

ORIGINAL ARTICLES

NOONAN SYNDROME PATIENTS WITH SHORT STATURE AT A SINGLE PAEDIATRIC ENDOCRINOLOGY CENTRE

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ABSTRACT

INTRODUCTION: Noonan syndrome (NS) is caused by mutations in RAS/MAPK signalling pathway genes. Growth hormone (GH) treatment is an established yet not fully standardized treatment.

AIM: The aim of this article is to assess the first 2 years of GH treatment in NS patients at a single centre.

PATIENTS AND METHODS: A total of 20 (16 males) NS clinically diagnosed regularly followed patients participated (2011–2020). Of these, 9 (45%) had cardiac defects, and 8 (40%) had short stature. Growth hormone deficiency (GHD) was confirmed in 5 patients who started GH treatment, and 2 were treated as short, small for gestational age children. Patients underwent anthropometry, clinical, laboratory and imaging investigations.

RESULTS: The mean age at NS diagnosis was 7.8 ± 3.4 years ($1.3 \div 10.5$), and at GH start 9.1 ± 1.5 years. At GH start, SDS_{height} was -3.42 ± 0.58 ($-4.1 \div -2.6$), SDS_{weight} -3.07 ± 0.58 ($-3.73 \div -2.27$), and SDS_{IGF1} -1.12 ± 0.98 ($-2.44 \div 0.25$). The mean BA at diagnosis was delayed by 2.6 ± 0.9 years. The GH starting dose was 0.035 ± 0.005 mg/kg/d, and changed little thereafter.

The growth velocity for the 1st year of treatment was 8.9 ± 1.4 cm, and for the 2nd year 6.9 ± 1.1 cm. The first year $\Delta SDS_{\text{height}}$ was 0.72 ($p=0.002$), $\Delta SDS_{\text{weight}}$ was 0.83 ($p=0.025$), the 2nd year increments being insignificant. The 1st and 2nd year ΔSDS_{IGF1} were 1.70 ($p=0.007$) and 0.25 (n.s.), resp. Bone age remained significantly delayed. No treatment side effects were observed.

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CONCLUSION: Our study showed that GH-treated NS patients follow the general growth patterns. In order to improve outcomes, the treatment should be further standardized.

Keywords: Noonan syndrome, growth hormone treatment, growth velocity, height gain

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INTRODUCTION

Noonan syndrome (NS) (OMIM#63950, ORPHA:648) is a congenital disorder presenting with characteristic facial features, heart defects, short stature, skeletal and ectodermal abnormalities, bleeding tendency and increased risk for tumour genesis (1). It was first reported in 1962 by Jacqueline Noonan. Noonan syndrome has a reported prevalence of 1:1000 to 1:2500 (2). It is caused predominantly by autosomal dominant mutations although most cases are sporadic. The genetic nature of the condition started to reveal since the first description of a mutation in 2001 (3). Before that, NS was often referred to as phenotypic Turner syndrome and similar terms. Different genes have been associated with NS such as PTPN11 (about 50% of cases) (1,4), KRAS, HRAS, BRAF1, SOS1, MAP2K1, MAP2K2, RAF1, etc. In about 15% of cases no mutation has been found yet (5). Mutated genes causing NS are involved in the RAS/MAPK signalling pathway, which is important for cell proliferation, differentiation, and apoptosis (6). This is also the reason to the frequent usage in the literature of the cumulative name RASopathies, all classified as rare diseases. Apart from NS, the RASopathies include LEOPARD syndrome (OMIM 151100), Cardio-facio-cutaneous syndrome (CFC) (OMIM 115150), Costello syndrome (OMIM 218040), Neurofibromatosis type 1 (OMIM 162200), Legius syndrome (OMIM 611431).

About 70% of the patients with NS have short stature (7) although its exact mechanisms have not yet been defined. Multiple studies (8–13) have shown the effectiveness of growth hormone (GH) treatment for these patients but it is not yet fully standardized.

