

Growth patterns of patients with Noonan syndrome: correlation with age and genotype

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Abstract

Background: Growth patterns of patients with Noonan syndrome (NS) were established before the involved genes were identified.

Objective: The goal of this study was to compare growth parameters according to genotype in patients with NS.

Subjects and methods: The study population included 420 patients (176 females and 244 males) harboring mutations in the *PTPN11*, *SOS1*, *RAF1*, or *KRAS* genes. NS-associated *PTPN11* mutations (*NS-PTPN11*) and NS with multiple lentiginos-associated *PTPN11* mutations (*NSML-PTPN11*) were distinguished. Birth measures and height and body mass index (BMI) measures at 2, 5, 10 years, and adulthood were compared with the general population and between genotypes.

Results: Patients with NS were shorter at birth (mean birth length standard deviation score (SDS): -1.0 ± 1.4 ; $P < 0.001$) and throughout childhood than the healthy population, with height SDS being -2.1 ± 1.3 at 2 years, and -2.1 ± 1.2 at 5 and 10 years and adulthood ($P < 0.001$). At birth, patients with *NS-PTPN11* were significantly shorter and thinner than patients with *NSML-PTPN11*, *SOS1*, or *KRAS*. Growth retardation was significantly less severe and less frequent at 2 years in patients with *NSML-PTPN11* and *SOS1* than in patients with *NS-PTPN11* ($P < 0.001$ and $P = 0.002$ respectively). Patients with NS had lower BMI at 10 years ($P < 0.001$). No difference between genotypes was demonstrated.

Conclusion: Determining the growth patterns of patients with NS according to genotype should better inform clinicians about the natural course of growth in NS so that they can optimize the follow-up and management of these patients.

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Introduction

Noonan syndrome (NS; Mendelian inheritance in man (MIM) #163950) is a relatively frequent (estimated prevalence of 1 in 1000–2500 live births) autosomal dominant disorder characterized by facial dysmorphic

features, heart defects (i.e. pulmonary valve stenosis and hypertrophic cardiomyopathy), developmental delay, and short stature (1). These distinctive traits are shared, with variable severity, by NS-related diseases, namely NS

with multiple lentigines (NSML; MIM #151100), NS with loose anagen hair (MIM #607721), cardiofaciocutaneous syndrome (MIM #115150), Costello syndrome (MIM 218040), and neurofibromatosis type I (MIM #162200). In the past decade, mutations in several genes encoding components of the RAS/MAPK signaling pathway were discovered to be involved in NS and NS-related diseases, and this group of disorders is now termed the RASopathies (2). They are one of the largest groups of multiple congenital anomaly diseases known.

The classical form of NS is principally associated with heterozygous missense mutations in four genes: *PTPN11*, *SOS1*, *RAF1*, and *KRAS* genes, accounting for 50, 10, 10, and <2%, respectively, of the NS cases (1). A dozen genes have been found to be implicated in NS, but genetic screening remains negative in about 30% of patients with NS.

NSML, previously referred as LEOPARD syndrome (acronym for multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth, sensorineural Deafness), is closely related to NS. Indeed, because lentigines appear with age, these two syndromes might be difficult to differentiate in early life. NSML is allelic with NS, being mainly caused by *PTPN11* (12q24.13) mutations (>80% of patients) and more rarely by *RAF1* (3p25.2) mutations (3, 4). So far, more than 60 mutations in *PTPN11* have been associated with NS, which mainly affect the interaction between the SH2 and the protein tyrosine phosphatase (PTP) domains, and about 20 mutations, all targeting specific residues within the PTP domain, have been linked to NSML (5). The *PTPN11* gene encodes Shp2, a widely expressed nonreceptor PTP that plays key roles in organism development and homeostasis by controlling major growth factors/hormone-triggered signaling pathways, notably the RAS/MAPK pathway (6). From a biochemical point of view, it has been shown that NS-causing mutations induce hyperactivation of the phosphatase (gain-of-function mutation), while NSML-associated mutations result in reduced Shp2 catalytic activity (inactivating mutation) (7, 8). How NS-Shp2 and NSML-Shp2 mutants can result in related syndromes while displaying opposite biochemical effects is still a matter of debate (9). Interestingly, a differential impact of these mutants on the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway has been causally linked to the development of hypertrophic cardiomyopathy, which is preferentially found in patients with NSML (10, 11).

Proportionate short stature, reported in more than 70% of affected patients, is one of the main clinical

symptoms of NS that lead to the diagnosis (12). Specific growth charts were established more than 25 years ago (13, 14) before the genes involved in NS were identified. The discovery of these genes made it possible to look for genotype–phenotype correlations in growth patterns. However, to our knowledge, only one study investigated the growth characteristics according to genotype in 127 patients with NS and 10 patients with NS-related diseases, with interesting preliminary results (15). In this study, growth parameters (height, weight, and body mass index (BMI)) were converted to age-specific and sex-specific standard deviations on the basis of children's growth standards, and then cumulated before searching for differences between genotypes. As a result, variations according to age and genotype could not be investigated.

