Height Outcome of the Recombinant Human Growth Hormone Treatment in Subjects with Noonan Syndrome: A Meta-Analysis

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Abstract

Children with Noonan Syndrome (NS) present variable growth impairment associated to facial dysmorphism and cardiovascular anomalies. Mutations in several genes of RAS/MAPK signaling pathway have been identified, likely impairing Growth Hormone (GH) sensitivity. If untreated, these patients often remain short in adulthood. Although Recombinant Human GH (rhGH) treatment improves short-term linear growth, poor data on the Final Height (FHT) of rhGH-treated subjects with NS are available.

After thorough search of the published literature for pertinent studies, a meta-analysis evaluation of the efficacy and safety of rhGH treatment in NS patients were performed. In total sample (n=885; 70.0% males), administration of rhGH progressively improved height pattern, but relevant catch-up growth was not shown. The rhGH-induced growth improvement appeared until FHT [n=168; −2.151 SDS (95% CI −2.792 to −1.511)]. During 1st year of rhGH treatment, height velocity gain meta-correlated with serum insulin-like growth factor 1 (IGF1) increment [n=31; r=0.685 (95% CI 0.419 to 0.843); P<0.0001] while negative meta-correlation was detected between age at rhGH start and height velocity [n=48; r=−0.608 (95% CI −0.765 to −0.383); P<0.0001]. Height gain after 1-yr rhGH treatment (dosage range 0.35–0.46 mg/kg/wk) was higher in NS patients without protein tyrosine phosphatase non-receptor type 11 (PTPN11) gene mutation [n=33; 0.903 SDS (95% CI 0.552 to 1.254)] than that observed in subjects positive for PTPN11 mutations [n=62; 0.606 SDS (95% CI 0.274 to 0.938); P<0.0001]. While few serious adverse events were reported during rhGH treatment, causal relationship to rhGH therapy was unlikely.

In conclusion, this meta-analysis indicates that rhGH treatment progressively improved height outcome of short children with NS. Future studies using carefully titrated rhGH protocols are need to optimize treatment protocols and establish possible risks, if any, to lead to clear indications for practice and regulatory agencies.

Keywords: Final height; Growth hormone therapy; Height outcome; Insulin-like growth factor 1; Meta-analysis; Noonan syndrome; Ptpn11 gene mutation

Abbreviations: Final Height-Fht; Growth Hormone Deficiency-GHD; Hypertrophic Cardiomyopathy-HCM; Insulin-Like Growth Factor 1-Igf1; Noonan Syndrome-NS; Protein Tyrosine Phosphatase Non-receptor Type 11-Ptpn11; Recombinant Human Growth Hormone- RHGH; Standard Deviation Score - SDS

Introduction

Noonan syndrome (NS) is a rare genetic disorder, characterized by specific facial features associated to cardiovascular anomalies, mild mental retardation, deafness, visual problems, hypogonadism with cryptorchidism, clotting disorders, and short stature [1]. Subjects with NS are typically born with appropriate size for gestational age, but postnatal growth impairment occurs leading to short adult height of −2.4 SDS for both men and women [2].

Currently, identified mutations explain genetic background of approximately 60% of NS subjects [1]. In about 50% of NS patients, mutations were found in the protein tyrosine phosphatase non-receptor type 11 (PTPN11) gene (12q24.1), which encodes the protein tyrosine phosphatase SHP-2 [3,4]. SHP2 is a ubiquitous protein recruited downstream of many tyrosine kinase-dependent receptors that, once activated, dephosphorylates phosphorylated tyrosines. The best-defined function of SHP2 is its positive role in the activation of the RAS/MAPK (mitogen-activated protein kinase) ERK1/2 pathway [5]. In addition, SHP2 regulates other key signaling pathways, including the phosphoinositide 3-kinase (PI3K) pathway, the Src Family Kinase (SFK), the target of rapamycin (TOR) kinase, or the Janus kinase 2/signal transducer and activator of transcription 5 (JAK2/STAT5) module.

