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Nutritional Aspects of Noonan Syndrome and Noonan-Related Disorders

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Rasopathies are a group of rare disorders characterized by neurocardiofaciocutaneous involvement, and caused by mutations in several genes of the RAS/MAPK pathway. In the present study, we characterized growth parameters, body composition, and nutritional aspects of children and adults (n = 62) affected by these disorders, mainly Noonan syndrome, using an indirect method—anthropometry—and a 24-hr recall questionnaire. The growth parameters in our cohort showed short stature, especially in individuals with *RAF1* and *SHOC2* mutations, lower obesity rates compared to the control population, and BMI scores highest in individuals with *BRAF* mutations and lowest in individuals with *SHOC2*. Body composition showed a compromise in the upper arm muscle circumference, with a statistically significant difference in the z-score of triceps skinfold ($P = 0.0204$) and upper arm fat area ($P = 0.0388$) between *BRAF* and *SHOC2* groups and in the z-score of triceps skinfold between *RAF1* and *SHOC2* ($P = 0.0218$). The pattern of macronutrient consumption was similar to the control population. Our study is the first to address body composition in RASopathy individuals and the data indicate a compromise not only in adipose tissue, but also in muscle mass. Studies using different techniques, such as dual-energy X-ray absorptiometry or imaging studies, which give a more precise delineation of fat and non-fat mass, are required to confirm our results, ultimately causing an impact on management strategies.

Key words: growth; body composition; anthropometry; food intake; Noonan syndrome; Noonan related disorders; RAS-MAPK pathway

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INTRODUCTION

Noonan syndrome (NS; OMIM 163950) is an autosomal dominant disorder of highly variable expressivity and complete penetrance, characterized by short stature, facial dysmorphism (ocular hypertelorism, downslanting palpebral fissures, proptosis, palpebral ptosis, malocclusion, and overfolded pinnae), short and/or webbed neck, sternal deformities, congenital heart disease (especially pulmonary valve stenosis and left ventricular hypertrophy), bleeding diathesis, and cryptorchidism [Romano et al., 2010].

Mutations in several genes acting in the RAS/MAPK pathway (mitogen activated protein kinase) underlie NS (*PTPN11*, *KRAS*, *SOS1*, *RAF1*, *NRAS*, *BRAF*, *SHOC2*, *MAP2K1*, *CBL*, *RIT1*, *RRAS*, *RASA2*, *A2ML1*, *LZTR1*, and *SOS2*) [Aoki et al., 2015], as well as other rare syndromes with characteristics similar to NS (Noonan-related disorders—NRD), including cardiofaciocutaneous syndrome (CFCS; OMIM 115150), Costello syndrome (CS; OMIM 218040), Noonan syndrome with multiple lentigines (NSML; OMIM 151100), Noonan-like syndrome with loose anagen hair (NSLAH; OMIM 607721). These disorders represent a group of genetic conditions known as RASopathies [Romano et al., 2010; Rauen, 2013].

Among the clinical findings in NS, the focus in the literature has been on descriptions and management of cardiac abnormalities and short stature. Less attention has been given to the nutritional aspects. Feeding difficulties have been described, especially in CS

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and CFCS, which tend to show a more severe failure to thrive [Gripp and Lin, 2012]. Sporadic reports of feeding difficulties in NS have been addressed prior to the molecular characterization of the disease [Sharland et al., 1992; Shah et al., 1999]. Recent studies have shown that dysregulation of RAS/MAPK pathway could play a role in metabolism and energy storage, as demonstrated by the low BMI in RASopathy patients and lean habitus in transgenic knock-in mice [Malaquias et al., 2012; Tajan et al., 2014].

Although a precise genotype–phenotype correlation in NS and NRD is not evident, some clinical findings could be more prevalent in individuals with specific gene involvement. However, there is no phenotypic feature exclusive to a specific genotype. For example, short stature is less frequent in patients harboring *SOS1* and *RIT1* mutations, compared to patients with mutations in *PTPN11* [Romano et al., 2010; Bertola et al., 2014]. The aim of this study was to evaluate the nutritional aspects in RASopathies, by analyzing anthropometric measurements in a large cohort of NS and NRD individuals with confirmed mutations. In order to establish genotype–phenotype correlations, the data were analyzed across subgroups categorized by gene involvement.

