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## ABSTRACTS FROM THE LITERATURE

### GH Resistance in Noonan Syndrome: From Cause to Clinical Outcome

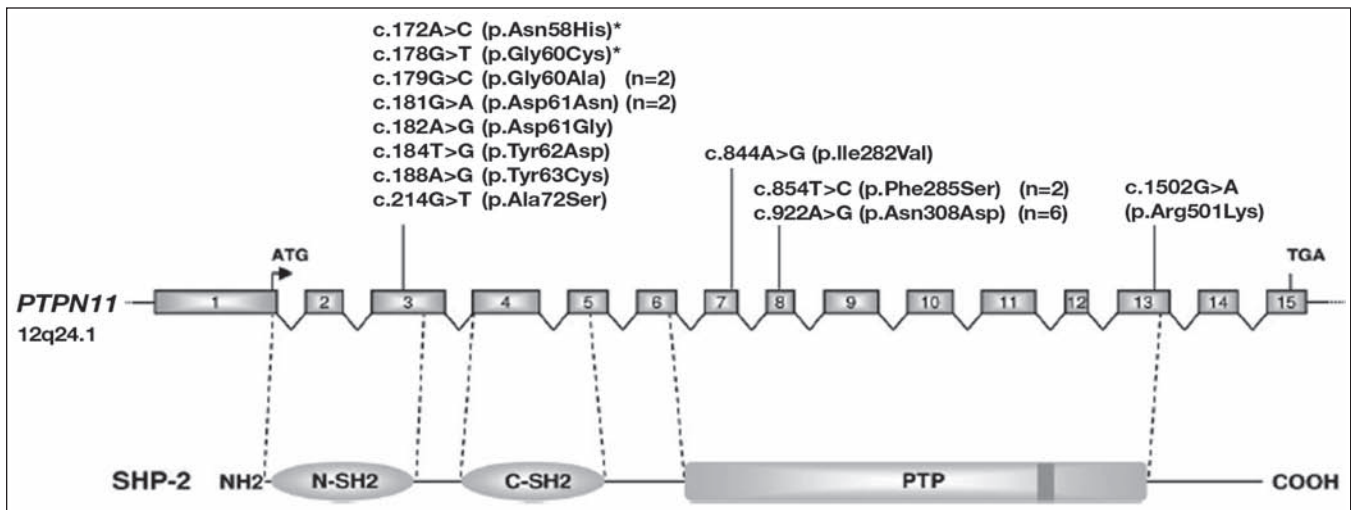
Proportionate short stature (SS) occurs in more than 70% of individuals with Noonan syndrome (NS), an autosomal dominant disorder found in 1:1000 to 1:2500 live births. NS is also characterized by typical facial dysmorphisms and cardiac defects, especially pulmonic stenosis and hypertrophic cardiomyopathy. Although prior growth hormone (GH) studies in these patients have shown mixed results (some normal, some abnormal, some suggesting neurosecretory deficiency), in general classic GH deficiency is a rare finding.

A causative gene for NS was identified in 2001: *PTPN11* (*protein tyrosine phosphatase, nonreceptor type 11*), which encodes Src homology region 2-domain phosphatase-2 (SHP-2). About half of individuals with NS harbor heterozygous missense mutations of SHP-2, the majority of which involve the amino SH2 (N-SH2) or the protein tyrosine phosphatase (PTP) domains (exons 3, 8, and 13). Both N-SH2 and PTP normally interact, keeping the ubiquitously expressed, cytosolic SHP-2 in a closed, inactive conformation. SHP-2 is activated upon binding of N-SH2 to a phosphotyrosine residue, such as those on activated receptors for GH, cytokines and other growth factors. By chronically stabilizing the SHP-2 open, and hence active, conformation, the missense mutations of NS would be expected to cause gain of function of this negative regulator of receptor signaling. SHP-2 can

not only dampen signaling through dephosphorylation of the receptor itself, it can also dampen downstream signals like dephosphorylating STAT5. Thus, SHP-2 mutations would be expected to cause GH resistance in patients with NS. Three recent papers studied this proposed hypothesis.