Biochemical studies have revealed that NS causing PTPN11 mutations result in hyperactivation of SHP2 catalytic activity by disrupting the inhibitory interaction between its catalytic and SH2 domains [6]. In this view, SHP2 binds to and dephosphorylates signaling molecules that are positive regulators of the cellular response to growth factors such as growth hormone (GH). Therefore, gain-of-function mutations of PTPN11 may be assumed to negatively regulate the cellular response to GH in NS subjects [5,7]. However, six other genes (i.e. KRAS, NRAS, SOS1, RAF1, BRAF, SHOC2) of RAS/MAPK signaling pathway were recently identified as causative for NS [8-12].

Albeit the etiology of short stature in NS subjects is not definitively known, Recombinant Human Growth Hormone (rhGH) therapy has been shown to improve growth pattern [13]. Despite the height gain showed during rhGH therapy by some authors, different response to treatment is reported. Factors such as rhGH dose, treatment duration,
age at therapy start and mid-parental height may affect height outcome [1]. Furthermore, genetic background may have major role, too [7]. Related to PTPN11 mutation status, some studies reported that growth responses to rhGH were significantly greater in mutation-negative subjects, consistent with the hypothesis of mild GH resistance in the mutation-positive patients [14-16]. However, data at Final Height (FH) after long-term rhGH therapy in NS children are poor [16-22].

Because the rhGH therapy could be advantageous in short individuals with NS, the aim of the present meta-analysis was to evaluate the long-term height outcome of rhGH treatment in these children. Safety aspects were also reviewed.

Patients and methods

The report of this protocol-based review was consistent with the PRISMA statement (Preferred Reporting Items for Systematic Review and Meta-Analyses) such as Cochrane Organization Criteria [23,24].

A computerized literature search using MEDLINE (PubMed) was conducted to identify previously published articles on the rhGH treatment of patients with NS throughout October 30th 2013. The used keywords were growth hormone, growth, somatotropin, somatropin, Noonan syndrome, PTPN11, KRAS, NRAS, SOS1, RAF1, BRAF, SHOC2 gene functions AND and OR during searches. We also screened the reference list of all published original articles and several reviews articles we found for additional references.

Two investigators independently examined the published manuscripts for possible inclusion and any discrepancies were discussed and resolved by consensus. Eligible studies were randomized controlled trials, published or unpublished, in any language who were allocated to either rhGH treatment or no active treatment. Only English full-text articles were included.

Two reviewers independently completed data extraction forms on each trial. These included data on the trial design, quality and outcomes. Where trial eligibility or methodological aspects were uncertain, authors were contacted for clarification. For cross-over trials, only data from the first phase of the study were included. For parallel studies, data were included up to the end of the randomized phase only. Calculations were performed by one investigator and checked by another. Discrepancies between the investigators were discussed and resolved by agreement.

The quantitative control of the bias was assured with the trim and fill method the sensitivity evaluation and heterogeneity analysis. The power concerning the significant differences was >0.8, assuring an appropriate sample size. Beg–Mazumdar’s tests were performed to measure publication bias, the tau b values calculated for all forest plot were always <0.2 with p-value <0.05. So there are no evidence for publication bias using Beg Mazumdar’s test. I^2 values were calculated to measure heterogeneity, the values of 3 forest plot were always <0.2 with p-value <0.05. So there are no evidence for publication bias using Begg–Mazumdar’s tests were performed the sensitivity evaluation and heterogeneity analysis.

The meta-analysis was performed considering sample sizes, standard errors and differences in means of continuous outcomes. Data were calculated with Comprehensive Meta-Analysis V2 software and summarized in the following forest plots.

Results

Of 116 eligible articles, 40 English-language reports evaluated growth outcomes of NS subjects using rhGH treatments. Seven case-reports were excluded from our analysis because used statistical software needs at least 2 patients for each groups while one study which reported NS height data including Turner syndrome ones. Because only clinical data expressed as arithmetic mean and Standard Deviation (DS) could be meta-analyzed, 27 studies were selected whose 5 articles also included genetic data.

From the 27 selected studies, 885 rhGH-treated children with NS were selected with male sex prevalence (n=808; 69.4% males; 30.6% females). It was not possible to separately analyze rhGH-induced growth outcome of NS subjects with growth hormone deficiency (GHD) for few and not standardized published data. Most of the enrolled patients were prepubertal (Tanner 1) at the start of rhGH treatment (median dosage 0.35 mg/kg/wk; range 0.17-0.46 mg/kg/wk).