MATERIALS AND METHODS

Study Protocol

Patients with confirmed molecular diagnoses of NS and NRD evaluated at the outpatient Genetics Clinic of the Instituto da Criança do Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo (ICr-HCFMUSP) from April 2012 to August 2013 were enrolled in this study.

This study was approved by the local Ethics Committee. Informed parental consent and/or patient assent were obtained before initiating the studies.

This is a descriptive cross-sectional study using a convenience sample, including 62 individuals (male = 34, female = 28) with the following diagnoses: NS (n = 47); NSLAH (n = 4), NSML (n = 3) and CFCS (n = 8). In NS, 35/47 harbored *PTPN11* mutations, 6, *SOS1*, 3, *KRAS* and 3, *RAF1*. In NSLAH and NSML, all patients had mutations in *SHOC2* and *PTPN11*, respectively. In CFCS, *BRAF* mutations were observed in 7/8 individuals and one had a *KRAS* mutation. The age ranged from 2 to 56 years, with a median age of 12 years. The main clinical findings of these individuals, categorized by gene involvement, are depicted in Table SI. None was treated with recombinant growth hormone.

Nutritional aspects and body composition were analyzed by an indirect method (anthropometric measurements), including height, weight, arm circumference and triceps skinfold thickness. A single set of measurements was obtained on each patient by a single, trained operator during Clinical Genetics outpatient visit. Birth length and weight were ascertained from available medical records. Body weight and height were measured using an electronic balance (PL200, Filizola, Brazil) and a fixed stadiometer (ES2020, Sanny, Brazil) or horizontal length board with a movable foot piece, respectively. Arm circumference and triceps skinfold thickness were measured with skinfold calipers (Lange, Cambridge Scientific Industries, Inc., Cambridge, MD), using standard anthropometric techniques [Frisancho, 1990].

Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Weight, height and BMI were calculated and classified according to the z-score for anthropometry using software licensed by WHO [2009, 2010] for children aged 2–19 years. Adults older than 19 years were classified by BMI according to WHO [2003]. To evaluate total fat mass and fat-free mass, upper arm muscle area, upper arm muscle circumference and upper arm fat area were derived by prediction equations from arm circumference and triceps skinfold measurement, and their centiles were calculated based on age and gender from appropriate reference data [Frisancho, 1990].

A 24 hr nutrition recall questionnaire was completed by asking about eating habits in order to monitor the intake of energy, carbohydrates, protein and fats. The questions were non-directive, leaving the patient or the legal guardian free to answer what was remembered, without being influenced by the researcher reviews. Necessary energy intake and the adequate intake of macronutrients were calculated for each subject according to age, as proposed by the Dietary Reference Intake (DRI) [IOM, 2005]. The patients were asked about gastrointestinal concerns, including questions about feeding problems, gastrointestinal symptoms and malformations. The latter were also obtained from medical records.

In an attempt to establish genotype–phenotype correlations, we compared the BMI and the body composition parameters in RASopathy individuals grouped by gene involvement.

Statistical Analysis

All data were recorded in a computer database and analyzed using SPSS statistical software (SPSS Version 13.1.1). Growth parameters and body composition data were expressed as mean and SD and compared using *t*-test, with a *P*-value below 5% considered significant.

RESULTS

Anthropometric and Body Composition Analysis

Growth parameters and classification of the nutritional status in patients with NS and NRD are shown in Tables I and II, respectively. Birth length and weight were within normal limits, but during development, short stature (z-score of −2) was present in our cohort as a whole and in the subgroups represented by *PTPN11*, *RAF1*, and *SHOC2*. Weight in patients below age 10 years was below the mean, but only one individual harboring a *SHOC2* mutation showed a significant compromise (z-score of −4.39). As a consequence of involvement of both height and weight, BMI scores showed values closer to the mean of the reference (Table I). In the subgroups analyzed, individuals harboring mutations in *BRAF* had the highest BMI scores while individuals with mutations in *SHOC2* had the lowest (Fig. 1). The majority of individuals (82%) was classified as normal or underweight based on BMI scores (Table I).

