CLINICAL STUDY

Long-term GH treatment improves adult height in children with Noonan syndrome with and without mutations in protein tyrosine phosphatase, non-receptor-type 11

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Abstract

Context: Noonan syndrome (NS) is characterized by short stature, typical facial dysmorphology and congenital heart defects. Short-term effect of GH therapy in NS is beneficial, reports on the effect on adult height are scarce.

Objective: To determine the effect of long-term GH therapy in children with NS.

Design: Twenty-nine children with NS were treated with GH until final height was reached.

Setting: Hospital endocrinology departments.

Patients: Children with the clinical diagnosis of NS, with mean age at the start of therapy of 11.0 years, 22 out of 27 tested children had a mutation in the protein tyrosine phosphatase, non-receptor-type 11 gene (*PTPN11* gene).

Interventions: GH was administered subcutaneously at 0.05 mg/kg per day until growth velocity was 1 cm/6 months.

Main outcome measure: Linear growth (height) was measured at 3-month intervals in the first year and at 6-month intervals thereafter until final height.

Results: At the start of treatment, median height SDS (H-SDS) was -2.8 (-4.1 to -1.8) and 0.0 (-1.4 to $+1.2$), based on national and Noonan standards respectively. GH therapy lasted for 3.0–10.3 years (median, 6.4), producing mean gains in H-SDS of $+1.3$ ($+0.2$ to $+2.7$) and $+1.3$ (-0.6 to $+2.4$), based on national and Noonan standards respectively. In 22 children with a mutation in *PTPN11* mean gain in H-SDS for National standards was $+1.3$, not different from the mean gain in the five children without a mutation in *PTPN11* $+1.3$ ($P=0.98$).

Conclusion: Long-term GH treatment in NS leads to attainment of adult height within the normal range in most patients.

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Introduction

Noonan syndrome (NS) is a multiple congenital abnormality syndrome characterized by short stature, a distinctive facial appearance, congenital heart defects (most frequently pulmonary valve stenosis or hypertrophic cardiomyopathy), thoracic deformities, bleeding diathesis and cryptorchidism in male patients (1–3). The incidence of NS is estimated to be between 1:1000 and 1:2500 live births, inheritance is autosomal dominant, although 60% of cases are sporadic. Tartaglia and colleagues have shown that missense mutations in the protein tyrosine phosphatase, non-receptor-type 11 gene (*PTPN11* gene) on the long arm of chromosome 12 encoding the protein tyrosine phosphatase, SHP-2, were the cause of NS in more than 50% of the cases they examined (4). Recently, mutations in Kirsten retrovirus-associated DNA sequences (*K-ras*), an

effector of activated receptor complexes downstream of SHP-2, have been found in NS and seem to be a rare cause of NS (5). Recently, mutations in Son of Sevenless, homolog 1 (*SOS1*), v-raf-1 murine leukemia viral oncogene homolog 1 (*RAF1*) and mitogen-activated and extracellular-signal regulated kinase kinase 1 (*MEK1*) have been found in clinical cases of NS (6–9). Altogether, mutations in these genes explain ~65% of clinical cases of NS. Therefore, NS remains a clinical diagnosis.

The median height of subjects with NS is reported to be slightly below -2 SDS for the normal population, with a mean adult height of 162.5 cm in men and 152.7 cm in women (10). Over the last two decades, therapeutic trials of growth hormone (GH) have been performed in patients with NS. Short-term results are encouraging, as is the first report on adult height after long-term GH treatment in a prospective study (11–13). With regard to safety of

long-term GH treatment, we showed that there were no changes in cardiac dimensions and insulin-like growth factor-I (IGF-I) levels remained in the normal range during short-term GH treatment (14, 15). Short-term results seem less favourable in children with NS with mutations in *PTPN11* than without mutations in *PTPN11* (16–18). Data on adult height after GH treatment in relation to the presence or absence of this mutation are not available.

We conducted a partly controlled, multi-centre trial of GH treatment in 37 children with NS. The 4-year results of this study were published in 2001 (11). We now report the adult height data in 29 children with NS in relation to the absence or presence of mutations in *PTPN11*, *SOS1* or *ras* genes.

