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

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Abstract (241/250 words)

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

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

Materials and Methods: The study population included 420 patients (176 females and 244 males) harboring mutations in the \textit{PTPN11}, \textit{SOS1}, \textit{RAF1}, or \textit{KRAS} genes. NS-associated \textit{PTPN11} mutations (NS-qPTPN11) and NS with multiple lentigines-associated \textit{PTPN11} mutations (NSML-qPTPN11) were distinguished. Birth measures and height and BMI measures at 2, 5, and 10 years and adulthood were compared with the general population and between genotypes.

Results: NS patients were shorter at birth (mean birth length SDS: $-1.0 \pm 1.4$; $p < 0.001$) and throughout childhood than the healthy population, with height SDS being $-2.1 \pm 1.3$ at 2 years, and $-2.1 \pm 1.2$ at 5 and 10 years and adulthood ($p < 0.001$). At birth, NS-qPTPN11 patients were significantly shorter and thinner than NSML-qPTPN11, SOS1, or KRAS patients. Growth retardation was significantly less severe and less frequent at 2 years in NSML-qPTPN11 and SOS1 patients than in NS-qPTPN11 patients ($p < 0.001$ and $= 0.002$, respectively). NS patients had lower BMI at 10 years ($p < 0.001$). No difference between genotypes was demonstrated.

Conclusion: Determining the growth patterns of NS patients 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.
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 characterised by facial dysmorphic features, heart defects (i.e. pulmonary valve stenosis and hypertrophic cardiomyopathy), developmental delay, and short stature. 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/mitogen-activated protein kinases (MAPK) signaling pathway were discovered to be involved in NS and NS-related diseases, and this group of disorders is now termed the RASopathies. 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. A dozen genes have been found to be implicated in NS, but genetic screening remains negative in about 30% of NS patients.

NS with multiple lentigines (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. So far, more than 60 mutations in PTPN11 have been associated with NS, that mainly affect the interaction between the SH2 and the PTP domains, and about 20 mutations, all targeting specific residues within the PTP domain, have been linked to NSML. The PTPN11 gene encodes Shp2, a widely expressed non-receptor protein-tyrosine
phosphatase that plays key roles in organism development and homeostasis by controlling major
growth factors/hormone-triggered signaling pathways, notably the RAS/MAPK pathway. 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). How NS- and NSML-Shp2 mutants can result in related
syndromes while displaying opposite biochemical effects is still a matter of debate. 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 NSML patients.

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. Specific growth charts were established more than 25
years ago 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. In this study, growth
parameters (height, weight, and body mass index) were converted to age- 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 the present study, we aimed to describe the growth characteristics (i.e. measurements at birth,
height, and body mass index) 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 \textit{PTPN11}, \textit{SOS1}, \textit{RAF1}, or \textit{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 (H. Cavé, A. Verloes), 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 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: \textit{PTPN11}, \textit{SOS1}, \textit{RAF1}, or \textit{KRAS}. Two types of \textit{PTPN11} mutations were distinguished: NS-associated \textit{PTPN11} mutations (\textit{NS-PTPN11}) (gain-of-function mutation), and NSML-associated \textit{PTPN11} mutations (\textit{NSML-PTPN11}) (loss-of-function mutation). For patients treated with growth hormone, growth data under treatment were excluded.

Between 2002 and 2012, germline mutations in the \textit{PTPN11}, \textit{SOS1}, \textit{RAF1}, or \textit{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: 1.3 to 13.6) in index patients, and 27.6 years (interquartile range: 5.6 to 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, Chi-squared 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%).

Growth hormone therapy was started 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 are expressed as standard deviation score (SDS) with reference to gestational age according to the Usher and McLean tables. Small for gestational age (SGA) was defined as birth height and/or birth weight SDS below -2.

Body mass index (BMI) was calculated as the ratio of weight in kg divided by the square of height in meters. Height and BMI measurements were converted to age- and sex-specific SDS on the basis of published reference data. Short stature was defined as height SDS below -2 and thinness as BMI SDS below -2.

