# The Effect of Growth Hormone Therapy on Cardiac Outcomes in Noonan Syndrome: Long Term Follow-up Results

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## What is already known on this topic?

Noonan syndrome (NS) is a multisystem disorder, with short stature and cardiac anomalies being the most common systemic effects. Theoretically, there is a risk of increased ventricular wall thickness and cardiac side effects associated with the use of recombinant human growth hormone (rGH).

## What this study adds?

This study aimed to investigate the effect of rGH use in patients with NS on ventricular wall thickness and a possible increased risk of cardiac side effects. Patients were divided into two groups based on whether or not they received rGH. There was no difference in this cohort between the rGH and non-rGH groups in terms of echocardiographic parameters, pre-and post-treatment.

## Abstract

**Objective:** Cardiac involvement is common in Noonan syndrome (NS). Concerns have been raised regarding the effect of recombinant growth hormone (rGH) use on ventricular wall thickness and a possible increased risk of cardiac side effects. This study aimed to investigate the effect of rGH on the development of hypertrophic cardiomyopathy and other cardiac findings in NS.

**Methods:** Patients under the age of 18 years and diagnosed with NS according to the Van der Burgt criteria, were included. Patients were divided into two groups according to those receiving rGH or not at the time of obtaining cardiac measurements. Before and after the treatment, electrocardiographic and echocardiographic (ECHO) assessments were made, including interventricular septal thickness, left ventricular internal diameter, and left ventricular posterior thickness. Results were expressed as Z scores.

**Results:** Twenty-four NS subjects (16 boys, eight girls) were included. At the beginning of the follow up, the overall height standard deviation score was -2.56 ± 0.94. Sixteen were on rGH. The mean rGH treatment duration was 8.3 ± 3.8 years, and the mean dose was 0.22 ± 0.04 mg/kg/week. The final height was 169 ± 8.2 cm, and 10 of 11 patients who reached the final height received rGH. There was no difference between the rGH and non-rGH groups in terms of ECHO parameters pre-and post-treatment.

**Conclusion:** In this cohort, there was no change in ECHO parameters on rGH and during follow-up. These results suggest that rGH is safe in NS patients with cardiac pathology under close follow-up.

**Keywords:** Recombinant growth hormone therapy, Noonan syndrome, hypertrophic cardiomyopathy, left ventricular dimension

## Introduction

Noonan syndrome (NS; OMIM 163950) is characterized by typical facial findings (hypertelorism, ptosis, downward-sloping palpebral fissures, epicanthus, depressed nasal bridge, low-set posterior-turned ears, and micrognathia), developmental delay, learning disability (usually mild), short stature, congenital heart disease, kidney anomalies, lymphatic malformations, and bleeding disorders (1,2,3). Mutations in the RAS-mitogen-activated protein kinase (RAS-MAPK) pathway cause NS by changing protein-coding genes (4). To date, 20 genes associated with NS (*PTPN11, SOS1, SOS2, KRAS, NRAS, RIT1, RRAS, RASA1, RASA2, MRAS, RAF1, BRAF, MAP2K1, MAP3K8, SHOC2, PPP1CB, SPRY1,*

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

*LZTR1, MYST4, A2ML1, CBL*) have been identified. Over the years, the identification of genes associated with NS has increased (1). Candidate genes may be associated with clinically diagnosed cases without molecular confirmation.

Congenital heart disease is frequently described in patients with NS. The syndrome is inherited mostly in an autosomal dominant pattern. It has an estimated incidence of 1:1000 to 1:2500 live births (2,3,4,5). More than 80% of the cases have cardiovascular system anomalies. The most common congenital heart defect is pulmonary stenosis (PS), with a rate of about 40%. Twenty percent of patients have hypertrophic cardiomyopathy (HCMP) with asymmetric septal hypertrophy. Other common cardiac anomalies in NS include atrial septal defect (ASD) in 6-10%, mitral stenosis (MS) in 6%, aortic stenosis (AS) and aortic coarctation in 9%, ventricular septal defect (VSD) in 5%, and patent ductus arteriosus in 3%. HCMP can be mild or severe and present from prenatal ages to late childhood. In some infants, HCMP resolves, while in others, it becomes rapidly progressive and can be fatal. Left obstructive lesions can also develop in adulthood (1,2,3,4).

Recombinant growth hormone (GH) therapy (rGH) has been given to patients with NS because of short stature, typical of NS. It has been reported that the use of rGH in NS can result in reaching the average height of normal healthy adult individuals (4). However, persistent high insulin-like growth factor-1 (IGF-1) levels may cause pathological cardiac hypertrophy and heart failure (2). Thus there are concerns about the use of rGH in NS because of the already existing cardiac involvement in the pathophysiology of the disease and the high frequency of cardiac hypertrophy. Theoretically, the risk of increased ventricular wall thickness and cardiac side effects associated with the use of rGH is predicted, but studies providing empirical evidence in this area are very limited (Table 1) (6-16).

The aim of this study was to describe the presence of accompanying cardiac anomalies and the effects of rGH use in NS patients. Other objectives were to investigate the cardiac effects of rGH treatment on ventricular wall thickness and other possible cardiac anomalies during follow-up in patients with NS who did not receive rGH, and to increase awareness of the importance of cardiac monitoring in pediatric NS.

