Boerma, E.P. Krenning, S.M.P.F. de Muinck Keizer-Schrama, Changes in Bone Mineral Density, Body Composition, and Lipid Metabolism during Growth Hormone (GH) Treatment in Children with GH Deficiency ¹, *The Journal of Clinical Endocrinology & Metabolism* 82(8) (1997) 2423-2428.
- [48] M. Misra, Effects of hypogonadism on bone metabolism in female adolescents and young adults, *Nature Reviews Endocrinology* 8(7) (2012) 395-404.
- [49] K.A. Reinker, D.A. Stevenson, A. Tsung, Orthopaedic conditions in Ras/MAPK related disorders, *Journal of Pediatric Orthopedics* 31(5) (2011) 599-605.
- [50] M. Takagi, Y. Miyashita, M. Koga, S. Ebara, N. Arita, S. Kasayama, Estrogen Deficiency is a Potential Cause for Osteopenia in Adult Male Patients with Noonan's Syndrome, *Calcified Tissue International* 66(3) (2000) 200-203.