In this study, we aimed to describe the growth characteristics (i.e. measurements at birth, height, and BMI) of patients with NS and NSML according to their age and genotype, and evaluate whether mutations in the different genes involved in NS and NSML may have differential impact in growth. To achieve this goal, we took advantage of a French national database and present the growth parameters at different ages according to their genotype in 420 patients with NS or NSML harboring mutations in the *PTPN11*, *SOS1*, *RAF1*, or *KRAS* genes.

Subjects and methods

Patient population

The majority of French patients with NS and NS-related disorders are referred for molecular testing to the Department of Genetics of Robert-Debré Hospital (Paris, France). The clinical data are recorded by the clinicians on standard datasheets. The data include, among other features, the gestational age and measurements at birth (weight, length, and head circumference), and the height and weight at the time of consultation, at 2, 5, 10 years of age, and in adulthood. Treatment with growth hormone (GH) is also recorded.

The study was approved by the Paris-Bichat-Claude Bernard Institutional Review Board (n°2003/15) and informed consent was obtained from legal guardians for genetic analyses and for building up the database.

Inclusion criteria for our study were a diagnosis of NS or NSML between 2002 and 2012, caused by germline mutations in one of the following genes: *PTPN11*, *SOS1*, *RAF1*, or *KRAS*. Two types of *PTPN11* mutations were distinguished: NS-associated *PTPN11* mutations

(*NS-PTPN11*) (gain-of-function mutation) and NSML-associated *PTPN11* mutations (*NSML-PTPN11*) (loss-of-function mutation). For patients treated with GH, growth data under treatment were excluded.

Between 2002 and 2012, germline mutations in the *PTPN11*, *SOS1*, *RAF1*, or *KRAS* genes were found in 999 patients. Among the NS cohort, there were 803 index patients and 196 related patients. The median age at the time of molecular testing was 6.2 years (interquartile range (IQR): 1.3–13.6) in index patients and 27.6 years (IQR: 5.6–36.5) in related patients. To ensure better homogeneity of the results, analyses were only performed in index patients. Data on the growth parameters were missing in numerous patients because of the retrospective study design. To be included in the study, patients had to have at least one height measurement among the four key ages recorded on standard datasheets (at 2, 5, and 10 years of age, and in adulthood). Ultimately, 420 patients (176 females and 244 males) were included. Gestational age and birth measurements were available in most patients of our cohort (339 of 420 patients, 81%). Simultaneous weight and height measurements, allowing BMI calculation, were available for 269 patients (64%).

To exclude a potential selection bias, we compared sex ratio and frequencies of different genotypes between our cohort ($n=420$) and index patients that were excluded ($n=383$). These two data were similar between the two groups ($P=0.433$ and 0.127 , respectively, χ^2 test).

In our cohort of 420 index patients, *NS-PTPN11* mutations were found in 300 (72%) patients, *NSML-PTPN11* mutations in 34 (8%), *SOS1* mutations in 41 (10%), *RAF1* mutations in 31 (7%), and *KRAS* mutations in 14 (3%) patients.

Growth hormone therapy was initiated in 58 patients (14%) at a mean age of 8.2 ± 3.9 years and for these patients the growth parameters obtained after the start of the treatment were excluded from the analysis. Moreover, data about GH treatment were missing in 90 patients (21%) and growth data were consequently excluded in these patients after the age of 2 years.

Auxological parameters

Weight, height, and head circumference at birth were expressed as standard deviation score (SDS) with reference to gestational age according to Usher and McLean (16). Small for gestational age (SGA) was defined as birth height and/or birth weight SDS below -2 .

BMI was calculated as the ratio of weight in kilograms divided by the square of height in meters. Height and

BMI measurements were converted to age-specific and sex-specific SDS on the basis of published reference data (17). Short stature was defined as height SDS below -2 and thinness as BMI SDS below -2 .

Molecular analysis

DNA samples were obtained from peripheral leukocytes. Mutation screening was performed by direct bidirectional sequencing of exons and their flanking intron–exon boundaries. The entire coding region of *PTPN11*, *SOS1*, *RAF1*, and *KRAS* was tested in all patients. Primers and PCR conditions are available on request.

The PCR products were sequenced (BigDye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems) and reaction products run on an automated capillary sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). Sequences were aligned using SeqScape Analysis Software (Applied Biosystems) and compared with the reference sequences for genomic DNA and mRNA. GenBank accession numbers for genomic and mRNA reference sequences, respectively, are as follows: *PTPN11* (NC_000012 and NM_002834), *SOS1* (NC_000002 and NM_005633), *RAF1* (NC_000007 and NM_004333), and *KRAS* (NC_000012 and NM_033360, isoform a) or (NM_004985, isoform b).