#### Mild GH Resistance

Binder and colleagues recruited all 29 children who presented to their center during the past 5 years with SS and at least 3 typical anomalies of NS or pulmonic stenosis. Blood lymphocyte DNA was extracted for PCR amplification and sequencing; 11 different missense mutations of *PTPN11* were found in 16 children from 14 unrelated families (55% of patients). Of these 11 mutations, 8 occurred in exons 3, 8 or 13. Comparing the mutation-positive (*mut*<sup>+</sup>) vs mutation-negative (*mut*<sup>-</sup>) subgroups, the former were found to have a higher incidence of pulmonic stenosis (81% vs 15%) and septal defects (63% vs 15%), and younger mean age at presentation (5.1 ± 2.7 vs 10.3 ± 5.2 years). Minor anomalies and height (−3.15 ± 0.92 vs −3.01 ± 1.35 SD) did not differ significantly, and all children were approximately 1 SD shorter in height than the mean for NS. While the higher spontaneous overnight and arginine-stimulated GH levels did not reach statistical significance, insulin-like growth factor (IGF)-I (−2.03 ± 0.69 vs −1.13 ± 0.89 SD) and IGF binding protein (BP)-3



Distribution of *PTPN11* missense mutations identified in 20 of the 35 NS patients. Mutations that have never been described are marked by an asterisk. The number of patients carrying the same mutation is indicated in parentheses.

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( $-0.92 \pm 1.26$  vs  $0.40 \pm 1.08$  SD) were significantly lower in the *mut*<sup>+</sup> group.

A subgroup of 11 prepubertal children received recombinant human (rh) GH for one year. Mean change in height SDS in the 8 *mut*<sup>+</sup> children ( $+0.66$  SD) was significantly lower than that in the 3 *mut*<sup>-</sup> children ( $+1.26$  SD). However, the *mut*<sup>+</sup> children received a lower mean rhGH dose (0.042 mg/kg/d vs 0.05 mg/kg/d).

Binder G, Neuer K, Ranke MB, Wittekindt NE. *PTPN11* mutations are associated with mild growth hormone resistance in individuals with NS. *J Clin Endocrinol Metab.* 2005;90:5377–5381.

### Response to 3 Years of rhGH Treatment

Ferreira and colleagues retrospectively analyzed the 14 children (10 male) followed at their Endocrinology Unit for NS; all had presented with SS (mean height  $-3.5 \pm 0.9$  SD) and were treated with (0.033–0.05 mg/kg/d) after a 6-month observation of baseline growth velocity. Eight of the children had been treated for 3 years, 4 for 2 years, and 2 for at least 1 year at the time of analysis. At the start of treatment, mean age was 12.3 years, bone age  $9.8 \pm 2.7$  years, and 10 were prepubertal. Seven children initiated puberty during treatment, and one received concomitant gonadotropin releasing hormone (GnRH) analog therapy. Treatment with rhGH was discontinued during the second year in one patient for increasing ventricular wall thickness; this patient had mild left ventricular hypertrophy before starting rhGH, and cardiac function continued to worsen afterwards despite cessation of rhGH.

Gene sequencing revealed 5 different, *de novo* heterozygous *PTPN11* missense mutations in 7 (50%) patients, 3 of whom were also seen among the children in the above Binder paper. At the start of treatment, the 7 *mut*<sup>+</sup> and 7 *mut*<sup>-</sup> patients did not differ in their GH secretory capacity (all had normal peak responses to clonidine stimulation; mean  $13.1 \pm 7.1$  ng/mL), nor

in their low IGF-I levels ( $-2.0 \pm 1.4$  SD). However, the rhGH-stimulated increment in IGF-I was significantly smaller in the *mut*<sup>+</sup> patients, as was the improvement in growth velocity, such that by the end of the third year of treatment, the *mut*<sup>+</sup> group had a significantly smaller gain in height SDS ( $+0.8 \pm 0.4$  vs  $+1.7 \pm 0.1$  SD;  $p < 0.01$ ). Bone age advancement did not differ between the 2 groups.

Ferreira LV, Souza SA, Arnhold IJ, Mendonca BB, Jorge AA. *PTPN11* (protein tyrosine phosphatase, nonreceptor type 11) mutations and response to growth hormone therapy in children with NS. *J Clin Endocrinol Metab.* 2005;90:5156–5160.

### Prospective Study of 2 years of rhGH Treatment

Limal and colleagues prospectively recruited 35 patients (19 boys) with NS and growth retardation (height  $< -2$  SD), excluding those with severe congenital heart malformations and/or hypertrophic cardiomyopathy. The 25 prepubertal children at study start (mean age  $10.4 \pm 3.1$  yr) were given rhGH 0.30 mg/kg/wk while the 10 pubertal children (mean age  $14.7 \pm 1.7$  yr) were given rhGH 0.46 mg/kg/wk to compensate for their late treatment start.