In total NS patients (n=885), the mean height at rhGH therapy start was subnormal [-3.112 SDS (95%CI -3.315 to -2.909)] in all the studies (Figure 1a). As shown in Figure 1 (Figure 1b-d), height progressively improved during first 3-yrs of rhGH treatment, although not major catch-up growth was not present: rhGH-induced growth appeared constant until FHT [n=168; -2.151 SDS (95%CI -2.792 to -1.511)], which approached normal range (Figure 1e).

In NS subjects, the height velocity gain during 1-yr rhGH treatment meta-correlated with increment of serum IGF1 concentrations [n=31; r=0.685 (95% CI 0.419 to 0.843); P<0.0001] while negative meta-correlation was detected between the age at rhGH start and height velocity during 1-yr of treatment [n=48; r=-0.608 (95%CI -0.765 to -0.383); P<0.0001]. No other correlations were possible meta-analyzed for few and not standardized published data.

The rhGH-induced growth phenotypes were also meta-analyzed regarding target genotypes. At rhGH start, NS patients with PTPN11 mutations showed higher mean height [n=63; -3.161 SDS (95%CI -3.897 to -2.425)] than those without PTPN11 mutation [n=45; -3.759 SDS (95%CI -4.006 to -3.512); P<0.0001] (Figure 2a and Figure 3a). On the other hand, height gain after 1-yr rhGH treatment (dosage range 0.35-0.46 mg/kg/wk) was higher in NS patients without PTPN11 mutation [n=33; 0.903 SDS (95%CI 0.552 to 1.254)] than that observed in subjects positive for PTPN11 mutations [n=62; 0.606 SDS (95%CI 0.274 to 0.938); P<0.0001] (Figure 2b). It was not possible to analyze other genotypes (e.g. KRAS, NRAS, SOS1, RAF1, BRAF, and SHOC2 genes) for few published data.

The bone age chronologically progressed during rhGH treatment in NS patients, although most of rhGH-treated NS subjects presented pubertal delay. Few serious adverse events were reported in NS patients during rhGH treatment. Deterioration of cardiac function for hypertrophic cardiomyopathy (HCM), cardiac arrhythmias, angina pectoris and supravalvular aortic stenosis, were reported in 15 patients (1.7%) during rhGH treatment [19,20,25-27]. Although rhGH administration was precautionary interrupted in most of these patients, the causal relationship to rhGH therapy was concluded to be unknown. In addition, kyphoscoliosis, a feature of NS, worse were in 7 children (7.9%) [19,20]. Other adverse effects associated with known co-morbidities of NS were episodically reported (no more than a single case for each ones).

Discussion

NS is an heterogeneous clinical disorder [1,7]. In childhood, impaired linear growth is the most constant clinical feature of the syndrome, leading to FHt about ~2.5 SD for both males and females [2].
Figure 1: Meta-analysis of height at baseline, after 12, 24 or 36 months of recombinant human growth hormone treatment until final height in patients with Noonan syndrome. (A) baseline; (B) 12 months; (C) 24 months; (D) 36 months; (E) final height. Each included study is represented by one square while square area is proportional to sample size (i.e., patient amount). The horizontal lines represent the confidence intervals (95%CI) while the vertical lines crossing zero value (i.e., the no-effect vertical line) mean the absence of significant difference (e.g., no treatment effect). If the study square or its horizontal line overlap the no-effect vertical line, there is no statistical significance. The meta-analysis summary measure is reported at the bottom of the left side, corresponding to a diamond or small vertical bar. If the diamond does not cross the no-effect vertical line, the result of the meta-analysis is statistically significant. The values (difference in means, p-values, confidence intervals, etc.) are indicated between the study names and the graphic.
The cause of short stature is poorly understood. Serum levels of IGF-1 were often found to be low in children with NS, while GH secretion has been found normal or increased [1]. Suggesting a mild form of GH insensitivity, we also reported by demonstrating a subnormal increase of serum IGF1 levels after GH stimulation test [28]. These findings and positive results of rhGH therapy in other non-GHD syndromes (e.g. Turner syndrome and SHOX haplo-deficiency) prompted experimental trials with rhGH in NS subjects. Most trials involved small numbers of patients with different chronological ages at enrollment, treatment durations, rhGH doses leading to varied clinical outcomes [1]. Positive response to short-term rhGH interventions are shown [13,25,29]. Albeit only few studies reported FHT data [16-22], these results determined the rhGH indication for NS by US Food and Drug Administration but not by European Medicine Agency, leading to different prescriptive behavior in USA and Europe.