Statistical analysis

Dichotomous variables are presented as percentages and compared between the five genotype groups using Fisher's exact test or χ^2 test when applicable. Birth measures, height, and BMI measures at age 2, 5, and 10 years (± 6 months) and in adulthood (>20 years of age) are described as mean \pm s.d. First, means SDS for the entire NS sample was compared with those of the general population. One-sample Student's *t*-test, assuming an average z-score of 0 in the general population, was used for normally distributed data; one-sample median test (Wilcoxon signed-rank test) was used otherwise, assuming a hypothesized median of 0 in the general population. $P < 0.05$ was considered to be statistically significant. Then, ANOVA was applied to compare the measures between the five groups at a 5% level. The normality of the measures was tested in each genotype group by Skewness and Kurtosis test, whereas the homogeneity of variances across groups was assessed using Levene's test. Given the robustness of ANOVA to nonnormality and heteroscedasticity, these tests were applied at a threshold of 0.001. Nonnormally distributed

Table 1 Birth measurements in patients with Noonan syndrome according to genotype.

	NS-PTPN11 n=244	NSML-PTPN11 n=25	SOS1 n=35	RAF1 n=25	KRAS n=10	P-value
Gestational age (weeks), median (IQR)	39 (37; 40) ^{c,2}	39 (38; 40)	38 (37; 39)	36 (36; 39)	37 (36; 38)	0.006 [†]
Prematurity (<37 GA), n (% (95% CI))	43 (18 (13; 22)) ^{c,3}	4 (16 (0; 31))	8 (23 (8; 37))	13 (52 (31; 73))	4 (40 (3; 77))	0.002 ^F
Head circumference (s.d.), mean (±s.d.)	-0.6 (1.5) ^{a,b,c,d,1}	0.5 (1.7)	0.5 (1.5)	1.1 (1.4)	1.1 (1.1)	<0.001 [†]
Birth length (SDS), mean (±s.d.)	-1.2 (1.3) ^{a,b,d,1}	-0.2 (1.4)	-0.4 (1.2)	-1.0 (1.5)	0.5 (1.3)	<0.001 [†]
Birth weight (s.d.), median (IQR)	-0.1 (-0.9; 0.5) ^{a,b,d,2}	1.3 (-0.3; 2.5)	1.0 (-0.2; 1.7)	0.5 (-0.4; 1.6)	0.7 (0.0; 3.0)	<0.001 [†]
SGA, n (% (95% CI))	66 (29 (23; 35))	4 (16 (4; 36))	6 (17 (7; 34))	5 (21 (7; 42))	0 (0 (0; 31))	0.129 ^F
Macrosomia, n (% (95% CI))	14 (6 (3; 10)) ^{a,b,d,F}	10 (40 (21; 61))	7 (20 (8; 37))	3 (12 (3; 32))	4 (40 (12; 74))	<0.001 ^F

P-values were calculated using the ANOVA ([†]) or Kruskal–Wallis ([‡]) tests for continuous variables and Fisher's exact test (F) for dichotomous variables. For pairwise group, comparisons with Šidák correction were done to adjust for multiple testing ($P < 0.013$ considered as significant).

^aSignificantly different between NS-PTPN11 and NSML-PTPN11 groups; ^bSignificantly different between NS-PTPN11 and SOS1 groups; ^cSignificantly different between NS-PTPN11 and RAF1 groups; ^dSignificantly different between NS-PTPN11 and KRAS groups.

¹Student's *t*-test; ²Mann–Whitney U test; ³ χ^2 test.

Adapted from Usher & McLean (16).

measures were expressed as medians and IQRs and were compared across groups using Kruskal–Wallis test.

In the case of significant results, pairwise comparisons between the *NS-PTPN11* genotype and each of the other groups were conducted using Student's *t*-test for normally distributed data, and Mann–Whitney U test. Because of the multiplicity of the tests, these comparisons were conducted at the 0.013 threshold (Šidák correction adjustment for four tests).

Statistical analyses were performed using Stata Statistical Software, version 11 for Windows (StataCorp).

Results

Birth measurements

Although most patients were born full-term, frequency of prematurity (<37 weeks of gestational age) was significantly higher (72 of 339 patients, 21%) in patients with NS compared with the general population in France (7%) (18). Significant differences were found in all genotypes ($P < 0.001$ for one-sample *t*-test of comparison with the general population), except for patients with NSML ($P = 0.094$).