Body composition analysis revealed z-score values of −2 for upper arm muscle circumference in all subgroups and in the RASopathies as a whole (Table III). Statistically significant differences were observed in the z-score of triceps skinfold ($P = 0.0204$) and upper arm fat area ($P = 0.0388$) between *BRAF* and *SHOC2* genes, and in the z-score of triceps skinfold between *RAF1* and *SHOC2* ($P = 0.0218$).

TABLE I. Growth Parameters at Birth and in the Evolution of Patients With NS and NRD

	Birth length (z-score), Mean ± SD	Birth weight (z-score), Mean ± SD	Height (z-score)[a], Mean ± SD	Weight (z-score)[b], Mean ± SD	BMI-for-age (z-score), Mean ± SD
NS + NRD	−0.8 ± 1.28, n = 45	−0.2 ± 1.19, n = 56	−2.4 ± 1.06, n = 62	−1.6 ± 1.18, n = 23	−0.4 ± 1.48, n = 62
Range	−5.22 to 1.53	−3.32 to 1.76	−4.70 to 0.47	−4.39 to 0.69	−4.09 to 3.22
PTPN11 (n = 38)	−0.8 ± 1.29, n = 24	−0.2 ± 1.22, n = 37	−2.3 ± 1.06	−1.9 ± 1.22, n = 10	−0.4 ± 1.50
Range	−5.22 to 1.53	−3.32 to 1.76	−4.30 to −0.57	−3.98 to −0.72	−4.09 to 2.98
BRAF (n = 7)	−1.2 ± 0.91, n = 6	−0.5 ± 1.21	−1.8 ± 1.07	−0.8 ± 1.08, n = 4	0.6 ± 1.48
Range	−2.58 to −0.08	−1.60 to 0.69	−2.80 to −1.81	−1.75 to 0.67	−1.10 to 3.22
SOS1 (n = 6)	−0.9 ± 1.24, n = 5	−1.0 ± 1.44, n = 4	−1.9 ± 1.11	−1.4 ± 1.14, n = 3	−0.9 ± 1.44
Range	−2.58 to 0.59	−2.41 to 0.43	−3.31 to −0.40	−1.71 to −1.12	−1.45 to −0.08
KRAS (n = 4)	−0.4 ± 1.35	−0.1 ± 1.18	−1.6 ± 1.07	−0.5 ± 0.86, n = 3	−0.7 ± 1.45
Range	−1.00 to −0.08	−0.62 to 0.21	−3.36 to −0.53	−1.86 to 0.69	−3.54 to 1.60
SHOC2 (n = 4)	−0.3 ± 1.33	0.9 ± 1.29	−3.6 ± 1.14	−4.39, n = 1	−1.9 ± 1.44
Range	−0.62 to 0.59	0.57 to 1.24	−4.60 to −2.62	—	−3.38 to −0.97
RAF1 (n = 3)	−0.9 ± 1.52	0.2 ± 1.31	−3.8 ± 1.19	−1.8 ± 1.39, n = 2	0.2 ± 1.29
Range	−3.30 to 0.59	−0.47 to 0.93	−4.70 to −3.00	−2.69 to −0.84	−0.87 to 1.70

NS, Noonan syndrome; NRD, Noonan related disorders; n, number of individuals; SD, standard deviation; BMI, body mass index.
Height and weight were measured during Clinical Genetics outpatient visit.
Whole cohort and subgroups categorized by the involved gene.
[a]Patients below 19 years of age [WHO, 2009, 2010].
[b]Patients above 19 years [WHO, 2003].