At our knowledge, this was the first meta-analysis on the rhGH treatment in NS. It differs from previous reports in using rigorous, systematic methods and meta-analysis: all published studies evaluating rhGH-therapy in patients with NS have been included and sensitive search strategy obtained a large number of patients. The use of meta-analysis permitted to estimate the size of rhGH treatment effect.

We meta-analyzed 27 studies comprising a large sample (n=885) of rhGH-treated children with NS, showing mean height gain of 0.813, 0.484 and 1.132 SDS after 12, 24 and 36 months respectively. Statistical significance at 0.001 level was just achieved at 12 months. Then, the growth rate was maintained until FHT filling mean 0.961 SDS (about 6 cm) improvement by rhGH therapy. When an increase of 5 to 10 cm in adult height is achieved, this can be considered as reasonable result, considering that it may allow reaching adult height in normal range in a large subset of patients. It is also noteworthy that this range of increase in FHT is similar with outcomes of rhGH therapy in other non-GHD conditions such as Turner’s syndrome or SHOX haplo-deficiency which usually gain a mean of 7 cm (range 5-15 cm) under rhGH therapy [30,31].

Despite the height gain varied largely (range 0.6–2.0 SDS, about 3-12 cm), the best results were found in studies that enrolled in younger [17,18]. When performing statistical analysis, either the younger the age at rhGH treatment is started [17] or the older the puberty onset [18], the better the results. In this regards, a negative meta-correlation between the age at rhGH start and height velocity during 1-yr of treatment was observed. No other correlations were possible meta-analyzed for few available data.

Figure 2: Meta-analysis of height at baseline and after 12 months of recombinant human growth hormone treatment in patients with Noonan syndrome and PTPN11 gene mutations. (A) baseline; (B) 12 months. Each included study is represented by one square while square area is proportional to sample size (i.e. patient amount). The horizontal lines represent the confidence intervals (95%CI) while the vertical lines crossing zero value (i.e. the no-effect vertical line) mean the absence of significant difference (e.g. no treatment effect). If the study square or its horizontal line overlaps the no-effect vertical line, there is no statistical significance. The meta-analysis summary measure is reported at the bottom of the left side, corresponding to a diamond or small vertical bar. If the diamond does not cross the no-effect vertical line, the result of the meta-analysis is statistically significant. The values (difference in means, p-values, confidence intervals, etc.) are indicated between the study names and the graphic.
Although rhGH dose did not seem to correlate with height velocity in NS [17], it is opposite to the growth improvement by rhGH which is generally dose-dependent in the other rhGH-treated conditions [32]. In this regards, we observed the height velocity gain positively meta-correlated with increment of serum IGF1 concentrations at least after 1-yr of rhGH treatment. Because serum IGF1 concentration usually positively correlates with rhGH dose [32], this finding may be due to the partial GH insensitivity related to the variable impairment of RAS/MAKP signaling pathway present in these patients. Further studies on this aspect should be performed in selected samples on the basis of genetic mutation, titrating rhGH dose according to serum IGF1 levels.