Compared with healthy neonates, patients with NS were shorter (mean birth length SDS: -1.0 ± 1.4 ; $P < 0.001$, one-sample *t*-test) and had smaller head circumference (mean SDS: -0.2 ± 1.6 ; $P = 0.010$, one sample *t*-test); birth weight was similar (median SDS: 0.0, IQR = $(-0.8; 0.9)$; $P = 0.265$, Wilcoxon signed-rank test). Birth measurements varied greatly according to the genotype. Patients with *NS-PTPN11* mutations had significantly smaller head

circumference compared with patients with other genotypes ($P < 0.001$ for all). Moreover, patients with *NS-PTPN11* were significantly shorter and thinner than patients with *NSML-PTPN11*, *SOS1*, and *KRAS* (birth length: $P < 0.001$ for all, birth weight: $P < 0.001$ for patients with *NS-PTPN11* compared with *NSML-PTPN11* and *SOS1*, $P = 0.008$ for patients with *KRAS* compared with patients with *NS-PTPN11*), but did not differ from patients with *RAF1* (Table 1).

In accordance with these data, frequency of SGA (birth height and/or birth weight SDS < -2) was significantly higher (81 of 339 patients, 24%) in patients with NS compared with the general population in France (5%) (18). Significant differences were found for all genotypes ($P < 0.001$ for patients with *NS-PTPN11*, *SOS1*, and *RAF1*, and $P = 0.012$ for patients with *NS-PTPN11*), except for patients with *KRAS* ($P = 0.468$) (Table 1).

As shown in Fig. 1 and Supplementary Table 1, see section on supplementary data given at the end of this article SGA predominated on length rather than weight.

Macrosomia was present in 38 patients (11%), especially in patients with *NSML-PTPN11* (40%) and *KRAS* (40%).

Height according to age and genotype

Compared with the age-specific and sex-specific reference ranges, patients with NS on average were significantly shorter throughout childhood, with height SDS being stable with age: -2.1 ± 1.3 at 2 years of age, and -2.1 ± 1.2 at 5 and 10 years of age and in adulthood ($P < 0.001$ for one-sample *t*-test of comparison with the general population at all ages).

Height (cm) values between 1 month and adulthood from male and female patients with NS were plotted

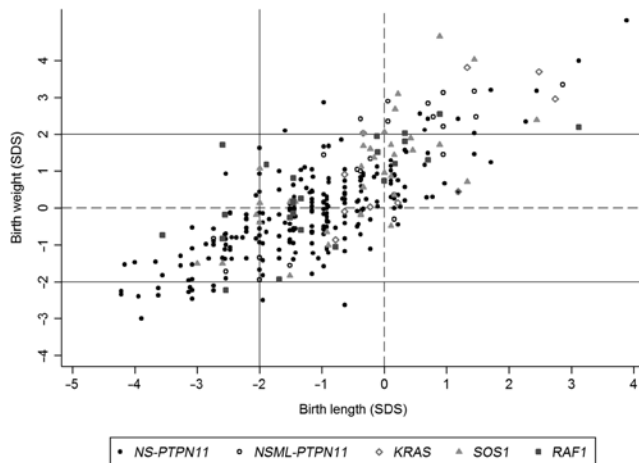


Figure 1

Distribution of birth length and birth weight SDS in patients with Noonan syndrome according to genotype.

Adapted from Usher & McLean (16).

according to age onto appropriate gender-specific WHO Child Growth Standards (Fig. 2A, all values ≥ 20 years were gathered together); a total of 619 data points were available from 420 patients. As previously described for patients with NS, mean height in both genders followed -2 SDS from childhood to adulthood.

To further investigate the evolution of height according to age and genotype, height values obtained at all ages (≥ 1 month) were converted to age-specific and sex-specific SDS on the basis of the WHO Child Growth Standards and were represented according to age and genotype (Fig. 2B). Interestingly, growth retardation was significantly less severe and less frequent at 2 years of age in patients with *NSML-PTPN11* and *SOS1* compared with patients with *NS-PTPN11* ($P < 0.001$ and $P = 0.002$, respectively) (Table 2 and Fig. 2B). Although a similar trend was observed at the other ages, no statistical difference was found, probably due to the small number of patients. No statistical difference was found between patients with *NS-PTPN11*, *RAF1*, and *KRAS*.

For patients with *NS-PTPN11*, median final height was 158 cm for males (IQR: (155; 160)) and 150 cm for females (IQR: (144; 153)), corresponding to height SDS of -2.5 and -2.0 , respectively.

Due to the small size of the subcohorts, it was not possible to precisely investigate gender differences between the genotypes. However, it is interesting to note that height at all time points (at birth, 2, 5, and 10 years of age, and adulthood) was higher in male patients with *NSML-PTPN11* compared with male patients with *NS-PTPN11*, whereas there was no difference between females.