Food Intake Analysis

The daily energy and nutrient intake were compared with Dietary Reference Intake (DRI) [IOM, 2005] and data from the control Brazilian population [FAO, 2015]. The results are depicted in Figure 1, showing a similar pattern of macronutrient consumption compared to the DRI [IOM, 2005] and to the Brazilian population.

Gastrointestinal Findings

Only one individual with CFCS reported feeding difficulty and required gastrointestinal tube and gastrostomy. Sporadic episodes of nausea and vomiting were reported by four individuals and ameliorated without treatment. Congenital malformations requiring surgical intervention were disclosed in two individuals, one with imperforate anus (harboring the mutation p.Ser257Leu in *RAF1*) and one with intestinal malrotation (*PTPN11* mutation p.Asp61Gly).

DISCUSSION

Growth impairment is a hallmark of RASopathies, present in 50% to 60% of individuals with NS, which is by far the most prevalent syndrome among the RASopathies. Growth impairment is characterized as proportionate and of postnatal origin. Our data are in accordance with these characteristics, showing a normal mean birth length and a deviation from the growth pattern of healthy children during development, with a mean z-score height of −2.4 in the postnatal period (Table I).

Long-term follow-up studies of large cohorts addressing the growth parameters were completed either prior to the discovery of the molecular basis or performed with partial molecular analysis [Shaw et al., 2007; Binder et al., 2012]. One study in the UK showed a mean z-score height of −2.07 in 107 individuals with NS at ascertainment and −1.88 at the follow-up of 56 patients (mean age of 25.3 years, mean follow-up interval of 12.02 years) [Shaw et al., 2007]. Although the group showed a low mean z-score, its range was very wide, from almost −7 to +1 SD. Such a wide range in z-score was also observed in our study (−6 to +1.6 SD) (Table I), even in patients harboring mutations in the same gene. The latter suggests that, while the NS genetic background is an important factor in causing short stature, other genetic, epigenetic and/or environmental factors influence this trait.

Binder et al. [2012] showed that the prevalence of overweight in patients with NS was lower in comparison to the general German population, both in males (30% vs. 66%) and females (14% vs. 50.6%). For the total German group (45), obesity was present in only 5 (11%) male participants. In our cohort, 8/62 (13%) of the individuals were classified as overweight (harboring mutations in *BRAF*, *KRAS*, *PTPN11*, and *RAF1*) and only 3/62 (5%), as obese (harboring mutations in *BRAF* and *PTPN11*). Accordingly, patients with NS/NRD in our cohort showed a lower prevalence of obesity than the Brazilian population (5% vs. 14%) [IBGE, 2010]. Recently, a low BMI score (median of −0.6) was observed in 28 individuals with NSML, with no obvious genotype–phenotype correlation [Tajan et al., 2014]. These data indicate that individuals with RASopathies tend to show a lean habitus in addition to a growth compromise. Of note, the pattern of an overrepresentation of the normal and underweight groups was unchanged during development, as both children and adults share similar figures—80% and 86%, respectively (Table II).

The possibility of a low macronutrient dietary intake was briefly assessed in our cohort using data gathered from 24-hr nutrition recall questionnaire. There was a similar pattern of energy and macronutrient consumption in patients with NS/NRD compared to the Brazilian population [FAO, 2015] and the DRI [IOM, 2005] (Fig. 1). Thus, it is possible that increased energy expenditure

TABLE II. Classification of the Nutritional Status in Patients With NS and NRD

Measurement	n	Mean ± SD	Range
Classification of nutritional status by BMI (z-score)[a]			
Underweight*	2	−4.0 ± 0.15	−4.09 to −3.88
Normal range	31	−0.7 ± 0.78	−1.93 to 1.30
Overweight	5	1.4 ± 0.28	1.00 to 1.70
Obesity	3	2.7 ± 0.65	2.98 to 3.22
Classification of nutritional status by BMI (kg/m^2)[b]			
Underweight**	5	16.4 ± 1.25	14.90 to 17.40
Normal range	13	20.8 ± 1.42	19.00 to 24.10
Overweight	3	26.9 ± 1.40	25.30 to 27.90
Obesity	0	—	—