AIM

The objective of the current study is to assess the characteristics of NS patients at a single Expert Centre of Rare Endocrine Diseases in Varna (VE-CRED) and to evaluate GH treatment prevalence and effectiveness.

PATIENTS AND METHODS

The design of the current study is retrospective cohort evaluation. The study includes patients diagnosed clinically with NS based on the van der Burgt criteria (14) at a tertiary university centre in Varna,

Bulgaria with an adjacent ECRED, for a 10-year period (2011–2021).

Patient's height and weight were measured in a standard manner using wall-mounted Harpenden stadiometer and professional TANITA measuring scale. Body mass index (BMI) was calculated. Two standard stimulation tests for GH deficiency (GHD) in some of the patients using glucagon, insulin, and/or arginine hydrochloride test substances. Standard protocols were followed for the tests. Growth hormone deficiency was defined by levels of GH <10 ng/mL at all measurement points during the tests. Bone age (BA) was estimated by experienced paediatric endocrinologists using the Greulich and Pyle method at the beginning of GH therapy and after 1 and 2 years of treatment, respectively. At each visit the patients had laboratory tests done to follow renal and hepatic function. Hormonal tests were performed including TSH, FT4, LH, FSH, estradiol/testosterone.

Patients who had structural heart defects were followed annually by a paediatric cardiologist and had echocardiography performed. DNA testing was performed at the Institute of Human Genetics, University Hospital Magdeburg, Germany in a sample of the patients.

Statistical analysis was conducted using IBM SPSS ver. 19.0. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 20 patients of whom 16 males (80%) and 4 females (20%) diagnosed with NS (one boy with LEOPARD syndrome and one boy with CFC) were included in the study. Seven patients (35%) received GH treatment due to short stature ($\text{SDS}_{\text{height}} < -2$ compared to standard growth reference). Four patients (20%) did not need therapy by the time of data collection due to lack of short stature. One patient (5%) with LEOPARD syndrome was diagnosed at 16.0 years of age with no further growth potential. Eight patients (40%), of whom one with short stature and 7 who were advised to visit the centre for reassessment after six months, were lost to follow-up.

Nine (45%) patients had structural heart defects. Six of them were annually followed by a paediatric cardiologist and had echocardiography performed. DNA test results showed that 3 patients (27.3%) had PTPN11 mutation, 1 (9.1%) had KRAS

mutation, 1 (9.1%) had BRAF mutation, one (9.1%) had A2ML1 mutation, in 2 patients (18.2%) no mutation was found, and 3 (27.3%) were still waiting for genetic results by the time of data collection.

Among our patients 7 (35%) received GH treatment. One patient is not included in the statistical analyses due to the short period of treatment (1 month) by the time of data collection and a total number of 6 patients were analysed. The DNA test results of the 7 GH-treated patients showed that 3 (43%) had PTPN11 mutation, in 2 patients no mutation was found, and in 2 (28.5%) the genetic work-up was not yet finalized (Fig. 1). Four (57.1%) of the treated patients had heart defects (2 with pulmonary stenosis, 1 with pulmonary and aortic stenosis and 1 with aortic regurgitation).

DNA test results of GH treated patients

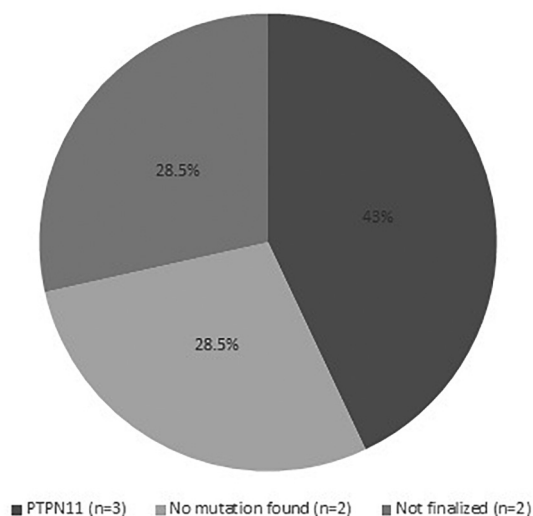


Fig. 1. DNA test results of GH treated patients

Two patients (10%) were treated under the label “small for gestational age without postnatal catch-up growth”. The other five children (25%) underwent at least two GH stimulation tests and all of them had peak levels of GH<10 ng/mL at all time points.