Sequence analysis revealed 12 different heterozygous *PTPN11* missense mutations in 20 children (57%) (Figure), 10 of which were previously reported; all but one occurred in exons 3, 8 or 13. The *mut*<sup>+</sup> subgroup had a higher frequency of small-for-gestational age (SGA [32%]) than the *mut*<sup>-</sup> (13%), though birth weight and head circumference were normal in all. At age 6 years, the *mut*<sup>+</sup> group was significantly shorter, as was their mean target height. Starting at age  $10.4 \pm 3.1$  years, 2 years of rhGH resulted in less catch-up growth among the prepubertal *mut*<sup>+</sup> children than the prepubertal *mut*<sup>-</sup> children; their end heights were  $-3.1 \pm 1.4$  SD (vs  $-2.0 \pm 0.9$  SD;  $p < 0.05$ ) and deficit from target heights were  $-2.5 \pm 0.9$  SD (vs  $-1.1 \pm 0.7$  SD;  $p < 0.01$ ).

At initiation, peak GH level following pharmacologic stimulation was  $15.4 \pm 6.5$  ng/mL (5–34.3) in all 35 children, though 5 of the *mut* had peaks of 5 ng/mL to 10 ng/mL. Of the 19 patients studied (11 *mut*<sup>+</sup> and 8 *mut*<sup>-</sup>), all had normal IGFBP-3, but they had IGF-I at or below the lower limit of normal, and acid-labile subunit (ALS) levels were extremely low in all 10 patients (5 *mut*<sup>+</sup>) tested at rhGH initiation. There was no difference between the 2 genetic subgroups in rhGH-stimulated increases in IGFBP-3 and IGF-I.

Limal JM, Parfait B, Cabrol S, et al. NS: Relationship between genotype, growth and growth factors. *J Clin Endocrinol Metab*. 2006;91:300–306.

**Editor's Comment:** *These 3 related papers offer intriguing glimpses into a possible mechanism of growth failure in NS. There are clearly additional mechanisms involved, since *mut* patients frequently also have SS. Nonetheless, as a group, these papers suggest new directions.*

### Mechanism

*In the idiopathic SS age of non-GH deficient growth failure, the quest has been on for molecular causes of post-receptor GH resistance. The search for individuals who harbor mutations in the signaling cascade directly downstream of the GH receptor has yielded fruitful results: novel GH receptor mutation that impairs GH receptor/STAT5 signaling but maintains normal STAT3 signaling,<sup>1</sup> mutations of STAT5b itself,<sup>2</sup> IGF-I gene partial deletion,<sup>3</sup> single copy number of the IGF-I gene,<sup>4</sup> and IGF-I receptor mutation.<sup>5</sup>*

*Yet these papers on NS serve as a reminder that a signaling cascade can be turned off (or down) not just by mutations from within, but also by mutations affecting molecules from without; gain of function mutations of*

*negative regulators of a cascade, such as SHP-2, can serve to augment the normal checks and balances and overly suppress the signaling cascade. This is not the first time that such possibility was shown. In 2001, 8 years after the FDA approved rhGH treatment for SS associated with chronic renal insufficiency, the molecular mechanism underlying the GH resistance was discovered. Comparing rats status-post partial renal ablation (chronic renal failure) and sham-operated, pair-fed rats (controls), Schaefer and colleagues found the former to have blunted hepatic induction of IGF-I expression by GH treatment despite unchanged GH receptor protein levels and GH binding to microsomal and plasma membranes.<sup>6</sup> Normal protein levels of JAK2, STAT5, STAT3, and STAT1 completed the cascade. Instead, these authors<sup>6</sup> found a 75% reduction in GH-induced tyrosine phosphorylation of JAK2, STAT5, and STAT3, due to over-expression of SOCS (suppressor of cytokine signaling)-2 and -3. The SOCS proteins normally function as a cellular internal feedback loop; they are induced by GH and in turn, inhibit GH-stimulated GH receptor/JAK2 complex activation to turn down the GH sensitivity of the cell.*

*The over-expression of SOCS in chronic uremia and the gain of function mutations of SHP-2 in NS may be just the beginning. Further search may reveal additional conditions involving augmented negative regulators, as well as loss of positive stimulators and enhancers, of the GH receptor/JAK/STAT signaling cascade. Thus, the quest for non-GH deficient causes of growth failure just got a whole lot broader.*

### Clinical Implications

*The increased GH resistance of PTPN11 *mut*<sup>+</sup> vs *mut*<sup>-</sup> patients reported in these papers suggests that a genotype-driven approach may be more effective*