The endogenous GH is essential for normal post-natal growth and exerts its action after binding to its specific receptor that phosphorylates several tyrosine residues located in the intracellular domain [32]. Tyrosine dephosphorylation leads to the physiological interruption of GH signaling pathway. It has been consistently documented that PTPN11 gene-encoded SHP-2 negatively regulates GH receptor-JAK2-STAT5 signaling [5]. Thus, increased tyrosine phosphatase action of the SHP-2 protein, observed in PTPN11-mutated NS subjects, is expected to cause decreased GH action and consequently negatively affect linear growth [33,34]. Although circulating IGF1 levels are frequently low, IGF1 and IGF1-BP were significantly lower in the PTPN11 mutation-positive than mutation-negative patients in some [14,15,33] but not in all studies [14,35]. In opposite to [33,35], NS children with PTPN11 gain-of-function mutations seem to present lower growth velocity and lesser height gain during rhGH therapy than NS patients without PTPN11 mutations [14-16]. Finally, this meta-analysis confirmed that NS-related PTPN11 mutations cause partial GH-resistance state which could explain the moderate efficacy of rhGH treatment at least during first yr-treatment, emphasizing the need for alternative growth promoting therapies in this subgroup of NS patients [34].

Although the present meta-analysis indicates that rhGH treatment in short NS patients may improve long-term height outcome, some aspects should be better addressed. Present results were limited by the weaknesses of some trials, which often included few patients. Other problems were poor reporting study design, unavailability of raw data in many studies, failure to analyze by intention to treat, and possible inadequate concealment of allocation. Although examination of funnel plots suggested that a small-study effect was unlikely, the subgroup analyses or meta-regression, using study level covariates, could not be performed because of the relatively small number of available data. In addition, the follow-up period was relatively short, with only 7 studies following patients to FHt, which have been contradicting in data [16-22].

As rightly observed by Kelnar [36], included studies were generally
observational with no randomization, or placebo or other control (rather than comparison) groups. Comparison groups have tended to be made up of patients who refused treatment, or who could not be included for ethical or social reasons or because they were too tall or had severe cardiac anomalies [36]. Compliance have rarely been assessed and there have been no systematic analyses of patients who withdrew from studies [36]. There has been also inconsistent validation of NS diagnosis, particularly in the larger multicentre studies, which may have used pre-existing database records that in turn may have included patients with other disorders with similar phenotypes. Ascertainment bias in subjects recruited (towards those who are most dysmorphic, are shortest or have obvious cardiac abnormalities) may be also present [36]. Furthermore, a wide range of rhGH dosages have been employed, ranging from physiological replacement to pharmacological doses. Finally, the definition of FHt was the achievement of a chronological age above 19 years [2]. As puberty is delayed in Noonan syndrome [1], the total duration of growth can be prolonged into the early twenties, mainly in males, who represent about the 70% of meta-analyzed sample. Therefore, FHt may have been underestimated.

As cardiac defects and cardiac hypertrophy are frequent features of NS, there were some concerns regarding the long term effects of rhGH therapy on hearth function of these children. In particular, concerns were both for an increase in ventricular wall thickness and for higher incidence of cardiac adverse events. Among combined 889 patients who were followed up until adult height [17-22], only 5 cardiac events (2 mild progressions of pulmonary valve stenosis, 1 HCM, 1 increased biventricular hypertrophy, and 1 cardiac decompensation) were reported during long-term rhGH treatment. In all these patients, clear relationship between these events and rhGH therapy was considered unlikely [27]. Although most of studies have excluded patients with HCM, left ventricular wall thickness remained normal when prospectively measured in NS patients on rhGH [13,26,37]. However, there are virtually no data on the safety of rhGH therapy in children with pre-existing hypertrophic cardiomyopathy [36]. In this view, the reported absence of negative effects of rhGH therapy on the heart in NS and especially on ventricular wall thickness is reassuring. Nevertheless, keeping in mind the current limited experience, the heart status should be carefully monitored during treatment. Finally, similar to heart disorders, scoliosis is another common finding of NS, but no data indicate an increased risk of developing or aggravating kyphoscoliosis during rhGH treatment so far.

The present meta-analysis indicate convincing benefits of rhGH treatment in short NS individuals without severe adverse events, but larger randomized studies are needed to assess the magnitude of height gain compared to contemporary generations of individuals with NS. Whether this benefit is linked to increased quality of life remains to be elucidated [38]. Furthermore, PTPN11 mutations appear to be pharmacogenetic predictor of rhGH response at least during 1-yr of treatment. Whether the PTPN11 mutation, or other mutations in the RAS/MAPK signaling pathways [3,10], correlate with the height outcome to rhGH treatment up to FHt merits further evaluation in larger prospective and representative studies.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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