The mean age at clinical diagnosis was 7.8 ± 3.4 (range 1.3–10.5) years. The mean age at the beginning of GH treatment was 9.1 ± 1.5 years ($7.5 \div 10.7$) and the mean period of treatment was 38.3 ± 15.3 months (range 17.0–59.0). At diagnosis, the mean SDS_{height} was

-3.42 ± 0.58 (range $-4.1 \div -2.6$), the mean SDS_{weight} was -3.07 ± 0.58 (range $-3.73 \div -2.27$) and mean SDS_{BMI} was -0.75 ± 0.17 (range $-1.09 \div -0.58$). The mean SDS_{IGF1} at the beginning of the treatment was -1.12 ± 0.98 (range $-2.44 \div 0.25$). The mean BA at diagnosis was 6.4 ± 1.3 ($4.6 \div 7.8$) years and was delayed by 2.6 ± 0.9 years compared to chronological age (CA). The starting dose of GH was 0.035 ± 0.005 mg/kg/d ($0.029 \div 0.045$). The dosage thereafter changed based on growth velocity, safety parameters and IGF-1 values for some of the patients and the mean dosage during the first year of treatment was 0.036 ± 0.002 ($0.034 \div 0.038$) mg/kg/d and was increased to 0.037 ± 0.003 ($0.034 \div 0.041$) mg/kg/d during the second year.

After one year of GH treatment, the mean height ΔSDS was 0.72 ($p=0.002$) and between the first and second year the mean height ΔSDS was 0.56 ($p=0.09$). The mean ΔSDS for weight was 0.83 ($p=0.025$) and 0.25 ($p=0.09$) at the end of the 1st year and between the 1st and 2nd year of treatment, respectively. The mean ΔSDS_{BMI} was 0.16 ($p=0.609$) and 0.35 ($p=0.801$) at the end of 1st year and between 1st and 2nd year of treatment respectively. The mean ΔSDS_{IGF1} was 1.70 ($p=0.007$) and 0.25 ($p=0.543$) at the end of the 1st year and between the 1st and 2nd year of treatment, respectively. Data are shown on Fig. 2 and in Table 1. The mean growth velocity during the first year of GH treatment was 8.9 ± 1.4 cm/year for the first year and 6.9 ± 1.1 cm/year for the second year of treatment (Fig. 3). By the end of the first year of treatment, the mean BA was 7.6 ± 2.2 years and was delayed by 1.9 ± 0.9 years compared to chronological age (CA). By the end of the second year of treatment the