## Methods

The study was approved by the Ankara University Faculty of Medicine Local Ethics Committee (decision number:

**Table 1. The studies, which were published earlier about the cardiac involvement in Noonan syndrome**

| Study | n | Mean rGH dose, mg/kg/week | Mean duration of therapy, years | Result | Cardiac safety of rGH |
|---|---|---|---|---|---|
| Brown et al. (6) | 23 | 0.33 | 3 | HCMP did not develop in the follow-up. | √ |
| Noordam et al. (7) | 27 | 0.35 | 3 | Long-term use of high-dose rhGH has no effect on left ventricular thickness. | √ |
| Ozono et al. (8) | 51 | 0.23 | 2 | No change in ventricular wall thickness with rGH | √ |
| Noordam BJOK (9) KIGS study | 85 | 0.25 | 3 | No relationship between these cardiac events and rGH | √ |
| Romano et al. (10) NCGS | 65 | 0.28-0.38 | 5.6 ± 2.6* | No cardiac side effects | √ |
| Osio et al. (11) | 25 | 0.33 (n = 10), 0.66 (n = 15) | 2 (1-9) | No cardiac side effects | √ |
| Lampit et al. (12) | 22 | 0.9** | 2 | No effect of rGH treatment on left ventricular wall thickness | √ |
| MacFarlane et al. (13) | 23 | 0.33 | 3 | One mild left ventricular hypertrophy at initial evaluation had no change. The other two subjects developed an increase in left ventricular wall thickness, close to the upper limit, without other features of HCMP. | √ |
| Apperley et al. (14) | 12 | 0.25 | 3 | No progression in findings or cardiac side effects | √ |
| Cotterill et al. (15) | 27 | 0.33 | 1 | No change in the cardiac mass in the treated group | √ |
| Romano et al. (16) | 412 | 0.33 | 3 | One abdominal aortic aneurysm, one PS, three unspecified cardiovascular event | √ |
| Our study | 24 | 0.22 | 4.5 | No worsening of cardiac findings, and no change in ECHO parameters in either the rGH group or the non-rGH group. | √ |

*Mean ± SD. **mg/m²/day.
rGH: recombinant growth hormone therapy, HCMP: hypertrophic cardiomyopathy, PS: pulmonary stenosis, NCGS: National Cooperative Growth Study, KIGS: The International Growth Database, ECHO: echocardiography, rhGH: recombinant human growth hormone

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

I2-93-21, date: 11.02.2021). Children and adolescents diagnosed with NS, either clinically according to the Van der Burgt (4) criteria, or genetically and were followed up in our clinic between January 1st, 2000, and January 1st, 2021, were eligible for inclusion. Exclusion criteria comprised pre-existing HCMP before starting rGH, and less than six months rGH treatment prior to detailed cardiac assessment.

Patient data were retrieved from the archive records and included anthropometry and physical examination findings at diagnosis and during follow-up, laboratory and genetic evaluations, systemic disease screening results, and treatment response. Clinical features, such as birth weight and length, weight standard deviation score (SDS), height SDS, body mass index, bone age at diagnosis, and target height were also recorded. Systemic problems at presentation and during follow-up were recorded. The karyotype of all female cases was 46, XX. The definitive diagnosis of NS was made according to the Van der Burgt (4) criteria as follows: 1) Typical facial appearance + 1 major or two minor clinical characteristic findings, or 2) Facial findings suggestive of NS + 2 major or three minor clinical characteristics verified for each case.

At presentation, all cases were evaluated in detail in terms of short stature. In addition, all of them had normal hemogram, liver, and kidney function tests, blood glucose, thyroid function test, total immunoglobulin A (IgA) level, and tissue transglutaminase IgA antibody were negative. Complete urine analysis was performed on all patients. Data retrieved from laboratory records included IGF-1 levels, IGF binding protein 3 (IGFBP3) levels, GH stimulation test results and bone age. Serum GH (ng/mL) level was measured by a chemiluminescence method. Serum IGF-1 and IGFBP3 were tested by immunochemiluminescence (MyBioSource, Inc. P.O. Box 153308 San Diego, CA 92195-3308, USA). An adequate response to GH stimulation tests was considered to be > 10 ng/mL. Annual growth velocity, change in height SDS (Δheight SDS), and growth velocity SDS values of each case were calculated. All of the cases were evaluated with electrocardiography (ECG) and echocardiography (ECHO) at presentation and during follow-up by the pediatric cardiology department.

Each case was recalled for physical examination and cardiac evaluation. Anthropometric measurements such as body weight (kg), height (cm), and head circumference (cm) were measured during the examinations. The weight was measured with a scale tool approved by the Turkish Standards Institute (TSE) with 0.1 kg intervals, and height measurements were made with a TSE-approved measuring