Patients and methods

The original study cohort consisted of 37 children with NS. All the children were referred to one of the participating hospitals (University Hospital Nijmegen; *n* = 29 and four Belgian Paediatric Endocrinology Departments; *n* = 8) for the evaluation of short stature or for the confirmation of the diagnosis of NS. In all cases, the diagnosis of NS was made or confirmed by a clinical geneticist experienced with NS on the basis of previously published criteria (19). Children were included if height SDS (H-SDS) was below –2 and they were prepubertal or in early puberty. Children with hypertrophic cardiomyopathy were excluded. Karyotypes (only performed in girls) were normal. Of these 37 children, nine were lost to follow-up. Table 1 reports relevant details of these nine children; that is, gain in H-SDS over the first 2 years, reasons for and age at withdrawal from the study. Genotypes were not available in these children.

Of the 28 children eligible for final height analysis, 26 reached final height, while 2 were still growing. Three further children with NS, receiving similar treatment in the same hospitals, were included in this analysis. Thus, from a cohort of 40 children with NS, final height can be reported in 29. In the original study protocol reported in 2001, GH treatment was discontinued after 2 years in eight children and restarted after 1 year (11). These children are included in the final height analysis.

Out of 29 children, 27 were tested for mutations in the *PTPN11* gene. As part of the cohort reported by Schubbert *et al.* the five children in which no mutation in *PTPN11* was found had the four *ras* genes (*K-ras*, *H-ras*, *N-ras* and *E-ras*) sequenced (5). Over the last year, v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) and *SOS1* genes were also sequenced in these children, for details on the molecular methods, see Zenker *et al.* 2007 (20).

Growth was measured by experienced observers at 3-month intervals in the first year and at 6-month intervals thereafter, using standard anthropometric techniques. Height was expressed as SDS for age according to national Dutch/Belgian (21, 22) and Noonan (10) standards. Pubertal stages, according to Tanner & Whitehouse (23), were recorded every visit. During the first-year blood counts, IGF-I levels, fasting glucose levels and HbA1c were measured every 3 months, thereafter blood counts, routine biochemistry, IGF-I levels and HbA1c once a year until final height. Echocardiography was performed every 6 months during the first 3 years of treatment and thereafter yearly in 12 children until 5 years of treatment (14). The 21 children with a congenital heart defect also had their regular follow-up at the paediatric cardiology with echocardiography during GH treatment. The results were reported to our hospital.

The GH dosage in all cases was 0.05 mg/kg per day subcutaneously, 7 days a week in the evening. Dosage was adjusted every 6 months for change in weight. Compliance to GH treatment was monitored by counting the remaining GH vials. If the height velocity was below 1 cm/6 months, GH treatment was discontinued. Final height measurements were taken at least 1 year after the discontinuation of GH treatment. The protocol was approved by the Medical Ethics Committees of the participating hospitals. The study was financially supported by Pfizer (New York, NY, USA).

Data analysis

Results are expressed as mean, followed by SDS and range, unless indicated otherwise. Differences between variables were tested with a paired *t*-test or unpaired *t*-test, when appropriate. Linear regression analysis was

Table 1 Data on patients who discontinued growth hormone treatment.

Patient number	Response first year	Response second year	Reason for leaving the study	Age at discontinuation
203	0.45	0.08	Withdrew voluntarily	8
213	0.34	0.01	Psychosocial	6
231	0.5	NA	Withdrew voluntarily	8
103	0.22	0.00	Withdrew voluntarily	5
104	0.62	0.31	Psychosocial	9
105	0.39	0.17	Withdrew voluntarily	8
106	–0.18	–0.26	Died in accident	5
107	0.83	0.35	Lost to follow-up	7
108	0.62	0.59	Withdrew voluntarily	7

Responses are given as change in height SDS; NA, data not available; age at discontinuation in years.

performed, with gain in H-SDS for national standards as a dependent variable and age (and bone age) at the start of treatment, gender, parental heights, age at the start of puberty and duration of GH treatment as independent variables (SPSS 14.0, Chicago, IL, USA).

Results

The clinical characteristics of the 29 children (21 boys and 8 girls) are shown in Table 2. Nine children had a parent with NS (three fathers and six mothers) and twenty cases were sporadic. Mean parental H-SDS was -0.9 (-2.8 to $+1.3$). At the start of treatment, mean age was 11.0 years (median, 11.3; range, 5.8–17.5 years). Mean H-SDS was -2.8 (median, -2.7 ; range, -4.1 to -1.8) for national standards and 0.0 (median 0.0; range -1.4 to $+1.2$) for Noonan standards. Mean bone age was 9.0 years (median, 8.9; range, 3.0–14.1 years). Twenty-five children were prepubertal, while four were in early puberty (one girl in Tanner stage B2, three boys in Tanner stage G2 with testicular volumes of 5, 6 and 7 ml).