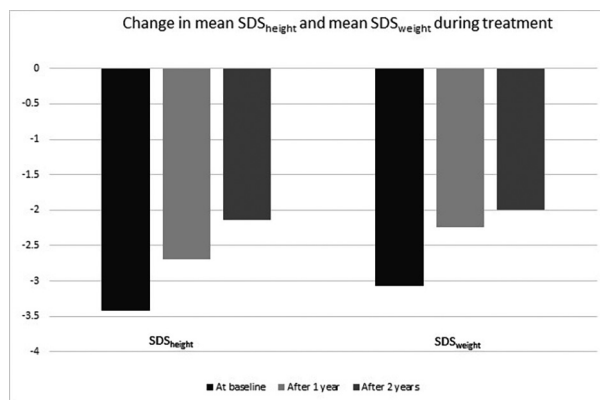


Fig. 2. Change in mean SDS_{height} and mean SDS_{weight} during treatment

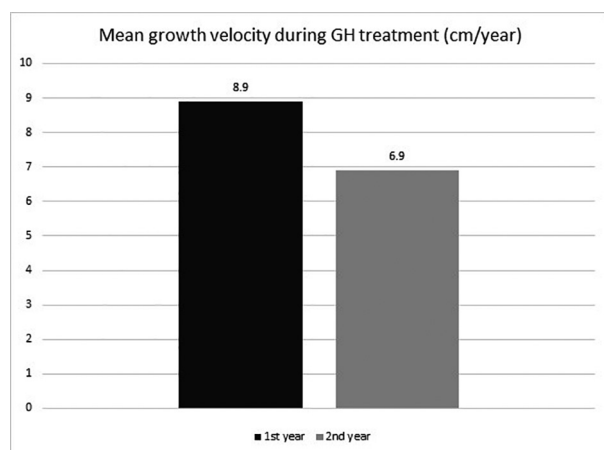


Fig. 3. Mean growth velocity during GH treatment (cm/year)

Table 1. Patient characteristics during the period of observation

Patient Characteristics	Baseline	1 st year	2 nd year
Mean SDS _{height}	-3.42	-2.70	-2.14
Mean SDS _{weight}	-3.07	-2.24	-1.99
Mean SDS _{IGF1}	-1.12	0.58	0.83

mean BA was 9.4 ± 2.3 years and was delayed 1.5 ± 1.1 years compared to CA.

No patient reached puberty for the first 2 years of GH treatment. In the patients with heart defects, no progression of the condition was observed. There was no significant change in BMI during the treatment period. None of the patients were diagnosed with a malignant disease throughout the period of observation.

DISCUSSION

Noonan syndrome is a relatively common genetic disorder within the group of rare diseases, with short stature as one of its main characteristic features. Although the precise pathogenetic mechanism of short stature has not been defined, multiple studies have shown the positive effect of GH treatment on stature. There are conflicting data regarding the long-term effectiveness of treatment. Some authors describe a significant increase in growth velocity and SDS_{height} during the first year of treatment and a waning effect afterwards (9,10). Other studies show consistent increase of SDS_{height} during long-term treatment (15). Horikawa et al. (13) showed that consistent increase in SDS_{height} is achieved only in NS patients

treated with higher doses of GH. Our study shows that during the first year of GH treatment there was statistically significant ($p < 0.05$) improvement in height, weight, GV and IGF-1 levels. Treatment during the second year shows less satisfactory and no statistically significant results. The unsatisfactory results may be due to the late age at beginning of treatment or low GH dosage.

Multiple studies have been performed on the relationship between genotype and GH treatment effectiveness. Noonan syndrome patients with a PTPN11 mutation are often reported to have normal to high levels of GH and lower levels of IGF-1 suggesting GH resistance (16). Some authors speculate that this might be the reason for the less satisfactory results from GH treatment seen in these patients compared to NS patients with mutations in other genes (12). In contrast, other studies have shown no significant difference in treatment effectiveness between different genotypes (17). The patient sample in the current study is small and such conclusions cannot be made.

The use of GH for NS patients has recently been approved by the European Medicines Agency (<https://mri.cts-mrp.eu/Human/Product/Details/14639>). So far there are no universal treatment protocols for NS patients. Zavras et al. (19) compared the effectiveness of similar doses of GH between patients with NS and with idiopathic growth hormone deficiency (IGHD) and showed that NS patients had less satisfactory results. Seok et al. (9) demonstrated that patients with NS needed higher doses of GH to achieve similar results on growth compared to IGHD patients. This may be due to the late age at the beginning of treatment and possible GH resistance (20) in NS patients. Some studies show no significant difference between the GH dosage used and the effects on growth in NS patients (19,21). In contrast, Horikawa et al. (13) described a significantly better response to a higher GH dose. In the current study the used doses of GH seem to be insufficient to achieve satisfactory results beyond the first year of treatment.