device with 0.1 cm intervals. Weight and height SDS values were calculated according to the norms of Turkish children, based on data by Neyzi et al. (17) and according to the standard curves from data by Ranke et al. (2) for cases with NS. The growth velocity was calculated. The growth velocity SDS calculation was evaluated in accordance with Baumgartner references (3). Puberty staging was assessed according to Tanner Staging (18). Left wrist radiographs of the patients were evaluated using the Greulich-Pyle Atlas, and the bone age was assessed (19). Interventricular septal thickness in diastole (IVSed), interventricular septal thickness in systole (IVSes), left ventricular internal end-diastolic diameter (LVIDed), left ventricular internal end-systolic diameter (LVIDes), left ventricular posterior wall thickness in end-diastole (LVPWed), and left ventricular posterior wall thickness in end-systole (LVPWes) were measured with ECHO through a parasternal long-axis view using M-Mode Doppler. All measurements were made in triplicate, and the mean was used for statistical analysis. Z scores of IVSed, LVPWed, LVIDed, and LVIDes were calculated (20). This detailed evaluation was carried out at the beginning of the follow-up, on follow-up, and at last control. Previous ECHO reports of the cases were classified according to the evaluated periods before, during, and after GH. In addition, the Z-score of pre- and post-treatment ECHO parameters (IVSed, LVPWed, LVIDed, LVIDes) were evaluated with regard to being > + 2, and if the finding was above + 2 this was considered significant in terms of hypertrophy.

All cases were divided into two groups: those who were on rGH and those who were not on rGH treatment. The study was designed to show the change in ECHO parameters of the rGH group at the beginning and end of treatment.

## Statistical Analysis

### Data Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences for Windows, version 22.0 (IBM Inc., Armonk, NY, USA). The conformity of the variables to a normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Differences between independent groups were analyzed with the Mann-Whitney U test. The study was longitudinal and included multiple, repeated measurements of parameters measured in each patient, so Repeated Measurements ANOVA test statistics were used to assess differences in outcomes of Z scores of ECHO parameters (IVSed, LVPWed, LVIDed, LVIDes) before and after treatment over time. Statistically, $p < 0.05$ was considered significant.

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

# Results

## Features at the Presentation of the Participants

Twenty-four patients (16 boys, eight girls) were included, of whom 16 were treated with rGH and eight were not. Their details are shown in Table 2. Congenital heart lesions were present in 11/16 of the rGH group and in 6/8 of the non-rGH group. The most common finding was short stature (n = 22, 91.6%). For the whole cohort, the mean age at presentation was $8.02 \pm 4.30$ years. Patients in the rGH group were significantly older than the non-rGH group (p = 0.02). At the beginning of the follow up, the overall height SDS was $-2.56 \pm 0.94$ [data by Neyzi et al. (6)], and $+0.25 \pm 1.07$ [Noonan specific data by Ranke et al. (2)].

Protein tyrosine phosphatase, nonreceptor type 11 gene (*PTPN11*) sequence analysis was performed in 10 cases, and mutation was present in five cases. One patient had normal *PTPN11* sequence analysis, and *MAP3K7* (NM_145331.3) heterozygous p.P22H (c.65c > A) missense mutation was detected in the WES analysis. In other patients, the diagnosis of NS was made according to the Van der Burgt criteria.

## Laboratory Features

There was an insufficient response to the GH stimulation test in the group receiving rGH, and mean serum IGF-1 was $-0.92 \pm 1.31$ SDS (n = 15) and IGFBP-3 was $-0.70 \pm 1.70$ SDS (n = 15).

## Growth Hormone Treatment and Long-term Follow-up Findings

Sixteen of the patients were on rGH. The follow-up period of the rGH group was $8.3 \pm 3.8$ years, and the age of rGH initiation was $9.7 \pm 3.2$ years. The mean rGH dose was $0.22 \pm 0.04$ mg/kg/week, and the mean duration of treatment was $4.5 \pm 2.1$. The mean first-year growth velocity was $1.13 \pm 0.83$ SDS (n = 14). First-year $\Delta$height SDS gain was $+0.50 \pm 0.32$, second-year $\Delta$height SDS gain was $+0.39 \pm 0.48$, and third-year $\Delta$height SDS gain was $+0.16 \pm 0.60$ (Figure 1).

In the follow-up, 10 of the 11 patients who reached final height had received rGH.

## Cardiac Features

In this cohort, cardiac pathology was present in 17 (70.8%) cases, and 11/17 (64.7%) were in the rGH group. PS (n = 6, 25%) and ASD (n = 6, 25%) were the most common findings at the beginning of the follow-up. Other findings in order of frequency were: VSD (n = 4, 16.7%), mitral regurgitation (n = 2, 8.3%), and one each (n = 1, 4.2%) with aortic regurgitation, coarctation of the aorta, bicuspid aortic valve, HCMP, interventricular septal hypertrophy, interatrial septal aneurysm, isolated left ventricular noncompaction cardiomyopathy (noncompaction CMP), patent foramen ovale, and right ventricular hypertrophy (Figure 2). Two patients with PS underwent pulmonary valvuloplasty, and one subject with VSD and coarctation of the aorta required surgical correction.

Pre-treatment ECHO evaluation was performed in 12 (66.7%) patients in the rGH group and in 5 (62.5%) patients in the non-rGH group. In terms of ECHO parameters, the Z scores of the rGH group at baseline were significantly higher compared to the non-rGH group: IVSed $Z_{score}$ p = 0.02; IVSes $Z_{score}$ p = 0.008; LVIDed $Z_{score}$ p = 0.05; LVIDes $Z_{score}$ p = 0.02; LPWed $Z_{score}$ p = 0.04; and LPWS $Z_{score}$ p = 0.01.