*for ameliorating the SS associated with NS. Two treatment strategies may be plausible, and additional studies designed to test these approaches will be needed to determine their relative efficacies and safety. First, to overcome the increased GH resistance, rhGH therapy may require higher doses, and an approach titrating rhGH dose to achieve desired IGF-I levels rather than a standard weight-based dosing scheme, may be the best way to gauge clinical requirements of *mut*<sup>+</sup> vs *mut*<sup>-</sup> individuals. Thus, we may discover 2 different*

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optimal dosing levels based on genetic subtype. On the other hand, we may discover that the degree of GH resistance in the *mut<sup>+</sup>* individuals is so great that cranking up the rhGH dose really cannot compensate effectively or may be associated with undesirable side effects. In this scenario (the second treatment strategy), treating with recombinant IGF-I and/or IGF-1/IGFBP-3 rather than rhGH, may be more appealing. These therapies have now become available and were recently approved by the FDA.

Adda Grimberg, MD

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## Signal Transduction and Cardio-Facial Syndromes

The cardio-facio-cutaneous (CFC) syndrome (OMIM 115150) presents with heart malformations, skin defects, and characteristic facies. It overlaps phenotypically with Noonan syndrome (NS) and Costello syndrome (CS). Gain-of-function mutations have been identified in the protein tyrosine phosphatase SHP-2 (PTPN11) in about half of patients with NS. Recently, mutations of one of the RAS proteins known as HRAS were identified in several patients with CS. Interestingly, several CS mutations had been previously identified as somatic oncogenic mutations in tumors. SHP-2 and HRAS are components of a well-known signaling cascade through which many receptor tyrosine kinases transmit signals to the nucleus. Illustrated in the figure, this pathway, which is often referred to as the RAS-MAP kinase pathway, is often associated with proliferative and growth signals in developing tissues and in cancer.

Based on the suggestion that NS and CS might reflect activation of this pathway, a group headed by Aoki speculated that CFC syndrome might be due to mutations in genes encoding other proteins in this cascade. They first sequenced the entire coding regions of 3 RAS genes (*HRAS*, *KRAS*, and *NRAS*) in genomic DNA from 43 individuals with CFC syndrome. Two *de novo* *KRAS* mutations were detected.

Next, they screened for mutations in the 3 isoforms of RAF (*CRAF*, *BRAF*, and *ARAF*), which is immediately downstream of RAS in the signaling cascade. Eight *BRAF* mutations were identified in 16 patients, 6 of which mapped to the kinase domain, where mutations had previously been found in tumors.

The investigators proposed that the mutations they had identified enhance MAP kinase signal activity and tested this notion by expressing the mutant genes and their normal control counterparts in reporter cells that would allow downstream signal output to be measured. They observed a significant increase in signal output for 1 of the 2 *KRAS* mutations and in 4 of the 8 *BRAF* mutations, supporting their contention and the idea that increase MAP kinase signaling is common to all of the disorders in this group.

They reasoned further that if all of the disorders share a common increase in RAS-MAP kinase signaling activity, then there may be mutational overlap as well. Accordingly,

they screened for *BRAF* and *KRAS* mutations in *PTPN11*-negative NS patients and for *PTPN11* mutations in CFC patients negative for mutations in *BRAF* or *KRAS*. No additional mutations were detected, suggesting that the 3 disorders are distinct entities.

In an accompanying editorial,<sup>1</sup> it is noted that a recent publication identified *BRAF* mutations in 18 of 23 individuals with CFC. This study also found mutations in *MAP2K1* and *MAP2K2*, which are downstream effectors of *BRAF* in the RAS-MAP kinase signal pathway. The editorial also points out that molecules in which mutations have been found typically participate in other signaling pathways in addition to the primary linear RAS-MAP kinase pathway, which probably explains why each syndrome has unique features.

Niihori T, Aoki Y, Narumi Y, et al. Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome. Nat Genet. 2006;38:294–296.

**Editor's Comment:** *MAP kinase signaling pathways are more complex than suggested in the figure, and there is extensive crosstalk between subpathways. Nevertheless, placing these syndromes into a group that results from enhanced RAS-MAP kinase signaling serves a useful purpose, especially as inhibitors of this pathway might potentially have therapeutic benefit for postnatal manifestations of these disorders, such as short stature.*

*In contrast to most cell types in which RAS-MAP kinase signaling is associated with cell proliferation and growth, such signals in growth plate chondrocytes, where they are generated downstream of *FGFR3*, inhibit both cell proliferation and growth. Thus, it is conceivable that achondroplasia, which is due to activating mutations of *FGFR3*, NS, CS, and CFC syndromes share a common pathogenetic mechanism that involves excessive output of the RAS-MAP kinase signaling cascade in growing bone.*

William A. Horton, MD

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