NS, Noonan syndrome; NRD, Noonan related disorders; n, number of individuals; SD, standard deviation; BMI, body mass index.
[a]Patients below 19 years of age [WHO, 2009, 2010].
[b]Patients above 19 years [WHO, 2003].
*Mutations: *PTPN11* (p.Tyr63Cys and p.Tyr279Cys).
**Mutations: *PTPN11* (p.Met504Val and p.Thr468Met), *KRAS* (p.Val14Ile), *SHOC2* (p.Ser2Gly in two individuals).

could play a greater role in reducing obesity rates than variations in dietary uptake. This is supported by a study by Tajan et al. [2014], in which a mouse model carrying the mutation p.Thr468Met in Ptpn11 presented a strong reduction of adiposity and resistance to diet-induced obesity, associated with overall better metabolic profile.

Gastrointestinal abnormalities, that could compromise the absorption of nutrients, were rare in our cohort. Only one patient required tube feeding and gastrostomy and few patients reported sporadic nausea and vomiting. Feeding difficulties are more prominent in patients with CS and CFCS [Roberts et al., 2006; Digilio et al., 2008; Pierpont et al., 2014]. In accordance with the literature, the patient in our cohort requiring gastrostomy had CFCS and a mutation in *BRAF* [Roberts et al., 2006; Pierpont et al., 2014]. Two other patients harboring mutations in *RAF1* and *PTPN11*, presented with gastrointestinal malformations, that is, imperforate anus and intestinal malrotation. As these malformations are extremely unusual in these syndromes, it is possible that this association is coincidental.

Noonan syndrome has the highest heterogeneity among the RASopathies, and although a precise genotype–phenotype correlation is not possible, some characteristics are more prevalent in

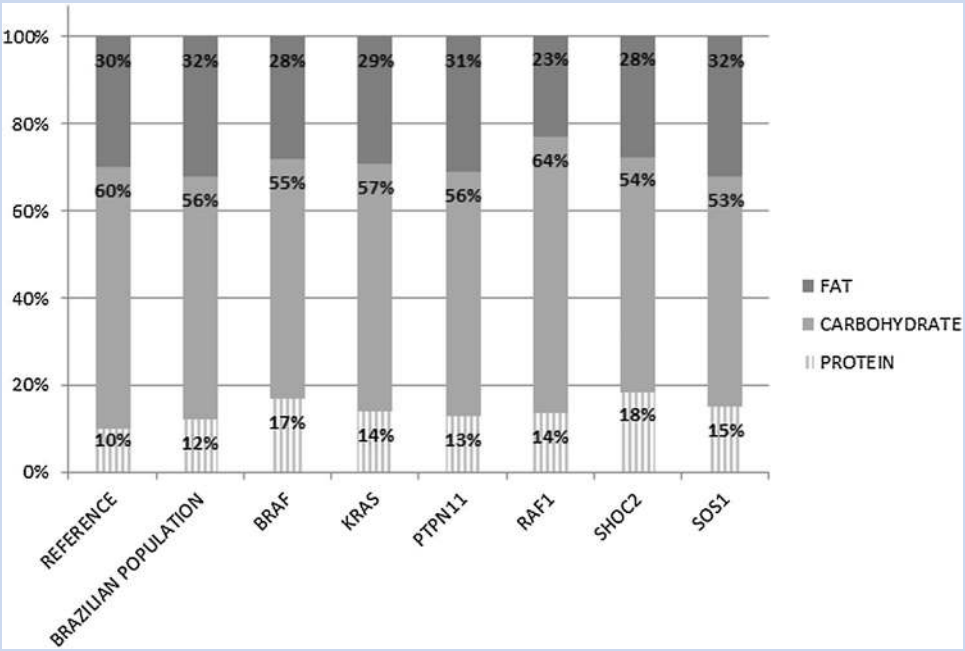

FIG. 1. Distribution of macronutrients according to daily energy consumption and gene mutation adjusted by age and compared to reference [IOM, 2005] and Brazilian population [FAO, 2015].