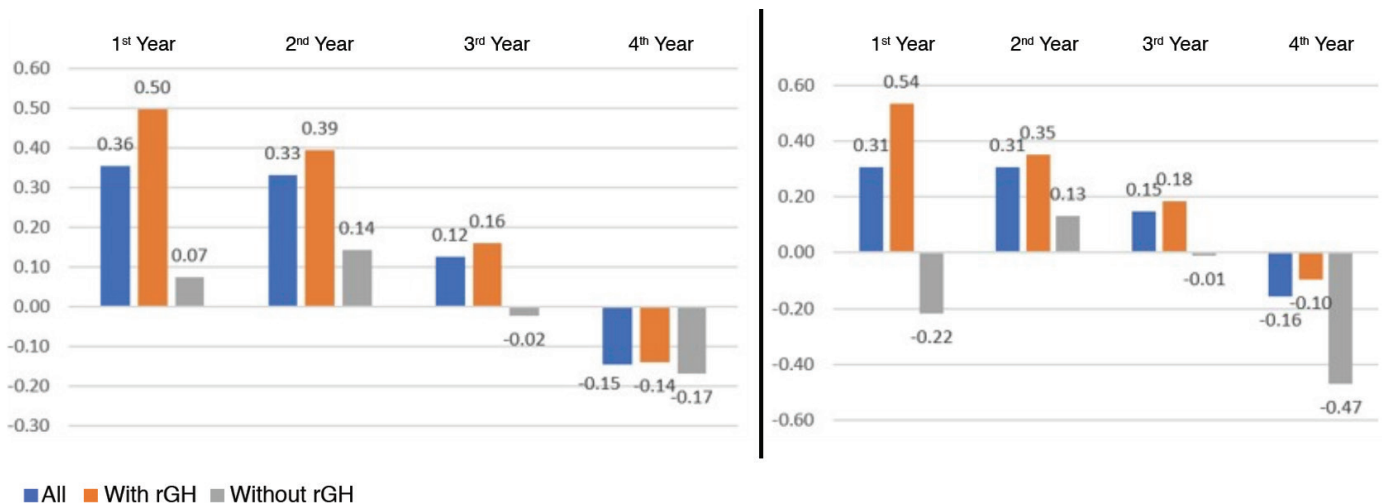**Figure 1.** $\Delta$Height SDS gain in all, the rGH group, and the non-rGH group according to data by Neyzi et al. (17) (left graph) and data by Ranke et al. (2) (right graph)

*SDS: standard deviation score, BMI: body mass index, rGH: recombinant growth hormone therapy*

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

When the post rGH therapy ECHO parameter $Z_{score}$ of the rGH group (n = 16) was compared to the non-rGH group (n = 8), no significant differences were observed between the two groups: IVSed $Z_{score}$ p = 0.5; IVSes $Z_{score}$ p = 0.3; LVIDed $Z_{score}$ p = 0.08; LVIDes $Z_{score}$ p = 0.2; LPWed $Z_{score}$ p = 0.27; and LPWS $Z_{score}$ p = 0.41.

When the ECHO parameters of rGH group at the beginning and the end of the treatment were compared, there was no
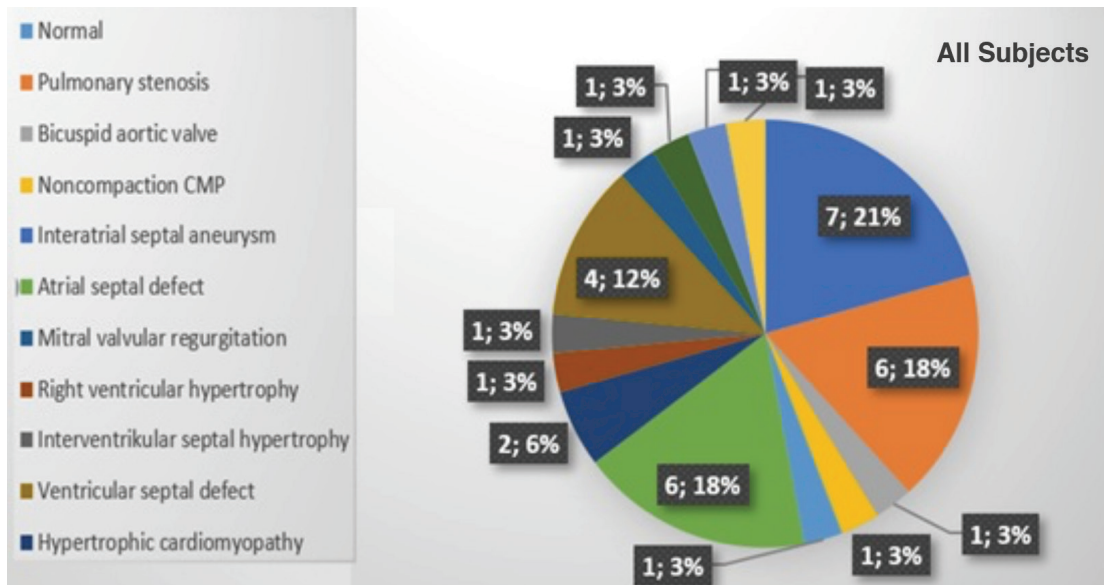