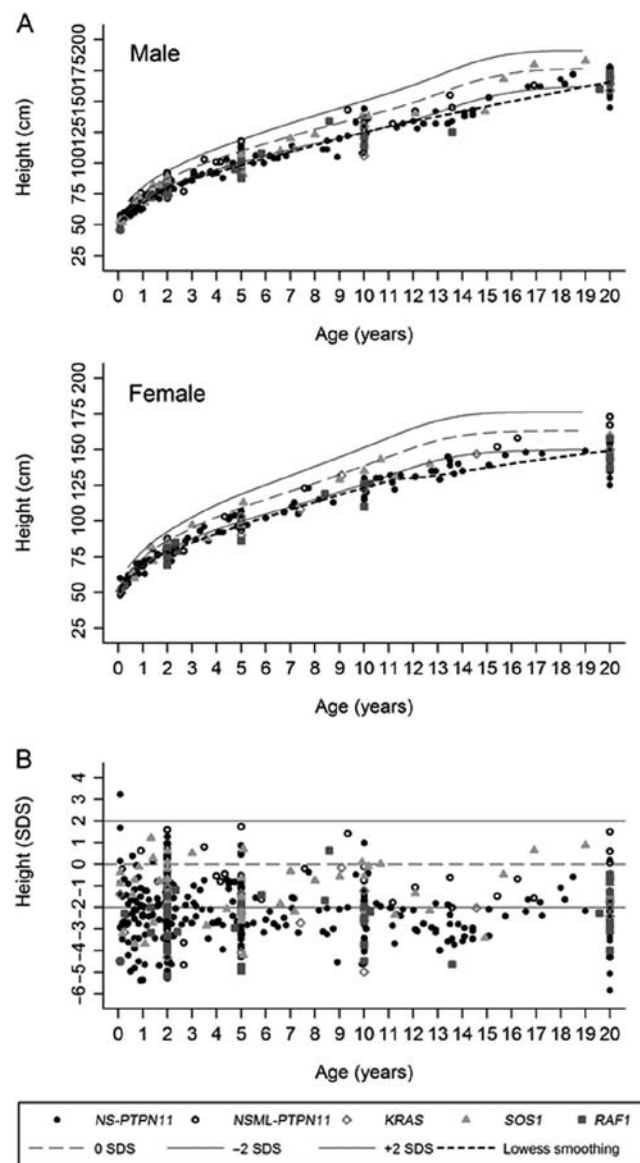


Figure 2

Evolution of height in patients with Noonan syndrome according to age, gender, and genotype. (A) Height (cm) values plotted according to age onto appropriate gender-specific WHO Child Growth Standards. (B) Height SDS values (WHO Child Growth Standards) according to age and genotype. SDS, standard deviation score.

BMI according to age and genotype

Given the very low numbers in certain genotype groups, Skewness and Kurtosis test could not be applied. BMI measures at 2, 5, and 10 years of age are given in Table 3 as median range and IQR. Compared with the age-specific and sex-specific reference ranges, the mean SDS of BMI

Clinical Study	C Cessans, V Ehlinger and others	Growth patterns of Noonan syndrome	174:5	646
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Table 2 Height (WHO Child Growth Standards) in patients with Noonan syndrome according to age and genotype.

	NS-PTPN11	NSML-PTPN11	SOS1	RAF1	KRAS	P-value
2 years						
<i>n</i>	153	15	12	10	5	
Height (SDS), median (IQR)	-2.1 (-3.0; -1.5) ^{a,b,3}	-1.0 (-2.0; 0.4)	-1.1 (-1.7; -0.2)	-3.2 (-3.8; -2.0)	-1.2 (-1.5; -1.0)	< 0.001 [‡]
Height < -2 SDS, <i>n</i> (% (95% CI))	90 (59 (50; 67)) ^{a,b,1}	4 (27 (8; 55))	2 (17 (2; 48))	8 (80 (44; 97))	1 (20 (0; 72))	0.001 ^F
5 years						
<i>n</i>	66	8	8	4	5	
Height (SDS), median (IQR)	-2.4 (-2.8; -1.3)	-1.7 (-2.6; -0.9)	-2.3 (-2.6; -1.1)	-3.8 (-4.8; -2.4)	-2.3 (-4.1; -2.2)	0.204 [‡]
Height < -2 SDS, <i>n</i> (% (95% CI))	38 (58 (45; 70)) ^{b,1}	2 (25 (3; 65))	5 (62 (24; 91))	3 (75 (19; 99))	4 (80 (28; 99))	0.325 ^F
10 years						
<i>n</i>	37	5	5	5	4	
Height (SDS), median (IQR)	-2.1 (-2.4; -1.5)	-1.2 (-1.7; -0.7)	-0.6 (-2.3; -0.1)	-2.6 (-3.7; -2.5)	-2.4 (-3.8; -1.7)	0.049 [‡]
Height < -2 SDS, <i>n</i> (% (95% CI))	20 (54 (37; 70))	1 (20 (1; 72))	2 (40 (5; 85))	4 (80 (28; 99))	3 (75 (19; 99))	0.338 ^F
Adulthood						
<i>n</i>	32	11	6	6	3	
Height (SDS), median (IQR)	-2.3 (-2.9; -1.8)	-1.2 (-2.2; 0.1)	-1.2 (-2.8; -0.6)	-2.0 (-3.1; -0.8)	-2 (-2.4; -1.7)	0.088 [‡]
Height < -2 SDS, <i>n</i> (% (95% CI))	21 (66 (47; 81))	3 (27 (6; 61))	2 (33 (4; 78))	3 (50 (12; 88))	2 (67 (9; 99))	0.161 ^F
Adult males						
<i>n</i>	13	4	3	2	2	
Height (cm), median (IQR)	158 (155; 160) ^{a,3}	169 (164; 175)	170 (166; 172)	170 (167; 173)	162 (159; 164)	0.015 [‡]
Adult females						
<i>n</i>	19	7	3	4	1	
Height (cm), median (IQR)	150 (144; 153)	155 (146; 167)	145 (145; 160)	144 (140; 152)	150 (150; 150)	0.606 [‡]