In 22 of the 27 tested children, a mutation in the *PTPN11* gene was found. In the five children in whom no mutation in *PTPN11* was found, sequencing of the

four *ras* genes (*K-ras*, *H-ras*, *N-ras* and *E-ras*), *SOS1* and *BRAF* genes showed a mutation in *SOS1* in one child and a mutation in *BRAF* in one child. The clinical characteristics of the child with a mutation in *BRAF* fitted the criteria of NS and not of cardio-facio-cutaneous syndrome or Costello syndrome (diagnosed by author IvdB). Thus, in three children, no mutation in one of the genes related to NS was found. Out of 29 children, 21 had a congenital heart defect.

Height

Median duration of GH treatment was 6.4 years (range, 3.0–10.3 years). The evolution of H-SDS during treatment is shown in Table 3. Expressed as national standards, mean gain in H-SDS was 1.3 (range, -0.2 to $+2.7$). Expressed as Noonan standards, mean gain in H-SDS was 1.3 (-0.6 to $+2.4$). Mean adult height in boys was 171.3 cm (median, 171.6; range, 162.2–182.5 cm). Mean adult height in girls was 157.3 cm (median, 156.4; range 150.8–166.0 cm). Out of the 29 children, 22 reached an adult height in the normal range (H-SDS > -2). In the 20 sporadic cases, mean H-SDS at start minus target height was -2.2 (s.d. 0.9) and mean adult H-SDS was -0.8 (s.d. 0.9).

Table 2 Clinical characteristics of patients with Noonan syndrome who reached final height.

Patient number	Sex	Age	Height-SDS at start	Pubertal stage	Mutation in <i>PTPN11</i>	Congenital heart defect	GH therapy (years)
201	M	12.0	-3.1	Prepubertal	<i>SOS1</i> ⁺ 1433 C>G (P478R)	N	5.5
202	M	13.3	-3.1	Prepubertal	922A>G (N308D)	Pulmonary valve stenosis	3.3
204	M	12.6	-2.7	Prepubertal	236A>G (Q79R)	Pulmonary valve stenosis	5.0
205	M	14.3	-3.8	5 ml testes	179G>C (G60A)	Pulmonary valve stenosis	5.0
206	M	8.0	-3.6	Prepubertal	<i>BRAF</i> ⁺ 1501G>A (E501K)	Pulmonary valve stenosis	9.9
207	F	9.7	-2.5	Prepubertal	184T>G (Y62N)	Aortic coarctation	3.9
208	M	12.3	-3.5	Prepubertal	N*	Pulmonary valve stenosis	5.0
209	F	10.5	-3.3	Prepubertal	922A>G (N308D)	N	5.3
210	F	6.9	-4.1	Prepubertal	182A>G (D61G)	Pulmonary valve stenosis	9.0
211	M	6.3	-2.3	Prepubertal	182A>G (D61G)	Pulmonary valve stenosis	9.5
212	M	10.5	-3.9	Prepubertal	179G>C (G60A)	Pulmonary valve stenosis	9.5
214	M	11.5	-2.5	Prepubertal	188A>G (Y63C)	N	5.0
220	M	12.6	-2.1	6 ml testes	922A>G (N308D)	N	4.0
221	M	10.4	-2.2	Prepubertal	853T>C (F285L)	N	7.6
222	M	17.5	-3.8	7 ml testes	N*	Atrial septal defect	4.0
223	F	11.3	-3.3	Prepubertal	188A>G (T63C)	N	6.0
224	M	12.5	-3.0	Prepubertal	922A>G (N308D)	Pulmonary valve stenosis	7.2
225	F	14.7	-2.8	Breast 2	NA	Pulmonary valve stenosis	3.0
227	M	8.0	-2.5	Prepubertal	922A>G (N308D)	Pulmonary valve stenosis	10.0
228	F	7.5	-3.5	Prepubertal	181G>A (D61N)	Pulmonary valve stenosis	10.3
229	M	13.2	-3.4	Prepubertal	1510A>G (M504V)	N	8.1
230	M	11.3	-2.1	Prepubertal	214G>T (A72S)	Pulmonary valve stenosis	7.5
233	M	9.3	-1.8	Prepubertal	236A>G (Q79R)	N	8.4
235	M	9.1	-2.2	Prepubertal	922A>G (N308D)	Atrial septal defect	7.1
5	M	13.3	-2.4	Prepubertal	N	Pulmonary valve stenosis	4.7
2	M	10.9	-2.5	Prepubertal	922A>G (N308D)	Pulmonary valve stenosis	7.5
372	M	12.3	-1.8	Prepubertal	NA	Pulmonary valve stenosis	3.7
6	F	11.6	-1.9	Prepubertal	844A>G (I282V)	Pulmonary valve stenosis	3.8
3	F	5.8	-2.6	Prepubertal	1502G>A (R501K)	Atrial septal defect	6.9