**Figure 2.** Distribution of cardiac pathologies

*CMP: cardiomyopathy*

**Table 2. Clinical characteristics of patients with Noonan syndrome and the effect of rGH**

|  | All (n = 24) | With rGH (n = 16) | Without rGH (n = 8) | p |
|---|---|---|---|---|
| At the beginning of the follow-up | 8.02 ± 4.30 | 9.72 ± 3.23 | 4.60 ± 4.15 | **0.02** |
| Gender (M:F) | 16:8 | 10:6 | 6:2 | 0.81 |
| %BMI | 91.19 ± 12.82 | 90.85 ± 13.64 | 91.86 ± 11.87 | 0.85 |
| Height SDS at admission | -2.56 ± 0.94 | -2.78 ± 0.89 | -2.12 ± 0.89 | 0.11 |
| Puberty Stage (prepubertal/pubertal) | 21/3 | 14/2 | 7/1 | 1 |
| Bone age | 6.73 ± 4.00 (n = 21) | 7.12 ± 3.65 (n = 16) | 5.50 ± 4.75 (n = 5) | **0.01** |
| Target height (cm) | 166.52 ± 8.31 | 166.06 ± 8.3 | 167.44 ± 8.82 | 0.6 |
| Target height SDS | -0.59 ± 0.78 | -0.52 ± 0.79 | -0.74 ± 0.76 | 0.39 |
| Target height SDS-Height SDS | -2.12 ± 1.08 | -2.49 ± 1.07 | -1.38 ± 0.61 | **0.02** |
| IGF-1 SDS | -1.00 ± 1.24 (n = 20) | -0.92 ± 1.31 (n = 15) | -1.25 ± 0.97 (n = 5) | 0.32 |
| IGFBP3 SDS | -0.87 ± 2.0 (n = 19) | -0.70 ± 1.70 (n = 15) | -1.53 ± 2.75 (n = 4) | 0.19 |
| Cardiac pathology (n, %) | 17 (70.8%) | 11 (64.7%) | 6 (35.3%) | - |
| rGH dose (mg/kg/week) | x | 0.22 ± 0.04 | x | - |
| rGH duration (year) | x | 4.5 ± 2.1 | x | - |
| ΔHeight SDS gain in the 1st year | + 0.36 ± 0.42 (n = 24) | + 0.50 ± 0.32 (n = 16) | + 0.07 ± 0.46 (n = 8) | **0.04** |
| ΔHeight SDS gain in the 2nd year | + 0.33 ± 0.46 (n = 20) | + 0.39 ± 0.48 (n = 15) | + 0.14 ± 0.32 (n = 5) | 0.16 |
| ΔHeight SDS gain in the 3rd year | + 0.12 ± 0.55 (n = 16) | + 0.16 ± 0.60 (n = 13) | -0.02 ± 0.12 (n = 3) | 0.09 |
| Subjects reached final height |  |  |  |  |
| Subjects reached final height (n) | 11 | 10 |  |  |
| Final height (SDS) | -0.94 ± 1.31 | -0.90 ± 1.37 |  |  |
| Target height SDS-Final height SDS | -0.40 ± 1.33 | -0.41 ± 1.39 |  |  |

*p < 0.05: Level of significance *Mann-Whitney U test.
SDS: standard deviation score, BMI: body mass index, rGH: recombinant growth hormone therapy, min-max: minimum-maximum, M: male, F: female, IGF-1: insulin-like growth factor-1, IGFBP3: IGF binding protein 3

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

significant increase in Z score of the IVSed, IVSes, LVIDed, LVIDes, LVPWed, LVPWes (Figure 3).

ECHO parameters of the rGH group and the non-rGH group (IVSed $Z_{score}$ p = 0.32, IVSes $Z_{score}$ p = 0.1, LVIDed $Z_{score}$ p = 0.23, LVIDes $Z_{score}$ p = 0.15, LPWed $Z_{score}$ p = 0.97, LPWS $Z_{score}$ p = 0.26) before and after treatment were not different (Table 3).

When each patient was evaluated individually, two cases in the rGH group had an IVSed Z score of > + 2 at the last follow-up visit, and an increase in the Z score of IVSed from + 0.59 to + 2.26 at the last follow-up was noticed in one of these. The IVSed Z scores of 2 of 3 patients with an IVSed Z score of > + 2 at presentation fell below < + 2 at the last follow-up. Although one of these patients had a Z score > + 2 at the last follow-up, the Z score of this patient also decreased from + 2.65 to + 2.09. There was only one case with a LVIDed $Z_{score}$ > + 2 at presentation. Afterward, the $Z_{score}$ dropped below < + 2. In the non-rGH group, there were no patients with LVIDed and LVIDes $Z_{score}$ > + 2 SDS at presentation or at final follow-up. LVPWed $Z_{score}$ of the only case with a Z score > + 2 at presentation decreased from + 2.81 to -0.35 during follow-up. In contrast to this, during follow-up, the Z scores of the two cases increased from 0.19 and 1.88 to 2.25 and 2.65, respectively, whereas both patients had a Z score < 2 at the last visit. When each group was analyzed on its own, in both groups, there was no significant difference

in the Z scores of IVSed, IVSes, LVIDed, LVIDes, LVPWed, LVPWes $Z_{score}$ between presentations (Table 3). At the final follow-up, none of the patients had a hemodynamically significant problem.