TABLE III. Body Composition in Patients With NS and NRD

	Arm circumference (z-score) Mean ± SD	Triceps skinfold (z-score) Mean ± SD	Upper arm muscle circumference (z-score) Mean ± SD	Upper arm muscle area (z-score) Mean ± SD	Upper arm fat area (z-score) Mean ± SD
NS + NRD (n = 62)	−1.5 ± 1.14	−0.3 ± 1.11	−2.1 ± 0.48	−1.1 ± 1.11	−0.5 ± 0.99
Range	−4.91 to 1.81	−2.19 to 3.67	−3.84 to −0.67	−2.96 to 4.90	−1.81 to 3.17
PTPN11 (n = 38)	−1.5 ± 1.08	−0.2 ± 1.27	−2.1 ± 0.48	−1.1 ± 1.17	−0.4 ± 1.12
Range	−3.28 to 1.81	−2.19 to 3.67	−2.82 to −1.29	−2.42 to 4.90	−1.81 to 3.17
BRAF (n = 7)	−0.7 ± −1.93	0.1 ± 0.94	−2.1 ± 0.34	−0.9 ± 0.70	0.1 ± 0.90
Range	−2.00 to 0.80	−0.83 to 1.54	−2.64 to −1.60	−1.71 to 0.12	−0.92 to 1.26
SOS1 (n = 6)	−1.8 ± 0.41	−0.7 ± 0.44	−2.1 ± 0.18	−1.4 ± 0.32	−0.8 ± 0.29
Range	−2.37 to −1.13	−1.16 to −0.06	−2.37 to −1.85	−1.77 to −0.85	−1.13 to −0.43
KRAS (n = 4)	−1.8 ± 1.16	−0.7 ± 0.50	−2.5 ± 0.50	−1.2 ± 0.66	−0.9 ± 0.47
Range	−3.43 to −0.71	−1.06 to 0.00	−2.97 to −1.78	−2.23 to −0.83	−1.22 to −0.20
SHOC2 (n = 4)	−2.4 ± 2.16	−1.3 ± 0.49	−2.4 ± 1.32	−1.3 ± 2.37	−1.3 ± 0.57
Range	−4.91 to 0.33	−1.83 to −0.85	−3.84 to −0.67	−2.96 to 2.24	−1.80 to −0.58
RAF1 (n = 3)	−1.0 ± 0.64	−0.1 ± 0.50	−2.1 ± 0.32	−1.2 ± 0.70	−0.3 ± 0.46
Range	−1.66 to −0.39	−0.43 to 0.52	−2.46 to −1.86	−1.98 to −0.77	−0.60 to 0.21

NS, Noonan Syndrome; NRD, Noonan Related Disorders; n, number of individuals; SD, standard deviation.
Whole cohort and subgroups categorized by the involved gene.

individuals harboring mutations in one specific gene. Individuals with mutations in *RAF1*, *SHOC2*, and *PTPN11* had short stature with the most significant effect in individuals with *RAF1* and *SHOC2* mutations. The main *RAF1* phenotype described in the literature includes hypertrophic cardiomyopathy and other heart abnormalities. While these reports primarily focused on cardiac phenotypes, other studies demonstrated a 90% prevalence of short stature with mutations in *RAF1* [Pandit et al., 2007; Razzaque et al., 2007]. Likewise, reduced growth is frequently described in patients with *SHOC2* mutations, often associated with proven growth hormone deficiency [Tartaglia et al., 2011].