Molecular analyses

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 were tested in all patients. Primers and PCR conditions are available on request.
The PCR products were sequenced (Big Dye Terminator Cycle Sequencing Ready Reaction Kit; Applied Biosystems, Foster City, CA, USA) and reaction products run on an automated capillary sequencer (ABI 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, SOSI 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 analyses**

Dichotomous variables are presented as percentages and compared between the five genotype groups using Fisher exact tests or Chi-square tests 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 means and standard deviations. First, means SDS for the entire NS sample were compared with those of the general population. One-sample Student tests, assuming an average z-score of 0 in the general population, were used for normally distributed data; one-sample median tests (Wilcoxon signed-ranks test) were used otherwise, assuming a hypothesized median of 0 in the general population. A p-value < 0.05 was considered as significant. Then, analyses of variance (ANOVA) were 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 tests, whereas the homogeneity of variances across groups was assessed using Levene's tests. Given the robustness of ANOVA to non-normality and heteroscedasticity, these tests were applied at a threshold of 0.001. Non-normally distributed measures were expressed as medians and interquartile ranges and compared across groups using Kruskal-Wallis tests.

In the case of significant results, pairwise comparisons between the NS-PTPN11 genotype and each of the other groups were conducted using Student t-tests for normally distributed data, and Mann-Whitney rank comparison tests otherwise. Because of the multiplicity of tests, these comparisons were conducted at the 0.013 threshold (Sidak adjustment for 4 tests).
Statistical analyses were performed using Stata Statistical Software, version 11 for Windows (Stata Corporation, College Station, TX, USA).

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 NS patients compared with the general population in France (7%)\(^1\). Significant differences were found for all genotypes (p < 0.001 for one-sample t-tests of comparison with the general population), except for NSML patients (p = 0.094).

Compared with healthy neonates, NS patients 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-ranks 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, NS-PTPN11 patients were significantly shorter and thinner than NSML-PTPN11, SOS1, and KRAS patients (birth length: p < 0.001 for all, birth weight: p < 0.001 for NS-PTPN11 patients compared with NSML-PTPN11 and SOS1, p = 0.008 for KRAS patients compared with NS-PTPN11 patients) but did not differ from RAF1 patients (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 NS patients compared with the general population in France (5%)\(^1\). Significant differences were found for all genotypes (p < 0.001 for NS-PTPN11, SOS1, and RAF1, and p = 0.012 for LS-PTPN11 patients), except for KRAS patients (p = 0.468) (Table 1).

As shown in Figure 1 and supplemental Table 1, SGA predominated on length rather than weight.
Macrosomia was present in 38 patients (11%), especially in NSML-PTPN11 (40%) and KRAS patients (40%).

**Height according to age and genotype**

Compared with the age- and sex-specific reference ranges, NS patients 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-tests of comparison with the general population at all ages).

Height cm values between 1 month and adulthood from male and female NS patients were plotted according to age onto appropriate gender-specific WHO Child Growth Standards (Figure 2A, all values ≥ 20 years were gathered together); a total of 619 data points were available from 420 patients. As previously described for NS patients, 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- and sex-specific SDS on the basis of the WHO Child Growth Standards and were represented according to age and genotype (Figure 2B). Interestingly, growth retardation was significantly less severe and less frequent at 2 years of age in NSML-PTPN11 and SOS1 patients compared with NS-PTPN11 patients (p < 0.001 and = 0.002, respectively) (Table 2 and Figure 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 NS-PTPN11, RAF1, and KRAS patients.

For NS-PTPN11 patients, 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, 2-5-10
years of age, and adulthood) was higher in male NSML-PTPN11 patients compared with male NS-PTPN11 patients, whereas there was no difference between females.

**BMI according to age and genotype**

Given the very low numbers in certain genotype groups, Skewness and Kurtosis tests could not be applied. BMI measures at 2, 5 and 10 years of age are described in Table 3 as median and interquartile range. Compared with the age- and sex-specific reference ranges, the mean SDS of BMI 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 NS patients were plotted according to age onto the appropriate gender-specific WHO Child Growth Standards (Figure 3A); a total of 352 data points were available from 269 patients. BMI was normal in NS patients until 10 years, after which it remained in the lower normal range.