## Discussion

Severe short stature is the major finding of NS. It is known that patients benefit from rGH treatment. *PTPN11,* the gene most associated with the syndrome, encodes the intracellular protein tyrosine phosphatase SHP-2. SHP-2 is a negative regulator of GH activity. Although the cause of GH deficiency is heterogeneous, in NS cases, IGF-1 is generally low, and the response to rGH is good (1,21,22,23). In the present study, IGF-1 SDS was low in the rGH group, and all of them had an insufficient response to the GH stimulation test. No resistance to rGH was observed in *PTPN11* positive cases.

Şıklar et al. (10) conducted the first national multicenter study in Turkey, and 124 cases with NS were evaluated retrospectively. Forty-seven of those had received rGH treatment and, comparable to our results, height gain in the first and second year of the treatment was $0.40 \pm 0.44$ and $0.75 \pm 0.55$ SD, respectively; however, a difference occurred in the third year. While an increase of $0.76 \pm 0.41$ SDS was observed in the third year in the Şıklar et al. (10) cohort, the third year Δheight SDS gain decreased to $+ 0.16 \pm 0.60$ SDS in the present study (21). This may be

### Table 3. Z score of ECHO parameters comparision in all groups

| ECHO parameters | Group with rGH | | Group without rGH | | p |
|---|---|---|---|---|---|
| | $\overline{X} \pm SDS$ | $\overline{X}$ [min-max] | $\overline{X} \pm SDS$ | $\overline{X}$ [min-max] | |
| IVSed | 0.45 ± 1.13 | 0.19 [-1.5-2.23] | 0.71 ± 0.85 | 0.48 [-0.44-2.46] | group: F = 0.032 p = 0.859 time: F = 3.403 p = 0.079 group*time: F = 1.033 p = 0.321 |
| IVSed | 1.01 ± 0.65 | 1.16 [-0.34-1.59] | 0.87 ± 0.92 | 0.67 [-0.75-2.53] | |
| IVSes | 0.73 ± 1.29 | 0.2 [-1.12-2.43] | 1.05 ± 0.93 | 0.78 [0-2.65] | group: F = 0.238 p = 0.631 time: F = 0.017 p = 0.896 group*time: F = 2.880 p = 0.104 |
| IVSes | 1.21 ± 0.66 | 1.28 [0.12-2.28] | 0.48 ± 1.57 | 0.99 [-2.9-2.5] | |
| LVIDed | -0.72 ± 0.85 | -0.44 [-2.31-0] | -0.67 ± 1.37 | -0.08 [-3.03-2.08] | group: F = 1.669 p = 0.210 time: F = 6.224 p = 0.021 group*time: F = 1.561 p = 0.225 |
| LVIDed | -1.98 ± 1.24 | -2.27 [-3.76-0.22] | -1.09 ± 0.95 | -1.06 [-2.75-0.39] | |
| LVIDes | -0.63 ± 0.89 | -0.04 [-2.21-0.03] | -0.73 ± 1.25 | -0.41 [-3.34-1.3] | group: WTS = 0.297 p = 0.586 time: WTS = 6.181 p = 0.013 group*time: WTS = 2.110 p = 0.146 |
| LVIDes | -1.7 ± 1.24 | -1.52 [-3.27-0.3] | -1.02 ± 1.05 | -0.92 [-3.11-0.31] | |
| LVPWed | 0.97 ± 1.09 | 0.65 [0-2.81] | 0.62 ± 1.23 | 0.19 [-1.47-3.18] | group: F = 1.277 p = 0.271 time: F = 0.197 p = 0.661 group*time: F = 0.001 p = 0.971 |
| LVPWed | 1.13 ± 1.28 | 1.47 [-1.06-2.65] | 0.76 ± 0.8 | 0.66 [-0.6-2.35] | |
| LVPWes | 0.64 ± 1.23 | 0.13 [-1.19-2.24] | 0.19 ± 1.02 | 0.01 [-1.87-1.98] | group: F = 0.042 p = 0.840 time: F = 0.039 p = 0.846 group*time: F = 1.354 p = 0.257 |

IVSed: interventricular septal thickness in diastole, IVSes: interventricular septal thickness in systole, LVIDed: left ventricular internal end-diastolic diameter, LVIDes: left ventricular internal end-systolic diameter, LVPWes: left ventricular posterior wall thickness in end-systole, SDS: standard deviation score, ECHO: echocardiography, rGH: recombinant growth hormone therapy, min-max: minimum-maximum

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

related to the fact that 70% of our patients had cardiac findings, so we could not increase the rGH dosage. No safe dose range in terms of cardiac exposure has been reported in studies conducted so far. Due to the lack of evidence, we believe that the results reported herein, under standard-dose treatment, are a valuable addition to the limited published data in this field.

Due to the common finding of cardiac involvement in NS and the frequency of cardiac hypertrophy, there are concerns about the risk of increased ventricular wall thickness and the frequency of cardiac side effects when giving rGH therapy. Studies investigating the cardiac effects of rGH consist of retrospective or short-term prospective studies. To the best of our knowledge, there is no other study investigating the changes in ECHO parameters in NS patients not on rGH and comparing these with the group who had undergone rGH treatment.