Height SDS, height standard deviation score; *PTPN11*, protein tyrosine phosphatase, non-receptor-type 11; ⁺, no mutation in *PTPN11*, but in gene stated; *, in these children the four *ras* genes and *SOS1* were also investigated; NA, data not available; N, no.

Table 3 Evolution of height SDS (H-SDS) during growth hormone treatment in 29 children.

	At start	After 1 year	At final height
National standards	−2.8 (0.7, −4.1 to −1.8)	−2.3 (0.7, −3.8 to −1.2)*	−1.5 (0.8, −3.0 to −0.3)*
Noonan standards	0.0 (0.8, −1.4 to +1.2)	+0.6 (0.7, −1.0 to +2.1)*	+1.2 (0.8, −1.1 to +2.9)*

Data are presented as mean (s.d., range). *Significantly different from H-SDS at start ($P<0.0001$).

Figure 1 shows graphically the heights at the start of GH treatment and adult height for boys and girls with NS.

Of the 11 children with a gain in H-SDS for Noonan standards below 0.5 in the first year, mean gain in H-SDS for Noonan standards over the course of treatment was 1.2, which was not different from the rest of the group (+1.3).

Median age at the onset of puberty was 13.8 years (range, 11.2–17.5 years) for boys and 13.3 years (range, 12–15.9 years) for girls. Median height at the onset of puberty was 145.1 cm (mean, 144.2; range, 136.8–156.7 cm) in boys and 144.5 cm (mean, 145.6; range 138.0–153.0 cm) in girls. Median increment in height during puberty was 25.6 cm (range, 19.6–33.7 cm) in boys and 13.8 cm (range, 4.6–22.2 cm) in girls.

Linear regression analysis, using total gain in H-SDS for general population standards as a dependent variable, showed that only age at the start of puberty ($r^2=0.41$, $P<0.01$) made a statistically significant contribution.

Response to GH treatment was not significantly different in children with mutations in the *PTPN11*, compared with children without mutations in *PTPN11*. Mean gains in H-SDS for national standards were 1.3 ($n=22$) and 1.3 ($n=5$) respectively ($P=0.98$, 95% confidence interval of the difference, −1.1 to +1.0), while mean gains in H-SDS for Noonan standards were 1.2 ($n=22$) and 1.5 ($n=5$) respectively ($P=0.39$, 95% confidence interval of the difference −1.1 to +0.5). Over the first year of GH treatment, mean gains in H-SDS for national standards were 0.50 in children with mutations in *PTPN11* ($n=22$) and 0.66 in children without mutations in *PTPN11* ($n=5$) respectively ($P=0.41$), while mean gains in H-SDS for Noonan standards were 0.56 ($n=22$) and 0.82 ($n=5$) respectively ($P=0.09$).

Fasting glucose levels were 4.8 mmol/l (s.d. 0.6) at the start of GH treatment ($n=29$), 4.7 mmol/l (s.d. 0.5) after 1 year ($n=25$) and 4.9 mmol/l (s.d. 0.6) at the end of GH treatment ($n=21$). HbA1c was 5.0% (s.d. 0.4) at the start of GH treatment ($n=29$), 4.9% (s.d. 0.3) after 1 year ($n=28$) and 4.8% (s.d. 0.4) at the end of GH treatment ($n=26$). Mean IGF-I SDS changed from 0.3 ($n=29$, range 1.5 to +0.7) to 0.9 ($n=29$, range −0.8 to +1.8) during the first year of GH treatment. Mean IGF-I SDS did not change significantly during the study. Blood counts did not change, in the differential blood count percentage monocytes remained unchanged: 4.7% at the start of GH treatment ($n=29$), 5.7% after 1 year ($n=28$) and 5.1% at the end of GH treatment ($n=27$).