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

**Discussion**

In this study, we described 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 NS-PTPN11 patients (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 NS patients compared with the general population in France (7 and 5%, respectively) \(^\text{18}\). NS patients were significantly shorter at birth compared than healthy neonates but had normal birth weight and head circumference, indicating an asymmetrical SGA. The subsequent evolution of postnatal growth in the NS patients was similar to that reported by Witt \textit{et al.} and Ranke \textit{et al.} 25 years ago \(^\text{13, 14}\), 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, NS-PTPN11 and RAF1 patients had more severely impaired growth than the other genotypes. This shorter size at birth of NS-PTPN11 patients was reported by Limal \textit{et al.} \(^\text{19}\) but not by Yoshida \textit{et al.} \(^\text{20}\). Conversely, we found that neonates with \textit{KRAS} mutations were often larger for gestational age and had an increased frequency of macrosomia. In the initial description of RAF1 patients, 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 polyhydramnios 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 NS-PTPN11 patients, 85% of RAF1 patients, 84% of KRAS patients, 35% of SOS1 patients, and 18% of NSML-PTPN11 patients (supplemental 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, NSML-PTPN11 and SOS1 patients were taller at birth and during infancy compared with NS-PTPN11, RAF1 and KRAS patients. Although a similar trend was observed at the other ages, no statistical difference was found, probably due to the small number of patients, so that definite conclusion could not be drawn. This less severe growth impairment was previously suggested for SOS1 patients \(^\text{15}\) but has never been shown for NSML-PTPN11 patients. These data may provide insight 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 \(^\text{21-23}\), the enhancement of RAS/MAPK activation seems to be crucial. Indeed, in several NS mouse models
carrying a PTPN11, SOS, or RAF mutation, chronic inhibition of the RAS/MAPK pathway has been shown to improve growth velocity\textsuperscript{24-26}. Regarding the mechanisms involved, it has been reported that a NS-PTPN11 mutant impairs the systemic production of insulin-like growth factor-I (IGF-I), the biological mediator of growth hormone (GH) acting on growth plate, through a hyperactivation of the RAS/MAPK signaling pathway\textsuperscript{25}. These experimental data are in accordance with clinical data suggesting partial GH insensitivity in NS patients\textsuperscript{19, 27}. Thus, different IGF-I levels may explain differences of height between genotypes. In addition, a possible direct effect (IGF-I 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/Erk kinase 1 (MEK1)\textsuperscript{28} and in a mouse model deficient in neurofibromin 1, a negative regulator of RAS/MAPK activation\textsuperscript{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\textsuperscript{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 phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway has been causally linked to NSML pathophysiology, notably hypertrophic cardiomyopathy\textsuperscript{10, 11}, and constitutional hyperactivation of the PI3K/Akt signaling pathway is associated with overgrowth syndrome\textsuperscript{31, 32}, suggesting a positive regulating role in growth. Thus, the hyperactivation of PI3K/Akt found in NSML, and possibly other RASopathies, may soften growth delay in these patients.

Another interesting observation in our study was that NS patients, with the exception of patients with \textit{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. and, in line with these data, a survey showed that adults with NS rarely develop overweight or obesity. This suggested that NS-associated mutations could modify energy metabolism regulation, and this hypothesis was recently confirmed in a mouse model expressing a NSML-PTPN11 (loss-of-function) mutation. 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 performed in the same Department of Genetics (Robert-Debré Hospital). However, except for the NS-PTPN11 patients, this study was limited by the small quantity of growth data in the other subcohorts, especially after the age of two years. Another important limitation was missing data due to the retrospective design of the study. For example, the presence of associated diseases than can modify growth (e.g., heart failure, failure to thrive) and the hormonal status (e.g., IGF-I levels) were unavailable. Last, from the initial cohort of index NS patients 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

To conclude, the present study is the first trying to describe the growth patterns of NS patients according to age and genotype. We provided some evidence that NSML-PTPN11 and SOS1 patients were taller at birth and during infancy compared with others 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 NS patients, thereby allowing them to adjust the follow-up and management of these patients.

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References


**Figure 1.** Distribution of birth length and birth weight SDS (Usher & McLean) in patients with Noonan syndrome according to genotype.

**Figure 2.** Evolution of height in patients with NS 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.

**Figure 3.** Evolution of BMI in patients with NS according to age, gender, and genotype.

A. BMI kg/m$^2$ 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.
Figure 1. Distribution of birth length and birth weight SDS (Usher & McLean) in patients with Noonan syndrome according to genotype.

705x513mm (72 x 72 DPI)
Figure 2. Evolution of height in patients with NS 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.