There are questions concerning the use of rGH in NS, which include "Would cardiac pathology occur with standard-dose rGH by close monitoring of IGF-1?" and "Is concomitant cardiac pathology an expected finding due to the nature of the syndrome?". Assessment of published studies and

the present study shows that there is no clear consensus on the effect of rGH use in NS on cardiac findings. Again, since HCMP cases were excluded in some studies, there is no clear consensus in the literature regarding the follow-up process of HCMP cases. One of the patients in the non-rGH group had HCMP, and thus rGH was not initiated due to hemodynamic instability.

There are several studies investigating the effect of rGH in NS cases (Table 1). Brown et al. (13) showed that HCMP did not develop in the follow-up. The effect of rGH could not be clarified in cases with HCMP, since they were excluded from the study (6). Seo and Yoo (14) showed that rGH was not a risk for HCMP progression and tumor development (24). Of the six articles evaluating adult height in NS patients receiving rGH (n = 889), cardiac adverse events were described in only five patients, including two mild PS progression, one HCMP, one increased biventricular hypertrophy, and one cardiac decompensation (25). Noordam et al. (16) evaluated left ventricular thickness in 27 patients with NS. Mild, non-progressive HCMP was detected in one. Patients were divided into group A (rhGH was started immediately and stopped for two years) and



**Figure 3.** Z score of echocardiography parameters in all groups during follow-up

*IVSed: interventricular septal thickness in diastole LVIDed: left ventricular internal end-diastolic diameter, LVIDes: left ventricular internal end-systolic diameter, LVPWed: left ventricular posterior wall thickness in end-diastole, GH: growth hormone*

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

group B (one-year treatment was started and two years of treatment was given), and patients were compared over three years of treatment. Although initially, the left ventricular internal diameter was smaller and the posterior wall thickness was thicker than normal, there was no difference between the pre-treatment and at the fourth year of treatment (7). Ozono et al. (17) reported serious cardiac adverse events in four of 51 (7.8%) patients on rGH (8). These authors concluded that the treatment had no cardiac side effects, and no progression or new HCMP cases were reported. In a review, it was reported that there was no change in ventricular wall thickness with rGH (26). In most studies, it was shown that the left ventricular wall thickness was normal in prospective evaluations of NS patients on rGH. Studies have investigated the effect of rGH on left ventricular thickness, but there are very few studies examining left ventricular thickness progression, as in our study. Since rGH may theoretically worsen HCMP, no study has examined the effect of rGH on HCMP. HCMP was detected in one patient in our cohort in the non-rGH sub-group, and improvement in HCMP was observed in the seven-year follow-up. However, since this improvement is seen on a case-by-case basis, studies of patients with NS with HCMP are needed to provide more robust data.

The International Growth Database (KIGS) study is the largest NS case cohort in the literature. According to the twenty-year KIGS database, cardiac side effects were found in seven of 429 children with NS who received rGH, and it was reported that there was no relationship between these cardiac events and rGH (9). At the end of 25 years (1987-2012) of the same KIGS database, pacemaker implantation was required in one of the cases due to arrhythmia. The most serious adverse events reported to date have been left ventricular hypertrophy (after 2.0 years) and CMP requiring cardiac transplantation (after 10.7 years). Cardiac side effects were reported in only four of the NS patients receiving rGH treatment (27). The National Cooperative Growth Study group also reported no cardiac side effects in 150 children (97 males) treated with rGH (10).

There are studies of treatment with a higher rGH dose than was used in our study. Osio et al. (22) investigated the effect of rGH in 25 prepubertal NS patients without major cardiac anomalies. No cardiac side effects were detected and the authors suggested that there was no risk for HCMP progression or tumor development (11). Lampit et al. (23) performed ECHO follow-up for two years in 22 cases (12 in the control group) and did not detect any effect of rGH treatment on left ventricular wall thickness (12). MacFarlane et al. (24) evaluated 23 patients with NS using ECHO before rGH treatment.

Cardiac anomalies were detected in 18 and moderate cardiac hypertrophy in three. There was no change in the findings after the twelfth month of treatment. The findings were re-evaluated three years later. At initial evaluation, no change was found in the wall thickness measurement in the patient with mild left ventricular hypertrophy. The other two subjects developed an increase in left ventricular wall thickness, close to the upper limit of normal for age and body surface area, without other features of HCMP. No cardiac side effects were detected in other cases (13). Apperley et al. (25) investigated patients with NS who received a mean of 0.037 mg/kg/day rGH and detected cardiac anomalies in 8 (88%) of 12 patients, including PS, two with ASD, and one with HCMP. There was no progression in findings or cardiac side effects over an average of three years of rGH treatment (14). Cotterill et al. (26) excluded cases with an average left ventricular wall thickness of more than 10 mm in their multidisciplinary study. At the end of the first year of treatment, no change was found in the cardiac mass in the treated group (15).

Recently, Romano et al. (27) assessed the cardiovascular safety of GH in patients with NS (n = 412). Of the 18 patients with PS and three with HCMP at baseline, they had no worsening during treatment. After the beginning of rGH, one ruptured abdominal aortic aneurysm, one PS, and three unspecified cardiovascular events were observed. Given the low prevalence of cardiovascular comorbidities, they reported a safe profile of rGH treatment in patients with NS (16).