Echocardiographic follow-up during the first 5 years of the study in 12 children did not show adverse effects on left ventricular dimensions (14). Regular follow-up in the 21 children with a heart defect did not show changes in ventricular mass. In two boys, there was

Figure 1 (A) Height of boys with Noonan syndrome at the start of growth hormone treatment (black dots) and at the final height (grey dots). Graph with dark lines: Dutch height standards, with grey lines Noonan height standards. (B) Height of girls with Noonan syndrome at the start of growth hormone treatment (black dots) and at the final height (smaller grey dots). Graph with grey lines: Dutch height standards, with dark lines Noonan height standards.

mild progression of pulmonary valve stenosis, in one of them balloon dilatation was necessary. A relation of these events to GH treatment was considered unlikely.

One girl showed mild progression of pre-existent scoliosis necessitating bracing.

Discussion

In this paper, we report on a prospective, albeit uncontrolled, study on the effect of GH treatment on adult height in a substantial number of children with NS. In the current study, there was a clear (+1.3 s.d.) mean gain in H-SDS, in relation to national or Noonan standards, corresponding to about 9.5 cm in boys and 9 cm in girls. When we compare the current data with the 4-year results we reported previously, gain in H-SDS after 4 years was maintained until adult height (11). Our previous concerns about bone age acceleration during the first years of GH treatment were not confirmed in the current study. We believe, therefore, that the best way to judge the effect of GH treatment in NS is by comparison with Noonan-specific standards, which is superior to assessing gain in H-SDS for bone age. Still, we need an update on Noonan standards from a contemporary cohort with known genotypes.

As in other studies, the wide range in gain in H-SDS is noticeable (12, 24). We tried to assess the predictors of a good response to GH treatment. In linear regression analysis, age at the start of puberty explained most of the variation of the effect of GH treatment. It would appear that the number of pre-pubertal years spent on GH treatment is very important, although in this study, especially in boys, pubertal growth was comparable with that in normal children. The same finding was made in the study of Osio *et al.* (13). As GH dosage was the same in these children, no effect of GH dosage was found; however, in line with GH treatment in other conditions, the results of GH treatment in NS from the Pfizer International Growth Study (KIGS), with a lower mean GH dose, showed a slightly smaller increment in adult height (24). In the study of Osio *et al.* no beneficial effect of a higher GH dose than the one we used was found. In that study, however, the gain in H-SDS was somewhat better, probably explained by the younger age at the start of GH treatment (13).

This study is the first to report adult height results after long-term GH treatment in children on whom genetic analysis for NS was performed. We could not confirm previously published observations of a greater gain in H-SDS in the short term in children without *PTPN11* mutations, compared with children with mutations in *PTPN11* (16–18). We only found a tendency to a better response in children without mutations in *PTPN11*, especially over the first year of GH treatment. The clear preponderance of children with mutations in *PTPN11* in this study is a limitation in this respect. Evidence is growing that *PTPN11*, and thus

SHP-2, mutations cause mild GH resistance by a post-receptor signalling defect (17). Perhaps, the difference in GH response between children with and without *PTPN11* mutations is only significant in the short-term and diminishes during long-term GH treatment. This might explain why Binder *et al.* did not find a difference in height at presentation between children with and without *PTPN11* mutations (17).

As the *ras* and *SOS1* mutations have effects downstream of SHP-2 and have no effect on GH receptor signalling, it is reasonable to suggest that the mild GH resistance due to *PTPN11*, and thus SHP-2, mutations is not present in 45% of individuals with NS with *ras*, *SOS1* and other, yet to be discovered, germ line mutations that deregulate K-*ras* signalling (5, 6).

The results of our study allow us to give children with NS due to mutations in *PTPN11* and their parents an idea of how much height they may gain with GH treatment. We hope that in a few years we will have the same information for children with NS due to mutations in K-*ras*, *SOS1* and other genes. Whether the benefits of GH treatment in NS balance the inconvenience of GH treatment is difficult to say. In our current cohort, we monitored the safety issues related to GH treatment, i.e. cardiovascular effects, IGF-I levels and effects on carbohydrate metabolism. This was reassuring. Still, our cohort is small and larger series of patients are needed to make definite statements on safety GH treatment in NS. We would recommend regular echocardiographic follow-up during GH treatment in children with NS and a heart defect.

This study has its limitations, as the study is not controlled. Therefore, we compared the data with disease-specific growth data and general population standards. The strength of the current study is that we verified the clinical diagnosis in all children, of which we performed mutational analyses in most and that treatment and follow-up were uniform.

In conclusion, we believe that long-term GH treatment in NS results in an increase in adult height. Children with NS due to *PTPN11* mutations and their parents can be counselled on the effects of long-term GH treatment.

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