All groups showed a weight below the mean, which influences the BMI scores (Table I). Previously, in the study of a growth curve for RASopathies [Malaquias et al., 2012], we showed that BMI scores varied with the gene involved. BMI scores were highest in individuals harboring mutations in *BRAF* and *RAF1*, and lowest in patients with *SHOC2* mutations. Our current cohort, which has a partial overlap with the previous study, showed the same pattern. Although the BMI scores for all groups were within normal values, individuals with *SHOC2* mutations had significantly lower (*P*-value of 0.0216) BMI scores than individuals with *BRAF* mutations. A trend for a low BMI score (−1.9) was observed in the *SHOC2* group (Table I), however, the number of individuals in the *SHOC2* group (n = 4) was too low to draw definitive conclusions. Studies in larger cohorts are required to confirm this association. Interestingly, the groups that showed positive BMI scores (*BRAF* and *RAF1*) differ in their clinical presentation. Individuals with *RAF1* mutations showed a striking height compromise, giving a stocky build appearance, whereas individuals with CFCS harboring *BRAF* mutations presented with a more diffuse pattern.

The mechanism underlying these gene-specific variations is poorly understood, but animal models have shed some light into this matter. Transgenic mice with mutations in Ptpn11 and Sos1 displayed growth impairment [Araki et al., 2004; Chen et al., 2010]. The associated increase in Erk signaling could be responsible for this phenotype. Nevertheless, Chen et al. [2010] demonstrated that mice with the Sos1 mutation p.E846K showed not only increased activation of components of the RAS/MAPK pathway, such as Ras and Erk, but also of Stat3 and Rac, indicating that the phenotype could be the result of different pathway deregulations. In this scenario, it is possible that distinct treatments will be required for NS based on genetic background.

Our study addresses body composition in RASopathy individuals. As was the case for BMI, all parameters were below the reference [Frisancho, 1990], including arm circumference, triceps skinfold thickness, arm muscle circumference, arm muscle area and arm fat area (Table III). The individuals with *BRAF* mutation had the highest values for these parameters, while the lowest were observed with *SHOC2* mutations. The role of deregulation of RAS/MAPK pathway in adipogenesis has been recently demonstrated in a knockin transgenic mouse model displaying the mutation p.T468M in Ptpn11, responsible for NSML [Tajan et al., 2014]. The mutant mice had a lean phenotype, caused by an impaired adipogenesis, associated with increased energy expenditure and enhanced insulin signaling. This phenotype could be ameliorated with a long-term treatment of lose-dose MEK inhibitor.

Interestingly, our data indicate that the biggest effect on body composition was observed in the parameters related to muscle mass, rather than adipose tissue (Table III).

In another RASopathy, neurofibromatosis type I, not included in our study, a decreased muscle cross-sectional area was depicted by peripheral quantitative computed tomography, a direct method for body composition analysis [Stevenson et al., 2005]. In our cohort, decreased muscle mass was observed for all studied populations when grouped by the gene involved, indicating that this is a feature of several RASopathies, including individuals with NS, NSML, and

NSLAH. It is possible that the decreased muscle mass plays a role in the low BMI scores, indicating that altered adipogenesis is not the only factor influencing the BMI scores in the cohort.

A limitation of our study is the use of one single, indirect method (anthropometric measurements) to quantify the body composition. A single technique is unlikely to be optimal in all circumstances, but anthropometry is a quick, easy and highly informative assessment of fatness [Wells and Fewtrell, 2006]). Although the prediction equations to obtain the fat mass and total fat-free mass are less reliable, there is a good correlation between anthropometric techniques and other methods such as dual-energy X-ray absorptiometry in children affected with rare genetic disorders [Motil et al., 2008]. Based on these equations, our study suggests a greater involvement in muscle mass than adipose tissue. This observation is highly relevant to the management of patients, which currently focuses on dietary orientation and does not usually include physical activities to improve muscle mass. Further studies in larger cohorts, using direct methods to access body composition, such as dual-energy X-ray absorptiometry or imaging studies, or combining these techniques with anthropometric analysis [Duren et al., 2008], are necessary to confirm the data obtained in our study. Moreover, 3-day diet records should be sought in order to obtain more complete information of the dietary intake. Confirmation of the findings reported in this study would stimulate the development of improved strategies for NS/NRD management.

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