There have been concerns regarding the possible side effects of GH such as progression of heart structural defects, particularly, hypertrophic cardiomyopathy, as well as increasing the risk for malignancy and negative metabolic effects. Most authors

show no progression of the structural heart defects during treatment (16,22). It should be noted, though, that NS patients with RAF1 mutation are at risk for developing progressive hypertrophic cardiomyopathy (17) and GH treatment in these patients should be carefully considered. Similar to previous data, the patients in the current study showed no progression of the structural heart defects.

Patients with NS are at increased risk for malignant diseases, particularly juvenile myelomonocytic leukaemia. There have been concerns regarding the risk for GH treatment to increase these patients' malignancy potential. So far GH treatment has not been associated with an increased risk for malignancy (8). None of the patients in the current study developed malignancy during the period of observation.

Growth hormone treatment has been associated with negative impact on lipid and carbohydrate metabolism (16). Studies of NS patients treated with GH show no negative effect on the metabolic profile, no change in BMI and body composition during treatment (15,19). Data from the literature suggest that disease-causing mutations in NS might have a protective role for the metabolism as NS patients rarely develop obesity and impaired carbohydrate metabolism (7). The current study is consistent with previous reports of stable BMI during GH treatment, but lipid levels were not assessed due to the low numbers of the participants.

There is still a large number of NS patients who do not reach a paediatric endocrinologist, and as a result are not diagnosed, treated or followed. This is a significant issue which could be overcome via unified efforts at different levels – education and training of medical students and medical staff, raising awareness among the society, increasing free resources for parents who seek advice, and more accessible genetic testing even for very subtle features. Early diagnosis and treatment of NS could significantly improve their final stature, quality of life, and late-term outcome.

A major disadvantage of our study is the small patient sample. Its strength is in the follow-up of patients at a single setting.

CONCLUSION

Our study showed that NS patients follow the general patterns for the first 2 years of GH treatment.

The applied doses seem insufficient to cause good height increment. In order to improve outcomes, the treatment should be further standardized.

REFERENCES

1. Van der Burgt I. Noonan syndrome. *Orphanet J Rare Dis.* 2007;2:4. doi:10.1186/1750-1172-2-4
2. Carcavilla A, Suarez-Ortega L, Rodríguez Sánchez A, Gonzalez-Casado CI, Ramón-Krauel M, Labarta J, et al. Noonan syndrome: genetic and clinical update and treatment options. *An Pediatr (Engl Ed).* 2020;93(1):61.e1-61.e14. Spanish. doi: 10.1016/j.anpedi.2020.04.008.
3. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet.* 2001;29(4):465-8. doi: 10.1038/ng772.
4. Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, van der Burgt I, et al. PTPN11 mutations in Noonan syndrome: molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. *Am J Hum Genet.* 2002;70(6):1555-63. doi:10.1086/340847.
5. Linglart L, Gelb BD. Congenital heart defects in Noonan syndrome: Diagnosis, management, and treatment. *Am J Med Genet C Semin Med Genet.* 2020;184(1):73-80. doi:10.1002/ajmg.c.31765.
6. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet.* 2013;381(9863):333-42. doi:10.1016/S0140-6736(12)61023-X.
7. Cessans C, Ehlinger V, Arnaud C, Yart A, Capri Y, Barat P, et al. Growth patterns of patients with Noonan syndrome: correlation with age and genotype. *Eur J Endocrinol.* 2016;174(5):641-50. doi: 10.1530/EJE-15-0922.
8. Apperley LJ, Ramakrishnan R, Dharmaraj P, Das U, Didi M, Blair J, et al. Effect of growth hormone therapy in patients with Noonan syndrome: a retrospective study. *Int J Endocrinol Metab.* 2020;18(4):e107292. doi: 10.5812/ijem.107292.
9. Seok EM, Park HK, Rho JG, Kum CD, Lee HS, Hwang JS. Effectiveness of growth hormone therapy in children with Noonan syndrome. *Ann Pediatr Endocrinol Metab.* 2020;25(3):182-6. doi:10.6065/apem.1938154.077.
10. Seo GH, Yoo HW. Growth hormone therapy in patients with Noonan syndrome. *Ann Pediatr En-*