Since NS individuals with HCMP were excluded from treatment, the effect of rGH on NS cases with HCMP could not be evaluated. Except for the KIGS study, the follow-up times of all reported studies are very limited. The present study includes one of the longer follow up periods, outside of KIGS, at $8.3 \pm 3.8$ years of follow-up in patients who received rGH. After long-term follow-up, we did not observe any worsening of cardiac findings in either the rGH group or the non-rGH group. Although we used the standard dose of rGH, the difference between the target and final height improved.

The genotype-phenotype correlation in cardiovascular disease in NS is well established (28). *PTPN11* is the most commonly implicated NS gene (~50% of cases) and is found in 59% of familial cases and 37% of sporadic cases. SHP-2 plays an important role in valvular morphogenesis and the development of heart defects (4,28,29). While *PTPN11* mutations are found in 80% of NS patients with pulmonary valvular stenosis and ASD, there is an inverse relationship with HCMP. However, in *RAF1* variants, HCMP

Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

J Clin Res Pediatr Endocrinol
2022;14(4):422-432

is inversely related to PS (7,28,29). In our study, genetic evaluation was performed in a very limited number of cases. Therefore, the statistical correlation could not be investigated. Although the genotype-phenotype correlation has been established, it should not be assumed that *PTPN11* mutation will never be associated with HCMP. Thus, in our cases with *PTPN11* mutation, there was a case with HCMP. Athota et al. (30) also reported that 84% of 117 NS individuals with *PTPN11* mutation had cardiac defects, and 8.5% of them had HCMP. This confirms the wide genetic variability observed in NS patients. A compound heterozygous mutation of *MYBPC3* and *PTPN11* was reported in a patient with NS and HCMP (31). Although a relationship between genotype and phenotype has been established, no study has revealed a definite mechanism (32,33,34,35). However, mutation positivity in the relevant gene for the diagnosis is a confirmatory diagnostic criterion for NS, a normal result does not exclude the syndrome. Therefore, in 1994 Van der Burgt et al. (35) developed a clinical diagnostic system, which remains current today (4).

A cardiologist should evaluate all individuals with NS with ECG and ECHO at diagnosis. Those who are found to have cardiac defects should be followed up regularly. It is recommended that cardiac reassessments should be performed every five years in individuals without any evidence of heart disease at their initial assessment. If congenital heart disease or HCMP is detected in early infancy, close ECHO monitoring is recommended. Periodic cardiac evaluations should be continued in adults, even if evaluations are normal during childhood or adolescence. It should not be forgotten that unexpected cardiac findings may develop over time (25,28,29). Recently, Rohrer et al. (36) also showed no higher prevalence of cardiac comorbidities in patients with NS who had been treated with rGH but close follow-up was recommended.

## Study Limitations

The limitations of our study include the low number of participants despite all patients diagnosed in a single center over a 21-year period being eligible. The number of participants is as many as one-fifth of the participants in the Turkish national study (21). Only five participants were included the national study. The number of participants in the non-rGH group was lower, and the follow-up period was shorter than the rGH group. Since rGH was given at a standard dose, the relationship between high-dose rGH and cardiac parameters could not be evaluated.

## Conclusion

In this study, rGH was effective in achieving good final height in patients with NS. The difference between final height SDS and target height SDS was small even when using standard dose rGH. Although cardiac pathology was observed in 70% of cases at presentation, there was no change in ECHO parameters from the point of left ventricular hypertropia or dilatation on rGH therapy and during follow-up. We, therefore, conclude that the use of rGH was safe in this small cohort of NS patients, most with cardiac pathology, under close follow-up.

## Ethics

**Ethics Committee Approval:** The study was approved by the Ankara University Faculty of Medicine Local Ethics Committee (decision number: I2-93-21, date: 11.02.2021).

**Informed Consent:** Published written consent was obtained from parents and in children also their assent.

**Peer-review:** Externally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: Sirmen Kızılcan Çetin, Mehmet Gökhan Ramoğlu, Zeynep Şıklar, Elif Özsu, Zehra Aycan, Hasan Ercan Tutar, Merih Berberoğlu, Concept: Sirmen Kızılcan Çetin, Zeynep Şıklar, Hasan Ercan Tutar, Merih Berberoğlu, Design: Sirmen Kızılcan Çetin, Zeynep Şıklar, Merih Berberoğlu, Data Collection or Processing: Sirmen Kızılcan Çetin, Mehmet Gökhan Ramoğlu, Elif Özsu, Zehra Aycan, Hasan Ercan Tutar, Analysis or Interpretation: Sirmen Kızılcan Çetin, Mehmet Gökhan Ramoğlu, Merih Berberoğlu, Literature Search: Sirmen Kızılcan Çetin, Mehmet Gökhan Ramoğlu, Elif Özsu, Zehra Aycan, Hasan Ercan Tutar, Merih Berberoğlu, Writing: Sirmen Kızılcan Çetin, Mehmet Gökhan Ramoğlu, Zeynep Şıklar, Merih Berberoğlu.

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Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

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Kızılcan Çetin S et al.
Growth Hormone Therapy Related Cardiac Findings in Noonan Syndrome

J Clin Res Pediatr Endocrinol
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