190x254mm (96 x 96 DPI)
Figure 3. Evolution of BMI in patients with NS 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.
Table 1. Birth measurements (Usher & Mc Lean) in patients with Noonan syndrome according to genotype

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

P-values were calculated using the ANOVA (\(^\dagger\)) or Kruskal-Wallis (\(^\ddagger\)) tests for continuous variables and Fisher test (F) for dichotomous variables. For pairwise group comparisons with Sidak's correction to adjust for multiple testing ( \(p < 0.013\) considered as significant) :  

- \(^a\) significantly different between NS-PTPN11 and NSML-PTPN11 groups,  
- \(^b\) significantly different between NS-PTPN11 and SOS1 groups,  
- \(^c\) significantly different between NS-PTPN11 and RAF1 groups,  
- \(^d\) significantly different between NS-PTPN11 and KRAS groups.  

\(^1\) Student t-tests  \(^2\) Mann-Whitney rank comparison tests  \(^3\) Chi-square test.
Table 2. Height (WHO Child Growth Standards) in patients with Noonan syndrome according to age and genotype

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

Pairwise group comparisons with Sidak’s correction to adjust for multiple testing (p < 0.013 considered as significant): 

- a significantly different between NS-PTPN11 and NSML-PTPN11 groups,
- b significantly different between NS-PTPN11 and SOS1 groups,
- c significantly different between NS-PTPN11 and RAF1 groups,
- d significantly different between NS-PTPN11 and KRAS groups.

Chi-Square test ² Fisher exact test ¹ Mann-Whitney rank comparison test
Table 3. BMI (WHO Child Growth Standards) in patients with Noonan syndrome according to age and genotype

<table>
<thead>
<tr>
<th></th>
<th>NS-PTPN11</th>
<th>NSML-PTPN11</th>
<th>SOS1</th>
<th>RAF1</th>
<th>KRAS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 years</td>
<td>n</td>
<td>66</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>BMI (SDS), median (IQR)</td>
<td>0.2 (-0.8; 1.0)</td>
<td>0.5 (-0.2; 0.9)</td>
<td>0.4 (-0.2; 1.7)</td>
<td>0.3 (-0.1; 1.1)</td>
<td>-1.4 (-1.4; -1.4)</td>
</tr>
<tr>
<td></td>
<td>BMI &lt; - 2 SDS, n (% [95% CI])</td>
<td>0 (0 [0 ; 5])</td>
<td>1 (20 [1 ; 72])</td>
<td>0 (0 [0 ; 60])</td>
<td>0 (0 [0 ; 60])</td>
<td>0 (0 [0 ; 98])</td>
</tr>
<tr>
<td>5 years</td>
<td>n</td>
<td>35</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>BMI (SDS), median (IQR)</td>
<td>0.0 (-0.5; 0.7)</td>
<td>-1.1 (-1.2; -0.9)</td>
<td>0.0 (-0.7; 0.7)</td>
<td>2.1 (2.1; 2.1)</td>
<td>-0.3 (-1.2; 0.6)</td>
</tr>
<tr>
<td></td>
<td>BMI &lt; - 2 SDS, n (% [95% CI])</td>
<td>1 (3 [0 ; 15])</td>
<td>0 (0 [0 ; 84])</td>
<td>0 (0 [0 ; 60])</td>
<td>0 (0 [0 ; 98])</td>
<td>0 (0 [0 ; 84])</td>
</tr>
<tr>
<td>10 years</td>
<td>n</td>
<td>23</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>BMI (SDS), median (IQR)</td>
<td>-0.9 (-1.5; -0.2)</td>
<td>-0.5 (-1.1; 0.1)</td>
<td>-0.4 (-0.9; 0.0)</td>
<td>-0.6 (-0.6; -0.6)</td>
<td>-2.2 (-2.2; -2.2)</td>
</tr>
<tr>
<td></td>
<td>BMI &lt; - 2 SDS, n (% [95% CI])</td>
<td>1 (4 [0 ; 22])</td>
<td>0 (0 [0 ; 84])</td>
<td>0 (0 [0 ; 60])</td>
<td>0 (0 [0 ; 98])</td>
<td>1 (100 [3 ; 100])</td>
</tr>
</tbody>
</table>

p-values were calculated using the Kruskal-Wallis (‡) tests for continuous variables and Fisher test (F) for dichotomous variables.