P-values were calculated using the Kruskal–Wallis ([‡]) test for continuous variables and Fisher's exact test (F) for dichotomous variables.

Pairwise group comparisons with Šidák correction to adjust for multiple testing ($P < 0.013$ considered as significant).

^aSignificantly different between NS-PTPN11 and NSML-PTPN11 groups; ^bSignificantly different between NS-PTPN11 and SOS1 groups; ^cSignificantly different between NS-PTPN11 and RAF1 groups; ^dSignificantly different between NS-PTPN11 and KRAS groups.

¹ χ^2 test; ²Fisher's exact test; ³Mann–Whitney U test.

was 0.2 at 2 years ($P = 0.185$; one sample *t*-test), 0.1 at 5 years ($P = 0.563$), and -0.7 at 10 years ($P < 0.001$).

BMI (kg/m^2) values between 1 month and 19 years from male and female patients with NS were plotted according to age onto the appropriate gender-specific WHO Child Growth Standards (Fig. 3A); a total of 352 data points were available from 269 patients. BMI was normal in patients with NS until 10 years, after which it remained in the lower normal range.

Then, the BMI values converted to age-specific and sex-specific SDS on the basis of the WHO Child Growth Standards were represented according to age and genotype (Fig. 3B). No difference between genotypes was found (Table 3 and Fig. 3B). Due to limited data, BMI could not be analyzed in the adult patients with NS.

Discussion

In this study, we describe the growth patterns of a large cohort of patients with NS according to age and genotype. The frequencies of the genotypes were similar to those previously described in patients with a genetically

confirmed diagnosis of NS, with a vast majority of patients with *NS-PTPN11* (about 70%) (1).

First, we compared characteristics of the entire NS cohort with the general healthy population. We found that frequencies of prematurity (21%) and SGA (24%) were high in patients with NS compared with the general population in France (7 and 5% respectively) (18). Patients with NS were significantly shorter at birth compared with healthy neonates, but had normal birth weight and head circumference, indicating an asymmetrical SGA. The subsequent evolution of postnatal growth in the patients with NS was similar to that reported by Ranke *et al.* (13) and Witt *et al.* (14), with a mean height following -2 SDS in males and females from childhood to adulthood. However, these growth patterns significantly varied according to the genotype. At birth, patients with *NS-PTPN11* and *RAF1* had more severely impaired growth than the other genotypes. This shorter size at birth of patients with *NS-PTPN11* was reported by Limal *et al.* (19), but not by Yoshida *et al.* (20). Conversely, we found that neonates with *KRAS* mutations were often larger for gestational age and had an increased frequency of macrosomia. In

Clinical Study	C Cessans, V Ehlinger and others	Growth patterns of Noonan syndrome	174:5	647
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Table 3 BMI (WHO Child Growth Standards) in patients with Noonan syndrome according to age and genotype.

	NS-PTPN11	NSML-PTPN11	SOS1	RAF1	KRAS	P-value
2 years						
<i>n</i>	66	5	4	4	1	
BMI (SDS), median (IQR)	0.2 (-0.8; 1.0)	0.5 (-0.2; 0.9)	0.4 (-0.2; 1.7)	0.3 (-0.1; 1.1)	-1.4 (-1.4; -1.4)	0.596 [‡]
BMI < -2 SDS, n (% (95% CI))	0 (0 (0; 5))	1 (20 (1; 72))	0 (0 (0; 60))	0 (0 (0; 60))	0 (0 (0; 98))	0.175 ^F
5 years						
<i>n</i>	35	2	4	1	2	
BMI (SDS), median (IQR)	0.0 (-0.5; 0.7)	-1.1 (-1.2; -0.9)	0.0 (-0.7; 0.7)	2.1 (2.1; 2.1)	-0.3 (-1.2; 0.6)	0.106 [‡]
BMI < -2 SDS, n (% (95% CI))	1 (3 (0; 15))	0 (0 (0; 84))	0 (0 (0; 60))	0 (0 (0; 98))	0 (0 (0; 84))	1.000 ^F
10 years						
<i>n</i>	23	2	4	1	1	
BMI (SDS), median (IQR)	-0.9 (-1.5; -0.2)	-0.5 (-1.1; 0.1)	-0.4 (-0.9; 0.0)	-0.6 (-0.6; -0.6)	-2.2 (-2.2; -2.2)	0.407 [‡]
BMI < -2 SDS, n (% (95% CI))	1 (4 (0; 22))	0 (0 (0; 84))	0 (0 (0; 60))	0 (0 (0; 98))	1 (100 (3; 100))	0.159 ^F