- doocrinol Metab. 2018;23(4):176-81. doi:10.6065/apem.2018.23.4.176.
11. Rohrer TR, Abuzzahab J, Backeljauw P, Birkegård AC, Blair J, Dahlgren J, et al. Long-term effectiveness and safety of childhood growth hormone treatment in Noonan syndrome. *Horm Res Paediatr.* 2020;93(6):380-95. doi: 10.1159/000512429.
12. Jeong I, Kang E, Cho JH, Kim GH, Lee BH, Choi JH, Yoo HW. Long-term efficacy of recombinant human growth hormone therapy in short-statured patients with Noonan syndrome. *Ann Pediatr Endocrinol Metab.* 2016;21(1):26-30. doi: 10.6065/apem.2016.21.1.26.
13. Horikawa R, Ogata T, Matsubara Y, Yokoya S, Oga-
wa Y, Nishijima K, et al. Long-term efficacy and
safety of two doses of Norditropin® (somatropin)
in Noonan syndrome: a 4-year randomized, dou-
ble-blind, multicenter trial in Japanese patients.
Endocr J. 2020;67(8):803-18. doi: 10.1507/endocrj.
EJ19-0371.
14. van der Burgt I, Berends E, Lommen E, van Beer-
sum S, Hamel B, Mariman E. Clinical and molec-
ular studies in a large Dutch family with Noonan
syndrome. *Am J Med Genet.* 1994;53(2):187-91. doi:
10.1002/ajmg.1320530213.
15. Lee PA, Ross J, Germak JA, Gut R. Effect of 4 years
of growth hormone therapy in children with Noon-
an syndrome in the American Norditropin Studies:
Web-Enabled Research (ANSWER) Program® reg-
istry. *Int J Pediatr Endocrinol.* 2012;2012(1):15. doi:
10.1186/1687-9856-2012-15.
16. Raynal, Patrick. Growth hormone and noonan syn-
drome: update in dysfunctional signaling aspects
and in therapy for short stature. *Horm Studies.*
2014;2. doi:10.7243/2052-8000-2-1.
17. Choi JH, Lee BH, Jung CW, Kim YM, Jin HY, Kim
JM, et al. Response to growth hormone therapy in
children with Noonan syndrome: correlation with
or without PTPN11 gene mutation. *Horm Res Pae-
diatr.* 2012;77(6):388-93. doi: 10.1159/000339677.
18. Rodriguez F, Gaete X, Cassorla F. (2021) Etiol-
ogy and Treatment of. Growth Delay in Noon-
an Syndrome. *Front Endocrinol (Lausanne).*
2021;12:691240. doi: 10.3389/fendo.2021.691240.
19. Zavras N, Meazza C, Pilotta A, Gertosio C, Pa-
gani S, Tinelli C, et al. Five-year response to growth
hormone in children with Noonan syndrome
and growth hormone deficiency. *Ital J Pediatr.*
2015;41:71. doi:10.1186/s13052-015-0183-x
20. Binder G, Neuer K, Ranke MB, Wittekindt NE.
PTPN11 mutations are associated with mild
growth hormone resistance in individuals with
Noonan syndrome. *J Clin Endocrinol Metab.*
2005;90(9):5377-81. doi: 10.1210/jc.2005-0995.
21. Noonan JA, Kappelgaard AM. The efficacy
and safety of growth hormone therapy in chil-
dren with noonan syndrome: a review of the evi-
dence. *Horm Res Paediatr.* 2015;83(3):157-66. doi:
10.1159/000369012.
22. Jo KJ, Kim YM, Yoon JY, Lee YJ, Han YM, Yoo
HW, et al. Comparison of effectiveness of growth
hormone therapy according to disease-causing
genes in children with Noonan syndrome. *Ko-
rean J Pediatr.* 2019;62(7):274-80. doi:10.3345/
kjp.2018.06842.