P-values were calculated using the Kruskal–Wallis ([‡]) test for continuous variables and Fisher's exact test (F) for dichotomous variables.

the initial description of patients with *RAF1*, macrosomia was found in about a quarter of patients (6 of 22 patients, 27%) and was associated with polyhydramnios (6 of 19 patients, 31%) (3). Polyhydramnios may also explain the higher frequency of prematurity in these patients.

Concerning postnatal growth, previous studies have reported differences in the frequency of short stature between genotypes. Thus, by reviewing the growth data available in published studies, we found that short stature affected 73% of patients with *NS-PTPN11*, 85% of patients with *RAF1*, 84% of patients with *KRAS*, 35% of patients with *SOS1*, and 18% of patients with *NS-PTPN11* (Supplementary Table 2). However, all these studies reported the frequency of short stature by pooling data expressed in SDS from patients of different ages, precluding the description of growth according to age. In our study, patients with *NSML-PTPN11* and *SOS1* were taller at birth and during infancy compared with patients with *NS-PTPN11*, *RAF1*, and *KRAS*. Although a similar trend was observed at the other ages, no statistical difference was found, probably due to the small number of patients, therefore definite conclusion could not be drawn. This less severe growth impairment was previously suggested for patients with *SOS1* (15), but has never been shown for patients with *NSML-PTPN11*. These data may provide insights into the mechanisms underlying growth retardation, which are still incompletely understood. Similar to the origins described for the heart and craniofacial defects observed in NS (21, 22, 23), the enhancement of RAS/MAPK activation seems to be crucial. Indeed, in several NS mouse models carrying a *PTPN11*, *SOS*, or *RAF1* mutation, chronic inhibition of the RAS/MAPK pathway has been shown to improve growth velocity (24, 25, 26). Regarding the mechanisms involved, it has been reported that a *NS-PTPN11* mutant impairs the systemic production of insu-

lin-like growth factor-1 (IGF-1), the biological mediator of GH acting on growth plate, through a hyperactivation of the RAS/MAPK signaling pathway (25). These experimental data are in accordance with clinical data indicating partial GH insensitivity in patients with NS (19, 27). Thus, different IGF-1 levels may explain the differences of height between genotypes. In addition, a possible direct effect (IGF-1 independent) of RAS/MAPK activation on growth plate and longitudinal bone growth may also be involved. Growth retardation related to abnormal differentiation of chondrocytes was reported in a mouse model expressing constitutively active mutant of the MAPK/MEK1 (28) and in a mouse model deficient in neurofibromin 1, a negative regulator of RAS/MAPK activation (29). Moreover, growth plate abnormalities were also observed in achondroplasia, a common skeletal dysplasia related to gain-of-function mutation in the fibroblast growth factor receptor 3 gene (*FGFR3*) leading to prolonged activation of RAS/MAPK at the growth plate level (30).

The difference in height between genotypes in our survey may thus be explained by differences in the degree of activation of the RAS/MAPK signaling pathway or by differential, genotype-dependent, tissue specificity. However, no genotype–phenotype correlation studies have been reported to quantitatively assess the level of RAS/MAPK hyperactivation or to document the tissue/organ pattern of RAS/MAPK dysregulation. Alternatively, some specific mutants may induce dysregulation of other signaling pathways, which might counteract or synergize with RAS/MAPK hyperactivation. In particular, hyperactivation of the PI3K/Akt signaling pathway has been causally linked to NSML pathophysiology, notably hypertrophic cardiomyopathy (10, 11), and constitutional hyperactivation of the PI3K/Akt signaling pathway is associated with overgrowth syndrome (31, 32), suggesting a positive regulating role in growth. Thus, the hyperactivation of PI3K/

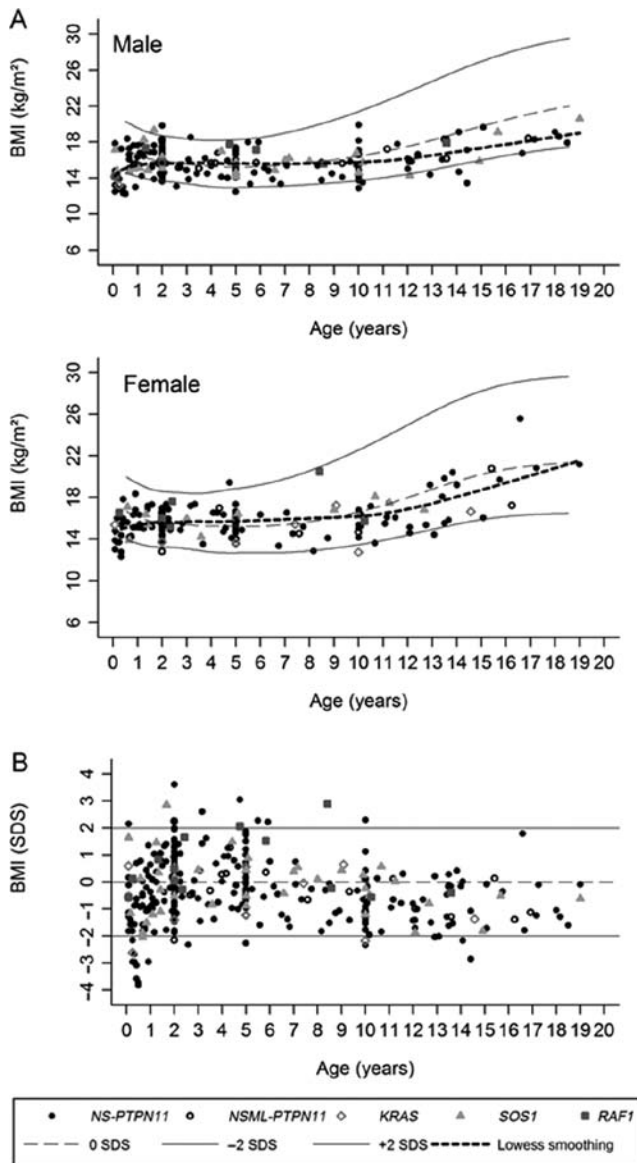


Figure 3

Evolution of BMI in patients with Noonan syndrome according to age, gender, and genotype. (A) BMI (kg/m²) values plotted according to age onto appropriate gender-specific WHO Child Growth Standards. (B) BMI SDS values (WHO Child Growth Standards) according to age and genotype.

Akt found in NSML, and possibly other RASopathies, may soften growth delay in these patients.

Another interesting observation in our study was that patients with NS, with the exception of patients with *RAF1* mutations, had normal BMI during the first 5 years of life and then displayed relative ‘thinness’ with a BMI in the lower normal range. Lower BMI values were also reported in children with NS by Malaquias *et al.* (15) and, in line with these data, a survey showed

that adults with NS rarely develop overweight or obesity (33). This suggested that NS-associated mutations could modify energy metabolism regulation, and this hypothesis has been recently confirmed in a mouse model expressing a *NSML-PTPN11* (loss-of-function) mutation (34). *NSML* mice display a lean phenotype associating reduced adiposity with resistance to diet-induced obesity and improved carbohydrate metabolism, which could be converted by the inhibition of RAS/MAPK and PI3K signaling pathway activation, respectively. Other studies are needed to explore the consequences of other NS mutants on metabolism and to understand how *RAF1* mutations, which also affect RAS/MAPK signaling, result in opposite phenotypes.

The strength of this study lies in the high number of patients and the absence of recruitment bias. Indeed, whatever the presenting sign (i.e. dysmorphic features, heart defects, developmental delay, or short stature) or the referring physician (i.e. geneticist, cardiologist, or endocrinologist), all molecular testing was carried out in the same Department of Genetics (Robert-Debré Hospital). However, except for the patients with *NS-PTPN11*, this study was limited by the small quantity of growth data in the other subcohorts, especially after the age of 2 years. Another important limitation was missing data due to the retrospective design of the study. For example, the presence of associated diseases that can modify growth (e.g. heart failure and failure to thrive) and the hormonal status (e.g. IGF1 levels) were unavailable. Finally, from the initial cohort of index patients with NS from Robert-Debré Hospital database, about half of them were included in our study. Despite similar sex ratio and frequencies of different genotypes between these two groups, a selection bias cannot be fully excluded.

Conclusion

This is the first study trying to describe the growth patterns of patients with NS according to age and genotype. We provided some evidence that patients with *NSML-PTPN11* and *SOS1* were taller at birth and during infancy compared with other genotypes.

Further prospective studies are required to better delineate the growth patterns of patients with NS from childhood to adulthood and perform more robust genotype–phenotype correlation analyses. These data are needed to better inform clinicians about the natural course of growth in patients with NS, thereby allowing them to adjust the follow-up and management of these patients.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-15-0922>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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